Ocular Compatibility of Hydrogel Contact Lenses:

Deposition & Clinical Performance

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Summary

Currently over 50 million people worldwide wear contact lenses, of which over 75% wear hydrogel lenses. Significant deposition occurs in approximately 80% of hydrogel lenses and many contact lens wearers cease wearing lenses due to problems associated with deposition. The contact lens field is not alone in encountering complications associated with interactions between the body and artificial devices. The widespread use of man-made materials to replace structures in the body has emphasised the importance of studies that examine the interactions between implantation materials and body tissues.

This project used carefully controlled, randomized clinical studies to study the interactive effects of contact lens materials, care systems, replacement periods and patient differences. Of principal interest was the influence of these factors on material deposition and their subsequent impact on subjective performance. A range of novel and established analytical techniques were used to examine hydrogel lenses following carefully controlled clinical studies in which clinical performance was meticulously monitored. These studies established the inter-relationship between clinical performance and deposition to be evaluated.

This project showed that significant differences exist between individuals in their ability to deposit hydrogel lenses, with approximately 20% of subjects displaying significant deposition irrespective of the lens material. Additionally, materials traditionally categorised together show markedly different spoilation characteristics, which are wholly attributable to their detailed chemical structure. For the first time the *in vivo* deposition kinetics of both protein and lipid in charged and uncharged polymers was demonstrated. In addition, the importance of care systems in the deposition process was shown, clearly demonstrating the significance of the quality rather than the quantity of deposition in influencing subjective performance.

Keywords: contact lenses, biomaterials, deposition, symptoms, proteins, lipids

Dedication

This thesis is dedicated to my wife, Debbie, whose patience, love and never ending support are far more than any one person deserves.

Acknowledgements

"The mediocre teacher tells. The good teacher explains. The superior teacher demonstrates. The great teacher inspires". [1]

While undertaking this thesis I have been privileged to collaborate, deliberate and speculate with many people.

Firstly I would like to thank Professor Brian Tighe and Ian Davies for their never ending help, enthusiasm and discussion. Without their faith and trust I would never have been given the opportunity to undertake this project, which has given me such immense pleasure - most of the time! I would also like to thank Valerie Franklin and Kathryn Evans for their help with the fluorescence, HPLC and SEM data collection. In addition, I am grateful for the help received from Reyhaneh Sariri and Aisling Mann for the UV and activity data, James Ma and Steve Tonge for the *in vitro* wettability data, Cecile Maissa for her help in calibrating the instruments with protein standards and all my other colleagues and friends at the Biomaterials Research Unit at Aston University for their helpful discussions over the past five years.

In the practice several people have helped with the organisation and collection of the clinical data, in particular Debbie Jones, Claire Langley, Penny Carter, Linda Hine and Diane Slade. I would also like to acknowledge Colin Collett for his patience with me during my time away from the practice.

I also owe a great debt of thanks to my late father-in-law, Walter Nixon, who for five years looked after me one night a week and made my travelling to Aston bearable.

Finally I would like to thank Vistakon, a Johnson & Johnson company, for funding the study. Without them this project would not have been conducted.

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[1] William Arthur Ward

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LIST OF SYMBOLS AND ABBREVIATIONS

1/52	One week
1/12	One month
3/12	Three months
%	Percentage
°	Degrees
λ	Wavelength
γ_{lv}	Liquid-vapour interfacial free energy
γ_{sl}	Solid-liquid interfacial free energy
γ_{sv}	Solid-vapour interfacial free energy
θ	Contact angle
Å	Angstrom
ACMO	N-Acryoylmorpholine
AMU	Atomic mass units
ANOVA	Analysis of variance
ARE	Acute red eye
AZBN	Azo-bis-isobutyronitrile
B&L	Bausch & Lomb
BUT	Break up time
c	Velocity of light
c	Concentration
C	Centigrade
CCLRU	Cornea and Contact Lens Research Unit
CH_3	Methyl
CHN	Elemental (carbon, hydrogen, nitrogen) analysis
Ciba	Ciba-Vision
COOH	Carboxyl
cm	Centimetre
Cyl	Cylinder
D	Dioptre
D	Diffusivity
Dk	Oxygen permeability
Dk/t	Oxygen transmissibility
DR40	Vistagel PLUS contact lens material
DTT	Dithiothreitol
ϵ_λ	Molar absorbance coefficient at wavelength λ
E	Energy
EDTA	Ethylenediamine tetraacetic acid
EEMA	2-Ethoxyethyl methacrylate
EGDMA	Ethyleneglycoldimethacrylate
ELISA	Enzyme linked immunosorbent assay
EOP	Equivalent oxygen potential
ESCA	Electron spectroscopy for chemical analysis
EWC	Equilibrium water content
EWDCL	Extended wear disposable contact lenses
FCS	Fetal calf serum
FDA	Food & Drug Administration
FITC	Fluorescein isothiocyanate
g	Gram
GMA	Glyceryl methacrylate
GPC	Giant Papillary Conjunctivitis
hr	Hour
h	Planck's constant
H	Hydrogen
H_2O_2	Hydrogen peroxide
HEMA	2-Hydroxyethyl methacrylate

Hg	Mercury
HPMA	2-Hydroxypropyl methacrylate
HPLC	High pressure liquid chromatography
I	Intensity of transmitted radiation
I_0	Intensity of incident radiation
IR	Infra-red
ISO	International Standards Organisation
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
J&J	Johnson and Johnson
k	Solubility coefficient
kd	Kilodalton
Kg	Kilogram
l	Path length
LE	Left eye
log	Logarithm
µg	Microgram
µl	Microlitre
µm	Micrometre
m	Metre
MA	Methacrylic acid
MAR	Minimum angle of resolution
Med 66	Medalist 66 contact lens material
MEMA	2-Methoxyethyl methacrylate
mg	Milligram
min	Minute
ml	Millilitre
mM	Millimole
mm	Millimetre
MMA	Methyl methacrylate
mN	Millinewtons
mol	Molar
mOsm	Milliosmoles
Na+	Sodium ion
NH_2	Amino
NIBUT	Non invasive break-up time
nm	Nanometre
NNDMA	N, N dimethyl acrylamide
NS	Not significant (statistical term)
NVP	N-Vinyl pyrrolidone
O_2	Oxygen
OD	Optical density
OH	Hydroxyl
P	Gas permeability coefficient
p	Statistical significance
PAGE	Polyacrylamide gel electrophoresis
PBH	Pilkington Barnes-Hind
PBS	Phosphate buffered saline
PC	Phosphorylcholine
PEG	Polyethylene glycol
PEO	Polyethylene oxide
pH	l/Log the hydrogen ion concentration
PHMB	Polyhexamethylene biguanide
PLTF	Pre-lens tear film
PLTFBUT	Pre-lens tear film break-up time
polyHEMA	Poly (2-hydroxyethyl methacrylate)
POTF	Pre-ocular tear film
POTFBUT	Pre-ocular tear film break-up time

pK	1/Log the rate constant of weak acid
PLNIBUT	Pre-lens non-invasive break-up time
PLTF	Pre-lens tear film
PMMA	Polymethyl methacrylate
POTFBUT	Pre-ocular tear film break-up time
PVA	Polyvinyl alcohol
PVP	Polyvinyl pyrrolidone
r	Correlation coefficient
RE	Right eye
RGP	Rigid-gas permeable
RID	Radial immunodiffusion
RM	Repeated measures (statistical term)
S_o	Electronic ground state
S_1	First electronic excited state
SiO_2	Silica (silicon dioxide)
sd	Standard deviation
SDS	Sodium dodecyl sulphate
sec	Second
SEM	Scanning electron microscopy
SNK	Student Neuman Keuls (statistical test)
T	Transmittance
TMH	Tear meniscus height
TLC	Thin layer chromatography
TRITC	Tetramethylrhodamine B isothiocyanate
TSP	Tear specific pre-albumin
UK	United Kingdom
USA	United States of America
USAN	United States Approved Name
UV	Ultra violet
v	Frequency
vs	Versus
VAR	Visual acuity rating
VDU	Visual display unit
VEG	Von Ebner gland
WJ	Wesley-Jessen

Chapter 1

Introduction

"There is nothing as remarkable as learning to think better". [1]

Deposits that occur on or within the matrix of contact lenses inconvenience both practitioners and lens wearers alike. Significant deposition occurs in 80% or more of soft lenses [1,2] and approximately 30% of all check-up appointments require remedial action relating to a deposit-related complication. [3] Approximately 35% of patients permanently cease wearing their lenses due to problems associated with discomfort, dryness and poor visual acuity, [4] possibly as a direct consequence of lens deposition. [5] These subjects remain lost to follow up, potentially resulting in an underestimation of the number of deposit related complications.

The contact lens field is not alone in encountering complications associated with interactions between the body and artificial devices. The study of interactions between implantation materials and body tissues is increasingly important, particularly since the widespread introduction of man-made materials to replace structures in the body. The implantation material or "biomaterial" undergoes various interactions with the host biological environment, with the true "biocompatibility" of any given material depending upon many factors, relating to both the host and the implanted material.

The underlying process occurring in contact lens deposition is common to many rejection processes occurring with biomaterials. These include the clotting of blood at foreign surfaces, the formation of dental plaque and the fibrous encapsulation of implantation materials. [6] In attempting to develop truly biocompatible materials it is necessary to understand and unravel the complex interactions that occur with currently available materials and the host environment. Obvious limitations occur in the investigation of implantation devices via *in vivo* studies, in that the subjects must be sacrificed or die before it is possible to remove and analyse the material in question. The ocular environment is an ideal site to study biocompatibility as it is readily accessible and the tear film, while complex, is simpler than blood and contains none of the cellular components and species such as platelets that complicate the study of blood contacting devices.

The aim of this project was to gain a greater understanding of the role of tears, care systems, extraneous factors and hydrogel materials in the mechanisms of spoilation of soft contact lenses. Hydrogel materials are principally of interest as they are the most commonly worn and are closer in design to current implantation devices than are lenses of a rigid nature. A major analytical complication with such a study relates to the minute quantity of deposited material that is present on single lenses. Much of the published work to date has involved the use of techniques that are more pertinent to the analysis of bulk quantities, involving the pooling

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[1] Unknown

of many lenses such that a sufficient quantity of the material of interest is produced. Furthermore, prior to the introduction of frequent replacement lenses in the late 1980's, sourcing lenses for analysis was extremely difficult, with studies being frequently undertaken on lenses at the end of their useful life. The history of such lenses was commonly unknown and they were typically received from uncontrolled sources, with varying wearing times, length of life and care system usage.

This project will concern itself with the detailed analysis of lenses harvested following carefully controlled clinical studies and will correlate the spoilation of such lenses with alterations in clinical performance. Finally, this project will involve itself in the development of new techniques and the enhancement of existing techniques, such that single contact lenses can be accurately and sensitively analysed non-destructively.

Chapter 2

Literature Review

"To treat your facts with imagination is one thing; to imagine your facts is another".[1]

Hydrogel lenses become rapidly spoiled with constituents from the tear film, particularly protein, [7] lipids [8] and mucin. [9] *In vivo* studies indicate that 50% of a lens becomes visibly spoiled within thirty minutes of insertion [10] and that protein deposits are detectable after one minute of wear. [7 11 12] The deposition of tear-derived substances onto contact lens surfaces is a complex subject, with no direct correlation between the degree of deposited protein and that which is measurable in the tear film. [13]

Contact lens deposits have many effects on the eye and visual adnexa. There is clear evidence that they:

- Reduce visual acuity [14 15] and contrast sensitivity. [16] Other factors related to measurable visual performance with hydrogels (such as forward light scatter) are not related to visible deposition. [17]

- Increase the incidence of Giant Papillary Conjunctivitis (GPC),[18 - 21] although the exact role of deposits in the aetiology of GPC is not entirely clear. It is proposed that a combined type I (immediate) and type IV (delayed) hypersensitivity reaction occurs, with the deposits acting as an antigen. [22 23] Additionally, mechanical trauma due to the lens edge and poorly wetting surfaces plays a significant role. Regular replacement of lenses can reduce the GPC rate from 6% to 0%. [24]

- Induce a greater predisposition to superior limbic keratoconjunctivitis. [25]

- Produce an increase in the level of the Acute Red Eye (ARE) Syndrome. [24 26]

- Reduce the levels of tear secretory IgA. [27] As this is part of the eye's defence mechanism it is theoretically possible that patients with significant deposition could be at potentially increased risk of developing eye infections.

In addition, the following factors may be directly influenced by deposition, although the evidence is less conclusive and the causative factors are probably multifactorial:

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[1] John Burroughs

- Reduced lens comfort, [5 28] although other studies suggest that there is no direct relationship between comfort and either laboratory assessment of protein deposition [29] or visible deposition. [30] These conflicting reports demonstrate the complexity of this issue and that comfort is influenced by more than one factor.

- Reduced oxygen transmissibility, [31 32] although other studies [33 - 35] report that deposition has no impact on transmissibility.

- Increased bacterial adherence to hydrogel materials by 30-45%, [36 - 42] potentially increasing the risk of microbial keratitis. This issue is a complicated and contentious one, with other studies finding that deposition plays no role in bacterial adherence, [43 44] a selective role [45] or actually helps to prevent bacterial adherence. [46 47]

2.1 CLASSIFICATION OF DEPOSITS

It is difficult to accurately categorise and compartmentalise deposits, either visually or biochemically. [48] The easiest method of classification is to divide them into "soft filmy" and "hard nodular" types and to consider whether they are surface related or distributed within the bulk of the lens matrix. One of the earliest and most comprehensive descriptive studies concerning deposits was that of Kleist, [49] whose broad descriptive outline is followed below. The figures accompanying the text were taken on a Nikon FS3 photo-slit-lamp or under a Leitz Dialux 20 microscope with a Wild MPS 15/11 camera.

2.1.1 Protein

Protein films are virtually invisible during the early stages of the spoilation process, but assume a thin, translucent, whitish appearance with time [50] (Figure 2.1) as the protein denatures. [51] It remains primarily on the surface, but may penetrate into the lens matrix with high water content lenses [52] and accounts for some 35% of visible lens deposits. [53] The film consists of several protein types, [54 - 56] of which lysozyme is often quoted as the major constituent. [51 57 58] However, this is a fallacy, [50 59 60] brought about by the fact that lysozyme is relatively easy to remove from lenses compared with other proteins such as lactoferrin and albumin, [61 62] preventing their analysis by destructive techniques such as electrophoresis, which relies on the removal of deposited species from the lens surface. Protein deposition onto hydrogel materials is a highly complex process, depending in part upon the size and charge of the protein, environmental pH, charge and water content of the substrate in question and competition between the various proteins present. [7 11 54 60 63] The protein requires removal before it disrupts visual acuity and possibly potentiates giant papillary conjunctivitis. [20 21] Cleaning systems are only partially effective at removing the deposited film, [62 64 65] removing only 30-50% of the deposited protein. [62 65] Biochemical bonds between the lens and the protein film generally necessitate the use of enzyme tablets to facilitate any significant degree of removal, [66 - 68] with such procedures producing significant improvements in both *in vivo* comfort and visible deposition. [64 67]

Figure 2.1 - Translucent Appearance of a Protein Film

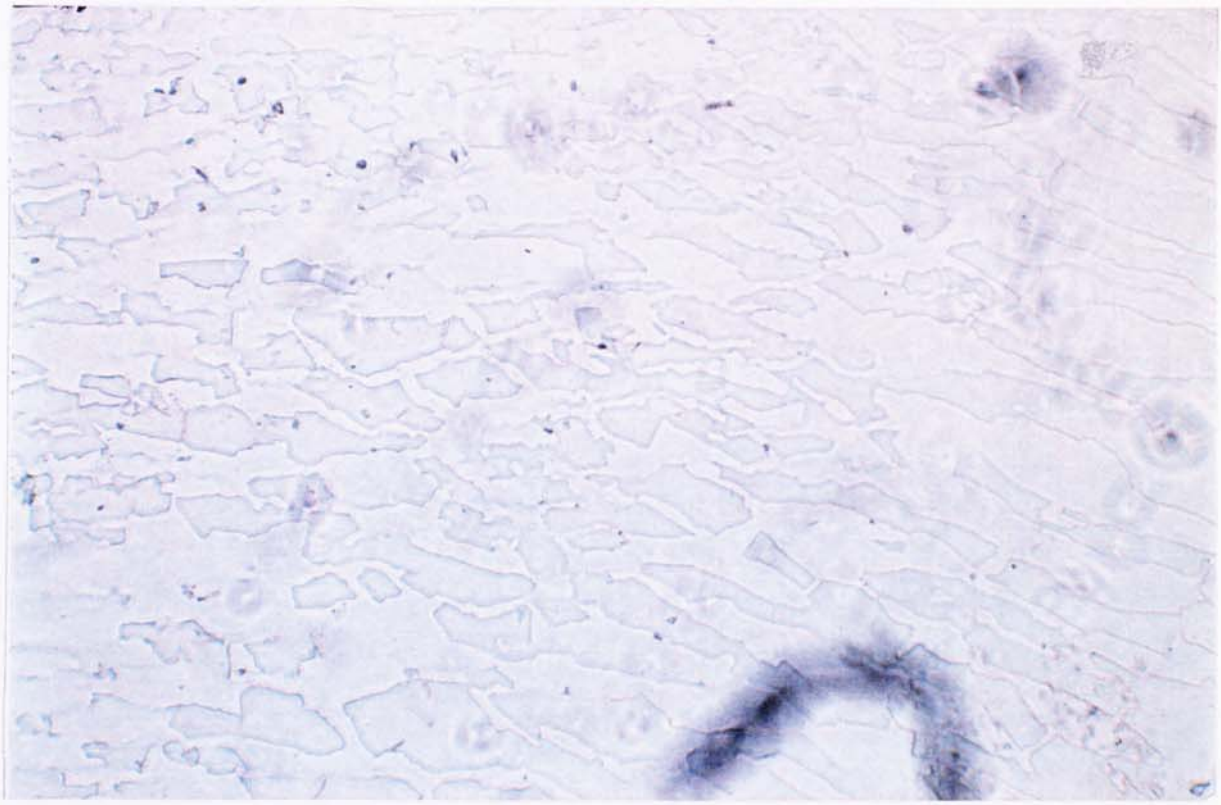


Figure 2.1 - Bright field optical microscopy picture of a protein film on a polyHEMA lens worn for 14 months

2.1.2 Lipid

Lipid deposits are clinically recognised by their greasy, shiny appearance (Figure 2.2). Lipoidal deposits arise from the meibomian glands, are removed by surfactant cleaning via a detergent action, [69] render the lens surface hydrophobic and may reduce visual acuity. [49] They occur more often in patients who exhibit a reduced tear volume [2] and when chlorhexidine containing solutions are used. [2 49]

The naturally occurring lipids biochemically identified include phospholipids, neutral fats, triglycerides, cholesterol, cholesterol esters and fatty acids. [70 - 72] Extraneous sources are often the cause of such deposits, particularly handling [57 72] and cosmetics. [57 73]

Figure 2.2 - Shiny Appearance to Lipoidal Deposits

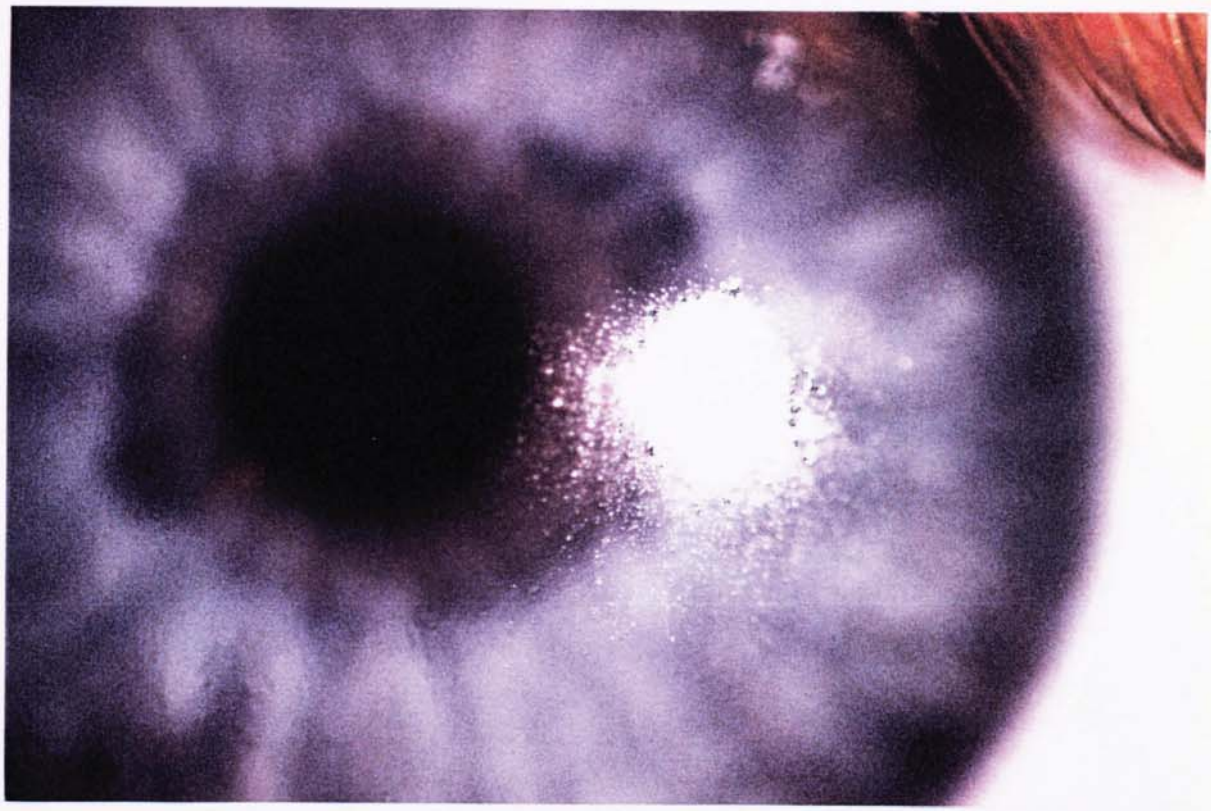


Figure 2.2 - Slit lamp appearance of a greasy soft lens. This polyHEMA lens was 9 months old and had been disinfected with hydrogen peroxide only, with no daily cleaner being used.

2.1.3 Calculi

These discrete, elevated, round or oval deposits are also termed "jelly-bumps", [74] "calcium deposits", [75] "mulberry spots", [76] mucoprotein lipid deposits [58] and calculi. [77] They occur singly or in clusters on the anterior surface of lenses (Figure 2.3), [49 78] exhibit birefringence, [49 58] range in diameter from 15μm to 1mm [57 78] and continue to grow until the lens is replaced. They are more common with high water content lenses, particularly if worn on an extended-wear modality, [78] and there is some individual patient predisposition. [77 79] As they physically grow into the lens removal leaves a "pit" in the surface, which acts as a site for further deposition. [49 73] The only solution to their presence is replacement of the lens.

Calculi penetrate the lens surface (Figure 2.5) and are formed by the successive laying down of globular structures that are tightly packed together (Figure 2.4). [80 81] They are the most intricate manifestation of ocular incompatibility and have a complex morphology [78] and chemical composition. [8 71 77 80 82] Several components are involved, principal among these being lipid, [8 77 80 82 - 85] calcium [2 82 84 86] and protein. [77 87] The variability in quoted composition is in part due to the quality and sensitivity of the analytical techniques used. The most widely accepted theory is that they consist of a combination of all the major components of the tear film, of which 90% is lipoidal in nature. [8 80 84 85 88] Cholesterol, cholesterol esters, mono-, di- and triglycerides, fatty acids and fatty alcohols are all present. [72 82]

They occur in 10 - 20% of visibly deposited lenses,[49 53 89] particularly being found in patients who have poor quality and/or quantity of tears [8 79 83] and/or meibomian gland dysfunction. [90] One commonly accepted theory [8 30 91] proposes that a destabilised tear film thins to a critical level, resulting in unsaturated fatty acid lipids contacting the lens surface. [88] These lipids attach via polymerisation and produce areas of non-wettability, which act as a further site for lipid attachment. Once a lipid nidus is formed then calcium soaps form secondarily through interaction with free fatty acid groups. This explains why most of these deposits occur within the inter-palpebral area, particularly in cases of inadequate or incomplete blinking as the diminished blinking results in drying, favouring the development of subsequent deposits. [28 70 83]

Figure 2.3 - Clinical Appearance of Lens Calculi

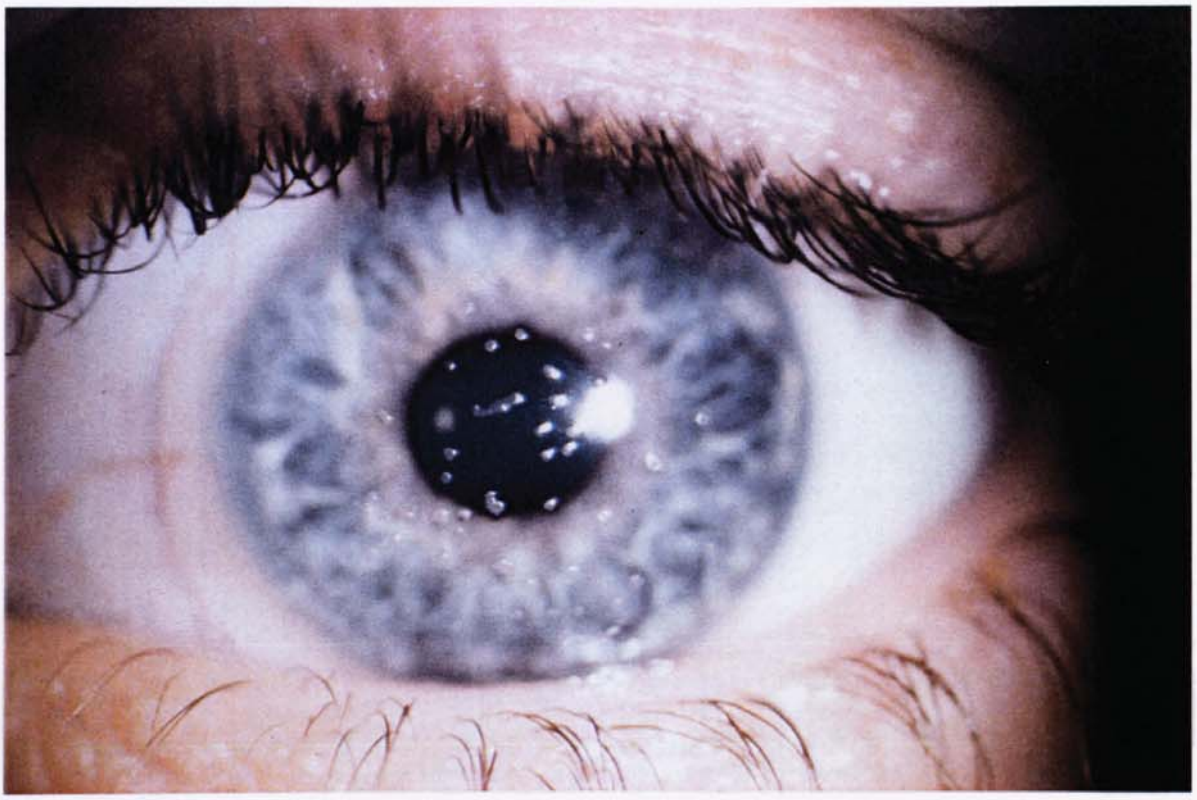


Figure 2.3 - Slit lamp appearance of lens calculi. This high water content lens was only 6 months old. Picture courtesy of Craig Woods.

Figure 2.4 - Gross Morphology of Lens Calculi (Aerial View)

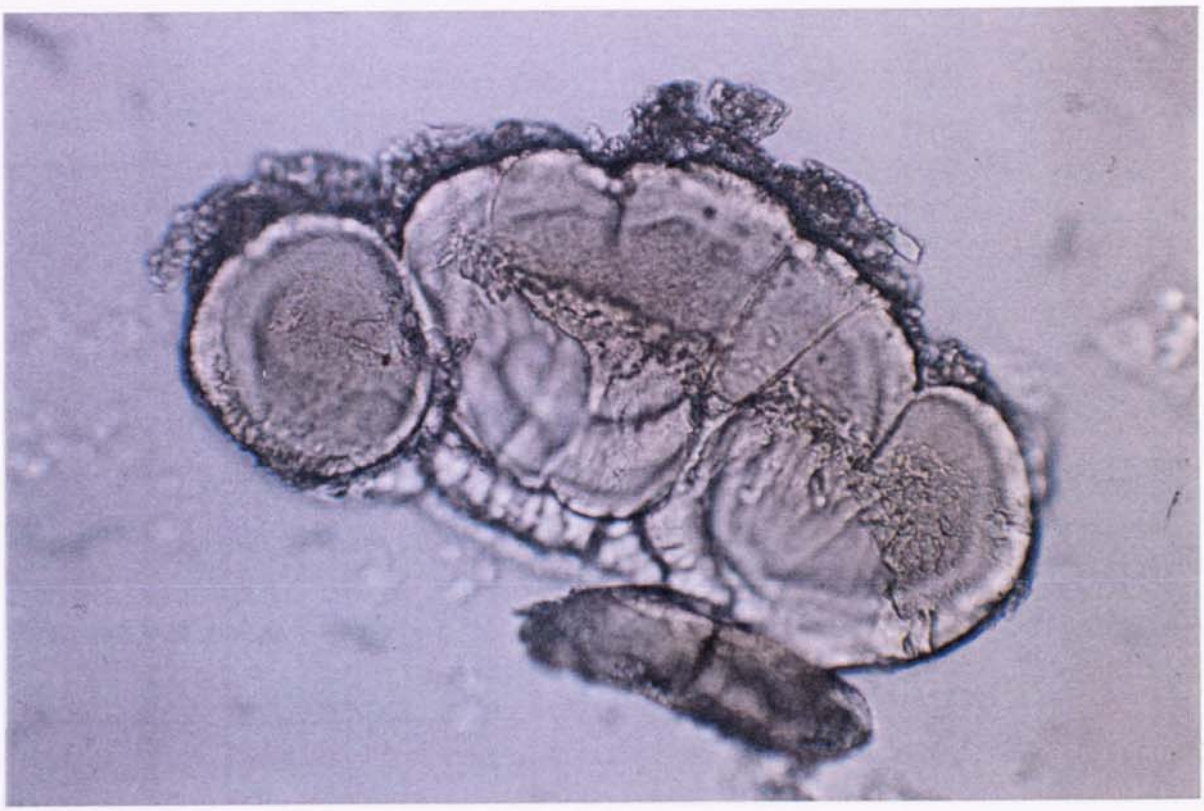


Figure 2.4 - Optical micrograph of lens calculi. The lobular structure is clearly seen.

Figure 2.5 - Gross Morphology of Lens Calculi (Sectional View)

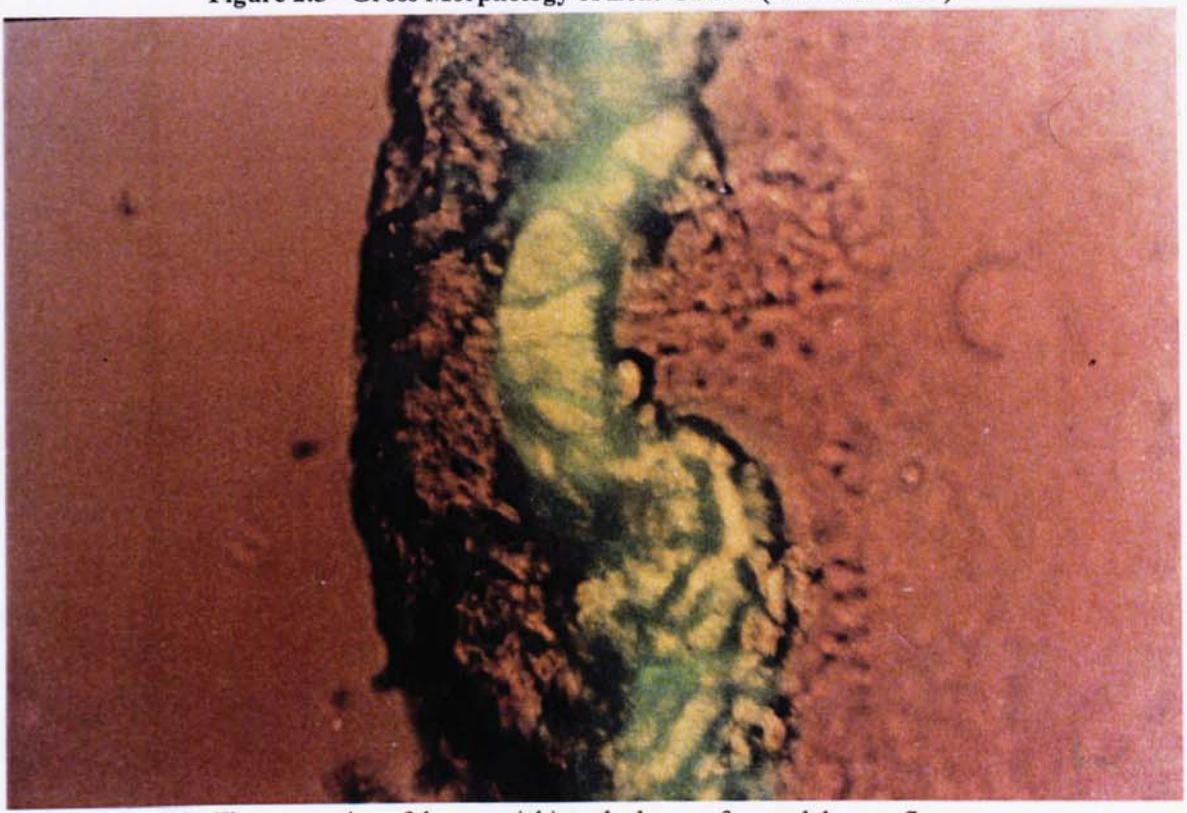


Figure 2.5 - The penetration of the material into the lens surface and the autofluorescent nature of the underlying lipoidal base is also clearly seen. Picture courtesy of Valerie Franklin.

2.1.4 Mucin

Conjunctival goblet cells produce mucin that may attach to lenses [2 54 57] and are identified in the laboratory using histochemical staining procedures. [54 92] On the lens they are often found associated with proteins and lipids [92] (particularly if heavy deposition is present [54]) and are often the base layer on which subsequent deposition of such materials occurs. [2 54] They are possibly involved in the binding of pathogenic microorganisms to lenses via the production of a polysaccharide-rich "biofilm". [73]

2.1.5 Calcium

Tears contain a significant amount of calcium. [93] In the early stages of calcium salt deposition (generally calcium carbonate or phosphate), inorganic calcium deposits may appear as a whitish film, which is frequently mistaken for a protein film. [57 94] They may also form more discrete, localised deposits that have a chalky-white appearance. They occur in 5-10% of visibly deposited lenses [53] and are often found overlaid by a lipid-rich organic layer, consisting principally of cholesterol esters. [95]

Evidence from *in vivo* and *in vitro* studies suggests that these deposits result from a simple mechanism in which the bulk and surface chemistries of the lenses play a negligible role. [95] They result from the imbibition and interaction of buffer-derived ionic species within the gel matrix during lens storage. On insertion into the eye nucleation sites develop following contact with calcium and phosphate rich tears. Atmospheric carbon dioxide and partial dehydration of the anterior lens surface produce a situation in which the calcium-phosphate-carbonate solubility product is exceeded and a surface film results.[73 95] Removal may be aided by heating gently in solutions that contain chelating agents such as sodium edetate (EDTA). [57 96]

2.1.6 Iron

Iron-containing metallic particles can become embedded in hydrogel lenses, oxidize into insoluble ferric oxide and form an orange-coloured "rust spot" on the lens surface. (Figure 2.6) [57 97 98] They are common in patients who live or work close to areas of building development, are extremely difficult to remove and necessitate lens replacement.

Figure 2.6 - Embedded Ferrous Body

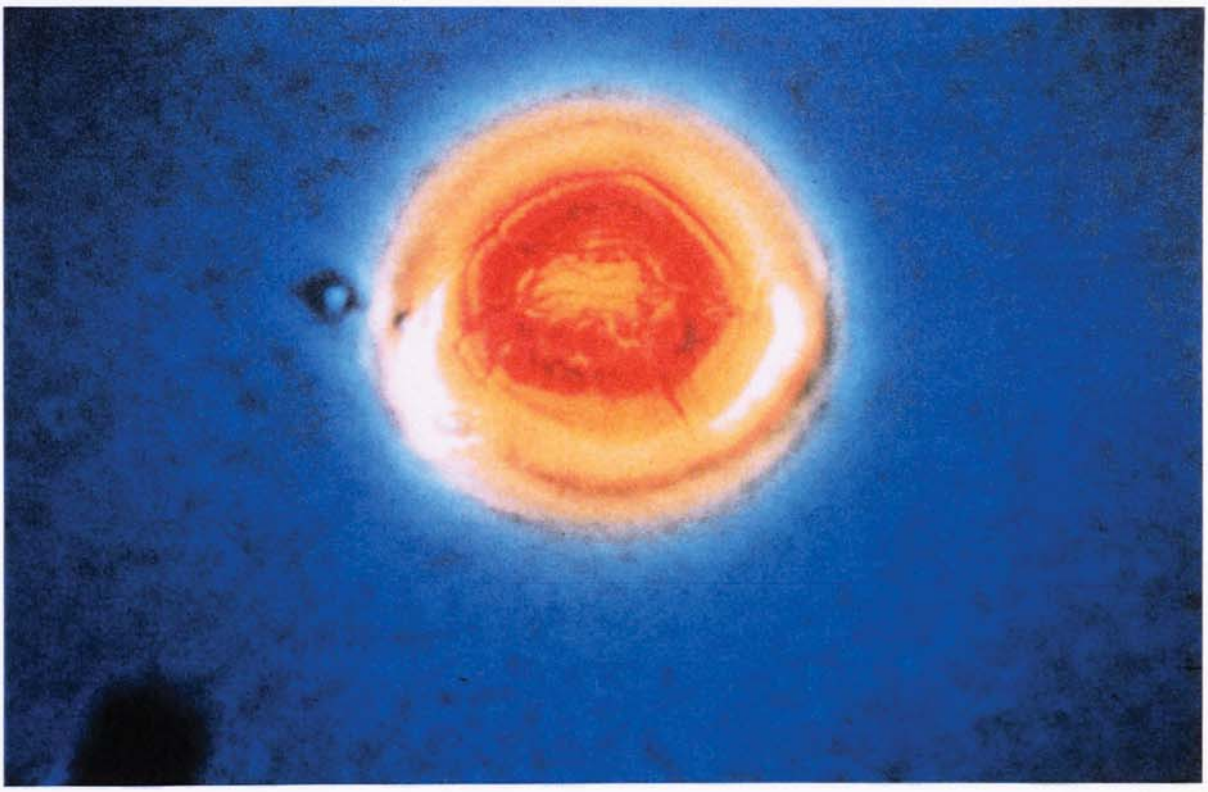


Figure 2.6 - Ferrous body embedded into hydrogel lens surface. Picture courtesy of Allergan.

2.1.7 Mercury

Mercurial deposits appear as a greyish film and are generally found in lenses stored in out-of-date soaking solutions containing thiomersolate (an organic mercurial antiseptic), particularly if heat sterilisation is employed. [99] The grey film consists of water insoluble mercuric sulphide [57] (Figure 2.7) and may produce an allergic reaction. [49] Treatment consists of lens replacement and re-commencement of wear with non-preserved cold disinfection systems.

Figure 2.7 - Mercurial Deposited Lens

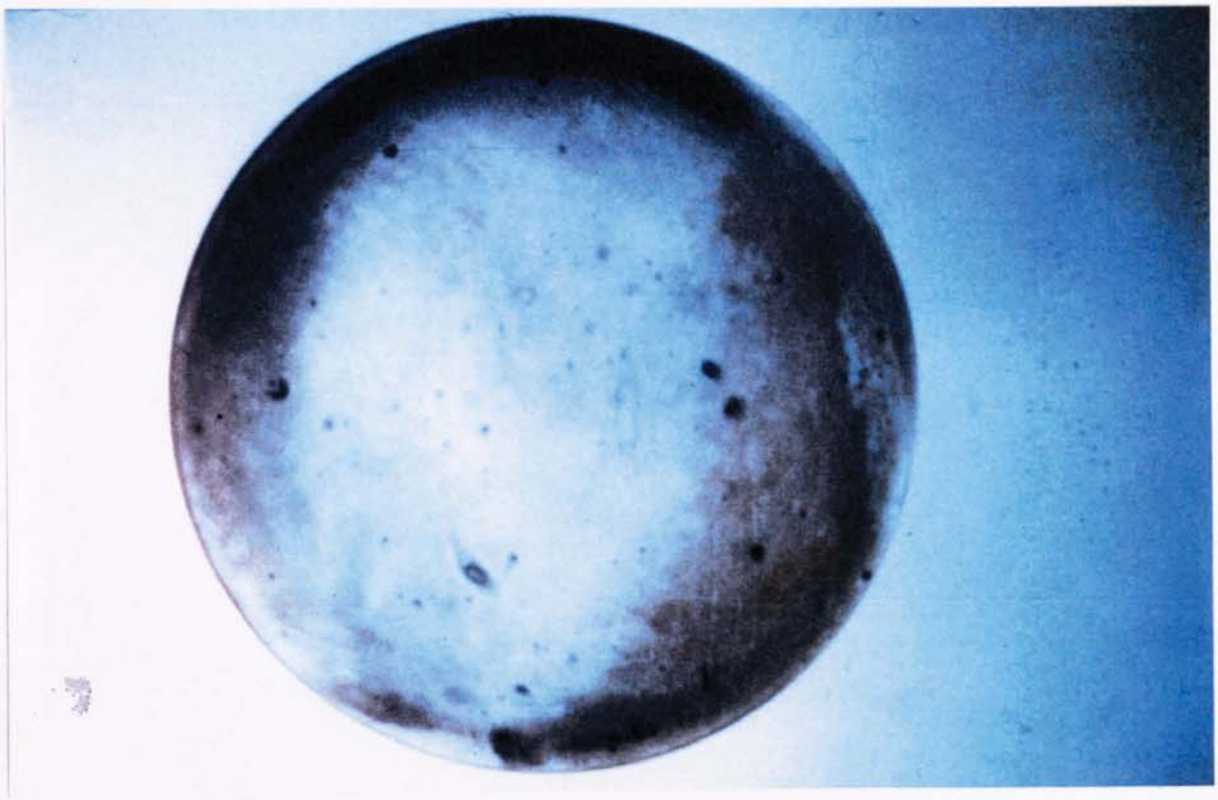


Figure 2.7 - Mercurial deposited lens. Picture courtesy of Allergan.

2.1.8 Discolouration

Discolouration in hydrogel lenses is relatively common, occurring in up to 50% of visibly deposited lenses, [53] and is particularly prevalent in high water content materials. [57 100] Many colours occur, including yellow-brown, orange, amber, blue, grey, pink and green (Figure 2.8). A yellowish or brown pigmentation in worn soft lenses may occasionally be due to direct absorption of nicotine from tobacco smoke. [49 101] Analysis of affected lenses reveals the pigmentation to have many characteristics of the skin pigment melanin and to occur just below the lens surface, the process resulting from the polymerization of aromatic compounds from the tears. [100] Discolouration may also result as a direct consequence of using cold disinfection systems preserved with chlorhexidine (yellowy-green), thiomersolate (grey) [99] or sorbic acid (yellowy-brown), particularly following heat disinfection. [57 100 102 103] The discolouration of lenses soaked in sorbic acid preserved systems is primarily due to the Maillard Reaction, a well-described organic reaction between aldehydes from the breakdown products of sorbic acid and amines from deposited proteins. [103] Other factors that may produce discolouration include the use of systemic and topical drugs, [58 104 105] cosmetics, diagnostic topical agents (such as fluorescein and rose bengal) and an increased concentration of manganese within the tear film. [106] Although strong oxidising agents can bleach the lens, badly discoloured lenses require replacement.

Figure 2.8 - Discoloured Lenses

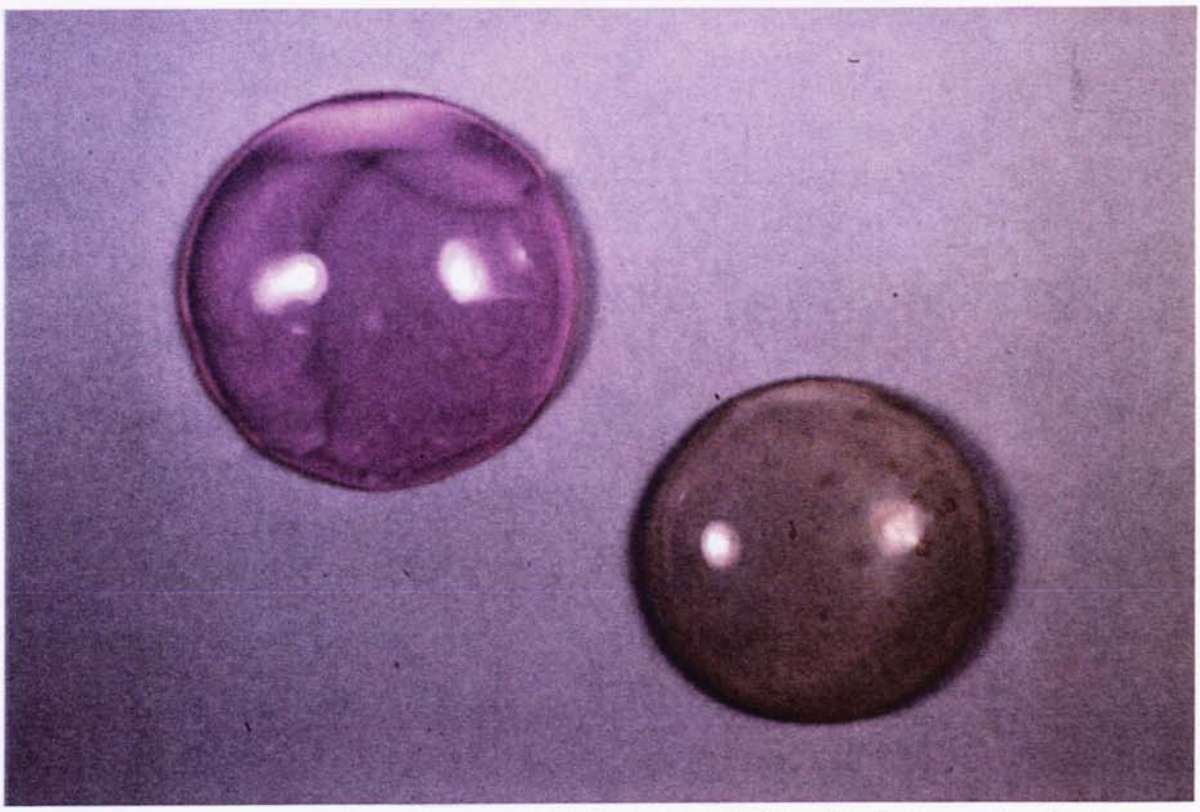


Figure 2.8 - The pink lens discoloured due to the patient mistaking "disclosing" tablets for enzyme tablets and the brown lens had been heat disinfected in Hydrocare solution and was 2 years old.

2.1.9 Fungi

Fungi (for example *Aspergillus*) and yeasts (for example *Candida albicans*) may use contaminants on the lens as a source of nutrients. The colonies are often coloured and exhibit filamentary growth both on the surface (Figure 2.9) and inside the lens matrix, particularly in high water content lenses. [73 107 - 109] They are enhanced in lenses deposited with protein, [110] are generally caused by inadequate disinfection procedures, commonly seen in part-time wearers and necessitate immediate lens replacement.

Figure 2.9 - Fungi Filaments

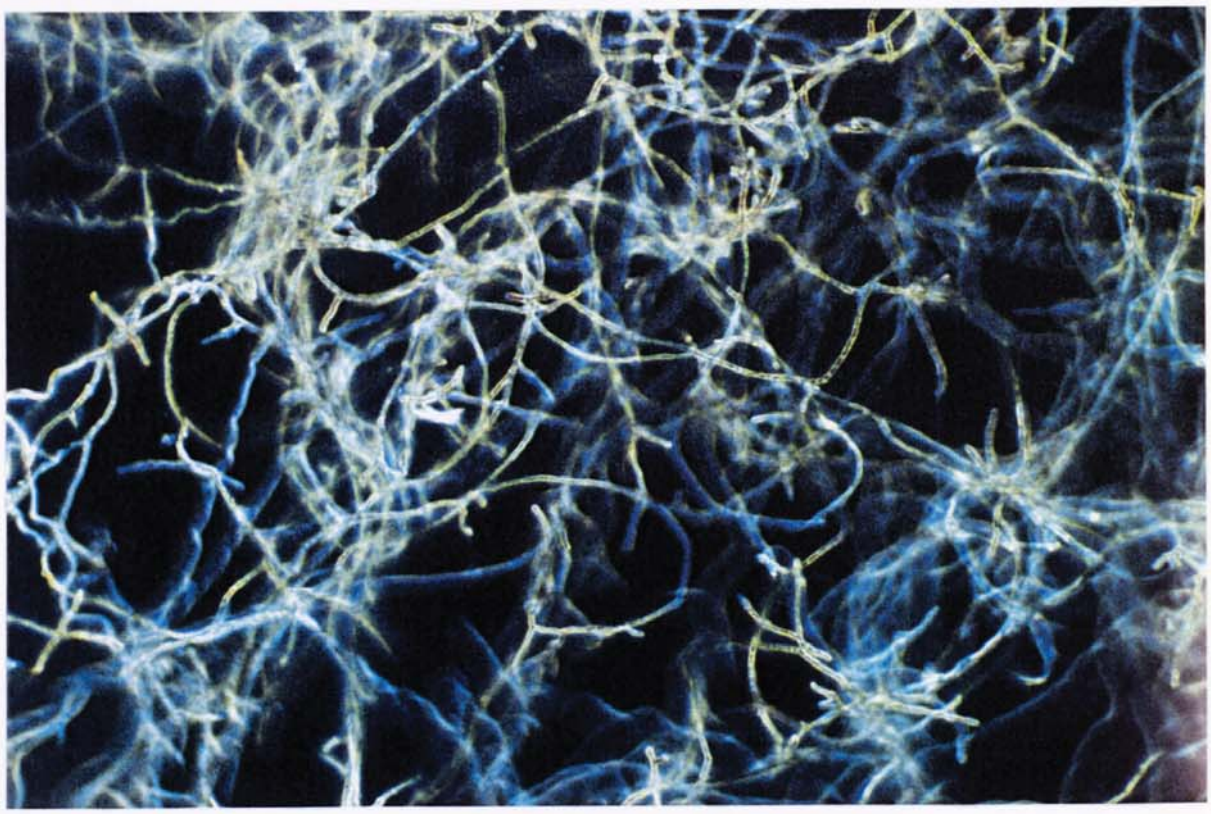


Figure 2.9 - Fungi filaments growing on the surface of a high water content lens. This lens was used on a part-time basis and had been disinfected with a chlorine release system.

2.2 QUANTIFICATION & CHARACTERISATION OF DEPOSITS

Practitioner analysis, quantification and identification of deposits generally consists of a cursory glance with the slit-lamp biomicroscope at 25-30x magnification. Whilst sufficient for the analysis, recognition and differentiation of "gross" surface deposits such as lens calculi from a filmy deposit, the lack of contrast between the deposits and the lens matrix makes it inefficient for the detection and quantification of more subtle levels of deposition.

The Rudko Method [111] allows practitioners to more accurately grade and identify deposits. This *in vitro* technique uses oblique light to illuminate the lens at low magnification (7-15x). The visualised deposit is classified into three areas, describing the deposit's general visibility, characteristic type or appearance and area of coverage. Despite the apparent advantages of the Rudko system over conventional slit-lamp based systems, the results are still subjective and at best only semi-quantitative in nature, even if improved with digital image processing. [112 113] It is unable to detect very low levels of deposition, [114 - 116] cannot biochemically identify the nature of the deposit and is poor at differentiating between lens materials containing markedly different levels of protein. [11 115 116]

The majority of publications concerning spoilation have used sensitive, laboratory-based techniques. [2 58] Most of these rely upon destructive techniques such as histochemical staining, [70 77 86 - 88 94 95 117] amino acid analysis, [51 59 118 119] dye-binding assays (eg Lowry assay; [119 120 121] Ninhydrin assay [65 115] or Bio-Rad assay [122]), ESCA,

[12] immunocytochemistry, [119 123 124] scanning and transmission electron microscopy (with or without electron probe microanalysis), [8 64 70 77 86 108 120 125 126] high pressure liquid [72] and thin layer chromatography [59 72 83] or electrophoresis. [7 11 32 54 118 119 121 125] While these techniques are relatively sensitive, many of them are unsuitable to analyse tear-derived deposits found in or on single contact lenses, being more useful for the analysis of bulk material. Further complications arise in sourcing the lenses for subsequent analysis. Many studies have used lenses from unknown sources, [8 49 57 59 88 125] combined lenses to produce sufficient material of interest [72 83] or spoiled lenses with surrogate tear solutions. [9 60 119 123] In attempting to analyse contact lens deposits it is possible to either study the deposits *in-situ* or to remove them and analyse the eluted/removed species. In attempting to remove deposited species there are several potential complications:

- Not all deposits may be removed by the extraction technique chosen. Subsequently it is impossible to ascertain the relative proportions of removed versus adherent species. [54 118 119 122 123]

- On removal components could be fragmented [118 125] and the conformation of the deposits will be almost inevitably changed. [119]

In conclusion, the complications of sourcing suitable lenses, using analytical techniques that, while sensitive, are usually destructive in nature and the inherent inability to remove all of the deposited species inevitably limits the inferences and conclusions that can be drawn from the results of the studies published to date.

2.3 FACTORS AFFECTING DEPOSITION

The factors relating to deposition are broadly divided into those attributed to the lens wearer (patient) and those related to the material/care system combination.

2.3.1 Patient Related Factors

2.3.1.1 Tear Film

Tears contain most of the constituents necessary to produce the major types of spoilation. The tear film consists of more than 98% water, in which are dissolved various electrolytes (sodium, chlorine, magnesium, potassium), metabolites (urea, uric acid, glucose) and several proteins and lipids. It has an osmotic pressure of approximately 304 $mOsmKg^{-1}$, [127 128] refractive index of 1.337, [127] mean pH of 7.5 (although significant inter-patient variations exist) [129 130] and surface tension of $43.6mNm^{-1}$. [131] The volume of tears present at any time is dependent upon patient age [132] and both production and evaporation/drainage rates. The mean volume is 5-10 µl, [133] with an average production rate of 1.2 µl min^{-1}. [134] Evaporation occurs at a rate of approximately 50 $gm^{-2}hr^{-1}$, [135] resulting in a tear turnover rate of 25% min^{-1}. [135]

The tear film is classically described as having a trilaminar structure of 6-10 μm thickness [136] or a six layered model if all the interfaces are considered, as shown in figures 2.10 and 2.11. [137]

Figure 2.10 - Classical tear film description. Taken from Holly & Lemp.

Figure 2.11 - Six layer tear model. Taken from Tiffany. [137]

The superficial lipid layer is mainly derived from the meibomian glands, contains phospholipids, neutral fats, fatty acids, cholesterol and cholesterol esters and is approximately 0.1 μm thick. The aqueous phase forms the bulk of the tear film, contains most of the dissolved tear film constituents and is derived principally from the lacrimal gland. The mucous layer consists of high molecular weight glycoproteins, lies against the epithelial surface of the cornea, is some 0.02-0.05 μm thick and is derived from the conjunctival goblet cells. More recently, other workers [138] [139] have proposed that the thickness of the tear film has been vastly underestimated and may be closer to 40 μm thick due to an increased thickness in the mucous layer. This topic is still debatable but current knowledge suggests that the mucous and aqueous layers should not be considered two separate entities but rather as two phases of the tear film that is under the overall "umbrella" of the lipid layer. Caffery [140] and Blades and Craig [141] provide fuller reviews of this topic.

When a contact lens is placed on the eye, the pre-ocular tear film (POTF) divides into two areas. The section behind the lens is termed the post lens tear film and that in front the pre-lens tear film (PLTF), whose major functions are outlined in Table 2.1. [142]

Table 2.1 - Role of the Pre and Post-lens Tear Film

Prelens Tear Film	Postlens Tear Film
Maintenance of vision	Lubrication - lens movement
Lubrication - comfort	Epithelial hydration
Maintenance of lens hydration	Debris removal
	Anti-microbial
	Epithelial nutrition
	Epithelial exfoliation

Insertion of a contact lens causes the tear film break-up time to significantly reduce compared with that measured without a lens in place and is thinnest in front of a rigid lens when compared with a soft lens. [143-145] The maintenance of a stable PLTF is vital to the long-term successful wear of contact lenses, as instability of the PLTF may increase tear evaporation and lens dehydration, promoting the formation of lens deposits, fluctuating vision and discomfort. [28] Studies show that significantly more contact lens wearers than non-wearers complain of dry eye symptoms (50% vs 22%), [146] possibly due to the reduction in the PLTF break-up time.

a) Tear Film Composition

Reports on the composition of tears are diverse, being dependent on the method used for collection, time of collection, age of patient and analytical technique used. [131] [147-150] Collection methods have included micro-capillary tubes, [147] [151-155] contact lenses, [156] plastic tape [157] and filter paper. [150] Analytical methods have included electrophoresis, [147] [151-154] enzyme linked immunoassay (ELISA), [148] [150] [153] high pressure liquid

chromatography (HPLC), [148 153 154] radial immunodiffusion (RID) [147 155] and thin layer chromatography (TLC). [158]

i. Tear Proteins

Proteins are polymers of L-α-amino acids linked together by peptide bonds and therefore termed polypeptides. They are large molecules with molecular weights ranging from 5×10^3 to 10^7 atomic mass units (AMU). All of the 20 amino acids found in the body consist of an NH_2 (amino) group and a COOH (carboxyl) group, with an additional "R" group, whose composition determines the amino acid. The general structure of a protein sub-unit (amino acid) is shown in Figure 2.12:

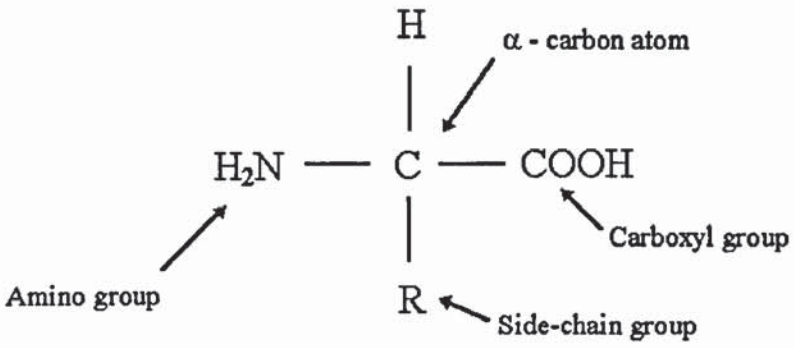


Figure 2.12 - General amino-acid structure

The "primary structure" of a protein describes the sequence of the amino acids along the polypeptide chain (usually formed from 100-1000 amino acid molecules) and the "secondary structure" describes the repeated section of the chains and specific structural features within the molecule. The latter is achieved by the formation of intrachain hydrogen bonds (α-helix) or interchain hydrogen bonds (parallel β-pleated sheets). The "tertiary structure" defines the overall folding of a polypeptide chain (its three-dimensional shape) and the "quaternary structure" describes the aggregation of monomeric protein subunits into oligomers (low molecular weight proteins). [159]

Traditionally proteins are classified into two groups - globular and fibrous. The former is generally spherical in shape, water soluble and contains a mixture of α-helix, β-pleated sheets and random structures (examples include the tear and blood proteins). Fibrous proteins are structural proteins consisting of long cable-like structures (examples include keratin and collagen). Protein molecules in their native conformation are globular and 20-100 Å in diameter and the interaction of several weak forces maintains the protein molecule in this native state. The native state of a protein describes its biologically active form, with the process of denaturation resulting in loss of its biological activity. This is brought about by heat and treatment with acids, alkalis, detergents, organic solvents and heavy metal cations such as mercury and lead. [159 160]

The natural habitat of most proteins is an aqueous environment. When a protein solution contacts a surface the protein molecules accumulate at the interface, with groups at the surface of the protein being the ones most likely to interact with the solid surface. The protein film may then act as a substratum for the subsequent

adhesion of other components. The surface of a protein is often complex in nature, with different characteristics such as varying hydrophilicity and charge, both of which control the way in which the protein adsorbs onto a surface. [162] Another major factor is the surface energy of the surface encountered, with an increased rate of adsorption to hydrophobic surfaces occurring. [163] Conformational changes occur in albumin, [164] lysozyme [165] and γ-globulin [166] upon adsorption to hydrogel surfaces, with a decrease of the α-helix content of the protein occurring. However, these changes are complex, being related in part to material surface charge, hydrophilicity and time. Denaturation increases with the passage of time, [164 165] and is significantly greater in structurally stable ("hard") proteins such as lysozyme than in unstable ("soft") proteins such as albumin when denaturation does occur. [167]

Amino acids vary considerably in chemistry, as shown in Table 2.2, with some being hydrophobic, some hydrophilic, some positively charged and some negatively charged. Proteins are often described as being either negatively or positively charged. Each protein consists of a number of amino acids of varying charges and the overall charge is a result of the net balance of these individual charges. As a result of these charges proteins contain the chemical structures necessary to bind with almost all materials that they contact, depending upon the charge of the surface and the structural arrangement of the protein. This feature is graphically described in Figure 2.13. [162]

Figure 2.13 - Protein Adsorption Fundamentals

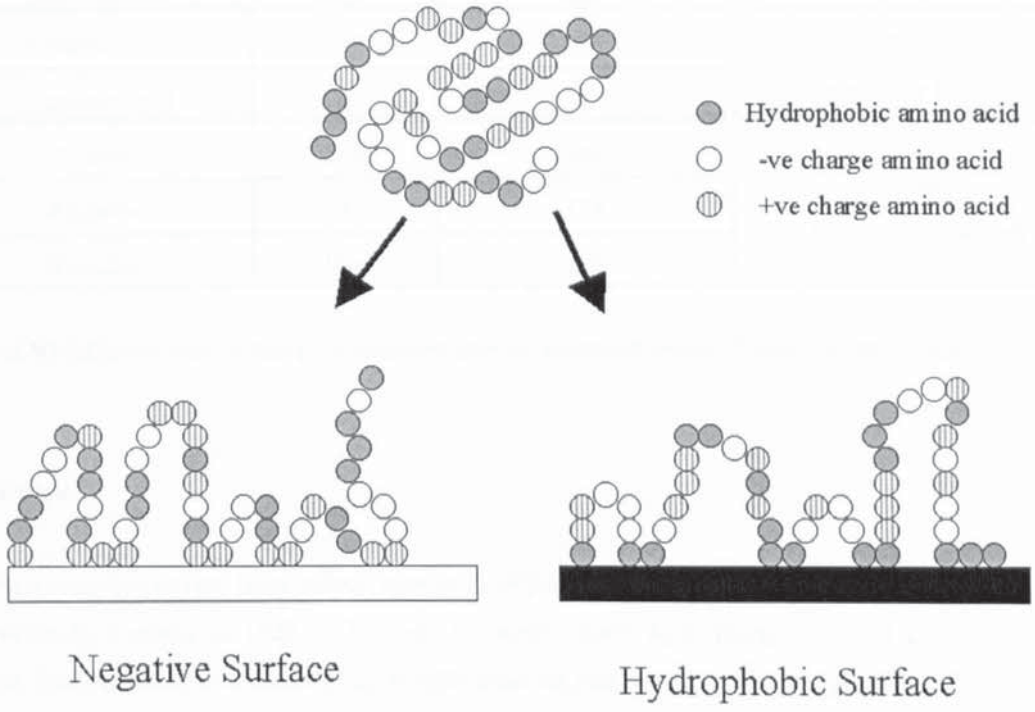


Figure 2.13 - Diagrammatic representation of the way in which protein molecules can re-orientate themselves depending upon the surface on which they contact. Adapted from Ratner et al. [162]

Table 2.2 - DNA Encoded Amino Acids Found in the Body [161]

Name	Symbol	Molecular Weight	Nature of the R Group
Alanine	Ala	89	Non Polar
Valine	Val	117	
Leucine	Leu	131	
Isoleucine	Ile	131	
Proline	Pro	115	
Phenylalanine	Phe	165	
Tryptophan	Trp	204	
Methionine	Met	149	
Glycine	Gly	75	Uncharged Polar
Serine	Ser	105	
Threonine	Thr	119	
Cysteine	Cys	121	
Tyrosine	Tyr	181	
Aspargine	Asn	132	
Glutamine	Gln	146	
Aspartic acid	Asp	133	Polar Negatively Charged
Glutamic acid	Glu	147	
Lysine	Lys	146	Polar Positively Charged
Arginine	Arg	174	
Histidine	His	155	

At least 60 different tear proteins are demonstrable by electrophoresis. [152] Of these, the major tear proteins are:

i. Lysozyme

This bacteriolytic enzyme (also called "muramidase") is derived from the lacrimal gland [168] and was first discovered by Fleming in 1922. [169] It works by preferentially hydrolysing the β-1,4 glucosidic linkage between N-acetylmuramic acid and N-acetyl-glucosamine that occurs in the mucopeptide cell wall of some gram positive bacteria. It is found in mammalian urine, saliva, tears, milk, cervical mucous, leucocytes and kidneys. Lysozyme is a very stable protein with a compact structure that is highly resistant to denaturation. It has a molecular weight of 14.6 kilodaltons (kd). There is a close cluster of basic groups (Arg 45 and 68 in one region, Arg 61 and 73 in a second and Arg 5, 125 and 128 in a third) which form the highly positively charged surface regions of lysozyme, which give it a very high isoelectric point of 11.1.

Lysozyme reduces in concentration with increasing age [150] and in patients with trachoma, [170] keratoconjunctivitis sicca [171] or herpes simplex keratitis, [172] is unaffected by contact lens wear [27 171 173] or the presence of inflammatory conditions (such as vernal conjunctivitis or giant papillary conjunctivitis [174]) and is present in similar quantities in stimulated and non-stimulated tears. [148] Several studies have reported an unidentified protein with a molecular weight of 30 kd accumulating on hydrogel lenses. [175 176] Further work [177] has proved that this protein is the homologous dimer of lysozyme and is more commonly found on group IV (high water content, negatively charged) lenses.

ii. Lactoferrin

The lacrimal gland synthesises this iron-binding glycoprotein. [178] It is found at high concentrations in milk and in exocrine secretions such as saliva, bile, tears, mucosal and genital secretions and in blood serum. [179] It is a single chain glycoprotein comprising 692 amino acids with two similar oligosaccharide chains and possesses two independent metal binding sites. It has a high isolectric point compared with other transferrins (8.5 compared with 5.7) due to the concentration of charged side chains on the molecule near the N-terminus.

It prevents bacterial colonization of the external ocular surfaces by depriving the ocular mucosa of free metal ions. [180] It may also play a role in inflammation, as lactoferrin is released into circulation by activated neutrophils during the early stages of inflammation. [179] It has several forms, is reasonably large (60 - 80 kd) [151 152] and binds to several other proteins and support media. [153 181] It reduces in concentration with increasing age [150] and in patients with keratoconjunctivitis sicca [171] and giant papillary conjunctivitis [182] or vernal conjunctivitis. [174] It is present in relatively similar quantities in stimulated and unstimulated tears [148] and is unaffected by lens wear. [171 173]

iii. Immunoglobulins

These are large molecules with molecular weights of 160-190 kd. Immunoglobulins are found in tears, saliva, nasal and bronchial fluids, colostrum, breast milk, perspiration and secretions of the gastrointestinal tract. Several immunoglobulins are found in tears, including IgA, IgE, IgG and IgM, of which IgA is the predominant immunoglobulin. [183 184] The lacrimal gland secretes IgA and it protects the mucosal surface by acting as an immunological barrier to the adherence and cell entry of microbial organisms. [185] The quantities of IgA, IgG and IgM are all greater in non-stimulated tears (as compared with stimulated tears) [148] and upon eye closure the level of secretory IgA increases to the point that it becomes the major tear protein present. [147]

The influence of lens wear on immunoglobulin levels is the subject of some discussion. The use of any lens type on an extended wear basis results in an increase in both IgA and IgG levels when compared with lenses worn on a daily wear basis. [186] One study [155] showed that rigid lens wear results in an increased level of tear IgA, possibly due to the increased mechanical stimulus of the lens edge compared with hydrogel lenses. Other studies demonstrate that lens wear results in a transient decrease in the measurable levels of IgA in the tears. [27 187] Differences in collection and analytical technique, lens type and wearing schedule explain these

discrepancies.

While the development of GPC in lens wearers is possibly linked to an allergic response to lens deposits, [18-21] lenses from such patients only show an increase in IgM deposition, with levels of IgA, IgG, IgE, lactoferrin and lysozyme showing levels of deposition commensurate with patients who do not exhibit GPC. [186 188] This difference in deposition of IgM confirms an immunological component to GPC.

iv. Serum Albumin

This is common in both plants and animals (ovalbumin in egg white, lactalbumin in milk). It is water soluble and is coagulated by heat. Albumin accounts for approximately 60% of total protein in blood serum, is composed of 580 amino acids and has a molecular weight of approximately 62 kd. [189] It is a single chain protein composed of three structurally similar domains and consists of 28 helices that range in size from 5 to 31 amino acids in length. Its isoelectric point is 4.9 at pH 7.0, leaving the protein with an overall small negative charge at this pH. It is a relatively unstable protein and may denature easily under conditions of changing temperature or pH. Trauma during collection can affect the levels of serum albumin in tears.

As with IgA, the closed eye environment has a higher level of albumin than is seen in the open eye situation. [147]

v. Tear Specific Prealbumin (TSP)

This is a unique protein that is never found in serum, [190] whose function remains largely unexplained. TSP is identical in its amino acid sequence to the human von Ebner gland (VEG) protein found in saliva and these proteins have recently been renamed "lipocalins". [191] It is much smaller than serum albumin, with a molecular weight of only 23 kd. [171] and exists in multiple isoforms. [189 192] Its concentration is unaffected by lens wear. [173]

Some controversy exists about the presence of a protein previously termed "Protein G". First reported by Gachon et al, [189] this 31 kD tear protein is irregularly found on SDS-PAGE patterns of tear proteins. It is likely that protein G is a dimer or aggregated form of TSP and is artifactually formed by the denaturing conditions of SDS treatment, a finding confirmed by several workers. [193 194]

vi. Various

Besides the major tear proteins described in detail above, other important proteins are found in the tear film:

a). Fibronectin - a 240 kd protein which is derived from plasma and increases in concentration in closed eye tear fluid due to leakage from dilated conjunctival blood vessels. [195] It is involved in corneal wound healing.

b) Plasmin - a non-specific serine proteinase resulting from the activation of plasminogen by plasminogen activators and an essential part of the fibrinolytic system due to its ability to cleave proteins. [196]

c) Vitronectin - a 75kd serum derived protein (also called complement-S protein) which may act to dampen complement activation in the tears and inhibit plasmin activity. [197] Its level increases in the closed-eye environment [197] and during extended wear of lenses. [198]

d) Complement - this system is involved in the inflammatory response by recruiting and activating phagocytic cells, lysing microorganisms and promoting immune complex solubilization. The level in closed eye tears increases, demonstrating the sub-inflammatory environment. [199]

vii. Relative Concentrations of Proteins

There is a wide variability in the published concentration of tear proteins. [131] Table 2.3 details the mean and range of concentrations of the major tear proteins found from a review of over 100 papers. [131] The variability encountered in these values is explained by many factors, including the collection technique and sensitivity of the final analysis. Additionally, several factors influence the protein concentrations found in any one individual patient. These include age, [148 150] sex, [106] health [178] and contact lens wear. [173]

Table 2.3 - Concentration Of Major Tear Proteins (mg/100ml) [131]

	Mean	Max	Min
Total Protein	786	960	652
Lysozyme	185.5	555	65
Lactoferrin	210.5	340	81
Albumin	130	390	1
TSPA	123	184	52
IgA	29	85	4
IgG	13	79	trace
IgM	1.4	5	0
IgE	0.02	0.02	0.003

The influence of collection technique and analytical technique on the measurement of lysozyme concentration is alluded to in Table 2.4, which outlines the values achieved with a variety of techniques. [131]

Table 2.4 - Range of Estimated Lysozyme Concentrations (mg/100ml) For Normal Tears By a Variety of Techniques [131]

Collection Method	Analytical Technique	Lysozyme Concentration
Basal tears	Crossed immuno-electrophoresis	71
Basal tears	Single radial immuno-diffusion	130
Basal tears	Enzyme activity assay	188
Basal tears	Enzyme activity assay	330
Micropapillary pipette	Enzyme activity assay	555

One point of interest is that, irrespective of collection technique, the ratio of lysozyme to lactoferrin remains fairly constant, as shown in Table 2.5. [131]

Table 2.5 - Concentration Ratios of Lysozyme and Lactoferrin in Tears [131]

Technique	Lysozyme	Lactoferrin
Crossed immunoelectrophoresis	1.0	1.6
Crossed immunoelectrophoresis	1.0	2.1
ELISA	1.0	1.4
SDS-PAGE	1.0	1.1
ELISA	1.0	0.5

The molecular weights and relative charges of the three principal proteins are considerably different, as outlined in Table 2.6. This is an important consideration as the factors have a significant impact on their interaction with hydrogel lenses.

Table 2.6 - Molecular Weight (kD) and Relative Charge Of Principal Tear Proteins

	Molecular Weight (kD)	Relative Charge
Lysozyme	14.6	+ve (+++)
Lactoferrin	60 - 80	+ve (+)
Albumin	65	-ve (-)

Small proteins (such as lysozyme) enter the matrix of hydrogel lenses, particularly those of a high water content nature, as they are smaller than the pore size. Highly positively charged proteins (such as lysozyme) will interact strongly with negatively charged polymers, as conditions will thermodynamically favour this.

ii. Tear Lipids

Tear film lipids are derived from the meibomian glands and glands of Zeiss and act to prevent evaporation of the underlying aqueous layer, thereby stabilising the tear film. [200 201] The common characteristic of all lipids is a large hydrophobic (oily) portion, which makes them relatively water insoluble, although they are all soluble in organic solvents such as hexane, chloroform and methanol. They fall into two broad categories, simple (non-polar/neutral) lipids and complex (polar) lipids. The simple lipids contain only fatty acids (eg ethanoic, oleic, linoleic) and alcohol components (usually glycerol). Examples include mono-, di- and tri-glycerides, fatty acids, cholesterol, cholesterol esters and waxy esters. Complex lipids are amphoteric in nature, in that they contain both hydrophobic and hydrophilic regions in the same molecule. They have a variety of important functions within the body, including structural components for membranes, storage and transport of metabolic fuels and components for cell recognition. These are termed "glycerophospholipids" and include phosphatidylcholine and the sphingolipids.

A wide range of lipids are detectable in the tear film, including waxy esters, cholesterol esters and triglycerides, with a significant inter-patient variation occurring. [158] A duplex structure describes the lipid layer in which the outer layer consists of cholesterol esters and the inner layer a monomolecular film of phospholipids, fatty acids and free cholesterol. [202] Mean quoted concentrations are 205.5 $mgml^{-1}$ for total lipid and 64.3 $mgml^{-1}$ for cholesterol. [131]

Lipid adsorption is a particular problem with rigid gas permeable (RGP) materials, [203 - 205] which deposit 2-3 times more lipid than soft lenses, [203] with deposition occurring in proportion to their silicone content. [203 205] Lipids adsorb to a greater extent on high water content materials [203 205] and their deposition is reduced by the presence of proteins. [205 206] The driving force for the deposition of these molecules onto hydrophobic contact lenses is the "oil-likes-oil" hydrophobic interactive force. [71 162 203]

iii. Tear Mucous

The principal sources of mucin are the goblet cells of the conjunctiva and the glands of Manz and the crypts of Henle. The 1.5 million goblet cells are found alone or in groups on the conjunctival surface and are most numerous in the nasal conjunctiva. The intimate linkage between the mucous layer and the microvillae and microplicae of the corneal epithelial cells help the superficial tear film to firmly "attach" to the cornea, [207] and removal of the mucous layer results in marked tear film instability. [208] Mucous "wets" the hydrophobic epithelium [209] and mucous threads within the tear film also help to remove entrapped debris and cells. [210]

b) Tear Film Assessment

The tear film plays an essential role in comfortable contact lens wear. Predictive tests estimate the stability and quantity of a patient's tear film and assess the likely success of contact lens wear. While a full critique is outside the scope of this review (and can be reviewed elsewhere [211]), a short overview is valuable.

i. Tear Film Quality

a. Break Up Time (BUT)

The tear break up time (BUT) estimates the rupture time of the pre-corneal tear film following the instillation of sodium fluorescein, while viewing the fluorescent film at 10-20x magnification with a slit-lamp and cobalt blue filter. Recording of the time taken in seconds to notice the first break following a blink assesses the BUT. While this technique has been used for many years, its repeatability has been criticised [212] due to inherent problems of variations in temperature and humidity, diurnal patient variations and changes induced by the amount of fluorescein instilled, which itself produces premature break up of the tear film. [213] A "normal" value is a BUT of ≥ 10 seconds. [214]

b. Non-Invasive Break Up Time (NIBUT)

More recent assessment methods of BUT use techniques which rely on interference, [215 216] or project various images (grids [217 - 220] or keratometer mires [221 222]) onto the tear film or contact lens surface directly. These techniques record the time taken for the pattern to break up without disturbing the tear film and give rise to non-invasive break up time measurements (NIBUT), which are invariably longer than the traditional fluorescein-derived BUT results. [213 223]

ii. Tear Film Quantity

a. Schirmer Test

Basal production rate is traditionally detected by using the Schirmer strip test, which estimates the amount of wetting of a sterile strip of Whatmann paper over a period of five minutes. This test is time consuming, uncomfortable and highly variable due to the reflex tearing induced, [224 225] with poor inter and intra-patient repeatability. [226] An average value is 15.3mm in 5 minutes. [227]

b. Cotton Thread Test (CTT)

The sensitivity of the Schirmer test is improved by using white cotton threads dyed with 0.05% phenol red, [228] 10% fluorescein [229] or commercially available orange-red thread. [212] These threads are placed in the lower tear meniscus and left for 15-30 seconds. When wetted the phenol red threads turn from yellow to red and the wetted length assesses the volume of tears. The test has the advantage that it is relatively comfortable, less invasive, rapid and less variable than the Schirmer test. [228 230] An average value in a "normal" patient is 27 mm in 15 seconds. [230]

c. Tear Meniscus Height (TMH)

The tear film divides into the preocular part (which covers the ocular surface) and that contained in the lower tear rivus. The tear rivus contains approximately 80% of the volume of tears. [133 231] Measurement of the height of this tear prism correlates well with symptomatic dry-eye patients if a TMH of <0.10mm is taken as a cut-off point. [232] An average value is 0.20mm. [232 - 234]

iii. Alterations in Quality and Quantity

Tear film quality and quantity may be affected by:

a. Age

Tear film production [132] and stability [235] declines with increasing age. As previously discussed, the collapse of a thinned tear film allows surface lipid to contact the hydrophobic lens surface and is likely to result in enhanced deposition.

b. Blinking

Patients normally blink 12 times per minute. [236] Any less than this will result in a subsequent increase in the rate of evaporation of the pre-lens tear film, with consequential drying of the lens surface and increased protein deposition. [3] Such reductions in blink-rate occur in situations where the patient's occupation involves "staring" at a particular target for any length of time (such as VDU work [237 238]) or in which the working environment has a low level of humidity or high temperature. Good evidence exists to indicate that the blink rate is modified by tear film break-up, with a blink occurring just prior to the disturbance of the tear film. [239 240] Patients with short tear film break-up times (BUT) develop deposits on their lenses faster than those with long BUT. [3 56 241]

c. Medication

Certain medications may affect the tear film [242 243] and ultimately result in changes to the deposition profile. These may result in reduced lacrimation (muscarinic antagonists, sympathetic agonists, beta receptor antagonists, diuretics and oestrogen/progesterone combinations), increased lacrimation or other changes in tear film composition. Certain drugs (for example Rifampicin, Sulphasalazine) are secreted into the tears, potentially resulting in the discolouration of hydrogel lenses. [242]

d. Diet

Diet may affect tear composition [140] and possibly result in an increased inclination towards deposition. [8] An elevated intake of protein, alcohol and cholesterol is correlated with an increased number of lens calculi. [8 83]

e. Ocular Surface Disease

Certain medical conditions will influence the production of the various constituent layers of the tears, with a consequential impact on the development of deposits, either directly or indirectly through alterations to tear film break up time. Examples include meibomian gland dysfunction and blepharitis (which alter lipid composition [90 244 - 246]), giant papillary conjunctivitis (which results in excessive mucous production [22]), mucin deficiency (which reduces tear film break-up times [209]) and aqueous tear deficiency (keratoconjunctivitis sicca) due to Sjogren's syndrome, rheumatoid arthritis and other disorders. [231 247]

f. Coating Inhibitory Factor

A surfactant-like factor is present in tears that prevents their deposition onto solid substrates. [154 248 249] Absence of this factor could theoretically result in a patient depositing their lenses to a greater extent.

2.3.1.2 Care Regimen

Contact lens care systems play an important role in controlling and modifying the deposited layer. Care products contain the following functional components:

a) Surfactants

Surfactant cleaning prior to disinfection is vital to remove the debris which accumulates during wear. [250] Surfactants have a dual role. Firstly, they soften the deposits, preventing them from becoming irreversibly adherent to the lens surface. [69] Secondly, they aid subsequent lens disinfection by reducing the bacterial bioburden by a factor of up to four log units (>99%). [251 252]

Little published information has concentrated on the relative cleaning effect of surfactant cleaners alone. The data currently published suggests that surfactants differ in their ability to remove deposits [253 - 255] and that disinfecting agents alone have a minimal surfactant effect, [250 255] particularly in their ability to remove lipid. [253] Simmons and colleagues [256] demonstrated the importance of adhering to the cleaning times recommended by the manufacturers, with shortened cleaning times resulting in increased deposition after only 10 weeks of lens wear.

b) Disinfectants

i. Heat

Heat disinfection offers the advantages of simplicity, low incidence of allergies, [257] low cost [258] and good efficacy. [259] However, it increases the level of spoilation and red eye reactions, particularly in high water content lenses. [260] Heat also irreversibly denatures protein, [54 124] which may subsequently act as an immunological agent.

ii. Preserved Disinfectants

a. Thiomersolate

Thiomersolate is an antibacterial mercurial compound with a broad range of activity, including good activity against *Acanthamoeba.* [261] It discolours lenses, [49 99] becomes concentrated in the lens matrix, [262] is associated with hypersensitivity reactions [263 - 266] in up to 35% of patients [267] (possibly following binding to the proteins on the lens surface) [265] and may result in a pseudodentritic keratitis. [268]

b. Chlorhexidine

Chlorhexidine digluconate binds strongly to hydrogel polymers and gradually leaches out. [269] It is usually combined with thiomersolate due to its weak action against fungi and *Serratia.* [267] It displays low level toxicity in humans and rabbits, [264 270] occasionally resulting in punctate staining and hyperaemia [267] or a dendritic-like keratitis. [268] A formulation marketed by Bausch & Lomb ("OptimEyes") provides chlorhexidine in a tablet format, which is subsequently dissolved in tap water. [271] While simple and cheap, adverse responses, [272 273] concerns regarding tap water quality and patients inadvertently using tap water as a rinsing agent [274] have been reported.

c. Polyquad

Polyquad is a high molecular weight antibacterial agent [275] that is reportedly too large to enter the matrix of hydrogel lenses, [276] as demonstrated in Table 2.7. Published information shows that the systems exhibit good antimicrobial efficacy [277 278] and minimal toxicity. [279] Polyquad systems do not contain a surfactant, as indicated in Table 2.8, but rely upon a citrate buffer for passive removal of proteins, particularly lysozyme. [280]

Table 2.7 - Approximate Weight and Size of Preservatives Used in Contact Lens Solutions

Preservative	Molecular Weight	Molecular Size (A°)
Polyquad	5000	225
Polyhexanide	1300	80 - 144
Chlorhexidine	359	32
Thimerosal	405	13
Sorbic acid	112	11
Hydrogen peroxide	18	5
Hydrogel lens pore size	-	30-50

Table 2.8 - Polyquad Systems

Solution	Manufacturer	Concentration (%)	Surfactant	EDTA %	Buffer
Opti-Free	Alcon	0.0011	None	0.5%	Citric acid
Opti-1	Alcon	0.0011	None	0.5%	Citric acid

d. Polyhexanide

Polyhexanide is found in a variety of care systems (Table 2.9). These are true "multi-purpose solutions" in that they are formulated to clean, rinse and disinfect, containing both preservatives and surfactants in one bottle. [275] The molecule is reportedly too large to enter hydrogel lenses. [281] However, it has an effect on rabbit corneas, [279] incidents of corneal epithelial deposits following its use have been reported [282] and it would therefore appear to accumulate in the lens matrix, a finding confirmed by Rosenthal and coworkers. [283]

Table 2.9 - Polyhexanide Systems

Solution	Manufacturer	Concentration %	Surfactant	EDTA %	Buffer
All-in-One	Sauflon	0.0005	Poloxamine	0.3	Boric acid
All-in-One Light	Sauflon	0.0001	Poloxamine	0.13	Boric acid
Complete	Allergan	0.0001	Tyloxapol	0.05	Tromethamine
Opticians Choice	Procare	0.0004	Polysorbate	0.01	Boric acid
ReNu	B&L	0.00005	Poloxamine	0.1	Boric acid
Solo-Care Soft	CIBA	0.0001	Poloxamer 407	0.025	Sodium phosphate

iii. Non-Preserved Disinfectants

a. Chlorine

These are oxidative systems that rely on the release of free chlorine from tablets dissolved in saline (Table 2.10). [284] It has a low ocular toxicity with reportedly good antimicrobial activity, [285 286] but some authorities question its efficacy. [278 287] Prior surfactant cleaning is vital to assist disinfection of the lenses, with several reports of keratitis in patients using chlorine-release systems without prior cleaning. [288 289]

Table 2.10 - Chlorine Release Systems

Solution	Manufacturer	% Free Chlorine Released
Softab	Alcon	3.5
Aerotab	Sauflon	6.0

b. Hydrogen Peroxide

Three percent hydrogen peroxide is an extremely effective antimicrobial agent, [290 - 292] killing bacteria in minutes. [278 293 - 295] Once disinfected the peroxide requires neutralisation by one of several methods before the patient can re-insert the lenses. Table 2.11 outlines the principal methods of neutralisation.

Table 2.11 - Hydrogen Peroxide Systems

Solution	Manufacturer	H_2O_2 %	Neutralisation Format	Neutralisation Method
Easy-Sept	B&L	3%	One-Step	Platinum Disc
10:10	CIBA	3%	Two-Step	Sodium Pyruvate
AOSept	CIBA	3%	One-Step	Platinum Disc
Perform	WJ/Pilkington	3%	Two-Step	Sodium Thiosulphate
Peroxide	Sauflon	3%	Two-Step	Sodium Thiosulphate
Oxysept II-Step	Allergan	3%	Two-Step	Catalase
Oxysept I-Step	Allergan	3%	One-Step	Catalase Tablet

In one-step systems (Figure 2.14, adapted from Christie & Meyler [296]) lenses are in 3% hydrogen peroxide for only short periods compared with the two-step systems. Long soaking times in 3% peroxide is a perceived advantage of peroxide systems as this enhances their antimicrobial efficacy. [293] With one-step systems this advantage is negated. However, if there is an enhanced immunological influence from denatured lysozyme [297] then such systems may induce fewer immunological problems than two-step systems, if time of soaking is crucial to the degree of protein denaturation.

The speed of neutralisation of the two-step systems is important as it relates to the simplicity and "user-friendliness" of the systems under test. Figure 2.15 (adapted from Gyulai et al [298]) clearly shows that the catalase system (Oxysept II-step) achieves the most rapid neutralisation of the three under discussion.

Figure 2.14 - Speed of Neutralisation of One-Step Peroxide Systems

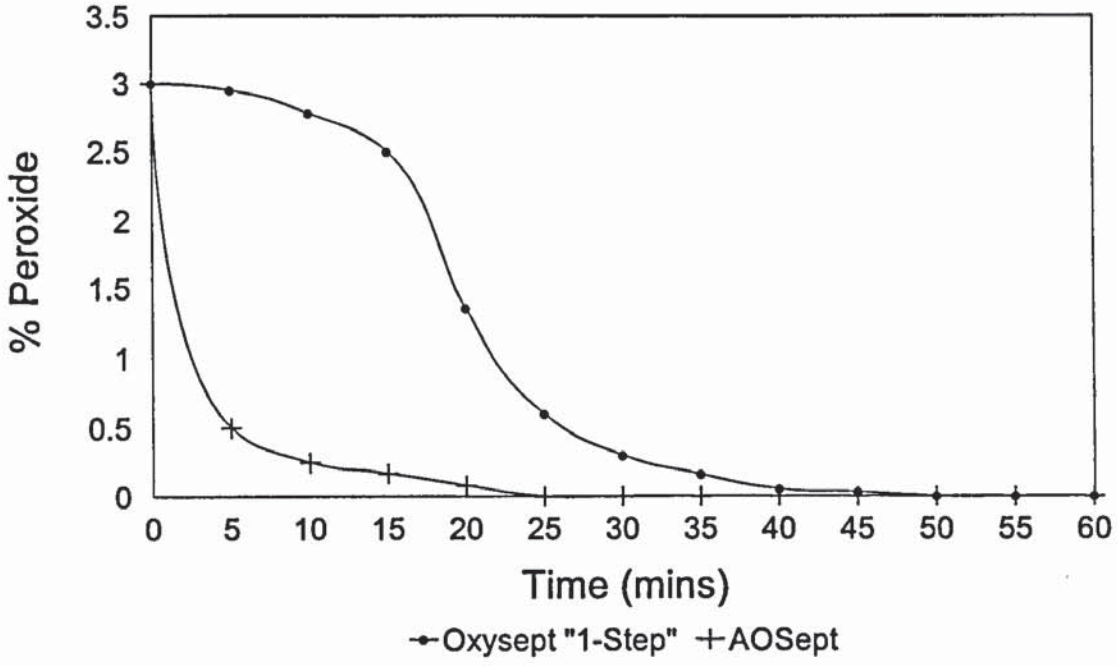
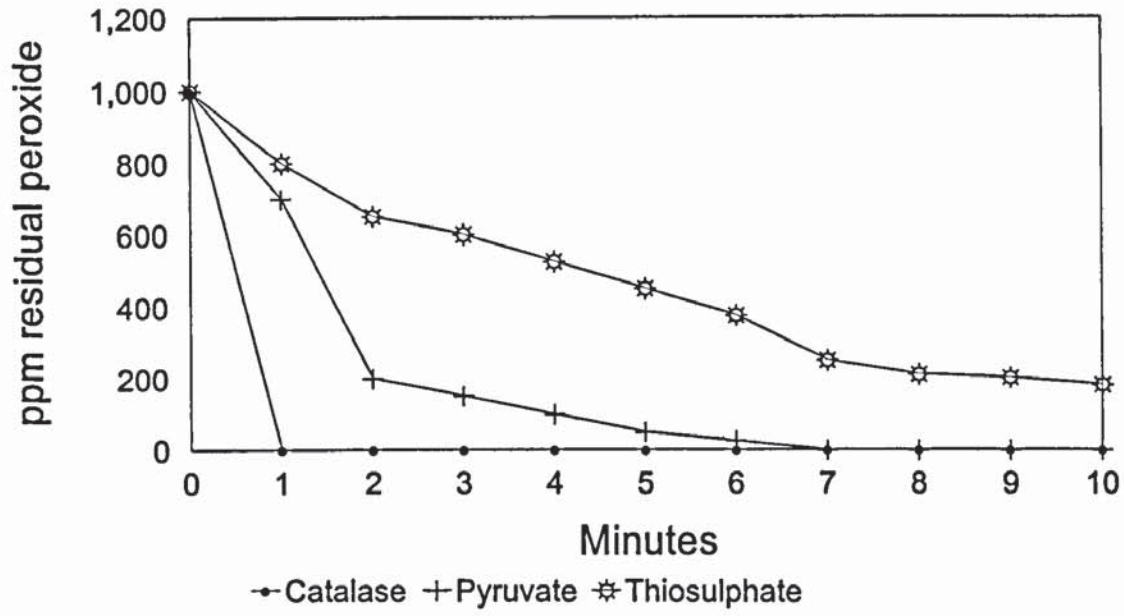


Figure 2.15 - Speed of Neutralisation of Two-Step Peroxide Systems



Complications with all peroxide systems include corneal staining following the instillation of incompletely neutralised lenses, [299] denaturation of lysozyme, [297] penetration of the cornea by residual hydrogen peroxide, [300] stinging on insertion, [301 302] diffuse corneal staining [303] and dehydration [304] and alteration of the parameters of ionic lenses. [305 - 307] In addition, the cost and complexity of such systems are often of concern to patients.

In comparative studies, AOSept displayed minimal toxicity on rabbit corneas compared with polyhexanide and polyquad preserved systems. [279]

iv. Enzyme Removers

Several authorities recognise the need for the periodic use of enzyme tablets to reduce both red eye incidence [260] and protein deposition, [51 67 68 250 308] removing some 75% of the deposited protein [123] and resulting in enhanced visual acuity. [15] However, little information is available concerning the frequency of their use, which of the 60 or so tear proteins are removed by the currently available enzyme preparations and how the conformational state of the proteins is affected. Patients may exhibit sensitivity to the enzymes, particularly papain [309] that adsorbs to the lens surface, [166] but papain is more effective at removing visible protein than pancreatin. [310 311]

Using both a surfactant and suitable disinfectant with frequent replacement lenses should obviate the need for enzyme tablets, as the lenses are replaced before the deposits build up to a significant level and the solution regime alone will remove 30-50% of the deposited protein. [62] This has been confirmed for low water content daily-wear planned replacement lenses worn for up to three months. [312]

v. Various

The disinfection efficacy of procedures such as ultrasonography, standing waves, microwaves and ultra-violet light has been questioned in several studies, [313 - 316] and their use cannot be advocated for the routine disinfection of hydrogel lenses due to their questionable microbiological efficacy and their influence on material parameters, particularly high water content materials. To date no studies have investigated their influence on deposit formation.

2.3.1.3 Compliance

Compliance is a complicated issue, with measures of compliance varying depending upon the criteria used to quantify it. [317] Studies show that 40-91% of contact lens patients are non-compliant in the use of contact lens care systems. [318 - 321] Intuitively it would appear that a simple system should result in better compliance than a complicated system. While this has been confirmed, [322 323] other studies have found that compliance levels were also poor with simple systems [320 321 324] and that cost is not an important factor. [325] This confirms the point that simple systems are not a substitute for careful training and frequent reinforcement on the need for adequate cleaning, rinsing and disinfection of all types of contact lenses, whether frequently replaced or traditional. Of particular concern is the fact that patients must adequately clean their lenses [288 289 326] and wash their hands before handling their lenses [327] to maintain safe, comfortable lens wear. However, it is these very factors that are frequently missed out. [319 - 323 328]

A number of factors relating to compliance may potentially influence deposition. These include inappropriate cleaning and rinsing of lenses, the use of contaminated tap water, the use of contaminated cases and

infrequent replacement of lenses. Indeed, in order that frequent replacement systems work adequately patients must be trusted to replace the lenses as requested. Patients using such systems are reportedly 97% compliant with replacement schedules when using re-usable frequent replacement lenses [329] and 95% compliant with single-use daily disposable products. [330]

2.3.1.4 Wearing Schedule

Deposit formation is greater with extended-wear than with daily wear of lenses, [78 83 331 332] with up to 80% of lenses becoming significantly deposited inside three months. [1] This is due to the lack of cleaning, length of wear and continual cyclical drying/wetting of the lens. [83]

2.3.1.5 Extrinsic Factors

Extrinsic factors such as make-up, [333 334] finger lipids, [72 334] nicotine, [101] various air-borne contaminants and bacteria [327] may all have an impact on lens spoilation. Most currently published data is anecdotal. The lens case is also a potential source of extraneous contamination. Patients should be encouraged to replace their lens case regularly, [335] to prevent the chance of contaminating their lenses with pathogens that remain attached to the biofilm in the case. Between 20-75% of cases exhibit microbial contamination. [336 - 339] Placing lenses in contaminated cases may contaminate the lens and negate one potential advantage of using frequent replacement lenses.

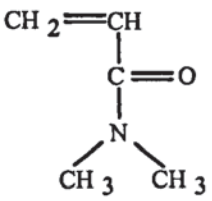
2.3.2 Lens Related Factors

Hydrogels are hydrophilic polymer molecules that are cross-linked by water. The amount of water adsorbed by a hydrogel is expressed as the equilibrium water content (EWC) and is defined as:

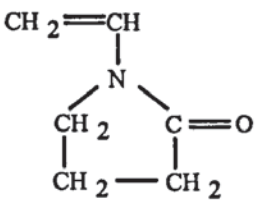
$$EWC = \frac{\text{weight of water in the gel}}{\text{weight of the hydrated gel}} \times 100\% \qquad \text{Equation 2.1}$$

The water in a hydrogel network exists in a continuum between two extremes - the "bound" or non-freezing water (which is strongly associated with the hydrogel network through hydrogen bonds) and the "free" or freezing water (which has much greater mobility and is free to partake of interactions with the surrounding environment). [340] The properties of any hydrogel depend upon both the water content and ratio of free to bound water. Many commercially available lenses are based on poly 2-hydroxyethyl methacrylate (polyHEMA), which has an EWC of 38%. Other monomers include methyl methacrylate, vinyl pyrrolidone, methacrylic acid and polyvinyl alcohol. Cross-linking agents (such as ethylene glycol dimethacrylate) are generally added to impart mechanical strength and thermal stability. Figure 2.16 outlines the chemical structures of these monomers.

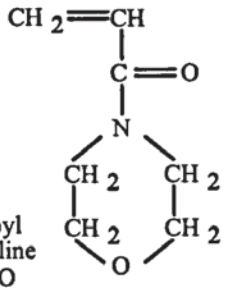
Figure 2.16 - Chemical Structure of Monomers Used in Hydrogel Synthesis



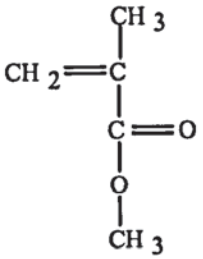
N,N-dimethyl Acrylamide NNDMA



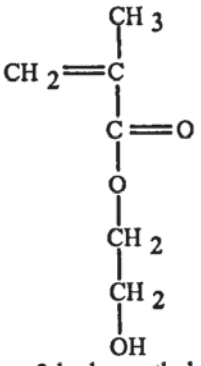
N-vinyl Pyrrolidone NVP



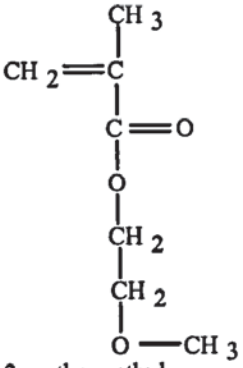
Acryloyl Morpholine ACMO



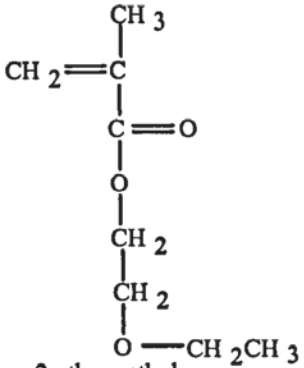
Methyl Methacrylate MMA



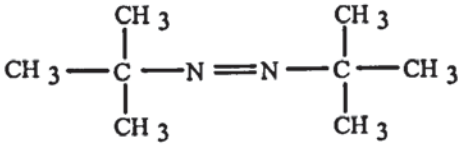
2-hydroxyethyl Methacrylate HEMA



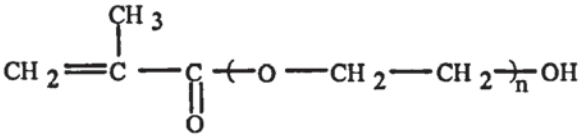
2-methoxyethyl Methacrylate MEMA



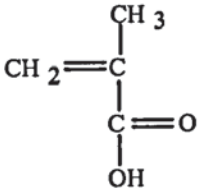
2-ethoxyethyl Methacrylate EEMA



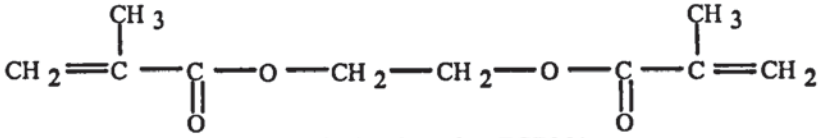
Azo-bis-isobutyronitrile AZBN



Polyethylene Glycol PEG



Methacrylic Acid MAA



Ethylene Glycol Dimethacrylate EGDMA

The cornea is avascular and derives most of its oxygen supply from the atmosphere. To prevent oedematous complications any contact lens must transmit sufficient oxygen to maintain as near normal corneal metabolism as possible. The gas permeability coefficient (P) of any gas and material is given by $P = D \times k$, where the diffusivity (D) is the amount of gas passing through the unit area of the material in the given direction in unit time and the solubility coefficient (k) is the volume of gas that dissolves in a unit volume of the material. [340 - 342] Gas transport (Dk) is directly proportional to the equilibrium water content of the material, with higher water content materials transmitting greater quantities of oxygen and carbon dioxide. [343 344] Currently available hydrogel contact lens materials have water contents ranging from 38% to 85%. The average level of oxygenation required at the anterior cornea to prevent oedematous complications is 10% EOP (Equivalent Oxygen Percentage), [345] although individual variations are marked [346] and age differences do arise. [347] To assess if currently available lenses provide patients with such levels of oxygen it is necessary to examine the transmissibility (Dk/t) of the lenses concerned. The most widely agreed figures for the minimum acceptable Dk/t are 24.1×10^{-9} (cm x mlO_2)/(sec x ml x mmHg) for daily wear and 87.0×10^{-9} for overnight or extended wear. [348]

Patients exhibit different corneal metabolic requirements [345 346 349] and some, notably those with higher prescriptions, may exhibit corneal swelling (oedema) and develop chronic hypoxic complications if only fitted with low water content materials due to the increased thickness of the lenses. [350 - 352] Such patients require high water content hydrogels to reduce such complications. Two principal strategies are available to increase the water content of hydrogels above that of polyHEMA. Small quantities of charged groups such as methacrylic acid or larger amounts of hydrophilic, neutral groups such as polyvinyl alcohol (PVA) or N-vinyl pyrrolidone (NVP) are added to polyHEMA or methyl methacrylate (MMA) to raise their equilibrium water contents to 60% or greater. Commercially available contact lens materials may be divided into various categories by considering their charge and water content. There are two classification systems available worldwide; the European ISO system and the American Food and Drug Administration (FDA) system. These are detailed in Tables 2.12 and 2.13.

Table 2.12 - ISO Classification System For Hydrogel Lens Materials

	a <0.2% weight of any ionizable chemical	b >0.2% weight of any ionizable chemical
Group 1	polyHEMA	polyHEMA
Group 2	copolymer of polyHEMA and/or other hydroxyalkylmethacrylates, dihydroxyalkylmethacrylates and alkyl methacrylates	copolymer of polyHEMA and/or other hydroxyalkylmethacrylates, dihydroxyalkylmethacrylates and alkyl methacrylates
Group 3	copolymer of polyHEMA with N-vinyl lactam and/or an alkyl acrylamide	copolymer of polyHEMA with N-vinyl lactam and/or an alkyl acrylamide
Group 4	copolymer of alkyl methacrylate and N-vinyl lactam and/or alkyl acrylamide	copolymer of alkyl methacrylate and N-vinyl lactam and/or alkyl acrylamide
Group 5	Hydrogels formed from polysiloxanes	None available

Table 2.13 - FDA Classification System For Hydrogel Lens Materials

Group I	Low water content[1], non-ionic[2]
Group II	High water content, non-ionic
Group III	Low water content, ionic
Group IV	High water content, ionic

2.3.2.1 Water Content

As described above, incorporating neutral polymers that are more hydrophilic than HEMA such as N-vinyl pyrrolidone (NVP) will increase the water content of hydrophilic materials. A major problem with such lenses is their rapid spoilation compared with low water content lenses. [10 115 122 123 126] High water content lenses have a greater pore size and/or number of pores and are therefore more susceptible to tear film constituents entering the lens matrix [115 122] but may denature protein to a lesser extent than low water content lenses. [124]

[1] low water content = ≤ 50% water

[2] non-ionic = ≤ 0.5% methacrylic acid

2.3.2.2 Surface Charge

To increase the hydrophilicity and water content of soft lenses acidic agents such as methacrylic acid may be added. [353] These charged substances produce lenses with an overall net negative charge. [85] These charged lenses attract higher levels of small, positively charged proteins such as lysozyme than lenses that are relatively inert. [7 11 115 116 119 123 122]

2.3.2.3 Manufacturing Technique

Manufacturing technique can influence deposit formation. [354 355] Lathe-cut lenses, due to their lathing marks, attract more surface deposits than those lenses fabricated by either spin-casting or moulding. [9] However, the results of several studies investigating this factor are contradictory [355 356] and, if there is any significance, it is only of minor importance when compared with water content and surface charge.

2.3.2.4 Age

As lenses age they attract more deposits. This occurs because the surface layer of spoilation acts as a site for further deposition. [357] Even when used correctly, currently available solution systems only remove 30-50% of deposited protein, leaving much of the deposited albumin and lactoferrin in place. [62]

2.4 CLINICAL MANAGEMENT OF DEPOSITS

Contact lens deposits cannot be entirely avoided. However, several methods are available to clinically manage heavy depositors.

2.4.1 Care Regimen

The use of a daily surfactant cleaner will solubilize the debris laid down on the lens throughout the day and prevent their immobilization. [69 255] Non-preserved cold disinfection systems will reduce discolouration and solution reactions. [358] Regular use of enzyme tablets will aid in protein removal. [66 - 68 250] Occasionally boiling the lenses in a solution containing ethylenediamine tetraacetic acid (EDTA) will remove excess calcium. [96] However, little information is available on the effectiveness of changing solutions in patients who are experiencing deposit-related complications.

2.4.2 Material Choice

Switching the patient to a low water content, non-ionic lens [7 11 115 116 119 123 122] or using reportedly deposit resistant polymers such as Tetrafilcon A [359] or Glyceryl Methacrylate [75 84 360] will reduce the incidence of protein deposits. However, little evidence exists regarding the effectiveness of changing materials within the same FDA group. [118]

2.4.3 Planned Replacement

The terminology regarding the wearing time for hydrogel lenses is confusing, with the terms "disposable" and "frequent replacement" being often interchanged. The terminology used in this thesis will be that adopted by the FDA: [361]

a) Conventional replacement/traditional wear - no specific replacement is scheduled. Lens replacement results when the lens becomes lost, deposited or due to a natural change in prescription.

b) Planned/frequent/programmed replacement - there is a specific period after which the lens will be replaced. Time periods usually cover 12 months to one week. These lenses are all reused (ie inserted, removed, cleaned and re-inserted) during their use.

c) Disposable - these lenses are all inserted only once; upon removal they are disposed of and never re-inserted. These currently only cover two specific types of lenses - daily wear daily disposable and extended wear lenses worn for at least one and up to six nights before being disposed of.

Switching the patient to a frequent replacement system will prevent deposit formation. [362] A review of deposition by Tripathi et al [2] almost 20 years ago led them to conclude that "...for may patients the only answer to deposition may be an inexpensive, disposable lens that can be replaced weekly or biweekly". However, little work has been published to date to enable practitioners to clinically assess the optimum replacement period required. [363]

2.5 SUMMARY

Clearly hydrogel lenses deposit rapidly with virtually all constituents of the tear film and these deposits produce clinical problems. The causes of deposition are multifactorial, involving factors relating to both the wearer and the worn material. While there are well-established methods of evaluating such deposition, much of the published work to date has revolved around the examination of contact lenses using destructive techniques on pooled samples, many of which have unknown history, wear schedules and care regimens. The results from such studies are often contradictory in nature and narrow in terms of their conclusions. To date it is difficult to decide if the variation that occurs is due to true variations or variations in the quality and sensitivity of the analytical work.

The selective role that various elements play in the spoilation of contact lenses has been only superficially studied. Clinically it would appear that certain components are more likely to produce spoilation in specific patients in particular material/care regimen combinations.

A review of the literature proves that to date much of the work conducted has evaluated deposition and its causes separately. There is a clear need for further investigation into the interactive effects between contact lens materials, wear periods, care systems and lens-wearing patients and the influence of deposition on clinical performance. These are the areas that will be addressed by this thesis.

Chapter 3

Experimental Methods and Materials

"The men who try to do something and fail are infinitely better than those who try to do nothing and succeed" [1]

To investigate the variables influencing deposition, several controlled clinical studies were conducted. Subjects used various combinations of lenses and solutions for specified periods, after which the lenses were collected and refrigerated at the clinical site (in London), before their transport to the laboratory for detailed deposition analysis (in Birmingham). All lenses were subjected to non-destructive analysis, avoiding the complications of species removal. Following this procedure other destructive techniques were employed as required to obtain further information.

3.1 ETHICAL APPROVAL

Subjects were enrolled at the clinical site following approval of the clinical studies by the Human Science Ethical Committee at Aston University

3.2 SUBJECT RECRUITMENT

Subjects were principally recruited from the private optometric practice in which the studies were conducted. A notice was displayed in the waiting area and potentially suitable subjects identified from the practice database were sent a letter to advise them of forthcoming studies. Additional subjects were recruited via a notice placed in the local newspaper, an example of which is provided in Appendix 1 (page 350).

3.3 SCREENING PROTOCOL

On presentation subjects were screened to ensure their suitability for each study. Subjects had ≤ 1.00 dioptre (D) of astigmatism and were successful wearers of daily-wear hydrogel lenses for at least three months. The subjects wore a mixture of high and low water content lenses and none had previously used any of the test lenses or solutions. Subjects with eye disease, allergies, insufficient lacrimal secretions, pre-existing ocular infections, any history of problems with lens wear and pregnant subjects were all excluded.

---

[1] Lloyd Jones

The full list of "Inclusion" and "Exclusion" criteria are detailed in Appendix 2 (page 351) and the "Screening Form" in Appendix 3 (page 352-353).

3.4 INFORMED CONSENT

Following an initial screening appointment suitable subjects were fitted with each lens type. If the lens fit was acceptable then the nature of the particular study was explained and an informed consent form signed, outlining the procedures required throughout the study.

Appendix 4 (page 354-355) contains a typical "Informed Consent" form.

3.5 STUDY DESIGN

All contact lens studies used either a randomised cross-over or contralateral eye design. In all studies both subjects and practitioners were unaware of the lens type (double-masked paradigm) and care systems were also masked from the practitioner (single-masked). Masking and randomisation was the responsibility of a clinical assistant, who was also responsible for instructing the subjects on the care regimens. The importance of concealing the randomization of clinical studies has been clearly shown [364] and for all studies the randomization schedules were kept under lock-and-key and were only accessible to the clinical assistant.

3.6 CLINICAL PROTOCOL

A standard clinical protocol was followed. The clinical routine used was that normally employed in a contact lens consultation, with the following order of events being strictly followed:

a) Subjective satisfaction - symptoms/problems, wearing times, compliance with care regimen.
b) Visual acuity.
c) Front surface wettability.
d) Lens fit.
e) Visible surface deposition.
f) Lens removal.
g) Slit-lamp examination with sodium fluorescein.

During the period of the clinical studies no enzyme tablets or rewetting drops were used at any time.

3.6.1 Subjective Satisfaction

Subjective satisfaction with lenses and/or solutions is frequently undertaken using interval scales. Conventional four point scales are too coarse for accurate assessment. [365] In these studies continuous 10 point interval scales with fixed end markers were used. Subjects graded their lenses and/or care systems for various factors on a 1-10 scale, where 1 represented the worst case and 10 the best.

Typical "Satisfaction Scales" are detailed in Appendix 5 (page 356-357).

3.6.2 Visual Acuity

Visual acuity measurements are important as part of the normal subjective routine and decrease with increasing lens spoilation and age, [14 15] although such changes are not seen with lenses replaced regularly. [366]

Visual acuity was recorded as logMAR acuity on Bailey-Lovie charts [367] (produced at the Multimedia Centre at University of California, Berkeley) at high (90%) and low (10%) contrast. [368] The system of recording used the Visual Acuity Rating (VAR) scale, [369] which records the actual number of letters read correctly on each line. The VAR scale represents 0.0 logMAR (Snellen = 6/6) when 100 letters are read, with 5 letters on each line. A difference of 1 VAR represents 1 letter less/more being correctly read, which is equivalent to 0.02 logMAR. Any letter read incorrectly on any line resulted in a reduced score of one letter. The total number of letters incorrectly read were recorded and an error score of "1" assigned to each. These error scores were added to the score for the last line on which any letters are read. This method regarded letters missed on "suprathreshold" lines (lines on which the majority of letters are read) as an additional indication of some deficit. In psychophysical terms these errors may be regarded as a reduction in the gradient of the "frequency of seeing curve", thereby increasing the range of letter sizes over which the subject experiences some uncertainty.

To reduce learning effects the subjects were instructed to read the charts from right to left with their right eye and vice versa with their left eye at each recording session. While statistical significance of change is recognised as requiring 7-10 letters change, [370 371] it is considered that this is too high for clinical significance, which may be closer to half a line of acuity change (2-3 letters). [366] Such precision may be achieved if a careful testing procedure is adopted. [372]

The illumination of the charts was recorded at each recording session. The illuminance during the studies, as recorded by an INS DX-100 Lux Meter was 442 ± 12 lux (range 453 - 426 lux).

3.6.3 Front Surface Wettability

As contact lenses age and become deposited their surface becomes contaminated. This contamination reduces the length of time over which the subjects' tear film remains adherent to the front surface of the lens. [363 373] The time taken in seconds for this front surface tear film to initially break up is termed the prelens non invasive break-up time (PLNIBUT) and may be used to provide some means of visually monitoring the lenses' surface wettability. In addition, short front surface break-up times may have an impact on visual performance, [374] particularly low contrast acuity. [375]

Front surface wettability of the lenses in this study was assessed using two methods.

3.6.3.1 Loveridge Grid

The "Loveridge Grid" [218] is a modified hand-held Klein keratoscope in which the conventional circular mires are replaced with a grid. The grid is projected onto the front surface of the contact lens and the time for the first square to distort is measured with a stop-watch. The time taken in seconds for the distortion to occur is recorded as the PLNIBUT.

The keratoscope is used without the normal magnifying lens in place. The grid is visualised via the viewing system of the slit-lamp, with a 20x eye-piece and 16x objective. The illumination system is turned off and placed nasally to enable easier positioning of the grid, with the internal illumination system of the keratoscope being used to illuminate the cornea (Figure 3.1).

Measurements were obtained by asking the subject to blink once and stare for as long as possible and the time taken for the projected grid to distort was recorded to the nearest second with a stop-watch, with the time being truncated after 30 seconds if no break occurred. This was repeated on two further occasions, with the subject being asked to blink five times between each measurement. The median of the three readings was recorded as the *in vivo* wettability (PLNIBUT).

Figure 3.1 - The Loveridge Grid

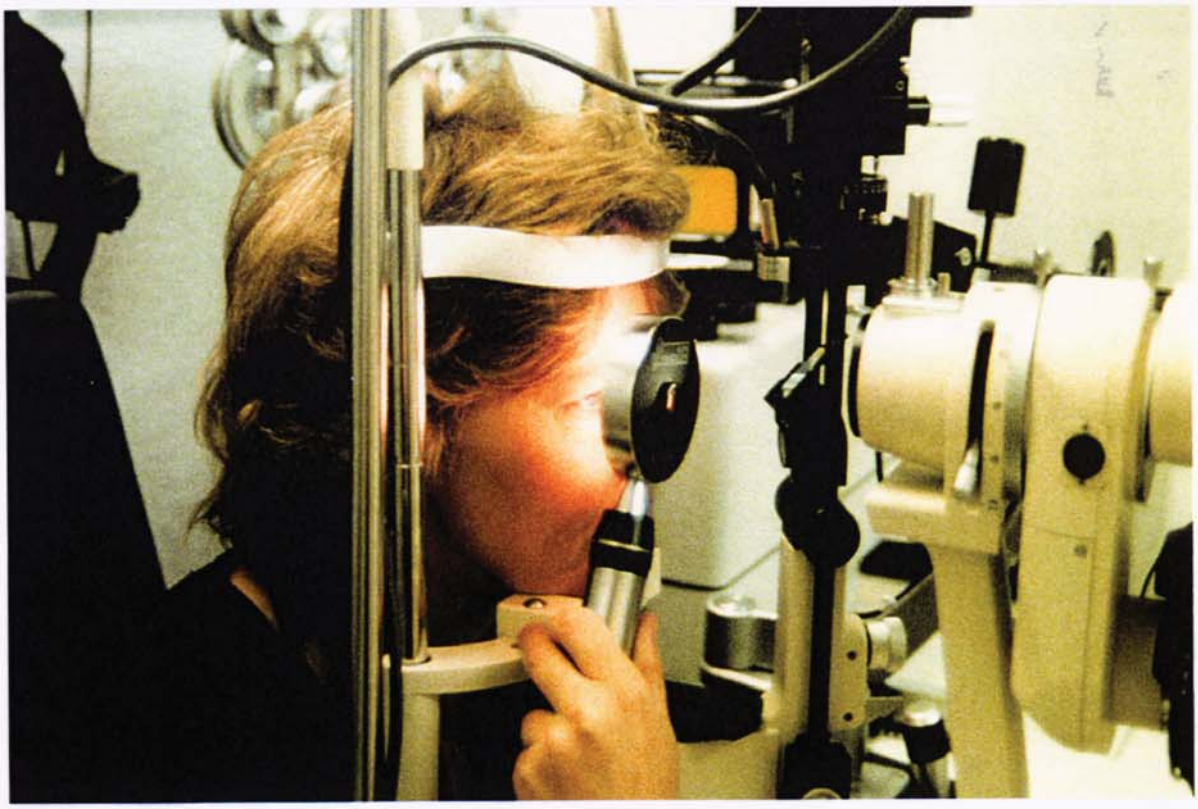


Figure 3.1 - Loveridge Grid in use

The disadvantage of this system is that the projected image only covers some 6-7mm of the cornea and it is possible that a break in the tear film will occur outside this area. While this technique has a distinct advantage over another widely accepted technique using a keratometer, [220] that only covers 3mm, studies have shown that the first break in the tear film often happens outside the central area, [214 376] with one study [363] indicating that the initial break in the tear film over the lens only occurs within the central area in 5% of cases. Whilst two other devices are available to assess PLNIBUT over the whole diameter of the lens (one based on a grid [217] and another on interference [143]), neither device was commercially available when this project commenced.

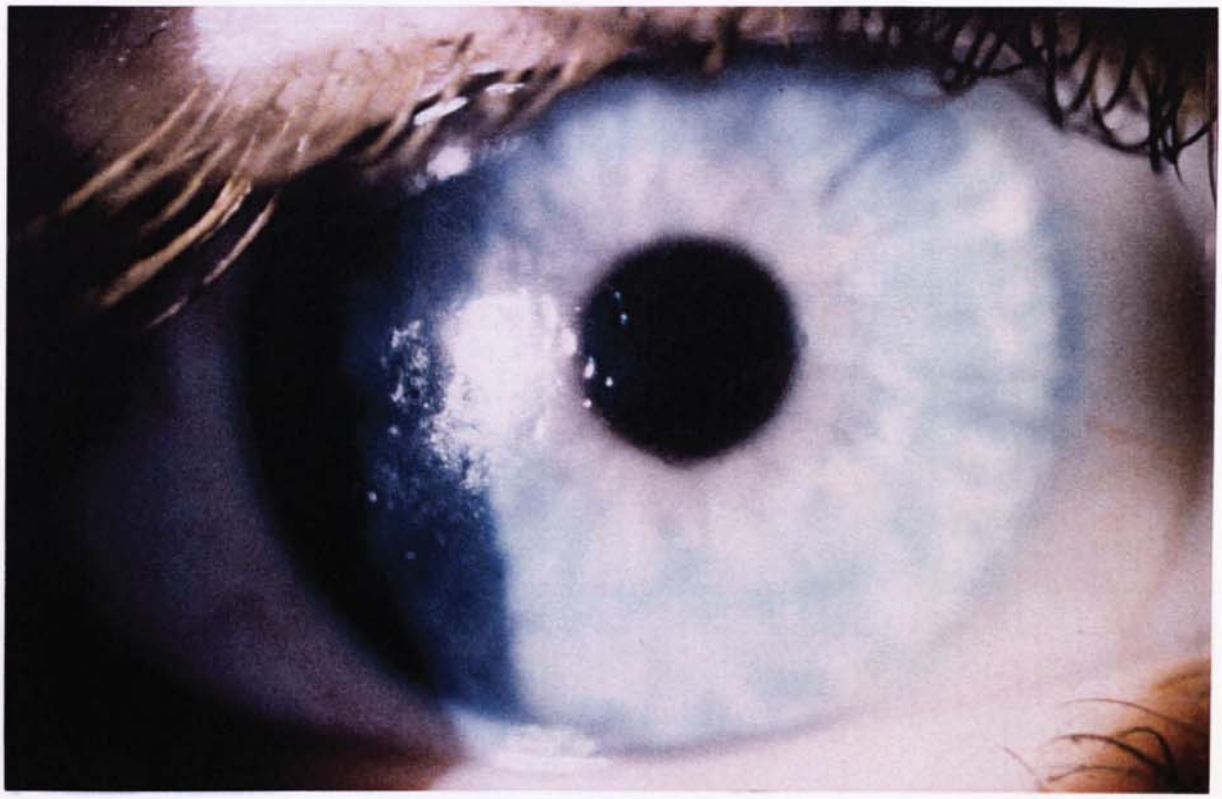
3.6.3.2 Slit-lamp

Subjects were asked to blink and stare wide for as long as possible. During this time the anterior lens surface was scanned with the slit lamp with a moderately bright, narrow slit and the time taken for a break to develop in the anterior tear film measured with a stop-watch. The visible wettability used an interval scale, as shown in Table 3.1. This technique has the advantage that all the lens surface is visible at all times. A typical view of a poorly wetting lens is shown in Figure 3.2.

Table 3.1 - Visible Wettability By Slit Lamp Assessment

GRADE	DESCRIPTOR
0	Perfect - complete tear coverage after 10 secs
1	Good - break-up after 5-10 secs
2	Moderate - hazy surface and break-up time < 5 secs
3	Poor - Non-wetting patches immediately after blinking
4	Very poor - hydrophobic areas associated with visual reduction

Figure 3.2 - Poorly Wetting Anterior Lens Surface



3.6.4 Lens Fit

Poorly fitting lenses could potentially influence spoilation, since such lenses may stimulate the ocular adnexa to produce more mucous or lipid. Additionally, conjunctival staining is influenced by lens movement. [377] Assessment of lens fit was important to ensure that compared lenses fitted in a clinically similar manner. Complete coverage of the cornea must occur in all positions of gaze and sufficient movement must occur such that debris is flushed from under the lens. Fitting was undertaken to agree with that recommended in standard texts. [378 379]

Lens fitting was assessed in four ways, using conventional methods previously described by others. [380 381] Lenses were allowed to settle for 20 minutes post insertion before any fitting assessment was undertaken.

3.6.4.1 Lens Movement

Lens movement in the primary position was recorded in mm using an eye-piece graticule engraved with 0.1 mm divisions. To aid the measurement (so that the lens edge was visible) each subject was asked to look at a point 40 mm above the horizontal (30°) at a fixation point attached to the objective of the slit-lamp while the degree of vertical movement was noted. A 12.5x eye-piece and 16x objective resulted in a movement of 1 mm being equivalent to 12 graticule units. Movements of one graticule unit are detectable, representing a movement of 0.08 mm. Within the bounds of measurement error this represents a resolution of ±0.1 mm. Movement was reported in graticule units for simplicity.

3.6.4.2 Total Diameter

This was also assessed *in-situ* by use of the graticule described in 3.6.4.1.

3.6.4.3 Percentage Tightness

Percentage tightness was assessed using a vertical push-up test. A continuous percentage scale was used, where 100% represented no movement, 50% a "perfect" fit and 0% a lens that totally decentred upon removal of lid tension. [382] This factor provides the most accurate single test of dynamic lens fit. [383]

3.6.4.4 Lens Centration

Lens centration was recorded in mm via a "grid system", where superior and nasal positions were recorded as positive values and inferior and temporal decentrations negative values. [384] Values were recorded from the limbus in four positions (superior, nasal, inferior and temporal) [381] and the decentration thus calculated. Total decentration was finally calculated from Pythagorean theorem.

3.6.5 Visible Deposition

The recording system used a modified Rudko technique [111] in which the lens surface was graded *in vivo* rather than *in vitro*, as suggested by Josephson & Caffery. [385] Subjects blinked and stared for 15-20 seconds. As the anterior lens surface dried out the type, coverage and degree of deposition became visible and was graded using the scale detailed in Table 3.2. Studies using this technique have clearly shown that deposition increases with increasing lens age. [14]

Table 3.2 - Visible Deposition Grading

TYPE	COVERAGE	DEGREE
0. None	0. 0%	0. Clear
1. Dots	1. ≤25%	1. Light
2. Haze/Film	2. ≤50%	2. Medium
3. Calculi	3. ≤75%	3. Heavy
	4. ≤100%	4. Very Heavy

As previously described in Chapter 2, such systems correlate only weakly with laboratory-estimated levels of deposition.

Figure 3.3 - Lens Calculi

Figure 3.3 - Lens calculi. Grading using the system used in this thesis would classify this degree of deposition as type 3, coverage 1, degree 2.

3.6.6 Physiological Performance

All slit-lamp examinations were undertaken on a Nikon FS3 zoom photo-slit lamp, with 20x eye-pieces at magnifications ranging from 7.5-30x. Fluorescein examinations were undertaken with a blue excitation filter and yellow barrier filter (Kodak Wratten #12) to enhance the contrast of any staining. [386] Observation of the

clinical signs in this study was used to ensure that no untoward complications were occurring with the lens/care system combination under test

Findings such as blood vessel infiltration into the cornea can be measured using a graticule eye-piece and multiple findings such as epithelial microcysts and stromal striae can be counted. However, most clinical findings cannot be recorded in this way. The generally accepted recording technique for use with features that cannot be measured depends upon the practitioner assigning scales to their clinical observations. Each observation may be regarded as part of a continuous scale that is assigned a grade based on the clinical observation. This grade serves as a standard by which any future observation can be judged.

A typical option is to use a 5 point "verbal" interval scale such as that in Table 3.3.

Table 3.3 - Clinical Severity Scale

Numerical	Descriptive	Meaning
Grade 0	Normal	No action
Grade 1	Slight	Not clinically significant
Grade 2	Moderate	May require intervention
Grade 3	Severe	Requires intervention
Grade 4	Very severe	Requires medical intervention

Confidence limits must be ascertained which will provide an indication as to the likely probability that a second observation will result in a grade that is different from the first when no real change has occurred. Confidence limits depend upon the clinicians consistency in assigning grades. [387] More sensitive results are found if the scale is expanded to 0-10. [365 388] One possible adaptation of the above scale is to use plus or minus increments (eg 0, -1, 1, 1+). [388] This changes the above scale from a 5-point scale to a 9-point scale and was the system used in these studies. In addition, where possible, dividing the ocular structure of interest into specific quadrants [389 390] will enhance such scales, [391] as each area can be assigned a severity scale and all areas added to give a global score. [392] The advantage of this scoring system is that the principal area of staining, in addition to its extent, can be detected.

Upon commencing this study no scales other than verbal ones were available. Over the past two years the subject of grading has received considerable interest. The use of picture scales (particularly when used with a decimalised scoring system) reduces inter and intra-observer variability. [387] The publication of such findings has resulted in the recent release of picture-based grading scales using either photographs (from the CCLRU) or drawn illustrations (from Efron [393]). Such scales offer better repeatability than verbal scales. [394] Conventional recording scales were used for all ocular features of interest, based on or around reports previously published. [395]

3.6.6.1 Corneal Staining

These studies used an adaptation of the CCLRU grading scale [396] and is described in Table 3.4.

Table 3.4 - Corneal Staining Severity Scale

EXTENT	DEPTH	AREA
0 - None	0 - None	A = Superior
1 - 1-20 punctate spots	1 - Not stromal	B = Nasal
2 - 21-40 punctate spots	2 - Stromal, slow diffusion	C = Inferior
3 - Light confluent area	3 - Immediate, moderate stromal diffusion	D = Temporal
4 - Abrasion/ulcer	4 - Widespread stromal diffusion	E = Central

The grading system used 0.5 steps and was scored by taking the staining score for each area (extent x depth) and adding all five areas together to achieve a global score.

3.6.6.2 Corneal Oedema

This was assessed using a clinical grading system [350] rather than physically measured using a pachometer. This technique correlates well with pachometry measures. [397]

Table 3.5 - Corneal Oedema Severity Scale

Grade	Signs
0	None
1	1 Striae
2	2-5 Striae
3	6-10 Striae
4	>10 Striae or any Folds

3.6.6.3 Bulbar Conjunctival Staining

The grading system was similar to that adopted by other workers. [390 398]

Table 3.6 - Bulbar Conjunctival Staining Severity Scale

Severity	Area
0 - None	A = Superior
1 - Mild	B = Nasal
2 - Moderate	C = Inferior
3 - Severe	D = Temporal
4 - Very Severe	

The grading scale was scored by adding the severity score for all four areas together, to achieve a global scoring index.

3.6.6.4 Bulbar Conjunctival Hyperaemia

A modification of the standard severity scale, broadly based on the photographic scale of McMonnies and Chapman-Davies, [399] was used.

Table 3.7 Bulbar Conjunctival Hyperaemia Severity Scale

Grade	Signs
0	None
1	Mild
2	Moderate
3	Severe
4	Very Severe

3.6.6.5 Palpebral Conjunctival Appearance

A grading system broadly based on that previously published was devised. [395]

Table 3.8 - Palpebral Conjunctival Grading System

Hyperaemia		Follicles	
Grade	Signs	Grade	Signs
0	None	0	None
1	Trace	1	Trace
2	Mild	2	Mild
3	Moderate	3	Moderate
4	Severe	4	Severe

3.7 SCHEDULED VISITS

To reduce differences between subjects they were informed that they should wear their lenses for a minimum of ten hours and maximum of sixteen hours daily. Clinical visits were scheduled for between 4 and 8 hours post-insertion and, whenever possible, within one hour of the initial appointment time. Clinical visits during the various studies occurred after 14 (±2), 28 (±3), 90 (±7) or 180 (±14) days.

3.8 DISCONTINUATION CRITERIA

Subjects were discontinued for any of the following reasons:

1. Clinically significant (>grade 2) biomicroscopic findings.
2. Persistent study related signs/symptoms.
3. Unacceptable vision.
4. Persistent poor fit with one of the lenses.
5. Inability to maintain the required wearing schedule.
6. Any prescription or topical ocular medications prescribed at any stage during the study.
7. Lack of motivation.
8. Failure to attend required visits.
9. Use of a non-study disinfection regimen.
10. Lost to follow-up.
11. Any non contact lens related ocular problem.
12. Pregnancy.

3.9 ANALYTICAL PROTOCOL

Upon completion of the four-week wearing schedule each lens was removed with plastic-tipped tweezers and placed in a glass vial containing sterile non-preserved non-buffered saline. The vials were capped, labelled with the subject's study number and refrigerated before spoilation analysis, which occurred within 28 days of collection.

Analysing contact lens deposition is not without its complications, as the sample sizes are small and the material of interest is bound to a solid substratum. Previous work has shown that variations between subjects are significant, so pooling of samples to increase the sample size is not ideal and decreases the relevance of the results obtained. Ideally, only analytical techniques that are sufficiently sensitive to allow for analysis of single lenses would be used. This is rarely possible. It was important in these studies to use a number of analytical techniques that were non-destructive in nature and could analyse the deposited substances *in-situ*. Finally, the complexity of the spoilation mechanism means that no single technique can presently characterise all species present and a battery of tests is required to elicit the basis behind the spoilation process.

The analytical techniques may be broadly divided into destructive and non-destructive methods.

3.9.1 Non-Destructive Analysis

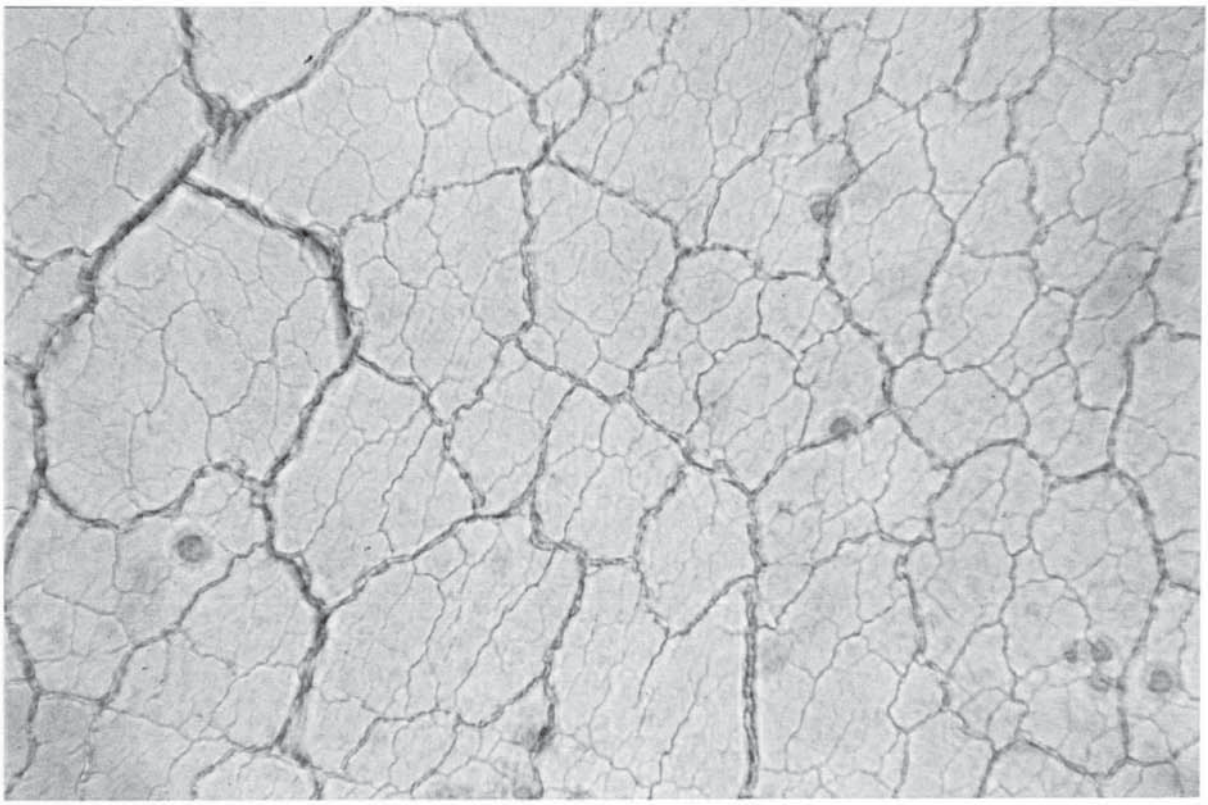
3.9.1.1 Light Microscopy

The gross morphology of the deposited species was examined under a Leitz Dialux 20 microscope with a Wild MPS 15/11 camera and photographed with Kodacolour 400 film.

The image of an object must differ in light intensity from that of the surrounding medium for it to be seen, the difference in intensity between object and medium being termed the contrast. Most biological specimens (including some deposits) are transparent, such that the contrast is near zero. In the past the solution to this problem was to stain the specimen. However, a variety of special illumination techniques have been developed to create contrast and obtain quantitative information. [400]

a) Bright field - the specimens under this type of monochromatic illumination change the amplitude of light so that different areas appear at different brightness (Figure 3.4).

Figure 3.4 - Bright Field View of Protein Film

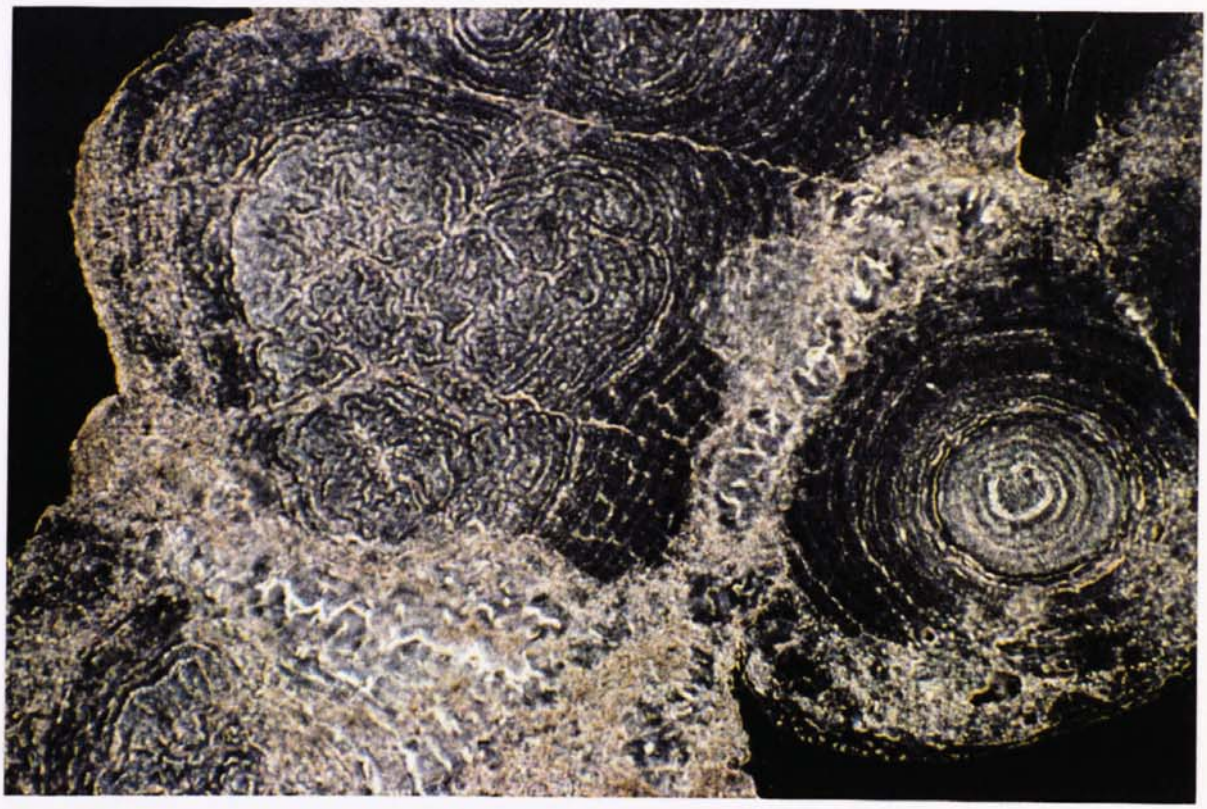


b) Phase contrast - used to render transparent objects visible, relying on their differences in refractive index. Viewing is achieved by converting the phase difference in the light passing through them into changes of wave amplitude (Figure 3.5)

c) Dark field - mainly used for highly transparent objects. The objects' edges are generally brightly illuminated against a dark background (Figure 3.6).

Figure 3.5 - Phase Contrast Picture of Lens Calculi

Figure 3.6 - Dark Field Picture of Lens Calculi



d) Fluorescence - a sensitive method for measuring minute quantities of fluorescent material. When a specimen is illuminated at an exciting wavelength of light (360nm) and observed through a filter that excludes the exciting light but transmits the fluorescence, components of the specimen that are difficult to detect are visualised (Figure 3.7).

Figure 3.7 - Fluorescence Microscopy Picture of Lens Calculi

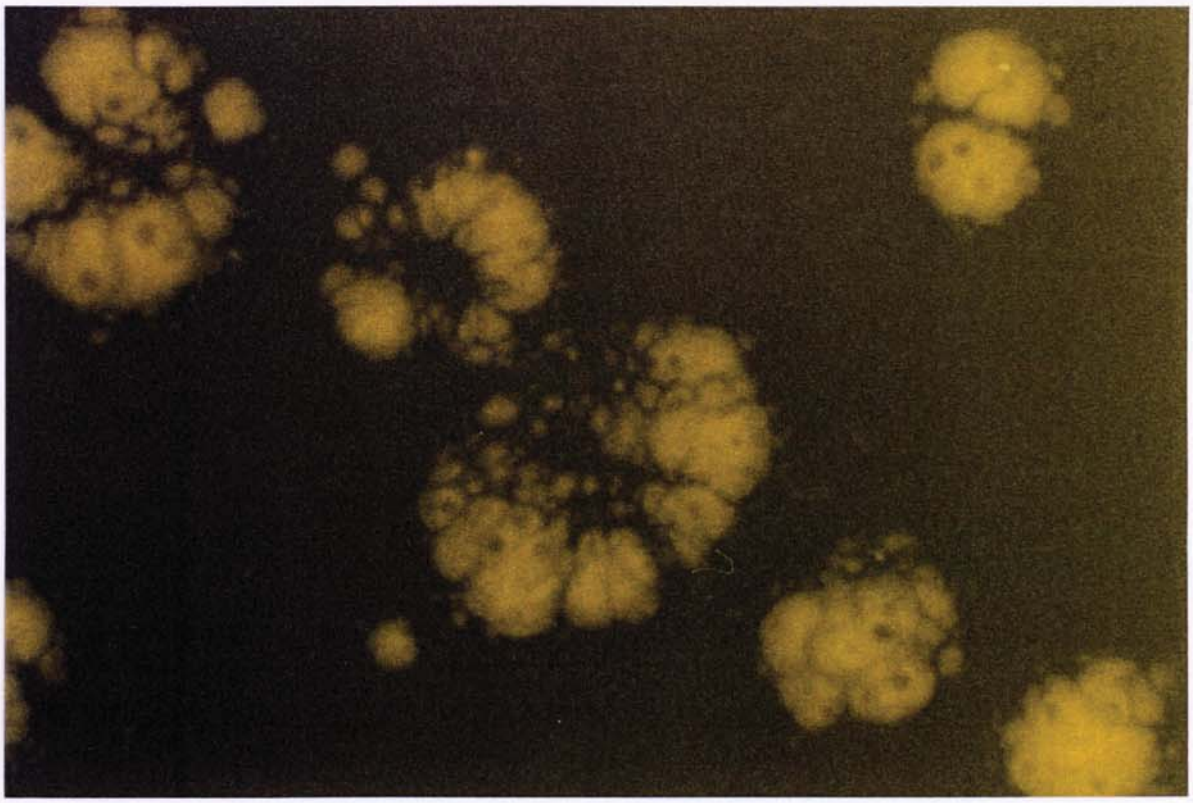


Figure 3.7 - Fluorescence photography reveals the autofluorescent nature of the lipids contained in the calculi. Photograph courtesy of Valerie Franklin.

3.9.1.2 Ultra Violet (UV) Spectroscopy

a) Background

All molecules absorb energy from at least one region of the spectrum of electromagnetic radiation. The wavelengths that are absorbed and the efficiency of absorption depend on both the structure and the environment of the molecule, making absorption spectroscopy a useful tool for characterizing both small and large macromolecules. Light consists of mutually perpendicular electric and magnetic fields, which oscillate sinusoidally as they are propagated through space. The energy (E) of the wave is calculated by:

$$E = hc/\lambda = hv \qquad \text{Equation 3.1}$$

in which h is Planck's constant (6.626×10^{-34} Js), c is the velocity of light (3×10^{10}cm sec^{-1}), λ is the wavelength and v is the frequency. When such a wave encounters a molecule it can either be scattered or absorbed, the relative probability of the occurrence of each process being a property of the molecule encountered. If the electromagnetic energy of the light is absorbed, the molecule is said to be excited. A molecule or part of a molecule that can be excited by absorption is called a chromophore. With some molecules the energy is reemitted as fluorescence (see 3.9.1.3, page 89). Light of wavelength λ can be absorbed only if:

$$\lambda = hc / (E_2 - E_1) \qquad \text{Equation 3.2}$$

in which E_1 is the energy level of the molecule before absorption and E_2 the energy level reached by absorption. A change between energy levels is called a transition. In the ground state electrons occupy the lowest levels of energy, as dictated by the laws of quantum mechanics. For most molecules the wavelengths corresponding to transitions between the ground state and the first excited state fall in the range of UV and visible light. Low energy transitions are also possible between vibrational levels within a single electronic level. These transitions produce radiation in the infrared (IR) range (see later). Absorption spectra in the ultraviolet region (200-400 nm) are due to energy transitions of both bonding and non-bonding outer electrons of the molecule. If energy is absorbed then the electrons move to a higher energy level, provided the energy level is of the correct value. Spectrophotometry involves the measurement of these absorptions.

The intrinsic chromophores present in proteins (the aromatic amino acid residues tyrosine and tryptophan) exhibit an absorption maximum at or near 280 nm. The method is relatively sensitive, measuring protein concentrations as low as 10 μgcm^{-3}. [401] The great advantage of this assay is that it is non-destructive and can be measured continuously, for example in chromatographic column effluents.

The actual measurement technique is based upon the Beer-Lambert law, which is a combination of two laws each dealing separately with the absorption of light related to the concentration of the absorber (the substrate responsible for absorbing the light) and the path length or thickness of the layer (related to the absolute amount of the absorber). If a substance is partially transparent it will transmit a portion of the radiation incident upon it.

The ratio of the transmitted and incident light gives the transmittance (T):

$$T = I / I_o \qquad \text{Equation 3.3}$$

where I_o is the intensity of the incident radiation and I the intensity of transmitted radiation. A 100% value of T represents a totally transparent substance, whereas a zero value of T represents a totally opaque substance. For intermediate values we can define the absorbance (A) or extinction (E) that is given by the logarithm of the reciprocal of the transmittance:

$$A = E = \log_{10} (1/T) = \log_{10} (I_o/I) \qquad \text{Equation 3.4}$$

Absorbance used to be called optical density (OD). It is a unitless parameter and has a range of values from 0 (=100%T) to ∞ (=0%T). The Beer-Lambert law states that the absorbance is proportional to both the concentration of absorbant and thickness of the layer:

$$A = \epsilon_\lambda cl \qquad \text{Equation 3.5}$$

where ϵ_λ is the molar absorbance coefficient for the absorber at wavelength λ, c is the concentration of absorbing solution and l the path length through the solution (or thickness of substance or cell size). Typically the holder for the substance under test (cuvette) has a path length of 1 cm.

The wavelength corresponding to a peak of maximum absorption is called λ_{max}. A list of some useful λ_{max} for this project is outlined in Table 3.9.

Table 3.9 - Absorption Maxima For Various Substances

Molecule	λ_{max} (nm)
Tryptophan	280
Tyrosine	274
Phenylalanine	257

Bulk protein concentration present in the contact lenses is measured using UV absorbance prior to the lenses being extracted for analysis by electrophoresis.

b) Instrumentation

Cuvettes (optically transparent cells) contain the material under study, along with a reference cuvette, which is used to set the spectrophotometer to read zero extinction. [402] These are made from UV transparent quartz (the low temperature form of silica, SiO_2) and are optically matched, with identical path lengths. The intrinsic absorbance of the unworn contact lens must be subtracted from the measurement obtained. This so-called "blank value" is obtained by using two matched cells. In the first method the reference cuvette contains a blank lens of approximately the same power. In the second method the reference cuvette contains only the fluid in which the test lens is suspended. Once the test lens is examined, the lens in the test cuvette is replaced with an unworn lens and the inherent absorbance is subtracted from the lens under investigation.

A set of standards is prepared in order to produce a concentration versus absorbance calibration plot. This appears as a linear plot because it satisfies the Beer-Lambert equation. Absorbances of unknown compounds are then measured and the concentration interpolated from the linear region of the plot.

While variable in type, all UV spectrophotometers essentially consist of the following:

- A tungsten filament lamp - produces wavelengths in the visible region (350 - 900 nm).
- A hydrogen or deuterium lamp - produces wavelengths in the UV region (200 - 400nm).
- Monochromator - selects a single wavelength of monochromatic radiation.
- Optical system - splits the multi-wavelength source into its component parts, such that light of the same reference illuminates both the sample and reference.
- Cuvette - transparent holder to hold test and reference samples.

- Photosensitive detector - measures incident and transmitted light.
- Recording device - chart recorder or computer.

This project used a Hitachi model U2000 spectrophotometer. This device features a Seya-Namioka monochromator with an Hitachi high resolution concave diffraction grating and uses a half mirror to separate the incident beam between the sample and reference cell. The cuvettes are square in cross-section (10 x 10 x 40 mm) and hold 3 ml of fluid.

Figure 3.8 - Line Diagram of Typical Double-Beam UV Spectrophotometer

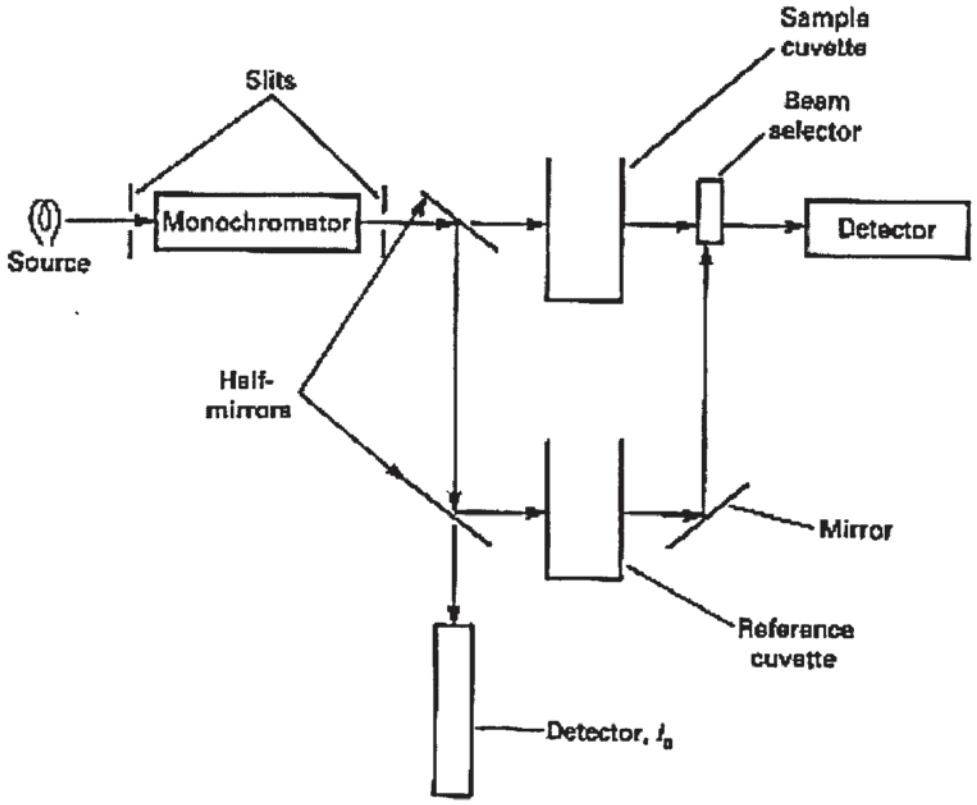
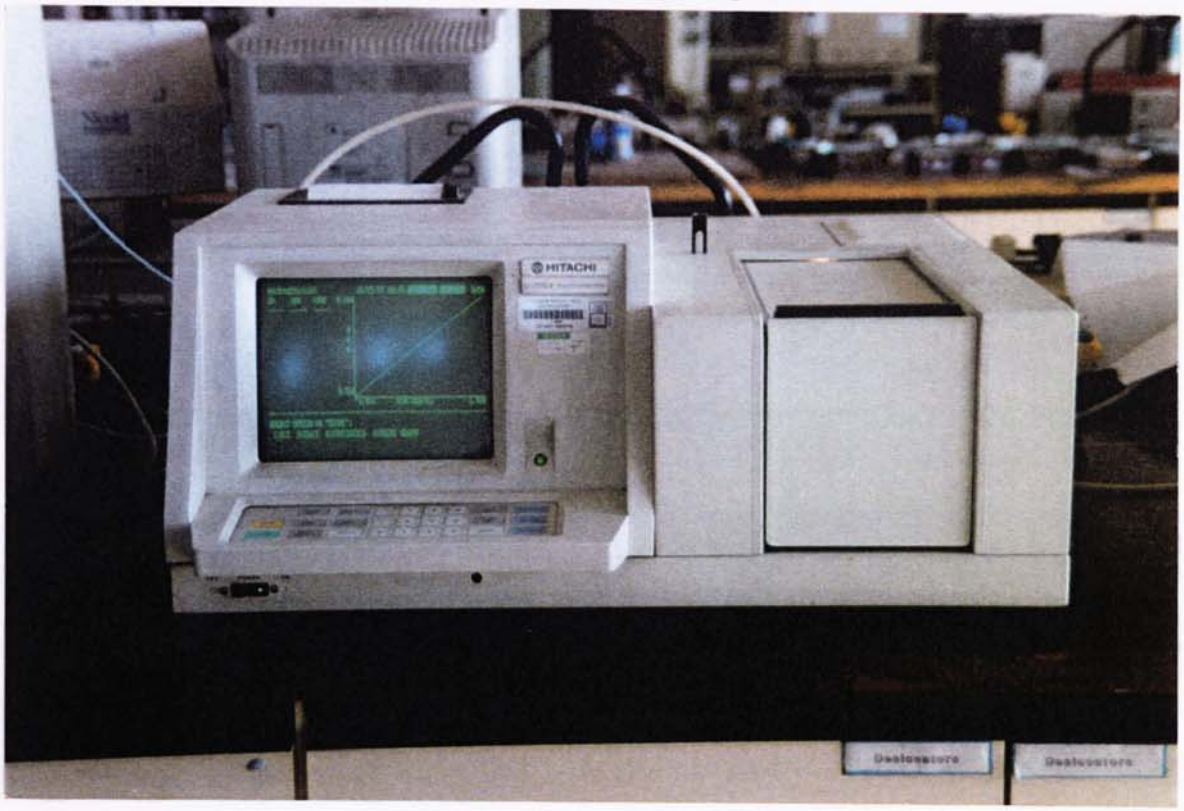


Figure 3.8 - Optical arrangement of typical UV spectrophotometer. Taken from Wilson & Walker. [401]

Figure 3.9 - Hitachi U2000 Spectrophotometer



c) Technique

- The cuvette was filled with fresh saline or distilled water and wiped dry, before placing in the test holder. The same procedure was conducted with the reference holder.
- The machine was auto zeroed.
- The test lens was placed in the test cuvette. The cuvette was marked to ensure that the lens always faced the same direction. The lens was always placed at the bottom of the cuvette, facing towards the direction of the detector.
- The measurement was conducted twice and the result noted as the mean of these two readings. The result was recorded as both "absorbance" and "concentration".
- A blank, unworn lens was measured in the same way and the result subtracted from that found above.

3.9.1.3 Fluorescence Spectrophotofluorimetry

a) Background

Luminescence phenomena include fluorescence, phosphorescence and chemiluminescence. Fluorescence is defined as the emission of radiation by molecules brought into an excited state after absorption of UV, visible or IR radiation. It is a form of spectroscopy, but is more sensitive than UV transmission. [403] With certain molecules the absorption of a photon is followed by the emission of light of a longer wavelength (ie lower energy). This emission is fluorescence (or phosphorescence if the emission lasts longer than 10^{-8} secs). Chemiluminescence requires excitation energy from a chemical reaction (such as that which occurs in a firefly). Because only very small amounts of material are required, fluorescence spectroscopy is frequently

of greater value than absorbance measurements. Absorption and fluorescence analysis are closely related, since absorption must precede fluorescence emission.

Fluorescence was originally described in 1833 by Sir David Brewster, who observed the "blood red" emission from chlorophyll when exposed to strong sunlight. Fluorescence spectroscopy was first reported in 1945. The principles are well understood. As previously described, a molecule can possess only discrete amounts of energy (absorption of energy taking place in discrete units only called quanta) and light energy can be absorbed only when the molecule moves from a lower to a higher energy level. If a molecule is initially unexcited (ground state, S_0) and the absorbed energy is greater than that required to reach the first electronic excited state (S_1) then the excess energy is absorbed as vibrational energy (Figure 3.10). This is rapidly dissipated as heat by collision with solvent molecules (if the excited molecule is in solution) and the molecule then drops to the lowest vibrational level within its new excited state (S_1). The excited molecule eventually returns to S_0 (normally within 10^{-8} secs) by either emitting light (fluorescence) or by non-radiative transition. Because energy is lost in dropping to the lowest level the emitted light will have less energy (longer wavelength) than the absorbed light (Stoke's shift); fluorescent light always has a longer wavelength then the absorbed (excitation) light.[404 405]

Figure 3.10 - Jablonski Energy Level Diagram

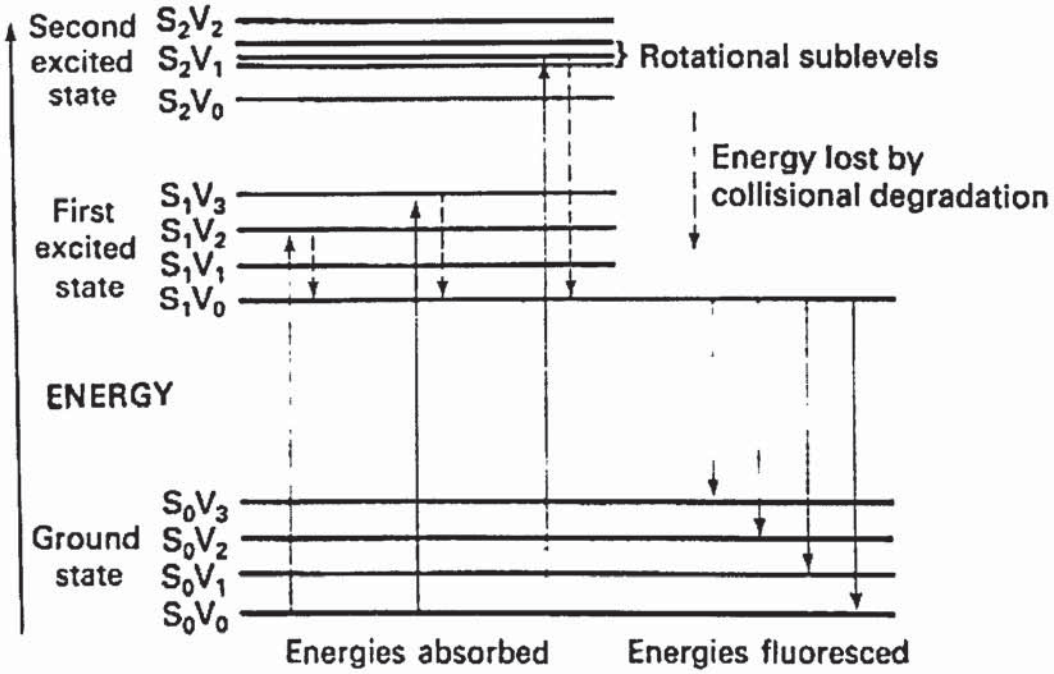


Figure 3.10 - Jablonski energy level diagram. Taken from Wilson & Walker. [401]

The amount of fluorescence emitted by a substance depends upon the excitation spectrum and fluorescence emission spectrum of the substance and the intensity and spectrum of the excitation radiation. The environment may also play a part (particularly pH and solutes), [406] possibly providing a radiationless process that competes with fluorescence and reduces it , a process termed "quenching". [407] Quenching relates to a reduction in fluorescence efficiency due to absorption of excitation energy by molecules that do not emit radiation [408] and is an important factor to consider in the study of complex systems such as that involving the multi-layering of intrinsically fluorescent proteins on surfaces. [407] Several processes can lead to quenching, [409] including energy transfer, complex formation and collisions. Both dynamic and static quenching occurs. Collisional quenching occurs when a fluorescent molecule in an excited state collides with a quencher, transferring energy and resulting in inter-system crossing. [407 410] Quenching explains why the fluorescence observed for a macromolecule is often different from the sum of the emissions of the component fluorophores (such as the three amino acid residues found in human proteins). Some residues may be protected inside the folded protein chain and others may be subjected to static quenching through interactions between the fluorophore and other parts of the folded protein chain.

Collisional quenching of fluorescence is described by the Stern-Volmer equation: [411]

$$F_0/F = 1 + k_q \tau_o[Q] = 1 + K_D[Q] \qquad \text{Equation 3.6}$$

where F_0 and F = fluorescence intensities in the presence and absence of a quencher
k_q = the bimolecular quenching constant
τ_o = the lifetime of the fluorophore in the absence of the quencher
$[Q]$ = the concentration of quencher
K_D ($k_q \tau_o$) = the Stern-Volmer quenching constant

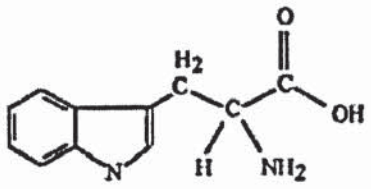
Fluorescent molecules (fluorophores) are relatively rare and are usually fairly rigid aromatic rings or ring systems. [403] Fluorescence may be expected from aromatic organic compounds having alternate single and double bonds in an unbroken sequence. [412]

There are two types of fluorophore - intrinsic and extrinsic. [407 413] Intrinsic fluorophores are contained within the macromolecules themselves (for example, the amino acid residues tryptophan, tyrosine or phenylalanine in proteins). Extrinsic fluorophores are added to the system, usually binding to a component (for example, fluorescein). For proteins there are only three intrinsic fluorophores; tryptophan, tyrosine and phenylalanine, [403] each of which contains an indole ring. In practice tryptophan fluorescence is most important (producing some 90% of the total fluorescence), as phenylalanine has only a very low quantum yield and tyrosine fluorescence is very weak due to quenching problems.[403 405 406] The fluorescent lifetime of the tryptophan residue is in the order of 1-6 nanoseconds. Proteins adsorb radiation at 280nm, with a fluorescence emission maxima in the region of 320-360nm. [414] The chemical structure of the three amino acid fluorophores is shown in Figure 3.11.

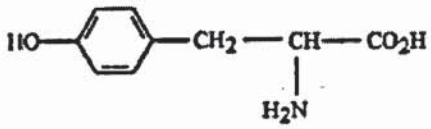
Table 3.10 - Excitation and Emission Maxima For Various Fluorescent Substances

Fluorophore	Excitation λ_{max} (nm)	Emission λ_{max} (nm)
Tryptophan	280	345
Tyrosine	280	308
Phenylalanine	280	282
Lipids	360	420-480
FITC [1]	490	517
TRITC [2]	550	580

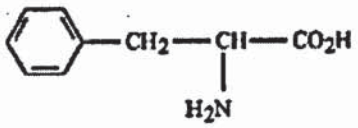
Figure 3.11 - Chemical Structure of Intrinsic Fluorophores



Tryptophan



Tyrosine



Phenylalanine

[1] Fluorescein isothiocyanate

[2] Tetramethylrhodamine B isothiocyanate

The dependence of fluorescence on the chromophore concentration can be derived from the Beer-Lambert law as follows:

$$I_t = I_o 10^{-\epsilon cl} \qquad \text{Equation 3.7}$$

where I_t is the intensity transmitted, I_o the incident intensity, ϵ the molar absorptivity of the compound under test, l is sample thickness and c is the molar concentration of the solute.

$$I_a = (I_o - I_t) = I_o * (1-10^{-\epsilon cl}) \qquad \text{Equation 3.8}$$

where Ia is the intensity absorbed.

$$I_f = (I_a * Q_f) = I_o * Q_f * (1-10^{-\epsilon cl}) \qquad \text{Equation 3.9}$$

where I_f is the intensity of fluorescence and Q_f (quantum yield) is the fraction of photoexcited molecules that lose their energy as fluorescence.

$$I_f = 2.30 * \epsilon cl * I * I_o * Q_f \qquad \text{Equation 3.10}$$

These equations show that $I_f \propto c$ for very low concentrations

As previously described, the autofluorescent nature of deposited tear film species is the result of intrinsic fluorophores that are present within lipids and proteins found within the tear film. Many of the fatty acids and lipids present in the tear film contain conjugated double bonds [414-416] and autofluorescent patterns are observable using an excitation wavelength of 360nm. [414,415]

These fluorophores are substances that possess delocalised electrons present in conjugated double bonds. As a result of the intrinsic fluorescent nature of lipids and proteins (and because the refractive index of hydrogels and water is almost equal) it is possible to monitor the lipids and proteins on the surface of a contact lens by using a fluorescence spectrometer. It is a particularly useful technique as it is non-destructive, highly sensitive and reproducible, quantifying proteins and lipids at levels substantially below their detection by fluorescence microscopy. The eye has been extensively studied using fluorescence techniques, using both endogenous (crystalline lens, [417-419] cornea, [420,421] retina [422]) and exogenous fluorophores. The addition of fluorescein and fluorometry has been used to investigate anterior chamber flow, [423] corneal epithelial [424] and endothelial [425] permeability and tear turnover rate. [426] Hydrogels have also been extensively characterized using luminescence spectroscopy, as reviewed by Rangarajan and colleagues. [427]

b) Instrumentation

The following components are required in a fluorescence spectrophotofluorimeter:

1. Intense light source - allows maximum sensitivity. This thesis used a 150 watt xenon lamp source.
2. Monochromator - for selection of excitation wavelength.
3. Sample compartment with cuvette - to hold lenses.
4. Monochromator - to select emission wavelengths.
5. Detector.
6. Output device - chart recorder or computer.

Figure 3.12 - Simple Line Diagram of Typical Fluorescence Spectrophotometer

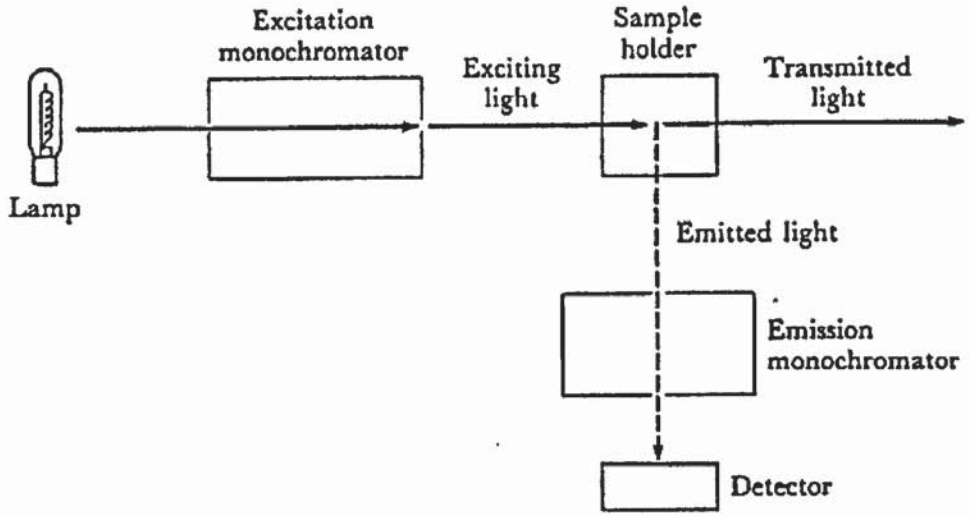
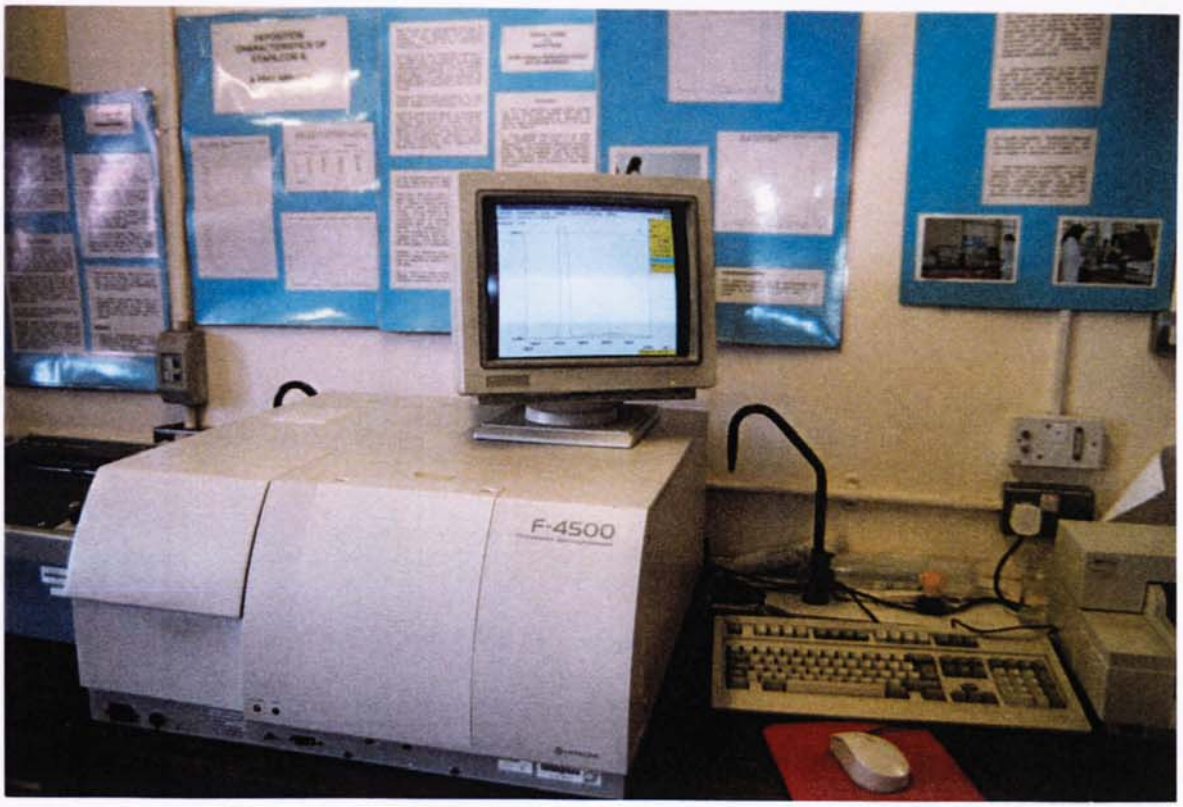


Figure 3.12 - Line diagram of typical fluorescence spectrophotometer. Taken from operational handbook of the Hitachi F4500 instrument.

To avoid detecting the incident beam use is made of the fact that fluorescence is emitted in all directions. Observation of the fluorescence is made at right angles to the incident beam. Fluorescence spectroscopy in this thesis was undertaken on a computerised Hitachi model F-4500 fluorescence spectrophotometer running MS-Windows.

Figure 3.13 - Hitachi F4500 Fluorescence Spectrophotofluorimeter

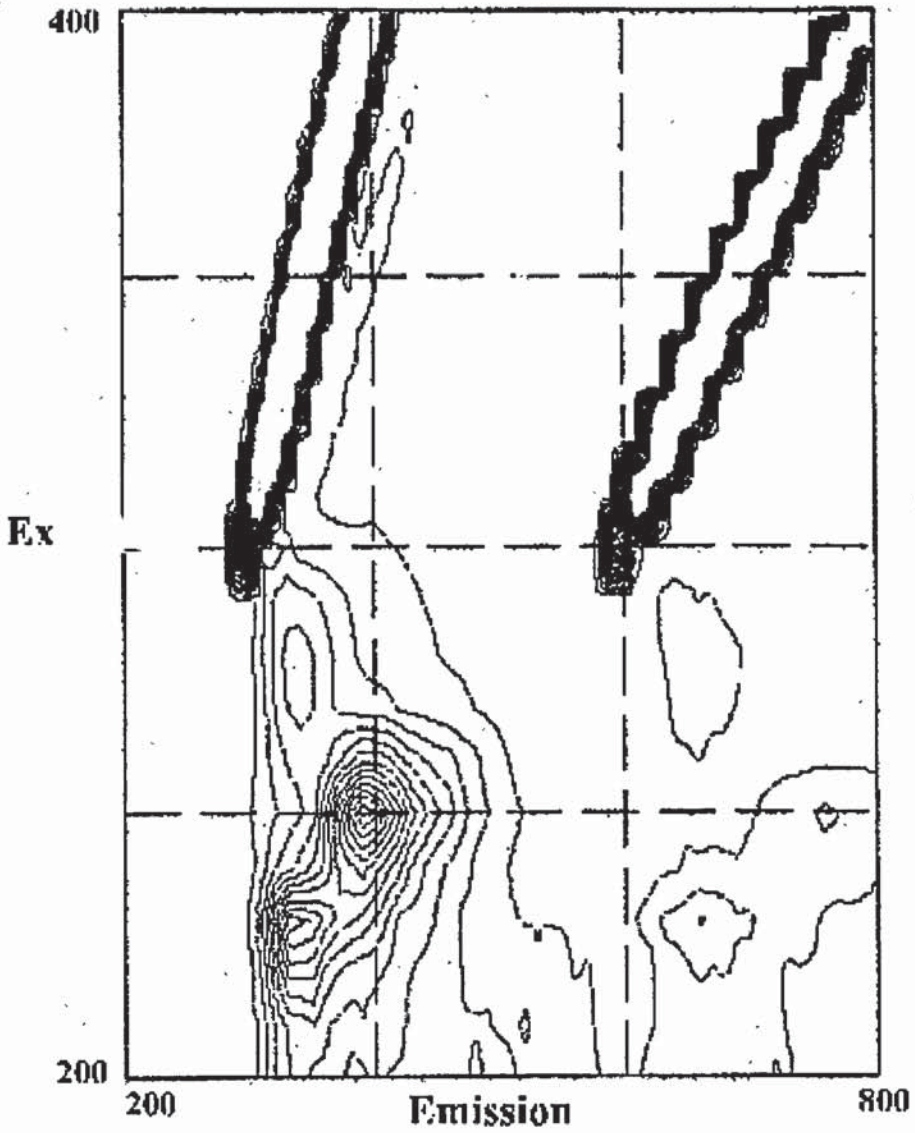


This machine is capable of four methods of sampling:

(i) 3D Scan

This produces a contour map of the sample and allows the optimum excitation and emission wavelength to be determined for subsequent use in single wavelength scans. Figure 3.14 displays a typical 3-D scan of an unworn polyHEMA lens. Second-order scatter peaks arise due to the fact that diffraction gratings pass harmonics of the wavelength of interest, with a grating monochromator selecting wavelength λ will also pass $\lambda/2$, $\lambda/4$ etc. For a spectrum recorded using an excitation wavelength of 280nm, a smaller peak will be observed at 560nm (the second order peak from the primary 280nm excitation). These are clearly visible in Figure 3.14.

Figure 3.14 - 3D Scan of unworn polyHEMA lens



(ii) Single wavelength

This allows exact photometric peak heights for protein and lipid deposition to be detected for spoiled lenses and was the principal type of scan used in the thesis. Previous trials using *in vitro* spoilt lenses found that a single scan at a scan speed of 1200 nm/min resulted in an emission spectrum that was unidentifiable from three or five multiple scans.

Runs on "blank" lenses allow for subtraction of background values from deposited lenses. The peak height for unworn lenses is minimal at both 280 nm and 360 nm excitation (Figures 3.15 and 3.16).

Figure 3.15 - Unworn, "Blank" Lenses Excited @ 280nm

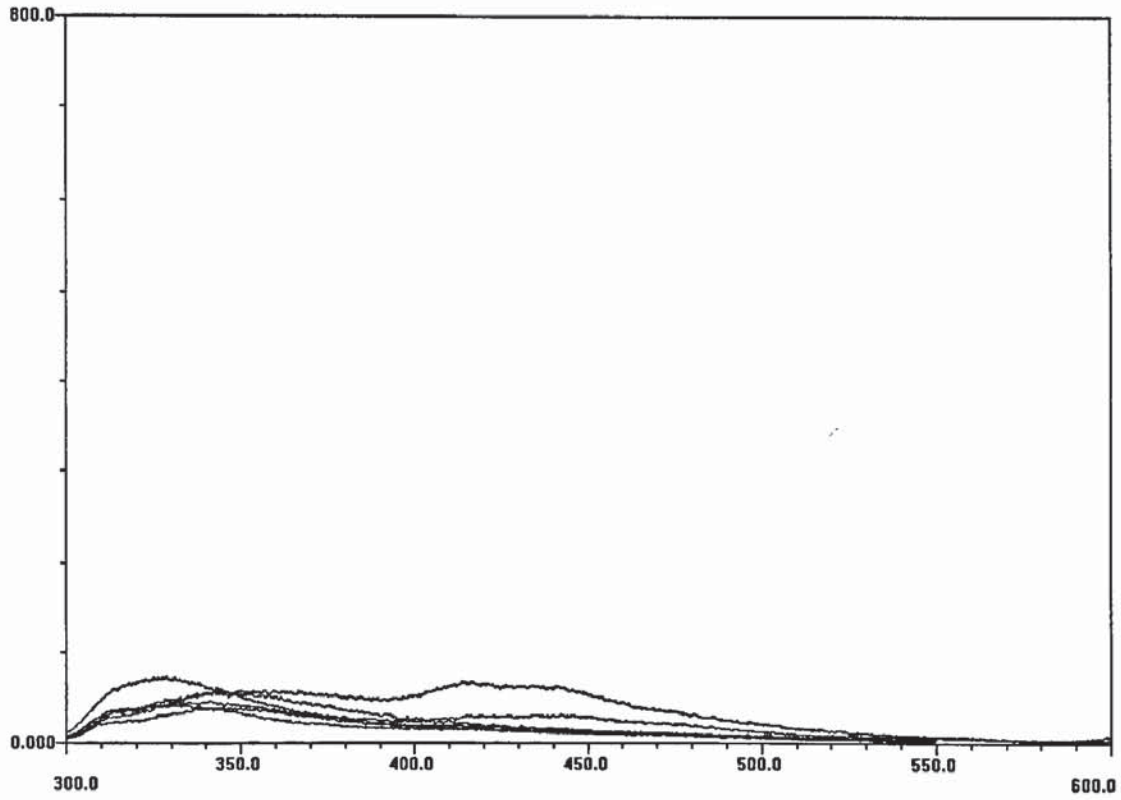


Figure 3.15 - Emission spectra of unworn lenses excited at 280nm. The lens materials were Acuvue, Surevue, Excelens, Lunelle ES70, Z6 and Vistagel PLUS

Figure 3.16 - Unworn, "Blank" Lenses Excited @ 360nm

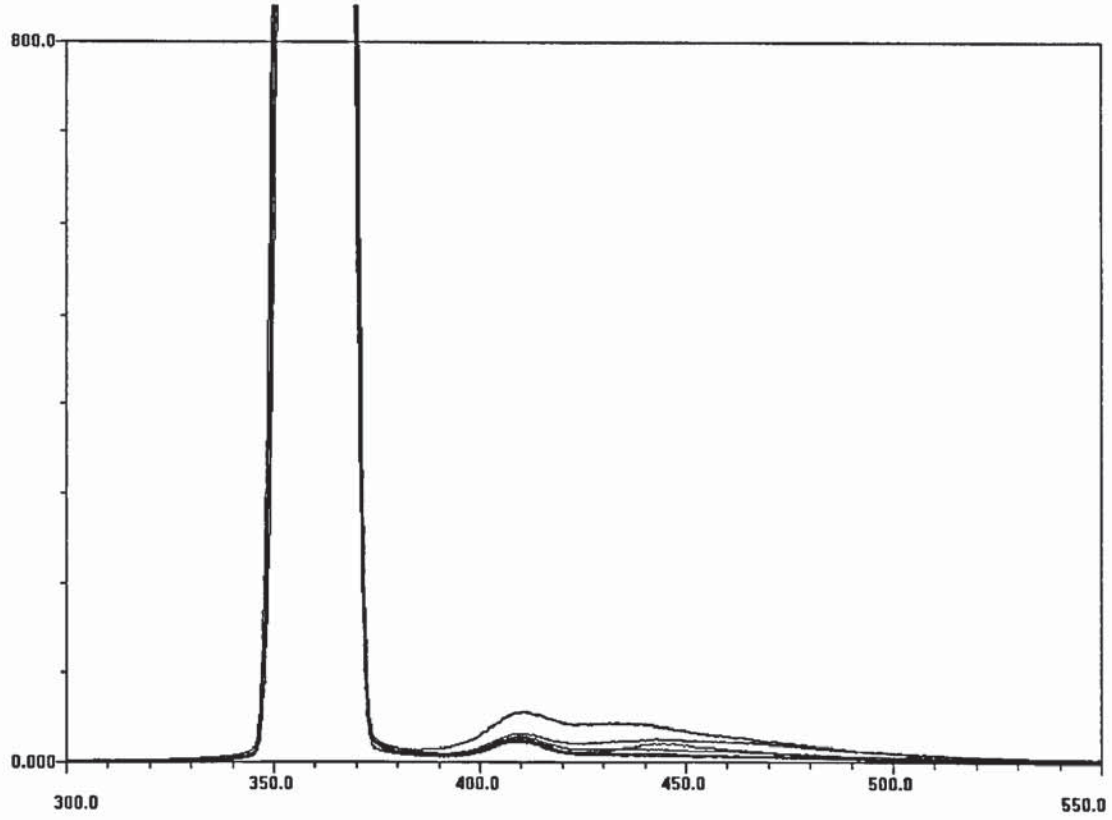


Figure 3.16 - Emission spectra of unworn lenses excited at 360nm. The lens materials were Acuvue, Surevue, Excelens, Lunelle ES70, Z6 and Vistagel PLUS

The excitation peak occurs at the wavelength used to excite the sample and the emission peak ("spoilation peak") occurs around 400-600 nm and is the result of biological deposition onto the contact lens surface. The spoilation peak intensity increases with increasing spoilation. Excitation at 280nm produces an emission peak centred around 340nm, which is characteristic of a proteinaceous deposit, with a broader peak on some lenses centred close to 440nm, which is characteristic of the soluble aqueous phase lipid component. Excitation at 360nm produces a peak centred around 440 nm, which is characteristic of the degree of lipoidal spoilation. Typical fluorescence emission spectrums of deposited contact lenses are shown in Figures 3.17 and 3.18 and in Appendix 6. Extensive work has been previously carried out with both *in-vitro* and *in-vivo* spoiled lenses using HPLC, electrophoresis, isotachophoresis and gas plasma etching to validate identification and quantitation procedures. [414]

Figure 3.17 - Typical Emission Plots of Lenses Worn For One Month Following Excitation @ 280nm

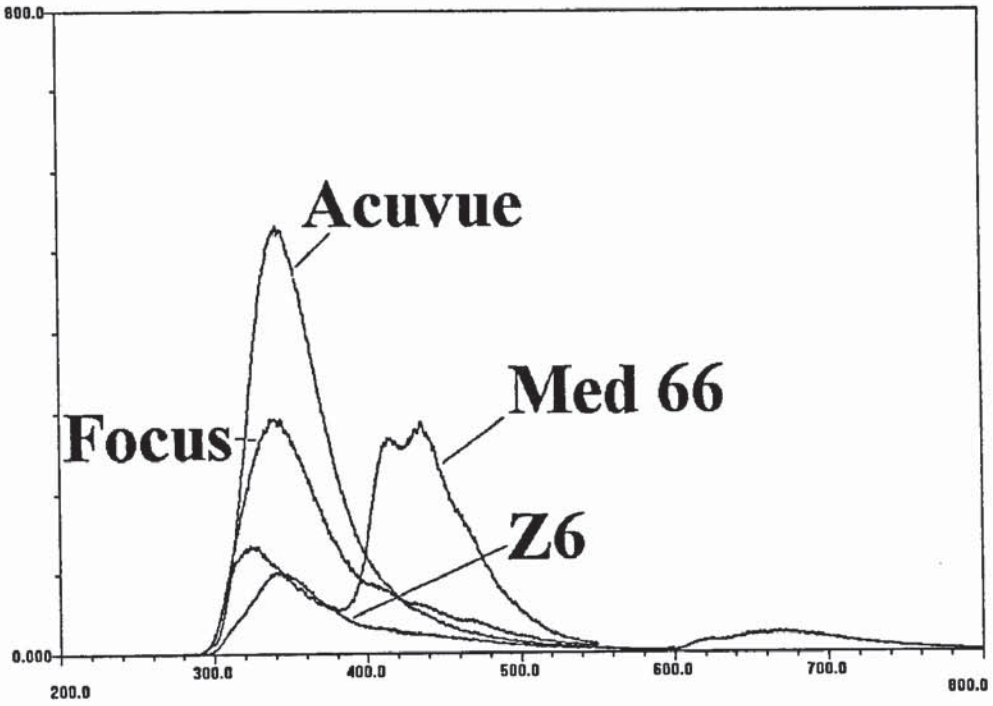
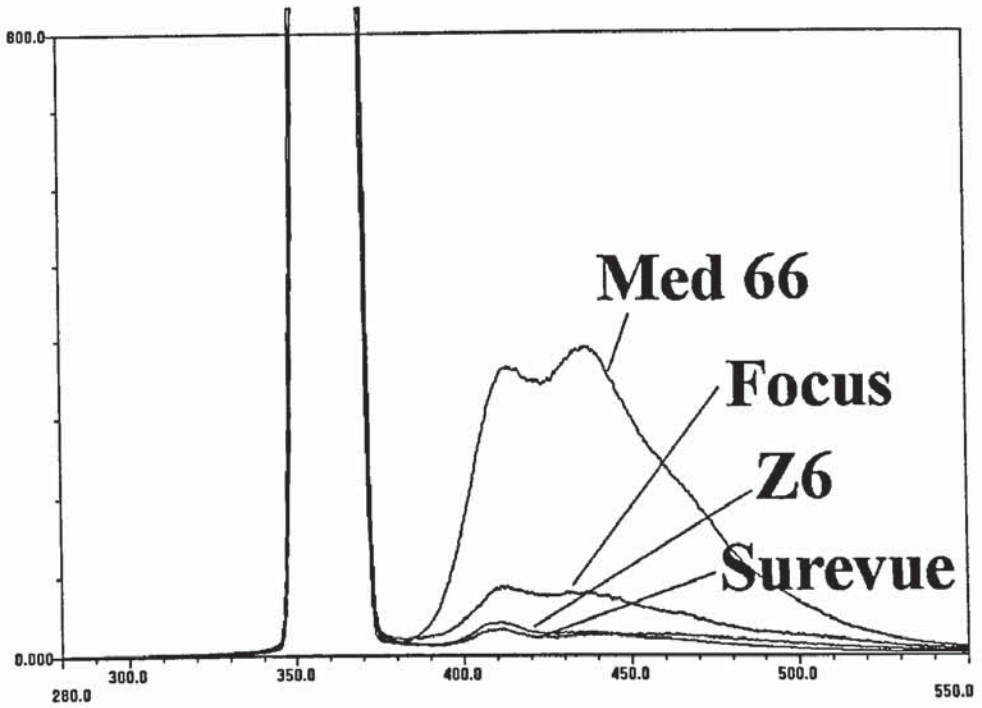


Figure 3.18 - Typical Emission Plots of Lenses Worn For One Month Following Excitation @ 360nm



(iii) Time scans

These are carried out over a period of time, allowing decay of a fluorescent species to be monitored.

(iv) Photometry

Scanning of a series of known concentration solutions to produce a calibration curve allows the concentration of an unknown sample to be determined.

c) Technique

- The round quartz cuvette was filled with fresh saline or distilled water and wiped dry, prior to placing in the test holder. The cuvette is 45 mm long, with an external diameter of 9 mm and internal diameter of 7 mm. It holds 1.4 ml of fluid when full.
- The test lens was placed in the cuvette, after ensuring that no bubbles were present in the examination fluid. The cuvette was marked to ensure that the lens always faced the same direction. The lens was always placed at the bottom of the cuvette, facing towards the direction of the light beam.
- The machine was set with the standard run conditions. These were a wavelength run of 300-550 nm @ 1200 nm min^{-1}, with vertical height set to 0-800 units, excitation slit width of 5 nm and emission slit width of 10 nm.
- Excitation wavelength was set to 280 nm (to scan for proteins) and the run initiated. Each run took 15 seconds to complete.
- The peak height was measured with a curser and the peak height and emission wavelength recorded.
- Excitation wavelength was changed to 360nm (to scan for lipids) and the run repeated.
- A blank, unworn lens was measured in the same way and the result subtracted from that found above.

3.9.2 Destructive Analysis

All lenses were subjected to at least one form of non-destructive analysis. They were subsequently subdivided into groups and exposed to destructive analytical techniques. Following this no further examination was possible.

3.9.2.1 Histological Staining Techniques

Unequivocal identification of deposits by optical microscopy is not possible. Improved results are obtained if the lenses are sectioned and stained with specific stains. [80] The staining techniques are employed for the chemical identification and localisation of components in the deposits.

The specific stains used included those detailed in Table 3.11.

Table 3.11 - Histological Stains

STAIN	SELECTIVITY
Oil Red O	Lipids
Digitonin	Cholesterol
Periodic Acid Schiff	Carbohydrates
Mucicarmine	Mucins
Millon's Reagent	Proteins
Von Kossa	Calcium

3.9.2.2 Scanning Electron Microscopy (SEM)

This is a non-invasive but destructive technique in which the lens surface is viewed at very high magnification (30-40 000x). [428] The sample is dehydrated, attached to an aluminium stub, gold or carbon coated in a sputter coater, placed in the Cambridge Instruments Stereoscan microscope and evacuated. A beam of electrons is shot at the sample and the reflected electrons are collected and an image formed on a cathode ray tube. Before examination lenses were fixed in buffered gluteraldehyde-picric acid for 90 minutes at 4°C. Post fixation in osmium tetroxide is also undertaken in certain cases, to improve lipid fixation. Details of the deposit morphology become visible which are not clear using light microscopy, as demonstrated in Figures 3.19 and 3.20.

Figure 3.19 - SEM of a Surface Film

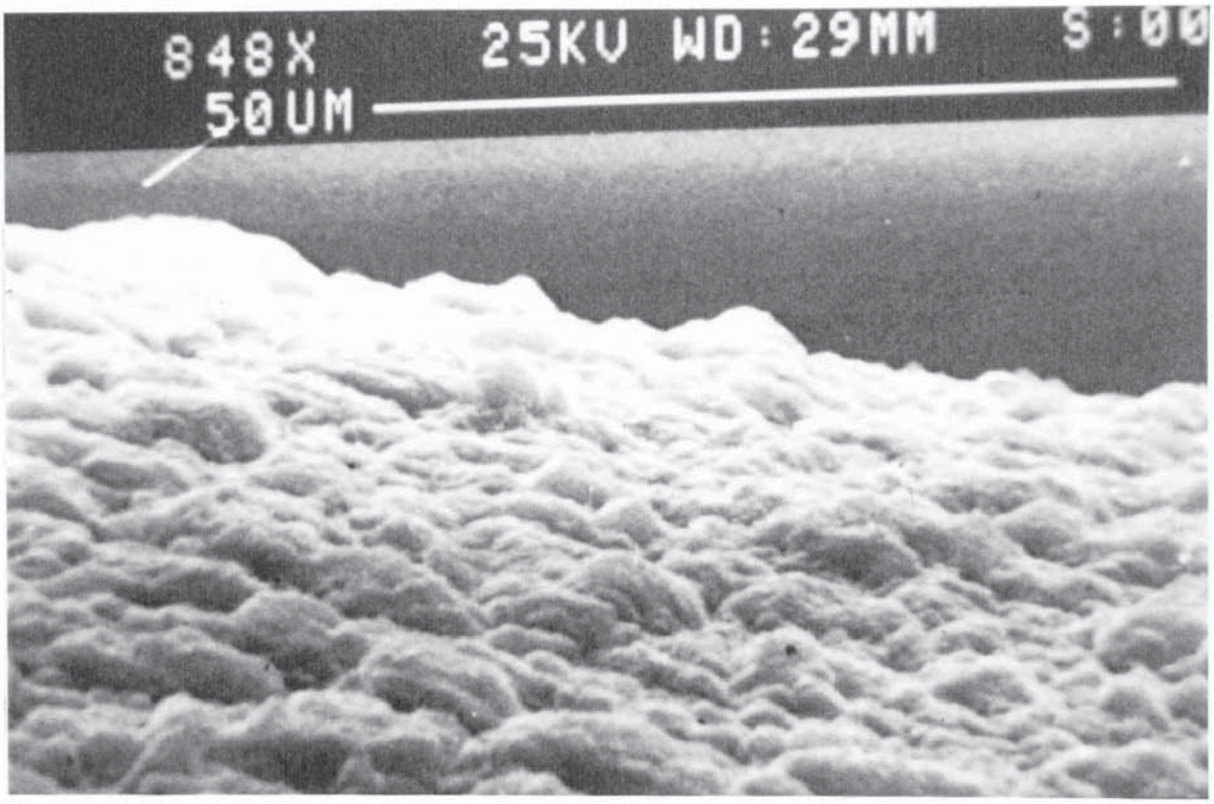


Figure 3.19 - SEM picture of deposited film on the surface of a two-year old high water content lens

Figure 3.20 - SEM of a Lens Calculi

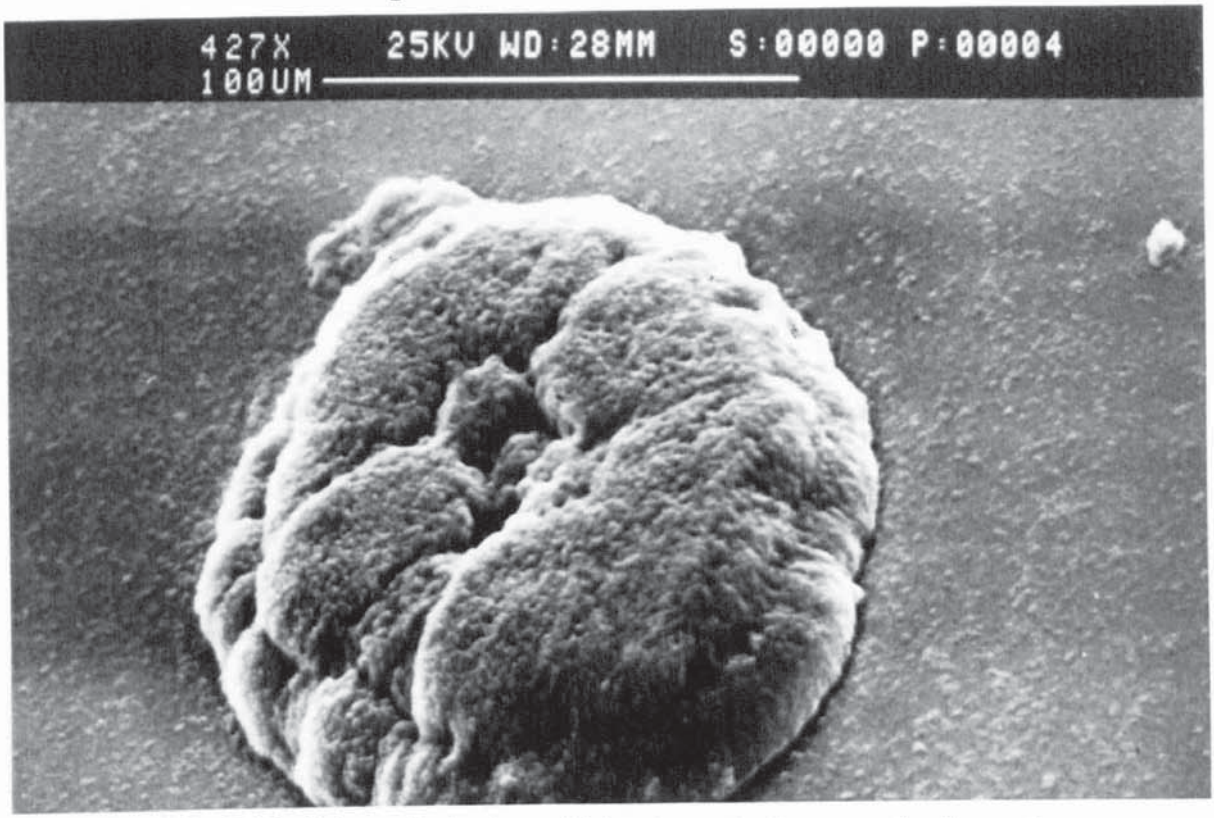


Figure 3.20 - SEM of a lens calculus from a high water content lens worn for six months

3.9.2.3 Lysozyme Activity

a) Background

Turbidimetry is associated with the estimation of the concentration of dilute suspensions [401 402] and measures the apparent absorption of radiation by the suspension. The apparent absorption should be measured at a wavelength where true absorption is not occurring; hence the Beer-Lambert law does not apply. When radiation is passed through a transparent medium (eg a solution in a cuvette), one or both of two distinct physical phenomena might occur. For extinction true absorption of energy occurs and allows changes in the energy states of electrons etc. When this is the only phenomenon occurring the medium is termed "optically empty". However, in a suspension, a quite distinct radiative phenomenon may occur, in which the light is scattered by the suspended particles. This scattering is due to reflection and refraction and produces the Tyndall effect, which occurs in all directions. In turbidimetry the incident and transmitted radiation may be measured in a spectrophotometer, but the contribution of true absorption is small and the Beer-Lambert Law is not strictly applicable.

This technique is difficult to employ but is extremely valuable.

b) Technique

The amount of bulk protein present is quantified by UV absorption @ 280 nm on the Hitachi Spectrophotometer. The deposited protein is extracted and the extraction solvent used to detect the activity of lysozyme using a method based on the decrease in turbidity (light scattering intensity) following lysis (because lysozyme dissolves the cell walls) of a suspension of Micrococcus lysodeikiticus (Sigma, M-3770) [63 119 148 153 183 429] The UV absorption is recorded @ 540 nm at 30 sec and 3 min intervals at 37°C.

The concentration of active lysozyme (mg/ml) in the lens is calculated for UV absorption values:-

Concentration of lysozyme in lens extract = $\Delta A_1 / \Delta A_s \times 0.4$ Equation 3.11

Where ΔA_1 = change in absorbance for lens extract
ΔA_s = the standard
0.4 = concentration of the lysozyme standard

3.9.2.4 Lowry Method

In some circumstances (for example where lenses incorporate an ultra-violet blocking agent) it was not possible to conduct non-destructive protein analysis. In such situations a colorimetric destructive protein assay was conducted (Lowry Method). Lenses were rinsed with sterile saline and the protein extracted using a 24-hour soak in 10% sodium dodecyl sulphate (Sigma-Aldridge) while on a low speed rotary shaker (IKA-Vibrax-VXR). Total extracted protein was measured using a modified Lowry technique on a Hitachi U2000

spectrophotometer. In this procedure the biuret agent is reacted with proteins followed by the phenol reagent. [430] The colour is developed after approximately 30 minutes and the absorbance read at 700 nm. The calibration curve was obtained by plotting the total protein (mg/ml) against the absorption at 700 nm. Protein calibration was obtained using chicken egg-white lysozyme (Sigma Chemical Company). The method is sensitive down to 10 μgcm^{-3}. [401]

As with any destructive technique it is impossible to completely remove all of the deposited protein. Separate experiments showed that with these materials and the extraction technique described above that 10±5 μg of protein remained firmly adherent to group II materials and that 80±10 μg remained adherent to group IV materials. [431] These results merely indicate the amount of protein remaining attached and in no way imply that the adherent protein types are similar in each case.

3.9.2.5 Electrophoresis

a) Background

Electrophoresis is the technique used for much of the basic identification and quantification of deposited proteins. [432] This separation technique uses the fact that biological species such as amino acids, peptides and proteins possess ionisable groups and can therefore exist in solution as electrically charged species, either as cations (+) or anions (-), depending upon the pH of the environment in which they are situated. At high (alkaline) pH they are negatively charged and at low (acidic) pH they are positively charged. Molecules that have a similar charge will also have different charge/mass ratios when they have inherent differences in molecular weight and may also have differing shapes. These differences form sufficient basis for a differential migration rate when the ions in solution are subjected to an electric field, as the protein complexes will move at differential speeds towards the anode.

Electrophoresis essentially consists of dissolving or suspending the protein mixture under examination in a buffer of suitable pH (for example barbitol) and then applying a direct electric current between two electrodes across a supporting medium (usually a gel of some description). The cations migrate towards the cathode and the anions towards the anode at a rate that is dependent on the balance between the impelling force of the electric field on the charged ion and the frictional and electrostatic retarding effects between the sample and the surrounding medium. The various proteins in a sample will separate out into distinct bands, which are subsequently visualised by staining with various dyes. These bands are then compared with various standards (such as albumin, lactoferrin and lysozyme) to facilitate their identification.

Despite the extensive use of this technique, the results are, unfortunately, still equivocal due to differences in the basic techniques employed during the various stages of its use.

The first problem encountered is that of removal of the protein from the lenses. Some investigators [122] use urea as the surfactant of choice, others NaOH. [119] However, the most sensitive method for effective removal of the protein film [121] is boiling gently in 1-5% sodium dodecyl sulphate (SDS) in the presence of

dithiothreitol (DTT). SDS is a strong anionic detergent routinely used to unfold proteins and DTT is a thiol reagent used to cleave or exchange disulphide bonds. This removal technique removes <50% of the deposited material. [118]

The second problem concerns the type of supporting medium or gel used. Most studies use either an agarose or acrylamide gel. The agar or agarose gels have a high water content, good fibre structure, large pore size and low frictional resistance. Consequently, movement of ions is very rapid and the subsequent bands are poorly separated and difficult to distinguish with any great accuracy. [119 122] Greater definition and separation are achieved with polyacrylamide gels [151] as they have minimal adsorption capacity and may be composed of various percentages of acrylamide - usually 3% to 30%, corresponding to pore sizes of 0.5 nm to 0.2 nm respectively - and this facility may be used to enhance the separation of whichever molecules are under investigation. [433]

The third variable concerns the dye used to stain the protein bands present on the gel. The most widely used dye is Coomassie brilliant blue [54 119 121 122] which complexes with proteins to give an absorption maximum in the region of 595 nm and provides a quantitative result. This reagent is simple to prepare, develops rapidly and is stable for approximately four hours. However, it is sensitive down to only 20 μgcm^{-3} [433] and is only a relative method as the amount of dye binding varies with the basic amino acid residues in the protein. It is available as a newer Coomassie blue R250 form that is approximately 10x more sensitive and has been used successfully to examine both *in vivo* and *in vitro* spoilt lenses. [444]

The most sensitive development technique [435] consists of a silver stain, which is 100x more sensitive than basic Coomassie. [436] However, these ultra-sensitive stains are generally more difficult to produce (often requiring 6-7 processes) and are only qualitative in terms of the results achieved, unless used with a densitometer. Modifications to the procedure are possible [437] (which reduces the time required), but these are still time consuming.

b) Instrumentation

Electrophoresis was conducted with an SDS extraction system, polyacrylamide gel electrophoresis (PAGE) separation gel on a Hoeffer system, visualising with either Coomassie Blue or silver staining. Gels were subsequently scanned using an LKB 2202 Ultrascan laser densitometer.

3.9.2.6 High Pressure Liquid Chromatography (HPLC)

a) Background

Chromatography is one of the most sensitive methods for separating and identifying chemical compounds. There are many kinds of chromatography (adsorption, partition, ion-exchange and molecular-sieving) and many specialized techniques for using them (column, paper, thin layer and gas chromatography). High pressure liquid chromatography (HPLC) is an extremely sensitive form of chromatography and is an example of the ion-exchange form.

An ion exchanger is a solid that has chemically bound charged groups to which ions are electrostatically bound; it can exchange these ions for ions in aqueous solution. Ion exchangers can be used in column chromatography to separate molecules according to charge density, charge distribution and molecular size. The basic principle is that the affinity of a substance for the exchanger depends upon both the electrical properties of the material and the relative affinity of other charged substances in the solvent. Bound material is eluted by changing the pH or by adding competing materials. HPLC uses very fine particles to produce a high resolution system and high pressure to maintain an adequate flow rate. [438]

HPLC is characterised by very rapid separation, excellent peak resolution and requires very little sample, making it ideal for the separation of both proteins and lipids eluted from contact lenses.

Figure 3.21 - Line Diagram of Typical HPLC Instrument

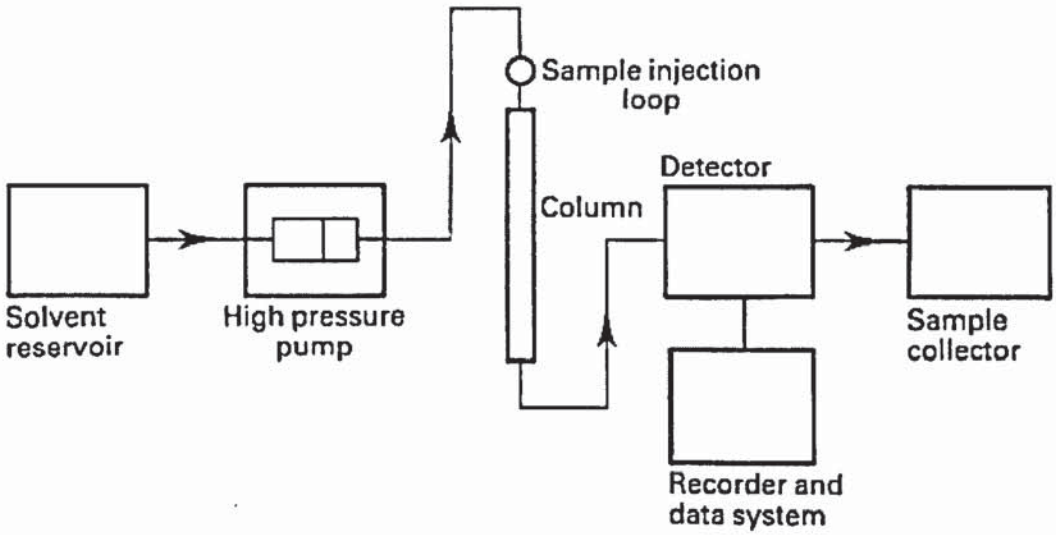


Figure 3.21 - Line diagram of typical HPLC instrument. Taken from Wilson & Walker. [401]

b) Instrumentation

In this project HPLC was used to classify lipid deposition. Material was removed from the lenses by soaking in methanol for 30 minutes while on a low speed rotary shaker. HPLC was carried out using Knauer pumps with Perkin Elmer LC-75 spectrophotometric detector and Perkin Elmer LS-1 fluorescence detector, driven by an Apple II Computer and U-Sci Ltd software.

3.10 *IN VITRO* DEPOSITION CHARACTERISATION

During these studies the test lenses were "spoiled" in an *in-vitro* model which mimics the tear/contact lens system. [255] Three to five lenses of each type were used for each experiment, one of each being used solely as a control (saline exposure only).

The artificial tear solution consisted of a 1:2 (v/v) solution of foetal calf serum (FCS) diluted with phosphate buffered saline (PBS) and finally spiked with lysozyme. The procedure used a "shaker" model, which consisted of several small glass beads in a vial, which provided an uneven surface for the lenses to sit on. This provided some contact between the air and tear solution (to mimic the *in vivo* situation), the latter being pipetted into the vial at a level just below the upper surface of the glass beads. The vials were then placed on a flat-bed rotary shaker to enhance the air-tear film contact with the lenses. The tear solution was replaced every 24 hours in order to maintain the supply of fresh protein and lipid components.

The procedures were carried out under sterile conditions in a tissue-culture experimental environment. The model was run for a total of 28 days and produced accelerated spoilation equivalent to approximately 6 months *in vivo* wear (unpublished data). The fluorescence data of the lenses was plotted every 3-4 days and provided results that charted the increase of deposition over time.

The use of the spoilation model enabled a direct comparison between lens materials and/or care systems without the complicating factor of inter- and intra-subject variability.

3.11 *IN VITRO* WETTABILITY

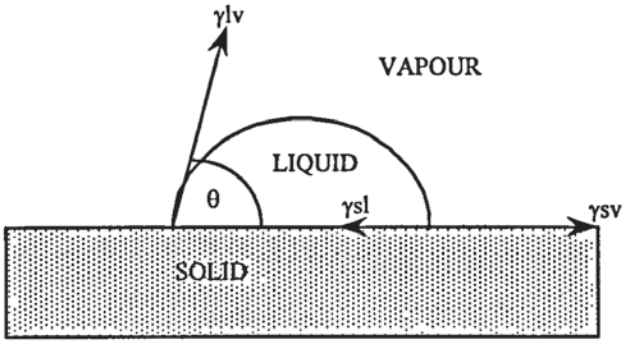
3.11.1 Background

When a liquid covers a solid surface the process is known as "wetting". The wetting of a solid by a liquid indicates the adherence of the liquid to the solid, despite the cohesive forces holding the liquid together. The coating of a contact lens with the tear film is an example of wetting.

Substances exist as solids and liquids because powerful attractive forces exist between the molecules, holding them together. Forces acting on the same type of molecules are called cohesive forces, as opposed to adhesive forces, which act on molecules of two different substances. Consideration of the cohesive forces in a drop of liquid provides some understanding of the concept of "surface tension". In the interior of a drop of fluid each molecule is surrounded by others on every side, resulting in no net attraction. However, on the surface there is no outward attraction to balance the inward pull. Due to this inward pull the surface molecules continually attempt to enter the bulk of the drop. The surface area contracts until it reaches a minimum size for a given volume, thus assuming a spherical shape. The molecules at the surface have an excess amount of potential energy compared with the molecules in the bulk. This potential energy per unit area is the surface tension.

The stronger the cohesive forces between molecules the higher the surface tension. The surface of a solid also has a surface tension but the overall bonding is so tight that the solid is rigid. When a liquid wets the surface of the solid the balance of forces at the surface changes. The surface molecules of the liquid are no longer attracted only towards the bulk, some will also be attracted to the surface of the solid. This has the effect of lessening the excess energy of the liquid surface molecules. This new lower surface energy (or surface tension) is called "interfacial tension". The greater the adhesion of the liquid for the solid the lower the interfacial tension. In incomplete wetting the liquid droplet will form a definite angle with the solid surface at the point of contact - the contact angle (Figure 3.22). In the contact lens industry the contact angle (θ) is called the wetting angle. A wetting angle of zero implies complete wetting of the solid by the liquid. [439]

Figure 3.22 - Wetting Angle Assessment



where γ_{lv} = liquid-vapour interfacial free energy

γ_{sl} = solid-liquid interfacial free energy

γ_{sv} = solid-vapour interfacial free energy

θ = contact angle

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} \cos \theta \qquad \text{Equation 3.12}$$

By measurement of the contact angles of a solid surface the surface energy of the solid can be calculated. Two angles may be measured - the "advancing" and "receding" angles. The advancing angle is the contact angle formed when the droplet is advanced onto a "dry" surface and the receding angle is that measured when the fluid is drawn off a previously wetted surface. The difference between these angles is termed the "contact angle hysteresis".

Examination of the definition of wetting and contact angle shows that enhancement of wetting may occur in three ways:

- lowering the surface tension of the liquid
- lowering the interfacial tension between the solid and liquid
- increasing the surface tension of the solid

Wetting angles are traditionally measured using a contact angle goniometer, which has a telescopic arrangement with cross-hairs to measure the wetting angle. [440] The angles may be assessed using one of three techniques - sessile drop, captive bubble or Wilhelmy plate. [441] Contact lens wetting angles are usually assessed using the sessile drop technique, in which a small drop of fluid is dropped onto the lens and the angle measured using a goniometer or laser beam. [442] Problems exist using this technique with hydrogels due to dehydration of the samples and mounting problems.

3.11.2 Technique

In this project wetting angles were assessed using a modification of the Wilhelmy plate. In this method a sample is slowly immersed into, or withdrawn vertically from, a liquid. A precision balance measures the force during the immersion and withdrawal procedure. An advancing angle is measured during immersion and receding angle during removal. [443]

Measurements of dynamic advancing and receding contact angles were made using a dynamic surface tensiometer (Nima Technology Coventry, Model DST 9025), using a modified Wilhelmy Plate method (Figure 3.23). This technique has the advantage that a larger surface area is investigated, thereby ensuring an homogenous measurement. Advancing contact angles can also be accurately and reproducibly assessed without the problems inherent with liquid evaporation found in conventional contact angle goniometry.

Figure 3.23 - Dynamic Wetting Balance

The quoted results are the force required to remove a 3.3 mm diameter strip of the lens material from HPLC grade water. The greater the force required the more wettable the lens is, as it "holds onto" the water and is more reluctant to be withdrawn from it.

3.12 ELEMENTAL (CHN) ANALYSIS

Elemental analysis to determine the relative concentrations of carbon, hydrogen and nitrogen was undertaken by the analytical and chemical consultancy service of Brunel University, Middlesex. Two lenses of each material of interest were placed in a coded bottle and sent for analysis. The concentrations of carbon, hydrogen and nitrogen were recorded as the mean of the results received.

3.13 HYDROGEL MATERIALS

The basic composition and features of the contact lens materials used in this project are described in Table 3.12.

Table 3.12 - Hydrogel Lenses Used During The Project

Manufacturer	Lens	EWC	Monomers	USAN	FDA Group
Hydron	Zero 6	38	HEMA	Polymacon	I
B&L	SeeQuence	38	HEMA	Polymacon	I
Aspect	Frequency 38	38	HEMA	Polymacon	I
Vista Optics	Vistagel PLUS	40	HEMA+MMA+ NVP+PEO	-	I
WJ/PBH	Classic	42	MMA+NVP+ HEMA	Tetrafilcon A	I
Vista Optics	Vistagel HI-PLUS	50	HEMA+MMA+ NVP+PEO	-	I
Ciba	Focus	55	HEMA + PVP + MA	Vifilcon A	IV
Ciba	Focus	55	HEMA + PVP + MA	Vifilcon A	IV
Aspect	Frequency 55	55	HEMA + MA	Ocufilcon D	IV
Vistakon	1-Day Acuvue	58	HEMA + MA	Etafilcon A	IV
Vistakon	Acuvue	58	HEMA + MA	Etafilcon A	IV
Vistakon	Surevue	58	HEMA + MA	Etafilcon A	IV
Ciba	Weicon CE	60	HEMA + NVP	-	II
Biocompatibles	Proclear	60	HEMA + PC	Omafilcon A	II
Ciba	Excelens	64	MMA + PVA	Atlafilcon A	II
B&L	Medalist 66	66	HEMA + NVP	Alphafilcon A	II
Hydron	Omniflex	70	HEMA + NVP	-	II
Lunelle	ES70	70	MMA + NVP	-	II
Lunelle	Rythmic	73	MMA + NVP	-	II
WJ/PBH	Precision UV	74	MMA + NVP	Vasurfilcon A	II

3.14 CARE SYSTEMS

The basic composition and features of the contact lens care systems used in this project are described in Table 3.13. Further details are listed in Tables 2.8 - 2.11.

Table 3.13 - Care Systems Used During the Project

Solution	Manufacturer	Disinfectant	Neutraliser
10:10	CIBA-Vision	3% Hydrogen Peroxide	Sodium Pyruvate
All-in-One	Sauflon	Polyhexanide	-
All-in-One Light	Sauflon	Polyhexanide	-
Complete	Allergan	Polyhexanide	-
Concerto	Lunelle	Polyhexanide	-
Hydrocare	Allergan	Thiomersolate	-
Optifree	Alcon	Polyquad	-
Opti-1	Alcon	Polyquad	-
Oxysept I-Step	Allergan	3% Hydrogen Peroxide	Catalase tablets
Oxysept II-Step	Allergan	3% Hydrogen Peroxide	Catalase
ReNu	B&L	Polyhexanide	-
Softab	Alcon	Chlorine	-
Solocare	Ciba	Polyhexanide	-

3.15 DATA ANALYSIS

Summary statistics were calculated for all variables. The grading scales, being ordinal measurements, were considered non-normal data. All other data were tested for normality of distribution.

The two eyes of a subject often yield correlated data.[444 - 446] Statistical analysis that treats correlated data as if it were independent is most likely to be biased towards statistical significance; ie the probability of a type 1 error is likely to be inflated. [446] Right and left eyes were compared using either paired Student T-test (normally distributed data) or Wilcoxon Signed-Rank test (non-normally distributed data). In all circumstances there was no difference between eyes. All graphical data, reported results and statistical analysis (unless otherwise stated) were therefore undertaken on the data from the right eye only.

Statistical analysis was undertaken using SigmaStat ® version 2.0 (Jandel Scientific, San Rafael, California).

Statistical analysis was undertaken using either a one-way repeated measures Analysis of Variance (ANOVA) for normally distributed data or one-way repeated measures ANOVA on ranks for non-normally distributed data. Where differences were found post-hoc testing was undertaken using the SNK method. Statistical analysis of subjective scores in which care system was also a factor were undertaken using a balanced design three-way ANOVA, with visit, lens type and care system as the factors. [447] [448] ANOVA analysis is not appropriate in this way for non-normally distributed data and factors which were not normally distributed were transformed prior to analysis. However, some authors point out that ANOVAs are very robust at dealing with data distributed in this way, [449] non-normally distributed data has little effect on the F-value and calculation of the p-value is dependant on the sum of the squares and not the mean value. [450]

Correlations that included non-parametric data were undertaken using Spearmans Rank Correlation.

In all cases a "p" value of <0.05 was taken as statistically significant.

3.16 SUMMARY

Due to the large number of results obtained during this thesis the results section will only detail those of significant importance. Analytical and statistical results are presented where the results are considered of value in understanding the general conclusions from the work conducted and in emphasising the previously stated objectives of the work.

Chapter 4

Pilot Studies and Calibration

"Hindsight is always twenty-twenty". [1]

Prior to the clinical and laboratory studies a series of pilot studies and calibration experiments were conducted.

4.1 WETTABILITY

Front surface wettability was measured using the Loveridge Grid, as previously described.

4.1.1 Repeatability

Repeatability was assessed as a subset of the study referred to in Chapter 9.2 (page 283). Twelve subjects (six males and six females) used three lens types (Ciba-Vision "Excelens", Pilkington B-H "Classic" and Vista-Optics "Vistagel PLUS"). *In vivo* wettability was assessed on collection with the Loveridge Grid as previously described in 3.6.3.1 (page 73). Each measurement was taken three times and the median of these was taken as representative of the recorded PLNIBUT, as recommended by Guillon & Guillon for the Tearscope instrument. [216]

To estimate the reliability of the measuring technique the approach of Reeves et al was followed. [371] The third measurement was discarded and the first two measures were used to assess reliability by estimating the standard deviation of the distribution of differences in value between the two measurements. When the difference between the two measurements (T_{diff}) is plotted against frequency of occurrence a normal distribution is obtained (Figure 4.1), whose mean was 0.4 ± 2.0 seconds. If a level of 1.96 x SD is taken as an indication of significant change (95% range of difference in scores) then a difference of four seconds is required to ensure a real change in measurement has occurred.

[1] Billy Wilder

Figure 4.1 - Loveridge Grid Repeatability

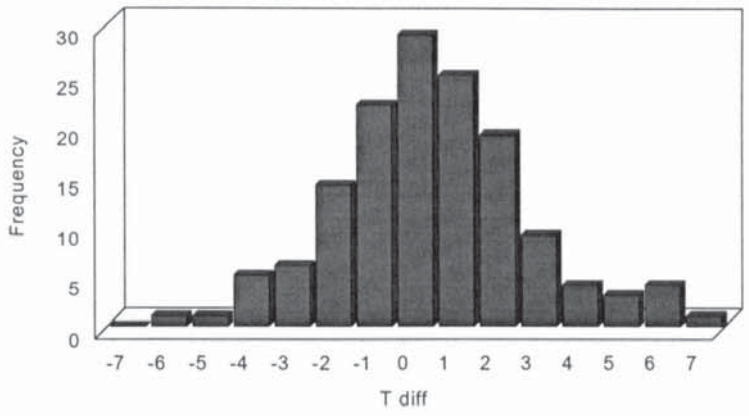


Figure 4.1 - Repeatability for Loveridge Grid on two readings. Graph plots the difference between repeat measures (T diff) against frequency

Analysis of the results indicates that the mean (±sd) PLNIBUT across all visits with all materials was 12.1 ± 7.4 seconds with a median of 11 seconds (range 0 - 30). This is similar (though slightly longer) than that found using other projection techniques that assess PLNIBUT. [376 455] Figure 4.2 details the results.

Figure 4.2 - Distribution of PLNIBUT Results

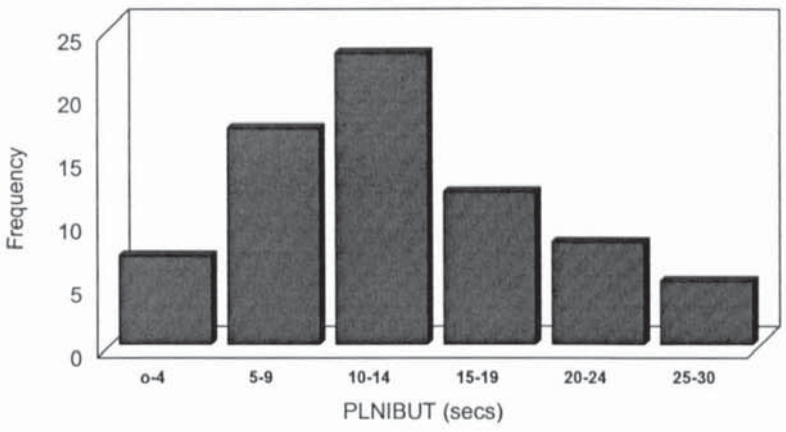


Figure 4.2 - Distribution of PLNIBUT results

4.1.2 Comparison with Slit Lamp Scale

If the mean Loveridge grid time in seconds is plotted against the grading scale (Figure 4.3) for all lenses then a significant correlation is seen (r = -0.72). However, the wide scatter of PLNIBUT scores by the Loveridge grid for each slit lamp grading score shows the coarseness of this latter scale.

Figure 4.3 - Correlation Between Loveridge Grid and Slit Lamp Scale

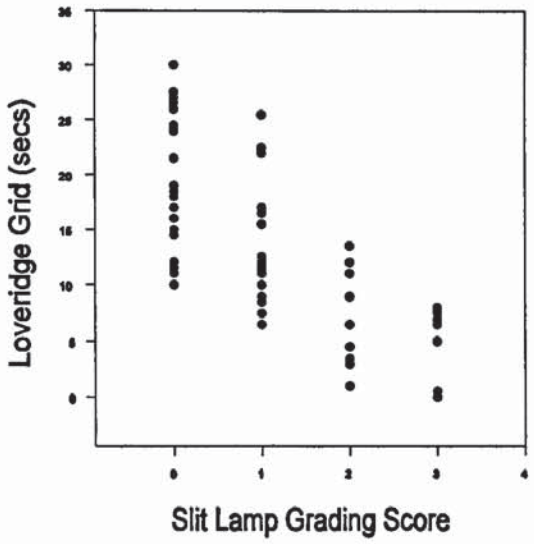


Figure 4.3 - Mean Loveridge Grid PLNIBUT (seconds) vs Slit lamp grading scale (0-4). A Spearman correlation of -0.72 ($p<0.0001$) indicates the degree of correlation between the two scales.

4.2 INSERTION AND REMOVAL TECHNIQUES

Initial wettability of lenses could potentially be altered by contamination of the lens surface with skin lipids during lens insertion. In addition, it is possible that deposition could be increased if lenses were inserted with a layer of deposition (of unknown etiology) from the practitioners fingers. While the latter of these would be expected to diminish in importance over the long-term period of the lenses life-span, it could be of significance in the short-term. Intuitively the deposition of lenses with extraneous sources such as cosmetics would appear a likely source of contamination on hydrogel lenses. However, little work has investigated this.[332 333]

As wettability on initial collection of lenses and deposition after short periods of wear were of interest during these studies the impact of insertion and removal on wettability and deposition was investigated.

4.2.1 Influence on Wettability

4.2.1.1 Methodology

Ten subjects (five male, five female) were enrolled. Lenses were removed at least eight hours before the study commenced. New polyHEMA lenses (Bausch & Lomb "SeeQuence") were inserted by a clinical assistant. After 20 minutes the PLNIBUT was assessed by a practitioner using the Loveridge Grid, as previously described. This procedure was repeated on another occasion, at least one day later. When inserting the lenses the clinical assistant either inserted them with ungloved hands or used non-powdered disposable

vinyl gloves (Oak Technical, Ohio, USA). The insertion technique was unknown to the practitioner conducting the PLNIBUT measurements and the insertion technique was randomised.

4.2.1.2 Results

Figure 4.4 details the results. The PLNIBUT with gloved hands was slightly longer than with ungloved hands (14.2 vs 13 seconds), and the standard deviation also smaller, although the difference was not significant (p=NS).

Figure 4.4 - PLNIBUT Comparing Gloved vs Ungloved Hands

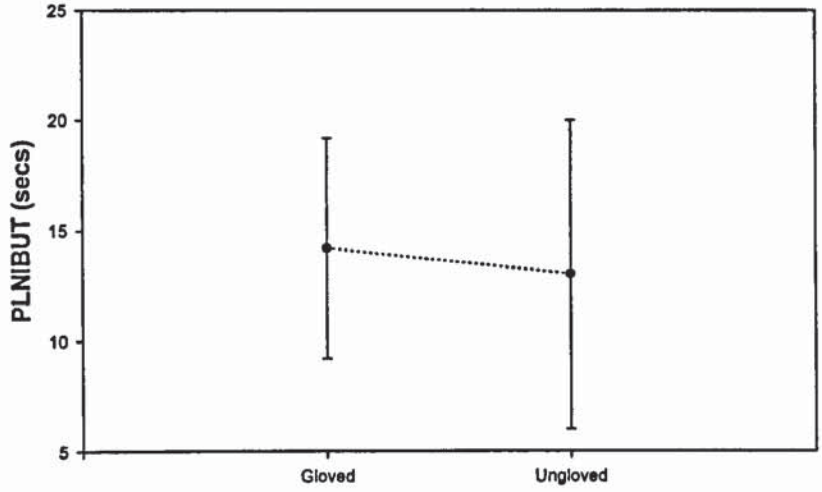


Figure 4.4 - PLNIBUT measurements (secs) following insertion with gloved and ungloved hands.

4.2.1.3 Conclusions

Insertion of lenses with ungloved hands by a "trained" handler does not significantly influence initial wettability.

4.2.2 Influence on Deposition

4.2.2.1 Methodology

Ten members of the laboratory staff were asked to handle 11 differing types of contact lens materials (all new, previously unworn -3.00D lenses) to assess their relative "handleability", as part of another study. All subjects washed their hands before handling the lenses and were asked to grade the lenses on a 1-10 scale, where 1 represented "impossible" and 10 "very simple". Following this the lenses were immediately examined by fluorescence and UV to assess the degree of surface contamination following their handling.

4.2.2.2 Fluorescence and UV Deposition

Figures 4.5 - 4.10 indicate the degree of deposition which occurs on lenses post-handling. Figures 4.5 - 4.7 reveal the results for all subjects for each material and figures 4.8 - 4.10 indicate the inter-subject differences. The results clearly show that measurable "spoilation" does occur with handling hydrogel lenses, even if they are handled with clean hands, and that there are differences both between subjects and between lens materials. Such findings are relevant in that the handling of lenses will influence the measured deposition unless suitable steps are taken to eliminate or reduce these complications. This is of particular significance when single-use, one-day disposable lenses are considered.

Figure 4.5 - 280nm Fluorescence Data For Handled, Unworn Lenses: Inter-Lens Differences

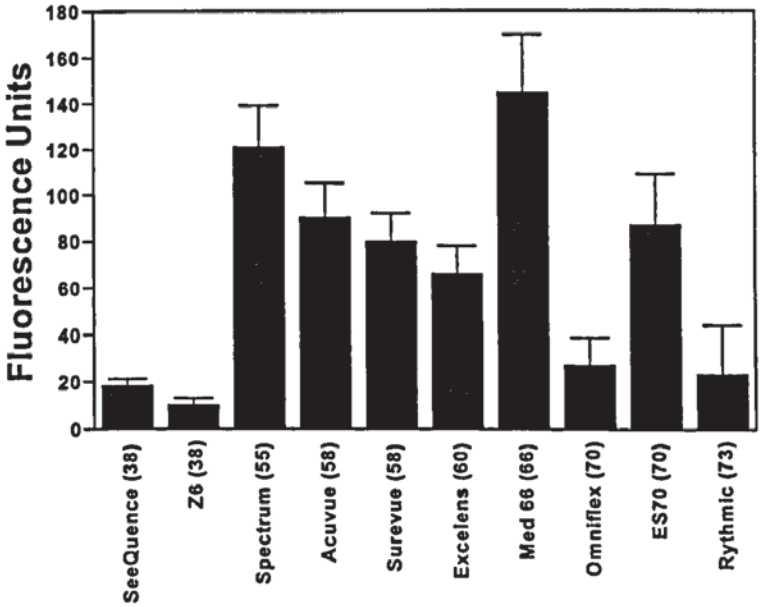


Figure 4.5 - Deposition measured using 280nm fluorescence excitation of lenses following handling by 10 subjects. Subjects had washed their hands prior to handling the lenses. The results are shown background corrected. The results were statistically different ($p < 0.002$).

Figure 4.6 - 360nm Fluorescence Data For Handled, Unworn Lenses: Inter-Lens Differences

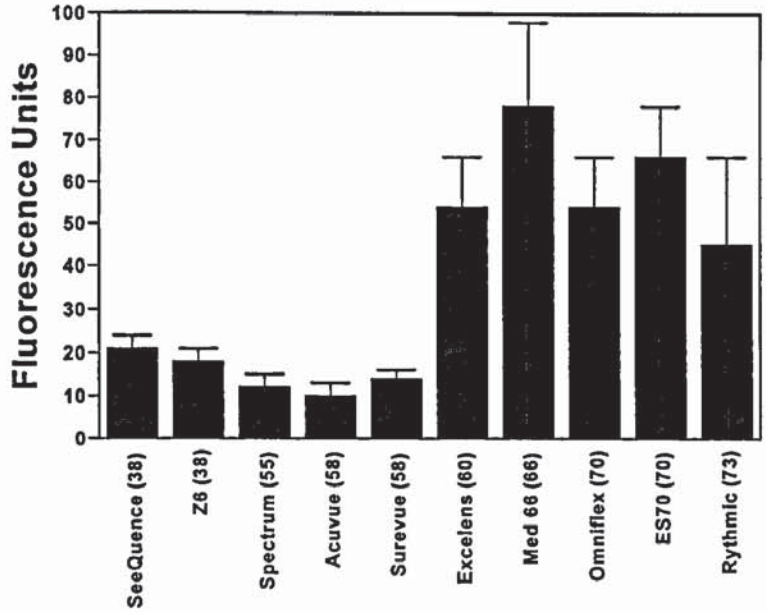


Figure 4.6 - Deposition measured using 360nm fluorescence excitation of lenses following handling by 10 subjects. Subjects had washed their hands prior to handling the lenses. The results are shown background corrected. The results were statistically different ($p < 0.001$).

Figure 4.7 - Transmission UV Data For Handled, Unworn Lenses: Inter-Lens Differences

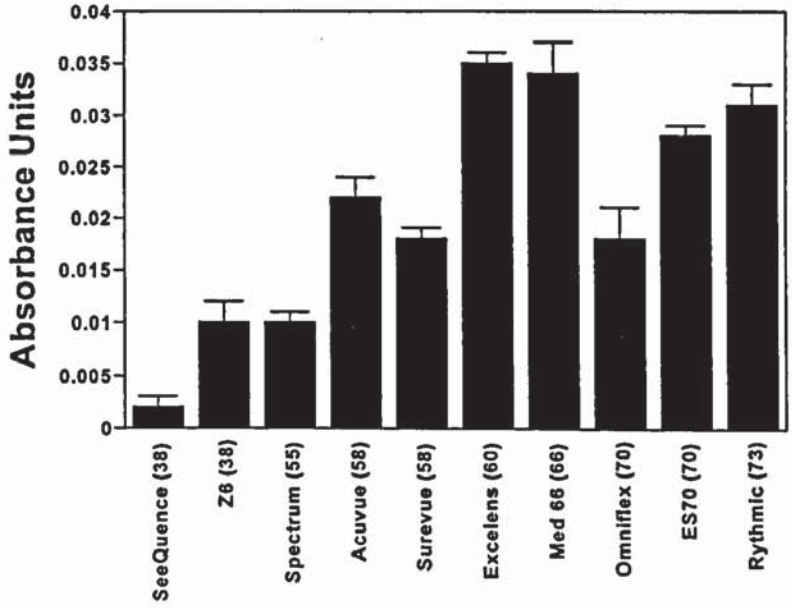


Figure 4.7 - Deposition measured using transmission UV of lenses following handling by 10 subjects. Subjects had washed their hands prior to handling the lenses. The results are shown background corrected. The results were statistically different ($p < 0.001$).

Figure 4.8 - 280nm Fluorescence Data For Handled, Unworn Lenses: Inter-Subject Differences

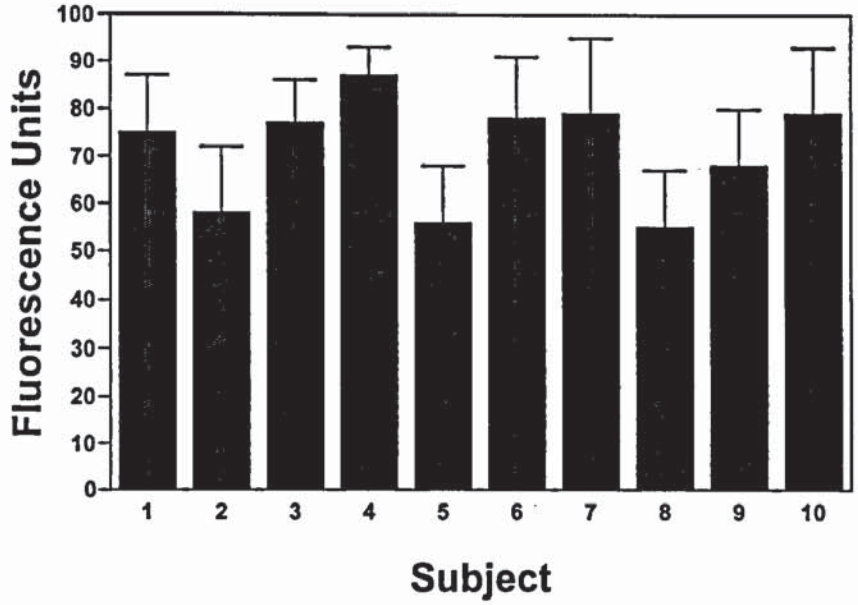


Figure 4.8 - Deposition measured using 280nm fluorescence excitation of lenses following handling by 10 subjects. Subjects had washed their hands prior to handling the lenses. The results are shown background corrected. The results were statistically different ($p < 0.01$).

Figure 4.9 - 360nm Fluorescence Data For Handled, Unworn Lenses: Inter-Subject Differences

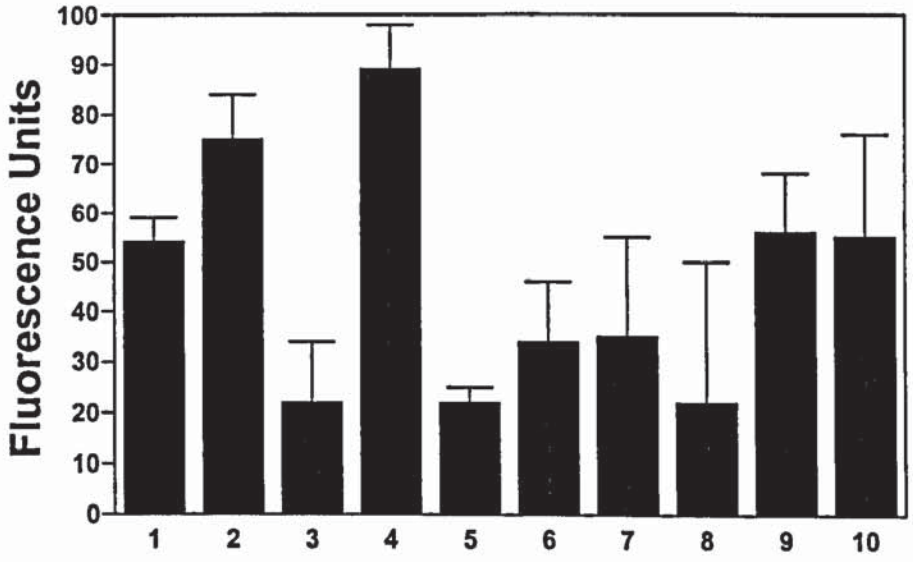


Figure 4.9 - Deposition measured using 360nm fluorescence excitation of lenses following handling by 10 subjects. Subjects had washed their hands prior to handling the lenses. The results are shown background corrected. The results were statistically different ($p < 0.005$).

Figure 4.10 - Transmission UV Data For Handled, Unworn Lenses: Inter-Subject Differences

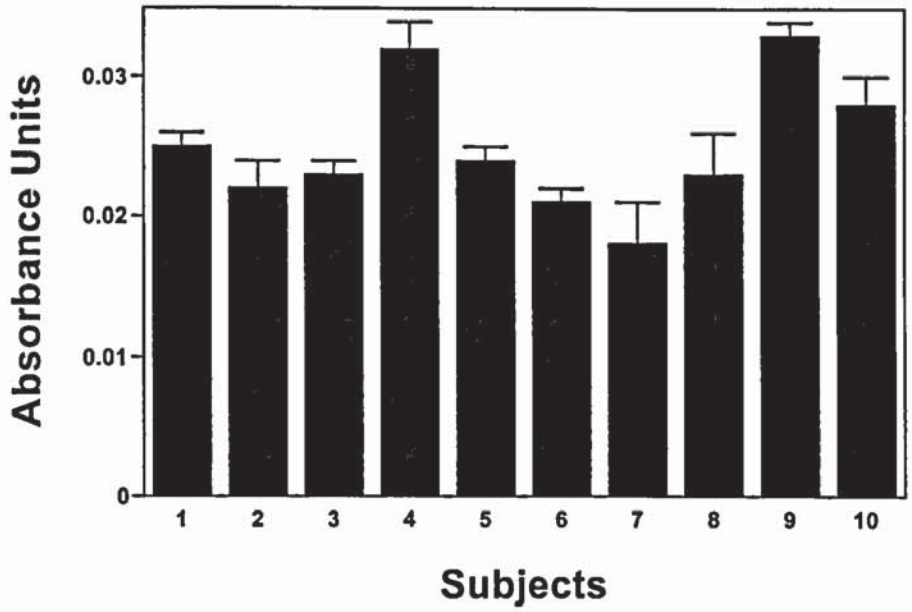


Figure 4.10 - Deposition measured using transmission UV of lenses following handling by 10 subjects. Subjects had washed their hands prior to handling the lenses. The results are shown background corrected. The results were statistically different ($p < 0.02$).

4.2.2.3 Conclusions

These results suggest that fluorescence is far more susceptible than UV to changes induced by handling and that 360nm fluorescence may be significantly affected by handling by certain subjects. Evidently a technique to remove lenses from subjects was needed which would not contaminate the lenses with skin lipids and other unknown chemicals. A technique was devised in which lenses were removed using plastic-tipped tweezers. The subject looked nasally, the lens was gently moved onto the temporal sclera, gently "pinched" with the tweezers and finally placed into saline in a labelled bottle, which was then refrigerated before taking to the laboratory for deposition analysis.

In some situations subjects had to remove lenses themselves and store them before attending the practice. This proved a particular problem with studies concerning one-day, single-use disposable lenses, in which it was impossible for the subjects to attend the practice each day for the lenses to be collected. In these situations the subjects were provided with alcohol wipes (Chauvin Pharmaceuticals) to remove lipid and other debris from their fingertips. They were instructed to wash their hands as normal, rub their fingers with the alcohol wipes, wait until their fingers were air-dry and then remove the lenses with clean fingers and place the lenses in glass vials containing saline solution.

In house examination of lenses removed in both ways by fluorescence and UV indicated that extraneous contamination was avoided using the above techniques.

4.3 GROSS MORPHOLOGY

Lenses were all visualised in the clinic with a Nikon FS3 photo slit lamp. Clinically obvious deposits were photographed. Lenses from the various studies were also periodically examined in the laboratory using microscopy with and/or without staining techniques to examine the gross morphology of the deposited material.

4.4 BLANK DETERMINATION

The measurement of deposition depends upon the accurate subtraction of the background absorbance or fluorescence from blank, unworn lenses.

Several points must be considered when evaluating the values for the blank, unworn lenses:

- The introduction of handling tints into lenses is likely to increase absorption.
- Lens thickness (in particular for plus powers) varies with prescription.
- Packing solutions may contain fluorescent material that may "coat" the lenses and influence the results.

Several laboratory studies were conducted to investigate these points:

4.4.1 Influence Of Lens Type

To check the differences between blank lenses of various types ten -3.00 dioptre unworn lenses of each type were measured in saline for differences in UV absorption and fluorescence.

4.4.1.1 Transmission UV

The results are shown in Table 4.1 and Figure 4.11.

Table 4.1 - Mean (± sd) UV Absorption Values of 10 x -3.00D Blanks

Lens	Handling Tint ?	Absorbance	Lens	Handling Tint ?	Absorbance
Acuvue	✔	0.067 ± 0.020	Medalist 66	✔	0.112 ± 0.023
1-Day Acuvue	✗	0.021 ± 0.020	Precision UV	✔	(uv blocker)
Frequency 55	✔	0.131 ± 0.030	Proclear	✗	0.027 ± 0.020
Classic	✔	0.041 ± 0.010	Rythmic	✗	0.021 ± 0.021
ES70	✗	0.028 ± 0.020	Surevue	✔	0.088 ± 0.026
Excelens	✔	0.166 ± 0.030	Vistagel PLUS	✗	0.030 ± 0.014
Focus	✔	0.226 ± 0.031	Zero 6	✗	0.018 ± 0.020

Figure 4.11 - UV Absorption For Unworn Lenses

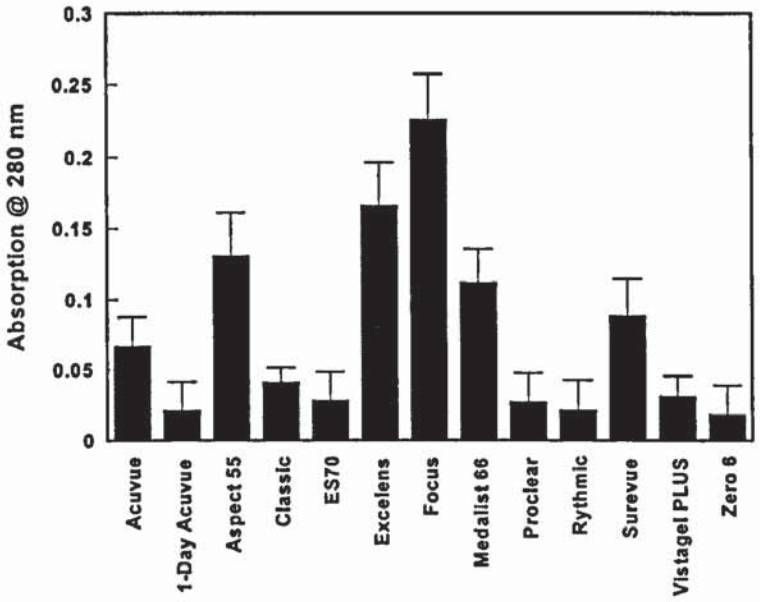


Figure 4.11 - UV absorption (mean ± sd) for 10 x -3.00D unworn lenses

Clearly differences exist between lens types with and without handling tints and, due to the large variation in the depth of the tint, differences between similarly tinted lenses are also observed. Background absorption values were deducted from blank samples based on the mean of ten samples.

4.4.1.2 Fluorescence

The results are shown for 280 nm excitation and 360 nm excitation in Tables 4.2 and 4.3 respectively.

Table 4.2 - Fluorescence Values of 10 x -3.00D Blanks @ 280nm Excitation

Lens	Emission λ (nm)	Peak Height	Lens	Emission λ (nm)	Peak Height
Acuvue	340.8 ± 1.2	68.5 ± 2.9	Frequency 55	321.2 ± 1.2	382.9 ± 97.2
1-Day Acuvue	338.8 ± 1.0	58.6 ± 4.8	Medalist 66	322.3 ± 2.0	436.4 ± 108.1
Classic	336.4 ± 1.2	92.3 ± 6.7	Proclear	329.6 ± 1.8	106.3 ± 14.3
ES70	333.4 ± 1.1	75.9 ± 8.2	Surevue	340.8 ± 1.1	47.4 ± 6.8
Excelens	325.2 ± 1.3	151.9 ± 15.3	Vistagel PLUS	340.4 ± 2.3	52.9 ± 5.6
Focus	332.1 ± 1.0	189.7 ± 28.5	Zero 6	340.2 ± 1.1	41.2 ± 3.4

Table 4.3 - Fluorescence Values of 10 x -3.00D Blanks @ 360nm Excitation

Lens	Emission λ (nm)	Peak Height	Lens	Emission λ (nm)	Peak Height
Acuvue	442.1 ± 1.7	15.0 ± 1.8	Frequency 55	442.3 ± 0.6	13.9 ± 1.3
1-Day Acuvue	440.4 ± 0.7	10.3 ± 1.3	Medalist 66	442.3 ± 1.3	38.8 ± 12.5
Classic	441.2 ± 1.3	28.4 ± 3.4	Proclear	441.0 ± 1.4	36.3 ± 2.3
ES70	439.8 ± 1.2	20.9 ± 1.2	Surevue	442.9 ± 2.2	14.0 ± 2.1
Excelens	443.4 ± 1.4	26.8 ± 2.1	Vistagel PLUS	439.4 ± 1.3	28.1 ± 1.3
Focus	440.2 ± 0.5	36.8 ± 3.4	Zero 6	440.2 ± 1.1	21.2 ± 1.4

Some lenses clearly exhibit high levels of fluorescence, particularly under 280 nm excitation. The results in some instances (notably Excelens, Focus, Frequency 55 and Medalist 66) are higher than the results exhibited with worn lenses of the same type. This is due to the presence of either fluorescent leachables (which have not been totally removed from the lenses following manufacture) or fluorescent species in the packing solution (see 4.4.3, page 126). To compensate for this, lenses were soaked in distilled water on a flat bed rotary shaker for seven days, with regular changing of the water, and then re-examined. This process resulted in a significant reduction in the 280 nm fluorescent signal and was the procedure adopted prior to measuring the blank lenses.

Leachables may also have influenced the results in Figure 4.5 - 4.10 in which the influence of handling on lenses was investigated. Fluorescent leachables in the handled lenses could have influenced the fluorescence results, as such lenses were only briefly rinsed before handling. However, there is no obvious correlation between the fluorescence results achieved in Figures 4.5, 4.6, 4.8 and 4.9 and Tables 4.2 and 4.3. In addition, as the conclusion from that study was that lenses should be removed using gloved hands then this factor will not have influenced the spoilation results reported throughout the rest of this thesis.

4.4.2 Influence Of Back Vertex Power

Six lens types were examined to investigate the influence of prescription on UV absorbance and florescence. Eighteen lenses of each type were examined, with a prescription range from -5.00D to +5.00D.

4.4.2.1 Transmission UV

The results are shown in Figure 4.12.

Figure 4.12 - Absorbance of Lenses Of Varying BVP

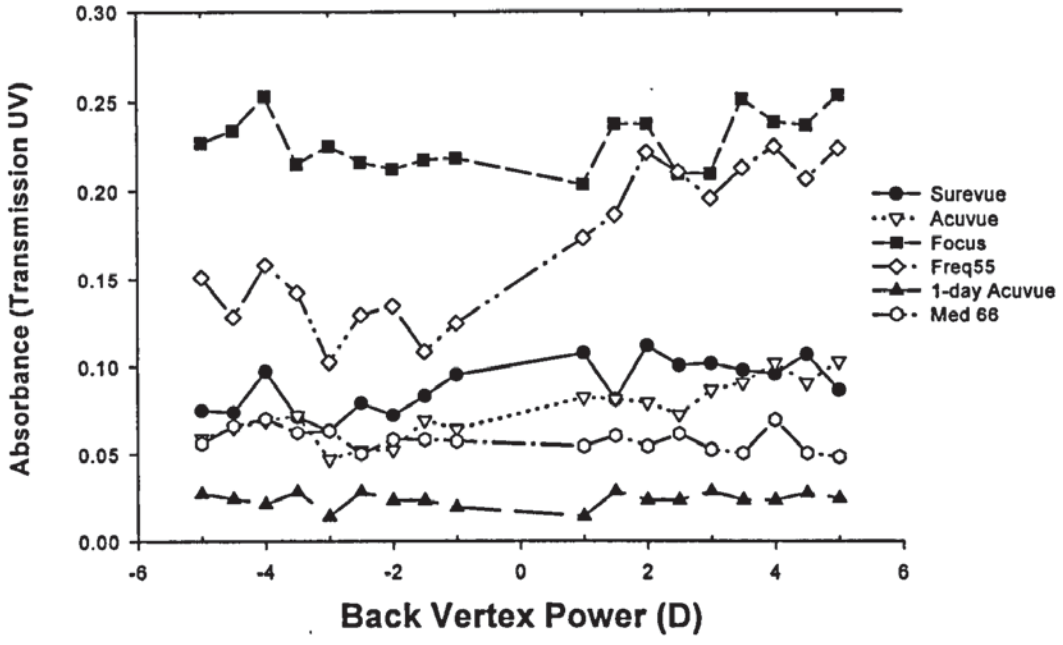


Figure 4.12 - Absorbance (transmission UV) for lenses of varying prescription (BVP)

The results suggest that there is only a small influence of prescription on UV transmission for negatively powered lenses and the value for a "standard" -3.00D lens is acceptable for blank subtraction of such lenses. The results also suggest that positive lenses should use a positively powered blank and that caution must be exercised with darkly tinted lenses (Focus and Frequency 55), which increase in absorption with increasing thickness. For these lenses blank determination should consider the prescription of the lenses under test.

4.4.2.2 Fluorescence

Examination of the lenses demonstrated that there was no influence of prescription on fluorescence.

4.4.3 Influence Of Packing Solution

The packing solution that lenses are stored in may influence the background fluorescence values for blank lenses. If the saline solution is augmented with fluorescent species (possibly to aid wetting on initial insertion) this will clearly influence the fluorescence of the blank.

To investigate this, five lenses (all -3.00D) of ten lens types were opened. Aliquots (1ml) of the shipping solution were removed with a disposable syringe and placed in the cuvette. Fluorescence was then undertaken at 280 nm and 360 nm. The fluorescence at 360 nm excitation for all solutions was negligible and the results in Table 4.4 detail the 280 nm results, including comparisons with saline and distilled water.

Table 4.4 - Fluorescence (Mean) of Packing Solutions at 280 nm Excitation

Lens	Emission Wavelength	Peak Height	Lens	Emission Wavelength	Peak Height
Acuvue	341.6	34.4	Medalist 38	321.6	579
ES70	333.8	46.5	Medalist 66	321.4	635.2
Excelens	342.1	39.6	Proclear	346.8	30.6
Focus	321.4	1600	Weicon CE	337.9	25.2
Frequency 38	322.8	1280	Water	332.6	10.6
Frequency 55	321.9	860.2	Saline	343.2	36.3

These results show that some packing solutions (for Focus, Frequency and Medalist) are highly fluorescent. This is either due to the incorporation of surfactants or the leakage of fluorescent leachables from the lens into the packing solution following their manufacture. These results help explain the high levels of fluorescence of blank lenses reported in Table 4.2 (page 124).

4.5 PROTEIN CALIBRATION

Calibration with protein solutions was necessary to investigate the relationship between transmission UV and fluorescence with increasing protein concentration. Four proteins (albumin, globulin, lysozyme and lactoferrin) were obtained in a pure form from Sigma Chemical Company and, via serial dilution, made into concentrations from 0.0125 - 0.1 mg/ml. These were then examined by transmission UV and fluorescence @ 280 nm. The linear regression results are detailed in Tables 4.5 and 4.6.

Table 4.5 - Linear Regression for Transmission UV vs Protein Concentration

Protein	Equation	R value	R^2 value
Albumin	0.0101 + (0.551* concentration)	0.917	0.841
Globulin	-0.0110 + (1.17 * concentration)	0.924	0.855
Lactoferrin	-0.00679 + (1.30 * concentration)	0.986	0.972
Lysozyme	-0.0168 + (2.35 * concentration)	0.963	0.928

Table 4.6 - Linear Regression for Fluorescence @ 280nm vs Protein Concentration

Protein	Equation	R value	R^2 value
Albumin	11.9 + (6174.5 * concentration)	0.979	0.958
Globulin	-94.3 + (16380.4 * concentration)	0.976	0.953
Lactoferrin	33.1 + (6981.9 * concentration)	0.985	0.970
Lysozyme	-139.9 + (43336.7 * concentration)	0.986	0.973

These results show that there is a linear relationship between increased protein concentration and measured protein with both techniques, over the range studied. This experiment is only of value as an illustration, only proving the relationship for single proteins over the concentration range studied. The situation in the tear film is significantly different as there is a mix of proteins present and the situation is a dynamic one, which constantly varies over time. There will be increased fluorescence quenching with an increased concentration of proteins and it is likely that this relationship will be non-linear outside the range studied. Further linear regression analysis on each solution reveals the relationship between transmission UV and fluorescence @ 280 nm. The results are tabulated in Table 4.7.

Table 4.7 - Linear Regression For Transmission UV vs Fluorescence @ 280 nm

Protein	Equation	R value	R^2 value
Albumin	UV = 0.00906 + (0.0000893 * 280 value)	0.936	0.877
Globulin	UV = -0.00578 + (0.0000733 * 280 value)	0.972	0.944
Lactoferrin	UV = -0.0116 + (0.000182 * 280 value)	0.984	0.968
Lysozyme	UV = -0.00988 + (0.0000545 * 280 value)	0.982	0.964

These results clearly show that there is an excellent correlation between the two instruments for single protein solutions. However, the tear film is a complex mixture of proteins and using a calibration curve of a single

protein would result in inaccuracies being interpolated of the volume of protein deposited on the contact lens. To overcome this problem a protein mixture was created, [431] with proteins in concentrations that approximately reflect that found in the tear film. [131] This was serially diluted and a final graph of absorbance versus concentration drawn (Figure 4.13). Calculation of the concentrations of lens bound proteins (total protein by UV) described in this thesis was based on this standard reference curve. This approach has drawbacks in that it assumes that the same ratio of protein deposits on all materials. However, of the approaches described above it was decided that this was the most appropriate. To convert the concentration in mg/ml to mg/lens the result is multiplied by Πr^2.

Figure 4.13 - Concentration vs Absorbance Plot For Protein Mixture

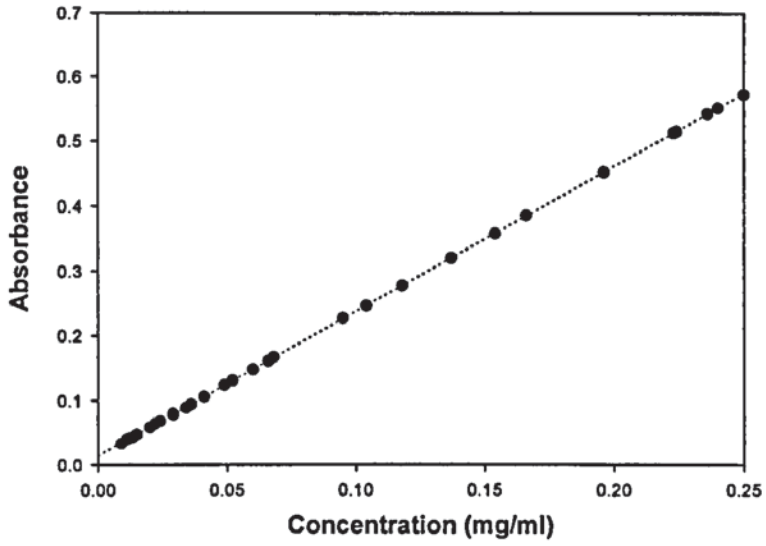


Figure 4.13 - Concentration vs absorbance plot for protein mixture. Linear regression analysis reveals that absorbance = 0.0139 + (2.237 x concentration) and $R^2 = 0.99$.

This linear relationship only exists for transmission UV as it is a bulk technique that relies upon absorbance. Due to fluorescence quenching there is a linear relationship for fluorescence only over low concentrations, which becomes non-linear as the protein concentration increases.

4.6 EMISSION WAVELENGTHS FOR FLUORESCENCE

The maximum excitation wavelength required for fluorescence is found using a 3-D scan, as previously described in Chapter 3 (page 95). The maximum emission wavelength for lenses is found by using a curser and noting the wavelength that occurs at the maximum peak height. Examples are found in Appendix 6 (pages 360-364).

The summary results for all the materials tested during this project are detailed in Table 4.8. The table lists the maximum emission wavelength for all materials before either wear or handling.

Table 4.8 - Maximum Emission Wavelength (Mean ± sd) For Study Lenses

Lens	@ 280 nm λ (max)	Range	@ 360 nm λ (max)	Range
Zero 6	343.6 ± 2.1	338.4 - 346.8	439.4 ± 3.4	430.8 - 443.6
Vistagel PLUS	340.4 ± 2.9	334.6 - 346.2	436.7 ± 3.6	432.2 - 446.4
Classic	332.5 ± 3.5	324.8 - 341	432.9 ± 2.8	413.0 - 437.2
Focus	340.1 ± 2.1	336.8 - 344.2	437.2 ± 1.6	434.4 - 442.4
Acuvue	341.5 ± 2.1	336.2 - 345.2	441.2 ± 2.7	435.8 - 446.4
Surevue	341.6 ± 1.7	338.8 - 344.4	441.9 ± 1.9	437.8 - 446.6
Proclear	345.5 ± 2.2	338.4 - 348.6	440.4 ± 2.8	433.6 - 447.6
Excelens	337.8 ± 3.9	332.2 - 345.2	437.8 ± 2.3	433.4 - 441
Medalist 66	326.1 ± 4.5	320.2 - 346.5	435.5 ± 1.5	423.2 - 438.6
ES70	342.4 ± 1.9	339.4 - 346.4	438.2 ± 1.5	430.6 - 436.6
Rythmic	340.2 ± 2.2	336.7 - 344.2	439.2 ± 1.9	432.2 - 445.2

The results indicate that for most materials exciting at 280 nm results in a maximum emission wavelength around 340 nm and exciting at 360 nm produces a maximum emission around 440nm. The exception to this occurs with Classic and Medalist 66, whose composition shifts the emission maximum to a shorter wavelength at both excitation values. This may be due to the cross-linking agents used in these compositions.

4.7 SAMPLING MEDIUM

Previous studies in these laboratories [431] have shown that protein (in particular lysozyme) is extremely mobile and can move out of group IV materials and into the surrounding solution with relative ease. This movement of protein is of particular significance as it will ultimately influence the amount of protein measured on lenses, resulting in a lowered estimate of the quantity of protein present.

Two areas are potential sites for complications; The media in which the lenses are placed following collection (transport media) and the media used during the deposition evaluation (examination media). Several points must be considered:

- The solution in which the lenses are immediately placed following removal from the eye should remove as little protein from the lenses as possible.

- The passage of protein into the solution will be influenced by the concentration gradient of protein (which will be influenced by the amount of protein in the lens; the greater the amount of protein the greater the passage of protein that is likely to occur).

- The pH of the solution will influence the movement of protein (particularly in group IV materials); an acidic pH will result in the pores of the material "shrinking" and potentially trap the protein in place.

- The solution used should not contain a surfactant as this may remove deposited material from the lens and result in a lower estimation of the deposition than is present immediately following removal.

- The solution used should not contain fluorescent species as this may bind to the lens surface and significantly influence the results.

These points are addressed below.

4.7.1 Fluorescence of Potential Transport Media

Table 4.9 indicates the fluorescent nature of a series of possible collection fluids. Aliquots (1ml) were removed from each bottle of previously unopened solutions and placed in the cuvette of the fluorimeter. The solutions were examined using fluorescence at 280 nm and 360 nm excitation.

Table 4.9 - Storage Solution Fluorescence

Solution	280 nm Excitation		360 nm Excitation	
	Emission Wavelength	Peak Height	Emission Wavelength	Peak Height
Distilled water	344.2	10.1	439.8	5.0
ReNu	344.2	54.5	442	16.7
Saline (Salette)	343.8	16.8	444	5.4
Oxysept II neutraliser	331.2	70.1	442.4	7.7
Complete	343	500.2	434.6	37.7
Opti-1	342	10.4	440.2	9.8
Hydrocare	344.4	425.4	443.4	200.4
Concerto	337.6	43.0	443	13.0
Solo-care	323	44.8	441.4	6.1
All-in-One	343.8	49.6	440.8	22.1
All-in-One Light	342.4	89.7	444.6	13.0

The results show that of all the possibilities, the ones most appropriate were saline, distilled water or Opti-1 as these gave the lowest background at both 280nm and 360nm excitation.

4.7.2 Influence of Examination/Transport Media On Parameters

It was important that lenses remained similar in dimension during the transport and examination process, as significant increases in, for example, diameter would show a lens that was "swelling" and would be more likely to leak protein into the surrounding media.

Three lenses of four types were placed in a temperature controlled Optimec analyser and their diameter measured in fresh buffered saline. The soaking solution was then changed to either fresh saline or distilled water. The pH of the distilled water was 6.2 ± 0.2 and that of the saline was 7.1 ± 0.1. After thirty seconds (the approximate time taken to obtain a deposition reading using either fluorescence or UV) the diameter was remeasured.

Table 4.10 details the mean (±sd) diameters and the % "swell" which occurred.

Table 4.10 - Influence of Examination Fluid on Diameter

Lens	FDA Group	Saline (mm)	Distilled Water (mm)	% Swell
SeeQuence	I	13.87 ± 0.05	13.93 ± 0.05	0.5
Medalist 66	II	14.23 ± 0.05	14.23 ± 0.05	0.0
Surevue	IV	13.83 ± 0.05	16.83 ± 0.12	21.7
Focus	IV	14.00 ± 0.08	15.10 ± 0.22	7.9

These results are easily explainable. Water will move into a hydrogel material due to osmosis, to an extent that is proportional to the concentration of the dissolved species, resulting in the volume of the lens increasing. The amount of MA present in the material will dictate the amount of fluid that will move in. PolyHEMA materials such as SeeQuence have a trace of residual MA left behind following their fabrication. Medalist 66 has no HEMA or MA and of the other two materials Surevue has a greater quantity of MA than Focus, and both have greater quantities of MA than SeeQuence. Consequently Etafilcon materials are stored in hypertonic saline rather than isotonic saline in order that their parameters remain stable.

The results indicate that examination or storage of group IV materials in distilled water may result in significant parameter changes and, potentially, loss of matrix bound protein.

4.7.3 Concentration of Species in Examination Media

Following examination of the lenses for deposition (using either fluorescence or transmission UV) the resulting amount of material that had moved into the cuvettes was examined. These lenses were taken from a variety of studies. All lenses had been worn under trial conditions, removed as previously described, placed in storage bottles in buffered saline and then placed into a cuvette for analysis. None had been cleaned before analysis taking place.

4.7.3.1 Transmission UV

Thirty lenses were examined by transmission UV after being left in the cuvette for two minutes. The baseline adjusted results are shown in Table 4.11.

Table 4.11 - Protein in Cuvette Following Transmission UV Analysis

FDA Group	Saline	Water
Group II	0	0
Group IV	4 ± 1 µg	14 ± 3 µg

4.7.3.2 Fluorescence

Thirty lenses were examined by fluorescence after being left in the cuvette for two minutes. The baseline adjusted results are indicated in Table 4.12.

Table 4.12 - Species in Cuvette Following Fluorescence Analysis

FDA Group	Saline		Water	
	@ 280	@ 360	@ 280	@ 360
Group II	8.5 ± 2.0	0	10.1 ± 2	0
Group IV	80.2 ± 22.1	0	135 ± 55	0

The results show that the movement of protein into the examination medium is greater with distilled water than with saline and that lipid does not readily desorb from lenses into either medium. Previous studies in these laboratories [431] have clearly shown the value of using an acidic solution such as 3% hydrogen peroxide to "lock" the protein into the lens material and prevent its movement during the examination process. However, this approach will denature the lysozyme present and so was rejected as a possible examination fluid during these studies.

4.7.4 Species in Transport Medium

The passage of protein and lipids into the surrounding media during the possible four-week period from collection in London to examination in Birmingham will significantly impact on the measured deposition on lenses. All lenses were stored in buffered saline following collection and before analysis in the laboratory. To examine the extent to which the deposited species had eluted from the lens into the surrounding medium 1ml aliquots of transport solution were removed from the collection bottles with disposable syringes and placed into the cuvettes. These extracts were then examined by fluorescence and transmission UV.

Initial results displayed markedly variable results both for repeatability and amount of desorbed species per patient. The most likely reason for this related to the variable amount of saline placed in each collection vial, which varied from 2-6.5ml. To eliminate this problem the amount of storage solution in each vial was standardised (5ml) and the vial placed on a slow shaker for two minutes before removal of the aliquots for examination.

The following results were found for thirty randomly chosen lenses.

4.7.4.1 Transmission UV

Table 4.13 details the mean (±sd) of protein found in the storage solution for a variety of materials.

Table 4.13 - Protein in Solution by Transmission UV (μg/ml)

Lens	Mean ± SD
Medalist 66	8 ± 6
Focus	53 ± 15
Acuvue	88 ± 20

4.7.4.2 Fluorescence

Table 4.14 details the means (±sd) of the species found in the storage solution for a variety of materials.

Table 4.14 - Storage Solution Species

Lens	Excitation @ 280	Excitation @ 360
Medalist 66	320 ± 126	44 ± 23
Focus	920 ± 238	38 ± 16
Acuvue	1500 ± 400	45 ± 24

These results show that there is a movement of protein into the surrounding solution, which is more marked for group IV materials, in particular those with considerable quantities of methacrylic acid. As a result of these findings it was decided that storage, transport and examination of lenses should take place using saline and not distilled water. During these studies lenses were always stored and examined in Salette, unit-dose saline (Alcon).

4.7.5 Technique Variability

The variation in blank values, fluorescence of storage and transport medium and movement of protein into the surrounding media will all act to influence the results of the deposition evaluation. Additionally, the repeatability of the instrumentation used to measure the deposited layer must be known.

Repeatability can be checked by taking, for example, thirty deposited lenses and measuring their deposition ten times. This would typically be conducted by taking each lens from the storage vial, positioning it in the cuvette, running the analysis once, removing the lens, placing it back into the vial and repeating the whole process a further x number of times. This would enable the investigator to arrive at a mathematically derived measure of repeatability. Preliminary examination revealed that the repeatability of measurement using this

technique was poor, with measured results for lenses usually diminishing over time, particularly the fluorescence results at 280nm excitation in group IV lenses. The reason for this was probably two-fold; lysozyme mobility resulted in the continued movement of protein out of the lens and into the examination fluid on each measurement and fluorescence "bleaching" may take place, which will take some time to recover.

An assessment of the variability in the instruments measurement capability can be obtained by leaving the lens *in-situ* and running the instrument on ten successive occasions, without disturbing the lens or turning the machine off. This technique yields 10 dependent readings of a single measurement. Table 4.15 details the percentage variation ([mean/sd]*100) found with four materials measured in this way, taking twenty randomly chosen lenses of each type. The results indicate that one reading is sufficient for each lens assessed.

Table 4.15 - Instrument Variability For 10 Dependent Readings (%)

Lens	Protein by UV	280 Fluorescence	360 Fluorescence
Medalist 66	< 1	< 3	< 4
ES70	< 1	< 1	< 2
Surevue	< 5	< 5	< 10
Focus	< 3	< 5	< 5

4.8 Key Points

- Four seconds difference constitutes a clinical change in PLNIBUT with the Loveridge grid device.
- PLNIBUT is not significantly influenced by inserting lenses with ungloved hands.
- There are inter-subject and inter-material differences that are influenced by inserting lenses without gloves.
- Lenses must be removed "aseptically" to prevent unknown influences on deposition through the transfer of skin lipids and other extraneous contaminants.
- To obtain an accurate blank subtraction value, blank lenses should be soaked before analysis and the mean value of ten lenses used as the true subtraction value.
- Back vertex power has a minimal effect on blank value unless the lens is heavily tinted.
- Buffered saline is the transport, storage and examination media of choice, in most circumstances.
- A "mass balance" approach to the analysis of deposition, with fluorescence and UV analysis of both the lens material and the collection fluid is important if all of the deposited species is to be evaluated.

Chapter 5

Inter-Subject Variability in Deposition Performance & Behaviour

"He who asks a question may be a fool for five minutes, but he who never asks a question remains a fool forever". [1]

Significant differences in spoilation behaviour between patients is frequently seen in clinical practice, with some patients visibly depositing their lenses in very short periods of time. Prior to the introduction of frequent replacement and disposable lenses in the late 1980's these patients posed a significant management problem, requiring often heroic measures to prolong the life of their lenses. The complicated and expensive multi-item care systems required and the unplanned frequent replacement of lenses resulted in high cost and low satisfaction to the patient.

Whilst generally recognised, the topic of inter-subject differences has been only scantily covered in the literature. The purpose of this chapter is to address this issue and to look at both inter- and intra-subject variability in deposition behaviour.

5.1 THE DEPOSITION OF A MONTHLY, DAILY-WEAR FDA GROUP II MATERIAL USED OVER A 12-MONTH PERIOD

The first study in this chapter investigated the inter- and intra-subject variability in deposition that occurs with an FDA group II material used over a 12 month period.

5.1.1 Materials and Methods

Ten subjects (five male and five female) were entered onto the study, whose demographic details are described in Table 5.1.1

[1] Tom J Connelly

Table 5.1.1 - Subject Demographics

	Mean ± SD	Range
Age (years)	29.0 ± 6.4	21 - 39
Flat Keratometry (mm)	8.01 ± 0.33	7.45 - 8.40
Steep Keratometry (mm)	7.95 ± 0.36	7.30 - 8.40
Sphere (D)	-3.00 ± 0.74	-4.25 to -2.00
Cyl (D)	-0.31 ± 0.26	-0.75 to 0.00

All subjects were fitted with Medalist 66 from Bausch & Lomb, details of which are given in Table 5.1.2.

Table 5.1.2 - Lens Parameters (Medalist 66)

Water Content (%)	66
Monomers	HEMA + VP
USAN	Alphafilcon A
FDA Category	Group II
ISO Category	Filcon 4a
Manufacture	Moulded
Back Optic Zone Radius (mm)	8.40
Total Diameter (mm)	14.20
Centre Thickness (mm)	0.10

The lenses were worn as monthly, frequent replacement lenses for twelve consecutive one month periods on a daily-wear basis whilst using ReNu (Bausch & Lomb) as the cleaning and disinfection system. Subjects were reviewed in the clinic on collection and after 4, 12, 24, 36 and 48 weeks. Visits were scheduled within ± 3 days of the expected date. No enzyme tablets or rewetting drops were used during the study.

5.1.2 Clinical Protocol

At the clinical visits subjective satisfaction, visual acuity, lens fit, wettability, visible deposition and physiological performance were assessed as previously described.

5.1.3 Analytical Protocol

At the end of the four-week wearing schedule each lens was either removed by the subject or practitioner, depending upon whether the replacement time coincided with a clinical visit or not. The practitioner collected the lenses using plastic tipped tweezers and the subjects collected them following the use of alcohol wipes, as previously described. The lenses were then placed in glass vials containing sterile non-preserved, non-buffered saline, the vials were capped, labelled and taken to the clinical centre. These were subsequently taken to the laboratory for spoilation analysis, which occurred within 28 days of collection.

The lenses were analysed for total protein using transmission UV and surface deposition using fluorescence spectrophotofluorimetry, as previously described.

5.1.4 Data Analysis

Summary statistics were calculated for all variables. The grading scales, being ordinal measurements, were considered non-normal data. All other data were tested for normality of distribution. Wilcoxon signed rank testing was used to compare non-parametric factors. Changes across time for clinical data were compared using a one-way RM ANOVA on ranks, with differences between visits being examined by Tukey Test. Inter-subject differences were compared using a two-way repeated measures ANOVA on two factors, with eye and subject as a factor and visit as the repeat. Correlation coefficients were undertaken using Spearman Rank Order Test.

In all cases a "p" value of <0.05 was taken as statistically significant.

5.1.5 Results

5.1.5.1 Subjective Factors

These results are summarised in Table 5.1.3. There was no significant difference between any measured factors across the study period (p=NS).

Table 5.1.3 - Subjective Factors (Median and 25% quartile)

Factor	1 month		3 months		6 months		9 months		12 month		p
Visual Quality	8	7	9	8	9	9	9	8	8	8	NS
Comfort	8	7	9	8	9	9	9	8.75	8	8	NS
Handling	8	5	9	8	9	8	9	8	9	8	NS
Overall Satisfaction	8	8	9	8	9	8	9	8.75	9	8	NS

5.1.5.2 Visual Acuity

Mean (±sd) high contrast visual acuity (VAR units) for all visits was 103 ± 5 (-0.06 logMAR; 6/5) and low contrast visual acuity was 93 ± 6. There was no significant difference across the study period (p=NS).

5.1.5.3 Lens Fit

Mean (±sd) lens fit by percentage tightness across all visits was 42.4 ± 6.2, indicating that the lens was assessed as a slightly loose fit. There was no change across visits (p=NS).

5.1.5.4 PLNIBUT

Figure 5.1.1 shows that while PLNIBUT reduced between collection and the four-week visit, the PLNIBUT after that was relatively static. The PLNIBUT change across time was not significant (p=NS). Figure 5.1.2 compares the PLNIBUT between the R eyes of subjects across all clinical visits.

Figure 5.1.1 - PLNIBUT For All Worn Lenses

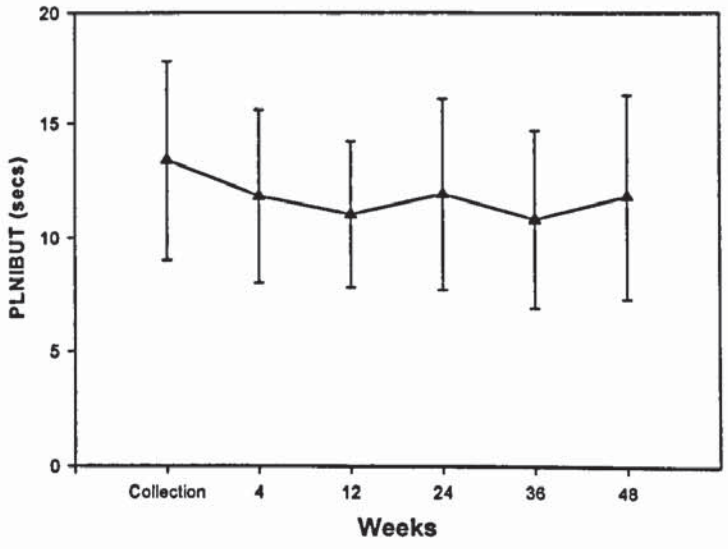


Figure 5.1.1 - PLNIBUT for monthly worn FDA group II lens across all appointment times for all right lenses for all subjects

Figure 5.1.2 - PLNIBUT Between Subjects Across All Appointments

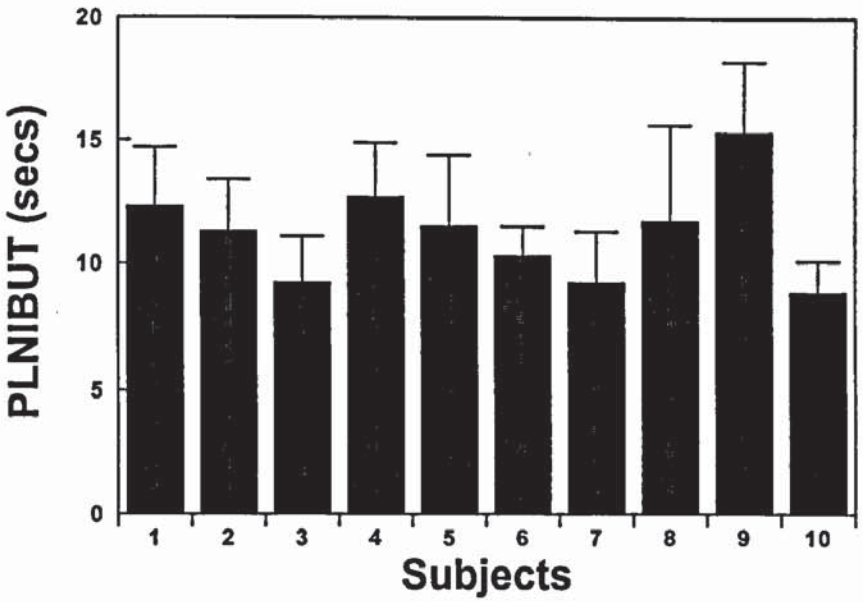


Figure 5.1.2 - PLNIBUT for monthly worn FDA group II lenses. The analysis included the R eyes only for all subjects across all appointments

One-way repeated measures ANOVA on ranks between subjects revealed that there was a significant difference between subjects ($p<0.04$). Post-hoc Tukey analysis revealed that subject 9 had a significantly longer PLNIBUT than all the other subjects ($p<0.05$), but all other subjects were not significantly different from each other (p=NS).

5.1.5.5 Physiological Appearance

There was no significant difference between the lenses at any follow-up for corneal staining, bulbar conjunctival hyperaemia or corneal oedema (p=NS). Most of the clinical findings were clinically insignificant. Mean corneal staining score was 0.9, median of 1 ("Minor"), with the principal area of staining (26%) being inferior "smile"type staining, which is a common finding with all high water content lenses. The overall incidence of any corneal staining was 49%. As with many moulded lenses the incidence of recorded conjunctival/limbal staining was fairly high, with 66% of lenses exhibiting some degree of conjunctival staining. However, this was clinically insignificant with a median score of "1" (mild) and mean of 0.7 for all lenses.

Palpebral lid changes are summarised in Table 5.1.4. While there was no difference in papillae appearance across time there was for hyperaemia grading ($p<0.005$). Post-hoc testing shows that these differences were insignificant between 1 and 6 months (p=NS), but then the hyperaemia increased ($p<0.05$), with no further change occurring between 9 and 12 months (p=NS).

Table 5.1.4 - Palpebral Lid Changes (Mean±SD)

	Hyperaemia	Papillae
1 month	0.1 ± 0.3	0.3 ± 0.5
3 months	0.2 ± 0.4	0.1 ± 0.3
6 months	0.2 ± 0.4	0.3 ± 0.5
9 months	0.8 ± 0.5	0.6 ± 0.7
12 months	0.9 ± 0.4	0.5 ± 0.6
p	<0.005	NS

5.1.5.6 Total Protein

The mean (±sd) total protein deposition for all worn lenses was 36 ± 6 µg. Table 5.1.5 describes the results for all worn lenses (right and left eyes) for all subjects across all visits. Figure 5.1.3 plots the mean (±sd) deposited protein for the right eye of all subjects. Intra-subject variation in deposition across the 12-month period was small (mean 30%, range 20-40 %), indicating the importance of the bulk characteristic of the material (water content and charge) in determining bulk protein deposition.

Table 5.1.5 - Total Protein (mg): Summary Statistics For All Worn Lenses

Subject	Mean	SD	Median	Min	Max
1	0.033	0.005	0.033	0.023	0.042
2	0.033	0.005	0.033	0.021	0.043
3	0.038	0.005	0.037	0.031	0.048
4	0.038	0.004	0.039	0.028	0.044
5	0.038	0.006	0.039	0.024	0.047
6	0.030	0.005	0.029	0.023	0.043
7	0.034	0.006	0.035	0.023	0.042
8	0.037	0.006	0.036	0.032	0.056
9	0.036	0.008	0.035	0.023	0.050
10	0.043	0.004	0.042	0.036	0.051

Figure 5.1.3 indicates the significance of inter-subject variation. A two-way RM ANOVA on the lenses worn by each subject during all months is detailed in Table 5.1.6 and confirms this, with significant inter-subject variation occurring ($p<0.001$).

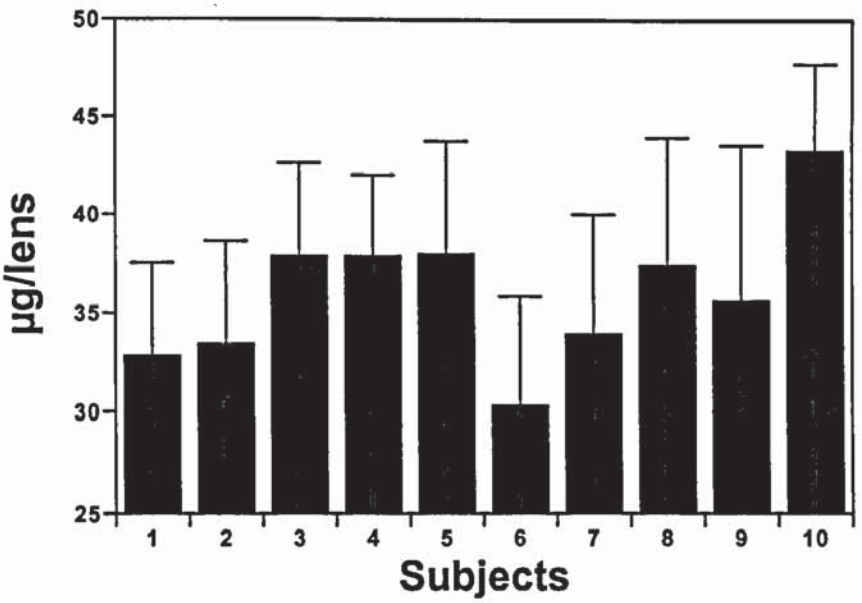


Figure 5.1.3 - Mean (±sd) total protein deposition (µg/lens) for all R lenses worn across all 12 month wearing periods

Table 5.1.6 - Subject Differences:
Two-Way RM ANOVA On All Worn Lenses For Total Protein

Source of Variation	DF	SS	MS	F	P
visit	11	0.000921	0.0000837		
subject	9	0.00269	0.000299	6.090	<0.001
subject x visit	99	0.00486	0.0000491		
eye	1	0.00000700	0.00000700	1.045	0.329
eye x visit	11	0.0000737	0.00000670		
subject x eye	9	0.000240	0.0000266	2.516	0.012
Residual	99	0.00105	0.0000106		
Total	239	0.00985	0.0000412		

Post-hoc testing using the Tukey test indicates the differences between subjects. All values joined by a continuous line are not statistically different.

Table 5.1.7 - Tukey Test For Differences Between Subjects

Subject	10	3	4	5	8	9	7	1	2	6
Mean	0.043	0.038	0.038	0.038	0.037	0.036	0.034	0.033	0.033	0.030

Further statistical analysis of the results (Table 5.1.8) using subject as the repeat indicates that there was no difference between eyes, visits or was there any interaction between the two factors.

Table 5.1.8 - 2-Way RM ANOVA Of UV Data

Source of Variance	DF	SS	MS	F	P
Subject	9	2747.5	305.28		
Eye	1	10.8	10.84	0.440	0.5238
Eye x Subject	9	221.7	24.63		
Visit	11	838.1	76.20	1.664	0.0929
Visit x Subject	99	4533.6	45.79		
Eye x Visit	11	71.1	6.46	0.621	0.8072
Residual	99	1030.8	10.41		
Total	239	9453.8	39.56		

5.1.5.7 Surface Protein

The mean (±sd) surface protein deposition by fluorescence @ 280nm for all worn lenses was 144.3 ± 47.7 units. Table 5.1.9 describes the results for all worn lenses (right and left eyes) for all subjects across all visits. Figure 5.1.4 plots the mean (±sd) deposited surface protein for the right eye of all subjects. Intra-subject variation in deposition across the 12-month period was larger than for total protein (50%, range 30-80%), indicating that surface protein deposition is more variable than bulk protein deposition.

Table 5.1.9 - Surface Protein: Summary Statistics For All Worn Lenses

Subject	Mean	SD	Median	Min	Max
1	126.7	24.6	119.5	81.6	182.6
2	139.1	37.2	134.2	94.2	280.7
3	131.4	33.2	132.9	84.8	234.7
4	149.2	45.4	148.6	99.1	320.1
5	154.0	44.4	144.7	84.3	268.8
6	134.9	51.5	133.4	70.6	350.7
7	163.3	51.1	151.6	104.5	271.9
8	149.4	51.9	142.5	59.5	325.6
9	164.2	69.5	144.0	90.4	368.7
10	138.9	38.7	125.5	96.3	258.0

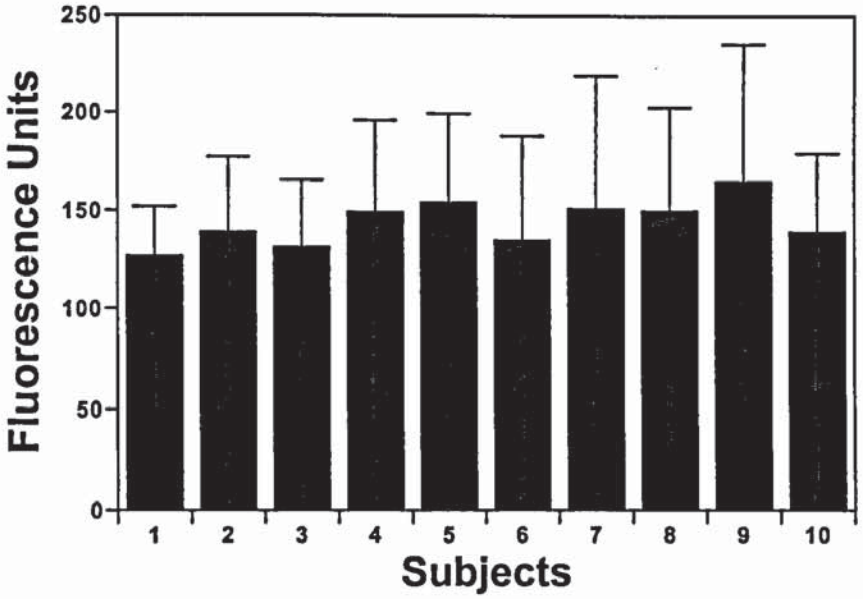


Figure 5.1.4 - Mean (±sd) surface protein deposition (fluorescence units) for all R lenses worn across all 12 month wearing periods

A two-way RM ANOVA on the lenses worn by each subject during all months is detailed in Table 5.1.10. The results indicate that no significant variation between subjects was present (p=NS). However, the power of the test (0.36) is below the desired power (0.8) and these negative findings should be viewed with caution. This is a valid criticism of studies investigating inter-subject differences using only small sample sizes.

Table 5.1.10 - Subject Differences:
Two-Way RM ANOVA On All Worn Lenses For Surface Protein

Source of Variation	DF	SS	MS	F	P
visit	11	44377.560	4034.324		
eye	1	2939.300	2939.300	0.775	0.398
eye x visit	11	41720.228	3792.748		
subject	9	34414.478	3823.831	1.782	0.081
subject x visit	99	212473.134	2146.193		
eye x subject	9	17953.825	1994.869	1.105	0.366
Residual	99	178690.851	1804.958		
Total	239	532569.378	2228.324		

Further statistical analysis of the results (Table 5.1.11) indicates that there while there was no difference between eyes, there was between visits and there was also a significant interaction between the two factors. Tukey testing showed that the differences were between months 10 vs 9 and 10 vs 5 ($p<0.05$).

Table 5.1.11 - 2-Way RM ANOVA Of Fluorescence @ 280nm Data

Source of Variance	DF	SS	MS	F	P
Subject	9	35026.1	3891.8		
Eye	1	5670.6	5670.6	2.53	0.1459
Eye x Subject	9	20143.6	2238.2		
Visit	11	45284.9	4116.8	1.94	0.0429
Visit x Subject	99	210070.2	2121.9		
Eye x Visit	11	54486.0	4953.3	2.92	0.0023
Residual	99	168214.4	1699.1		
Total	239	538895.8	2254.8		

5.1.5.8 Lipid Deposition

The mean (±sd) lipid deposition by fluorescence @ 360nm for all worn lenses was 374.6 ± 264.3 units. Table 5.1.12 describes the results for all worn lenses (right and left eyes) for all subjects across all visits. Figure 5.1.5 plots the mean (±sd) deposited surface protein for the right eye of all subjects. Intra-subject variation in deposition across the 12-month period was larger than for total or surface protein (96%, range 60-150%).

Table 5.1.12 - Lipid: Summary Statistics For All Worn Lenses

Subject	Mean	SD	Median	Min	Max
1	262.9	123.2	253.3	116.3	662.9
2	647.6	338.7	550.1	202.1	1468.0
3	206.2	105.5	170.5	105.4	622.0
4	335.9	133.7	306.6	125.1	717.2
5	245.6	163.9	184.1	86.4	793.1
6	383.3	238.0	329.4	170.1	1368.0
7	223.9	73.8	207.4	112.3	415.1
8	305.5	222.8	234.7	69.9	1011.0
9	615.2	354.9	501.0	62.6	1574.0
10	467.5	121.5	450.3	273.0	726.6

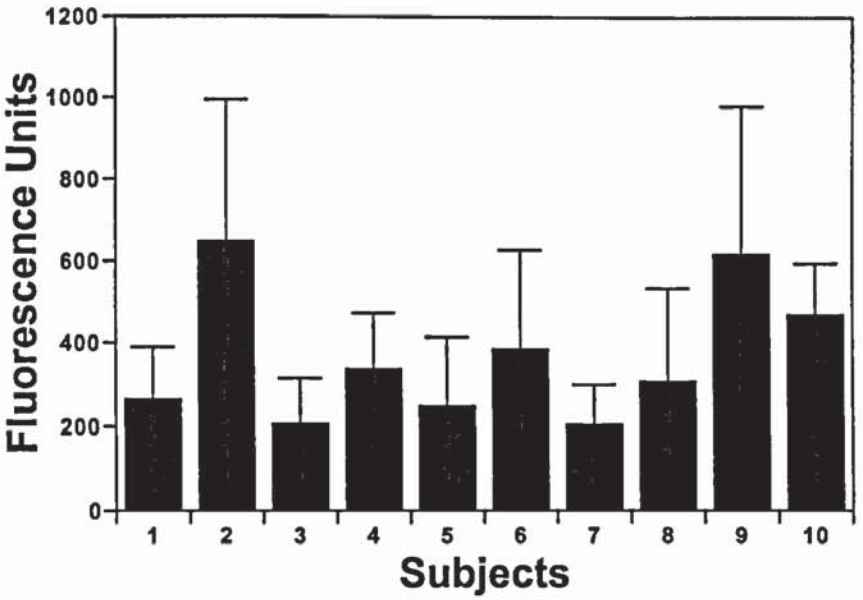


Figure 5.1.5 - Mean (±sd) lipid deposition (fluorescence units) for all R lenses worn across all 12 month wearing periods

A two-way RM ANOVA on the lenses worn by each subject during all months is detailed in Table 5.1.13 and shows significant differences between subjects, with certain subjects depositing significantly more lipid than others (eg subjects 2, 9 and 10 versus 1,3, 5 and 7), indicating the presence of marked inter-subject variation.

Table 5.1.13 - Subject Differences:
Two-Way RM ANOVA On All Worn Lenses For Lipid

Source of Variation	DF	SS	MS	F	P
visit	11	1071183.195	97380.290		
eye	1	212813.837	212813.837	4.835	0.060
eye x visit	11	482188.924	43835.357		
subject	9	5393576.472	599286.275	10.795	<0.001
subject x visit	99	5496098.076	55516.142		
eye x subject	9	1079919.455	119991.051	5.516	<0.001
Residual	99	2153457.488	21752.096		
Total	239	15889237.448	66482.165		

Post-hoc testing using the Tukey test indicates the differences between subjects (Table 5.1.14). All values joined by a continuous line are not statistically different.

Table 5.1.14 - Tukey Test For Differences Between Subjects

Subject	2	9	10	6	4	8	1	5	7	3
Mean	647.6	615.2	467.5	383.3	335.9	305.5	262.9	245.6	223.9	206.2

Further statistical analysis of the results (Table 5.1.15) indicates that while there was no difference between eyes, there was between visits and there was also a significant interaction between the two factors. Tukey testing indicated that the differences were between months 2 vs 3, 2 vs 4 and 2 vs 9 ($p<0.05$).

Table 5.1.15 - 2-Way RM ANOVA Of Fluorescence @ 360 nm Data

Source of Variance	DF	SS	MS	F	P
Subject	9	5393653.1	599294.8		
Eye	1	212840.0	212840.0	1.77	0.2156
Eye x Subject	9	1079876.6	119986.3		
Visit	11	1071150.8	97377.3	1.87	0.0421
Visit x Subject	99	5495940.8	55514.6		
Eye x Visit	11	482150.5	43831.9	2.02	0.0346
Residual	99	2153469.7	21752.2		
Total	239	15889081.4	66481.5		

5.1.5.9 Correlations

a) Protein vs Lipid by Fluorescence

Figure 5.1.6 indicates that there is only a weak positive correlation (0.33) between surface protein and lipid. This is undoubtedly due to the fact that lipid deposition is more related to inter and intra-subject variation and that protein deposition is predominantly controlled by material charge and water content.

Figure 5.1.6 - Correlation Of 280nm vs 360nm Fluorescence

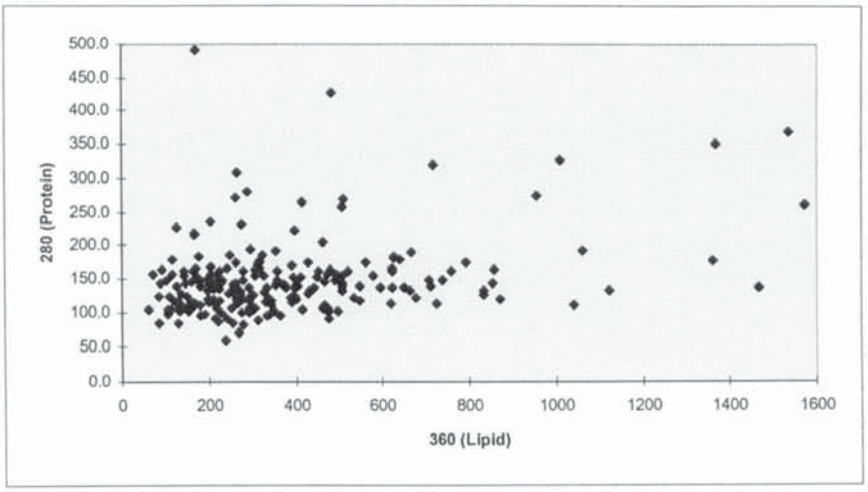


Figure 5.1.6 - Correlation of 280 nm fluorescence vs 360 nm fluorescence. Correlation coefficient was 0.33.

b) Surface Protein (280 nm fluorescence) vs Total Protein (transmission UV)

Figure 5.1.7 indicates that there is only a very weak positive correlation (0.28) between surface protein and total protein.

Figure 5.1.7 - Surface vs Bulk Protein

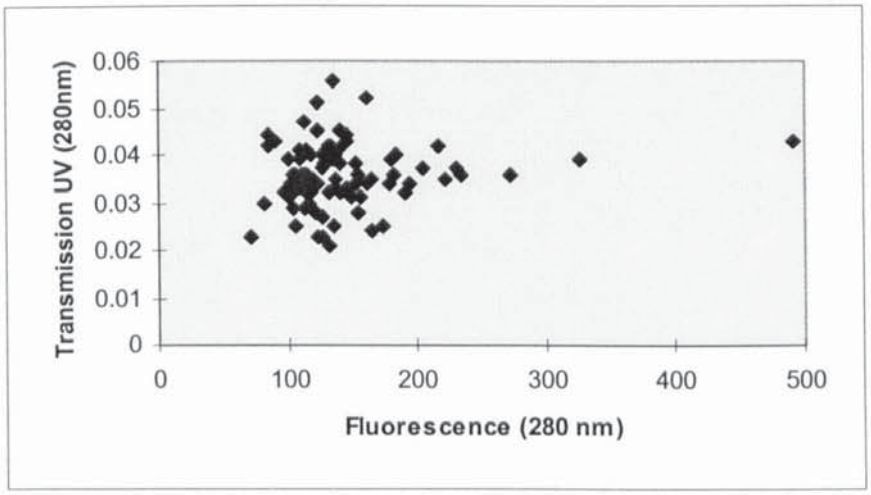


Figure 5.1.7 - Correlation of 280 nm fluorescence vs 280 nm transmission UV. Correlation coefficient was 0.28.

c) Right vs Left Eyes

Correlation coefficients between eyes for the three deposition types were 0.23 for surface protein, 0.48 for lipid and 0.66 for total protein. These results indicate that care must be undertaken when planning contralateral eye projects if surface deposition is to be evaluated. Of the three, total protein deposition is more

closely correlated between eyes.

d) Deposition vs PLNIBUT

Correlation coefficients between the PLNIBUT at clinical visits and the laboratory assessed levels of deposition of the lenses worn at those appointments indicated a weak negative correlation for surface protein ($r = -0.31$) and no significant correlation for either total protein ($r = -0.02$) or surface lipid ($r = 0.07$).

5.1.5.10 Removable/Mobile Deposition

Previous work in these laboratories has revealed the degree of mobility of deposited species, particularly protein. To investigate this factor the degree of lipid and protein deposition in the storage solution (saline) was measured by fluorescence and the ability with which deposits could be easily removed by use of a surfactant cleaner was also studied.

a) Storage solution deposition

Following 30 seconds on a rotary shaker 1ml of storage fluid was removed with a disposable pipette and placed into the cuvette of the fluorimeter. The mean (±sd) fluorescence units in the storage solutions were 220 ± 78 units (range 202 - 270) for protein and 34 ± 14 units (range 28 - 45) for lipids.

b) Immobilised deposition

The degree of removable deposition from the lens surface was evaluated by measuring the percentage of deposits which could be removed by cleaning the lens with a surfactant cleaner. Twenty lenses were randomly chosen and the degree of deposition calculated by fluorescence. The lenses were then cleaned with two drops of Allergan "LC65", thoroughly rinsed with saline and re-fluoresced. The degree of removable deposition was 15 ±30% for lipids and 34 ±22% for proteins.

5.1.6 Discussion

This was the longest study of all studies conducted during this thesis and provides valuable information on the changes in subjective factors, deposition, wettability and physiological performance which occurs with frequent replacement materials over a one year period.

In this study subjective factors and visual acuity remained unchanged across time, showing the stable nature of these factors with planned replacement lenses. Visual acuity with conventional lenses reduces with the passage of time [14-16] and the findings in this study that visual acuity remains stable with lenses replaced monthly supports the clinical premise that the general performance of such lenses is enhanced compared with conventional lenses. During the year no changes were measured in corneal staining, bulbar hyperaemia or

oedema. However, there were significant changes in palpebral hyperaemia in the second half of the study. It is plausible that such changes were induced by the preservative (PHMB), surfactant (poloxamine), overall formulation of ReNu or the lenses themselves, as all subjects were refitted with the Medalist lenses and had not previously been exposed to them. Such changes warrant further observation in a larger sample over a longer period. The incidence of recorded corneal staining, at approximately 50% of all appointments, agrees with other authors regarding this observation. [391 392] The degree of conjunctival staining present at any level was 66%, which is lower than the level recorded by Lakkis and Brennan, [390] who observed conjunctival staining in 98% of subjects. Their subjects were examined on only one occasion and the differences in result may be attributed to differences in methodology or reflect the fact that conjunctival staining is temporal in nature and varies across time.

Assessment of the PLNIBUT results suggests several important findings. Figure 5.1.1 indicates that PLNIBUT reduces between collection and after four weeks, but then remains relatively stable across the year, suggesting that there are no seasonal changes of any significance in this factor. Visual inspection of these results suggests the large degree of inter-subject variability encountered in lens wettability, as evidenced by the large standard deviations present. These are confirmed in Figure 5.1.2, which demonstrates that, despite the wide variation in the measurement technique itself, there are significant inter-subject differences in PLNIBUT. These results are similar to those recently published concerning inter- and intra-variability in PLNIBUT using other techniques. [451 452] A review of these results suggests that a larger scale study with many more subjects to monitor changes in wettability over time is certainly warranted. In addition, more sophisticated methods of assessing the PLNIBUT and PLTF thickness/quality (such as the Tearscope) would be beneficial.

Examination of the deposition results over the year enables some estimate of the intra-subject variation across time that occurs with this material worn for one month. These results are important as they indicate how likely a single lens worn for one month will indicate the degree of deposition that occurs in subsequent months. The intra-subject variability was ± 30% for total protein, ± 50% for surface protein and ± 96% for lipid. This would appear to suggest that total protein deposition is less variable in degree over time than surface deposition, suggesting that material characteristics such as water content and charge are of greater significance than patient factors. However, the repeatability of the total protein assay (transmission UV) is greater than that which occurs with fluorescence, which was used to assay surface deposition (Table 4.15, page 135). These factors will inevitably influence the results. However, not withstanding this argument, the differences do suggest that there is a greater variation in surface deposition across time than occurs in bulk protein deposition.

The results also demonstrate that there are significant inter-subject differences between subjects for total protein and lipid, but not for surface protein. However, the sample size of subjects was not sufficiently large enough to exclude the fact that this latter finding was due to a sampling size error. This is a valid criticism of such small subject groups. However, to calculate sample sizes some idea of the likely variability in result is required and no such information existed prior to these studies. Analysis of tables 5.1.7 and 5.1.14 indicate quite clearly there is no correlation between those subjects who are heavy lipid depositors and those who

deposit most protein. This would suggest that while some subjects would benefit from materials that are resistant to protein deposition, they may be more susceptible to lipid deposition and both types would inevitably produce deposited lenses.

With this lens material no correlation could be found between surface and total protein. This is an unusual finding, and will be further discussed in Chapter 6.4 (pages 210-212). Additionally, no correlation between eyes occurred. This has obvious implications for contralateral eye studies in which differing materials are placed in opposite eyes of the same individual. If the results from this study are replicated then differences may be found which are attributable more to the ocular surface than to material characteristics. Consequently cross-over, bilateral eye studies are infinitely preferable to contralateral eye paradigms.

Finally, examination of the degree of removable deposition following wear showed conclusively that proteins are more easily removed than lipids, which progressively become partitioned and immobilised in group II materials. These results will be discussed further in Chapter 6.2 (pages 191-192) and Chapter 9 (pages 293-295 and 308-310).

The recent introduction and rapid growth of single-use, one-day daily disposable lenses suggests that further studies investigating the variability in performance and deposition of lenses worn for very short wearing periods may be of some value.

5.2 THE DEPOSITION OF DAILY-WEAR FDA GROUP II & GROUP IV MATERIALS USED AS DAILY DISPOSABLE LENSES

The second study in this chapter investigated the inter- and intra-subject variability in deposition that occurs with an FDA group II and group IV material when used as single-use, daily disposable lenses over a 28 day period.

5.2.1 Materials and Methods

Twenty subjects (three male and seventeen female) were entered onto the study, whose demographic details are described in Table 5.2.1.

Table 5.2.1 - Subject Demographics

	Mean ± SD	Range
Age (years)	35.3 ± 6.4	22 - 45
Flat Keratometry (mm)	7.78 ± 0.23	7.35 - 8.40
Steep Keratometry (mm)	7.65 ± 0.29	7.10 - 8.40
Sphere (D)	-3.67 ± 1.40	-6.00 to -0.50
Cyl (D)	-0.44 ± 0.33	-1.00 to 0.00

All subjects were fitted with the two test materials, which were Medalist 66 from Bausch & Lomb and Acuvue from Vistakon, details of which are given in Table 5.2.2.

The lenses were worn as daily-wear, single-use daily disposable lenses for twenty-eight consecutive one day periods. The lenses were inserted in the morning, worn for a minimum of 10 hours and then removed. Subjects were reviewed in the clinic on collection and after 24 days (±3 days). The subjects were divided into two equal groups, each of which wore a pair of lenses every day for 28 days. At the end of the 28 days a cross-over took place.

Table 5.2.2 - Lens Parameters

	Medalist 66	Acuvue
Water Content (%)	66	58
Monomers	HEMA + VP	HEMA + MA
USAN	Alphafilcon A	Etafilcon A
FDA Category	Group II	Group IV
ISO Category	Filcon 4a	Filcon 1b
Manufacture	Moulded	Moulded
Back Optic Zone Radius (mm)	8.40	8.80
Total Diameter (mm)	14.20	14.00
Centre Thickness (mm)	0.10	0.07

5.2.2 Clinical Protocol

At the clinical visits visual acuity, lens fit and physiological performance were assessed as previously described. Additionally, visual quality and lens comfort were recorded by the subjects every day using the previously reported 10 point grading scale.

5.2.3 Analytical Protocol

At the end of each day the subjects collected their lenses following the use of alcohol wipes to clean their fingers, as previously described. The lenses were then placed in glass vials containing sterile non-preserved non-buffered saline, the vials were capped, labelled and given to the clinical centre. The lenses were analysed in the laboratory for total protein using transmission UV and surface deposition using fluorescence spectrophotofluorimetry. Due to the number of lenses involved it was decided to analyse lenses from days 1,3,5,7, 9,11,13,14,15,17,19,21,23,25,27 and 28 of 10 patients (chosen at random) and lenses from days 1,7,14,21 and 28 for the other patients, of both lens types. This resulted in 840 lenses being analysed.

5.2.4 Data Analysis

Summary statistics were calculated for all variables. The grading scales, being ordinal measurements, were considered non-normal data. All other data was tested for normality of distribution. Wilcoxons Signed Rank Test was used to compare sets of non-parametric data and Students paired t-Test was used to compare normally distributed data. Correlation coefficients were undertaken using Spearman Rank Order Test. Inter-subject differences were compared using a 2- or 3-way RM ANOVA, with visit as the repeat. Post-hoc testing was conducted using Tukey's test.

In all cases a "p" value of <0.05 was taken as being statistically significant.

5.2.5 Results

5.2.5.1 Subjective Results

The visual quality and comfort results for all lenses for all days are summarised in Tables 5.2.3 - 5.2.5 and Figures 5.2.1 and 5.2.3.

Table 5.2.3 - Subjective Scores

	Acuvue			Medalist 66		
	Mean ± SD	Median	25% Percentile	Mean ± SD	Median	25% Percentile
Visual Quality	9.1 ± 1.1	10	8	8.2 ± 1.7	8	7
Comfort	9.1 ± 1.2	10	8	8.4 ± 1.7	9	8

Figures 5.2.2 and 5.2.4 demonstrate the day-to-day variability in comfort and vision with each lens type in three "typical" subjects during the period of the study. It is quite clear that, while there is variability with both lens types, the greater day-by-day variation occurs with the Medalist material.

Figure 5.2.1 - Mean Subjective Vision Scores For Subjects

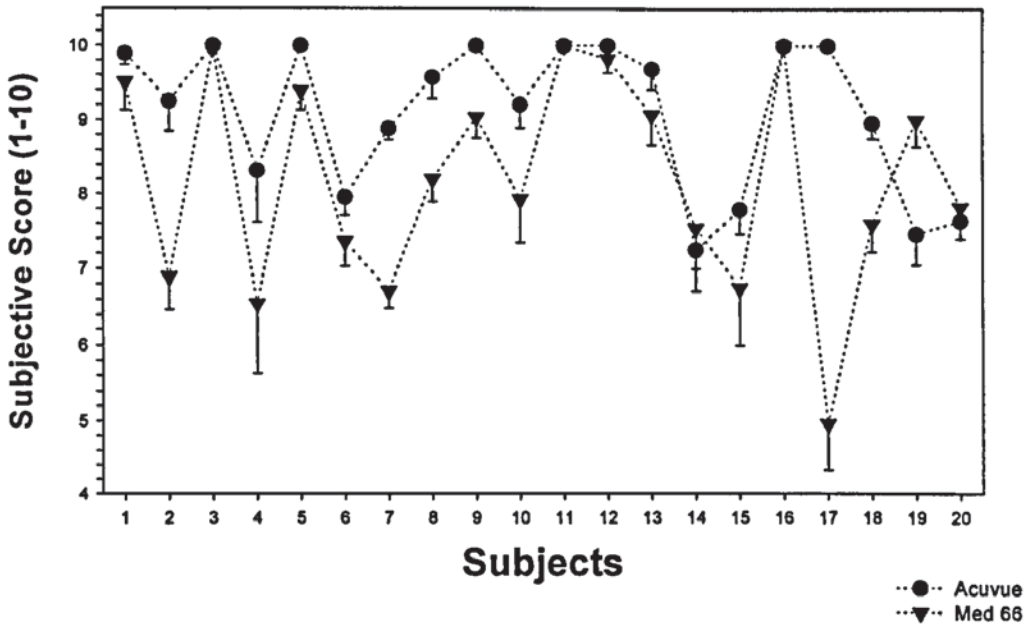


Figure 5.2.1 - Mean (±sd) subjective vision scores for all subjects for every day. The dotted lines join results for each lens type for all subjects.

Figure 5.2.2 - Day-to-Day Variation In Subjective Vision Scores

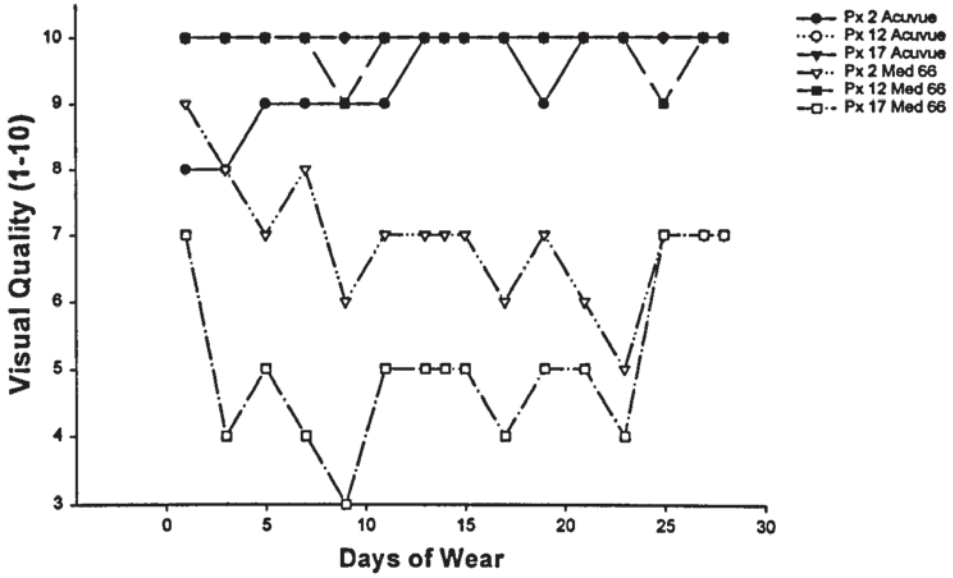


Figure 5.2.2 - Subjective vision scores for 3 subjects across all days.

Figure 5.2.3 - Mean Subjective Comfort Scores For Subjects

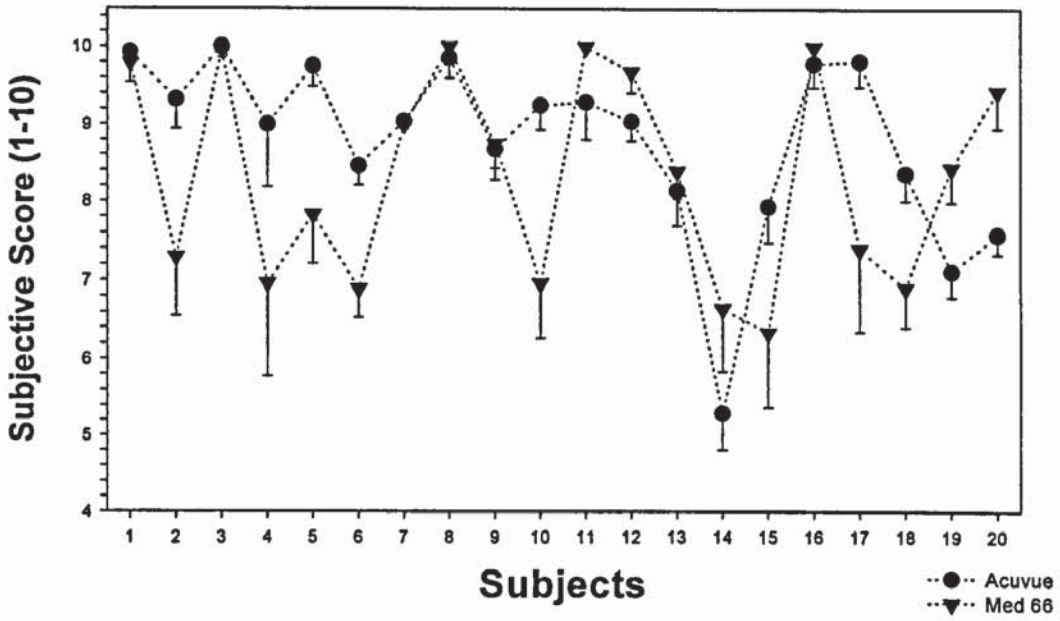


Figure 5.2.3 - Mean (±sd) subjective comfort scores for all subjects for every day. The dotted lines join results for each lens type for all subjects.

Figure 5.2.4 - Day-to-Day Variation In Subjective Comfort Scores

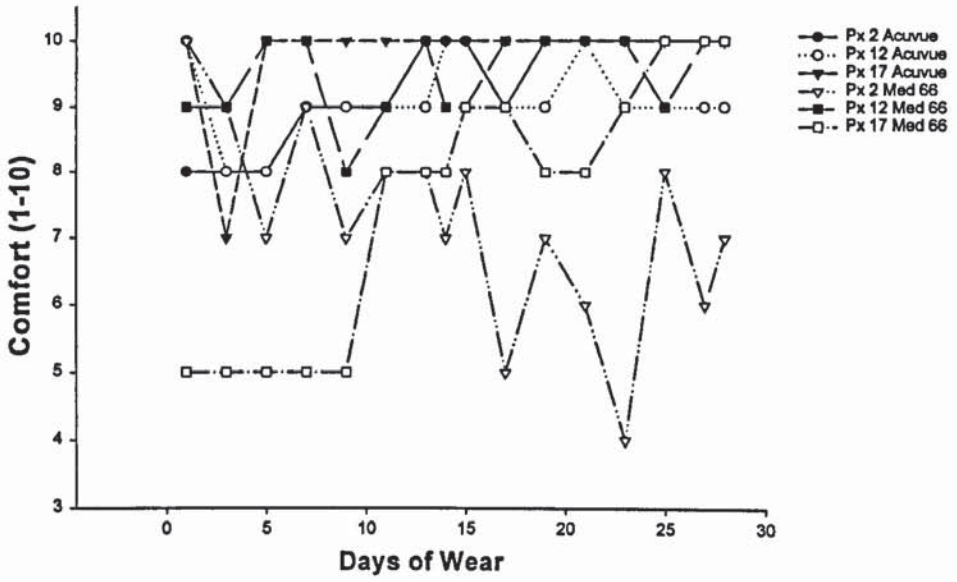


Figure 5.2.4 - Subjective comfort scores for 3 subjects across all days.

Inter-subject and lens differences were analysed by running a 2-way repeated measures ANOVA with visit as the repeat and lens and subject as the factors, using the results from days 1, 7, 14, 21 and 28 as these were the only days that results were obtained for all subjects. The results are detailed in Tables 5.2.4 (visual quality) and 5.2.5 (comfort).

Table 5.2.4 - 2-Way RM ANOVA - Visual Quality

Source of Variation	DF	SS	MS	F	P
visit	4	7.120	1.780		
lens	1	39.605	39.605	47.717	0.002
lens x visit	4	3.320	0.830		
subject	19	217.495	11.447	13.166	<0.001
subject x visit	76	66.080	0.869		
lens x subject	19	78.095	4.110	6.068	<0.001
Residual	76	51.480	0.677		
Total	199	463.195	2.328		

Table 5.2.5 - 2-Way RM ANOVA - Comfort

Source of Variation	DF	SS	MS	F	P
visit	4	11.780	2.945		
lens	1	6.845	6.845	3.923	0.119
lens x visit	4	6.980	1.745		
subject	19	231.255	12.171	6.944	<0.001
subject x visit	76	133.220	1.753		
lens x subject	19	108.455	5.708	4.372	<0.001
Residual	76	99.220	1.306		
Total	199	597.755	3.004		

There was a significant difference between the lens types for visual quality (p=0.002), but there was no difference between the lenses for comfort (p=NS). For both factors there was a significant difference between subjects (p<0.001) and there was a significant interaction between lens and subject (p<0.001).

5.2.5.2 Visual Acuity

There was no significant measurable difference at high or low contrast between the lens types at either appointment or was there any detectable change in acuity during the study with either lens type (p=NS). Mean (±sd) high contrast visual acuities (VAR units) were 103 ± 7 for Acuvue and 102 ± 7 for Medalist 66 and low contrast results were 92 ± 6 for Acuvue and 91 ± 7 for Medalist 66.

5.2.5.3 Lens Fit

Mean (±sd) lens fit by percentage tightness on the two clinic appointments was 54.3 ± 6.4 for Acuvue and 40.8 ± 6.1 for Medalist. Lens differences were analysed by running a 2-way repeated measures ANOVA with subject as the repeat and right lens and visit as the factors. The results are detailed in Table 5.2.6.

Table 5.2.6 - 2-Way RM ANOVA - Percentage Tightness

Source of Variation	DF	SS	MS	F	P
Subject	19	1343.438	70.707		
Lens	1	1240.313	1240.313	18.990	<0.001
Lens x Subject	19	1240.938	65.313		
Visit	1	25.313	25.313	0.706	0.411
Visit x Subject	19	680.938	35.839		
Lens x Visit	1	52.813	52.813	2.097	0.164
Residual	19	478.438	25.181		
Total	79	5062.188	64.078		

There was clearly a difference between lens types ($p<0.001$), with Acuvue being assessed as being a tighter fit.

5.2.5.4 Physiological Appearance

There was no significant difference between the lenses at the 28-day follow-up for corneal staining, bulbar conjunctival hyperaemia or palpebral conjunctival appearance. There was a significant difference for conjunctival staining ($p=0.003$), with Acuvue exhibiting a greater frequency of conjunctival staining. Most of the clinical findings were clinically insignificant. Mean corneal staining score was <grade 1 ("Minor") with most of the staining for each lens type (43% for Acuvue and 56% for Medalist 66) being inferior "smile"type staining due to incomplete blinking and dehydration, which is a not uncommon finding with all high water content lenses. The overall incidence of corneal staining was 48% with Acuvue and 40% with Medalist 66.

As with many moulded lenses the incidence of recorded conjunctival/limbal staining was fairly high, with 92% of Acuvue and 70% of Medalist 66 lenses exhibiting some degree of conjunctival staining. However, this was considered clinically insignificant, with a median score of "1" (mild) and mean of 1.6 for each lens type.

5.2.5.5 Deposition Results

a) Total Protein By UV

The results for all worn lenses are summarised in Table 5.2.7, with the results for the R eye being graphically illustrated in Figure 5.2.5. The dotted lines join the results for each lens type for all subjects.

Table 5.2.7 - Total Protein (Mean±sd) By UV (µg/lens) For All Worn Lenses

Acuvue	Medalist 66
137 ± 46	23 ± 10

Figure 5.2.5 - Mean Total Protein By UV For All Subjects

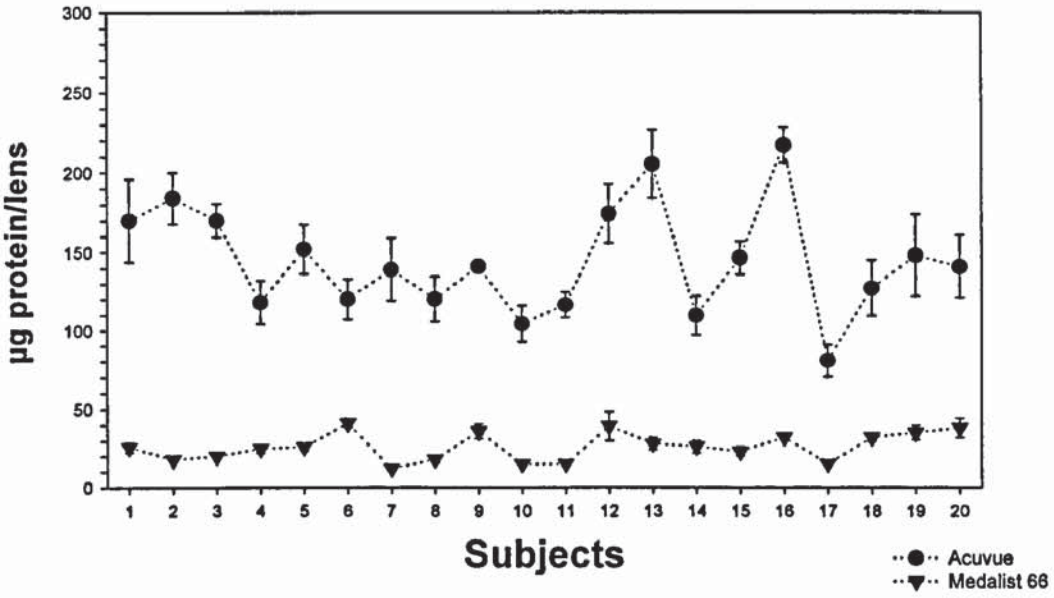


Figure 5.2.5 - Mean (±sd) total protein by UV for all subjects for all worn R lenses

Inter-subject and lens differences were analysed by running a 3-way repeated measures ANOVA with visit as the repeat and lens, eye and subject as the factors, using the results from days 1, 7, 14, 21 and 28 as these were the only days that results were obtained for all subjects. The results are detailed in Table 5.2.8.

Table 5.2.8 - 3-Way RM ANOVA - Total Protein By UV

Source of Variation	DF	SS	MS	F	P
visit	80	0.073	0.001		
lens	1	1.297	1.297	1926.6	<0.001
subject	19	0.137	0.007	7.918	<0.001
eye	1	0.000	0.000	0.594	0.442
subject x lens	19	0.095	0.005	7.447	<0.001
subject x eye	19	0.008	0.000	0.653	0.862
lens x eye	1	0.000	0.000	0.655	0.419
subject x lens x eye	19	0.008	0.000	0.597	0.9069
Residual	240	0.162	0.001		
Total	399	1.781	0.004		

The results demonstrate that the group IV lens (Acuvue) deposited significantly more total protein than the group II lens ($p<0.001$), there was no difference between eyes (p=NS) and that there were significant inter-subject differences ($p<0.001$). For Acuvue there was a significant difference ($p<0.05$) between subjects 2, 12, 13 and 16 compared with 10 and 17 and all other subjects were not significantly different (p=NS). For Medalist there was a significant difference ($p<0.05$) between subjects 6, 9, 12, 16, 19 and 20 compared with 7, 10, 11 and 17 and all other subjects were not significantly different (p=NS).

The day-to-day deposition traces for three of the patients' R eyes (subjects 2, 12 and 17) are graphed in Figure 5.2.6. Inter-subject differences are clearly seen in Figures 5.2.5 and 5.2.6. The standard deviation bars in Figure 5.2.5 indicate the degree of intra-subject variation. The variation was ± 34% (range 16-56) for Acuvue and ±24% (range 10-36) for Medalist.

Figure 5.2.6 - Day-to-Day Variation in Total Protein Deposition

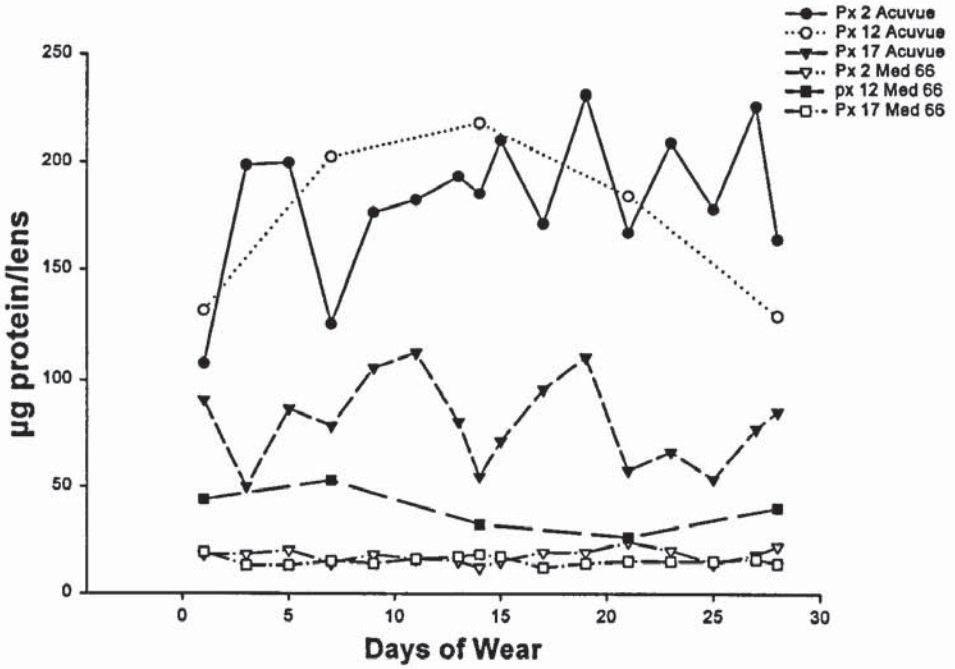


Figure 5.2.6 - Total protein by UV for 3 subjects right eyes for all measured days

b) Surface Protein By Fluorescence @ 280nm

This is summarised in Table 5.2.9, with the R eye being graphically illustrated in Figure 5.2.7. The dotted lines join the results for each lens type for all subjects.

Table 5.2.9 - Surface Protein (Mean±sd) By Fluorescence (fluorescence units)

Acuvue	Medalist 66
314 + 86	77 + 45

Figure 5.2.7 - Mean Surface Protein By Fluorescence For All Subjects

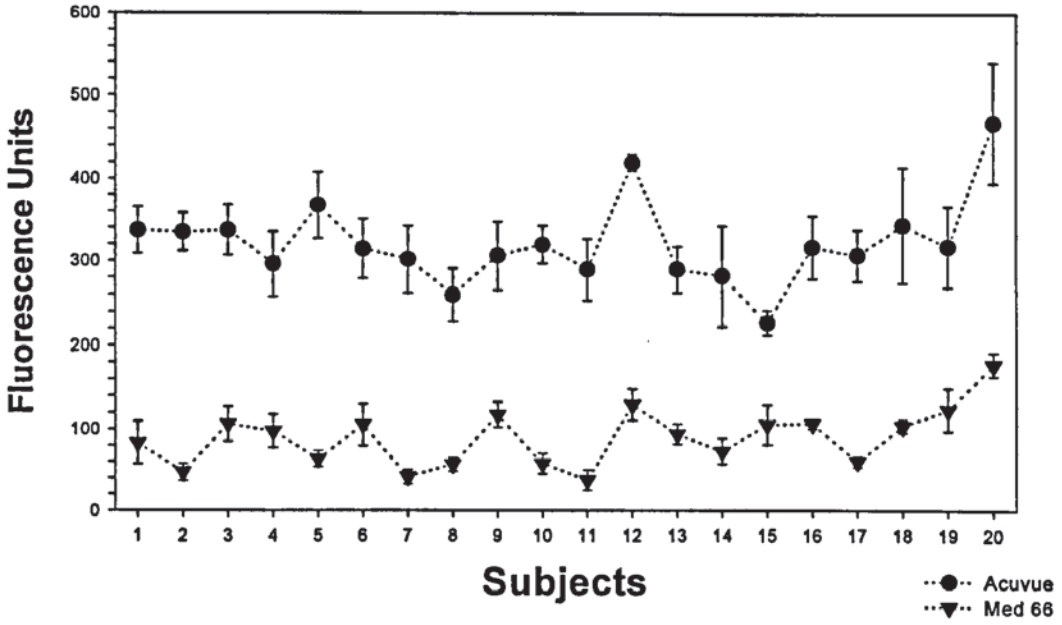


Figure 5.2.7 - Mean (±sd) surface protein by fluorescence for all subjects for all measured lenses from R eye

Inter-subject and lens differences were analysed by running a 3-way repeated measures ANOVA with visit as the repeat and lens, eye and subject as the factors, using the results from days 1, 7, 14, 21 and 28 as these were the only days that results were obtained for all subjects. The results are detailed in Table 5.2.10.

Table 5.2.10 - 3-Way RM ANOVA - Surface Protein By Fluorescence @ 280nm

Source of Variation	DF	SS	MS	F	P
visit	80	554050.082	6925.626		
lens	1	5508878.410	5508878.410	1283.47	<0.001
subject	19	555521.267	29237.961	4.222	<0.001
eye	1	1.664	1.664	0.00	0.984
subject x lens	19	158240.316	8328.438	1.940	0.0122
subject x eye	19	66465.158	3498.166	0.815	0.6883
lens x eye	1	0.194	0.194	0.00	0.995
subject x lens x eye	19	98310.150	5174.218	1.206	0.254
Residual	240	1030120.758	4292.170		
Total	399	17971587.999	19978.917		

The results demonstrate that the group IV lens (Acuvue) deposited significantly more surface protein than the group II lens ($p<0.001$), there was no difference between eyes (p=NS) and that there were significant

inter-subject differences ($p<0.001$). For Acuvue there was a significant difference ($p<0.05$) between subjects 12 and 20 compared with 8 and 15 and all other subjects were not significantly different (p=NS). For Medalist there was a significant difference ($p<0.05$) between subjects 9, 12 and 20 compared with 2, 7 and 11 and all other subjects were not significantly different (p=NS).

The day-to-day deposition traces for three of the patients' R eyes (subjects 2, 12 and 17) are graphed in Figure 5.2.8. Inter-subject differences are clearly seen in Figures 5.2.7 and 5.2.8. The standard deviation bars in Figure 5.2.7 indicate the degree of intra-subject variation. The variation was ± 40% (range 20-76) for Acuvue and ±36% (range 12-64) for Medalist.

Figure 5.2.8 - Day-to-Day Variation in Surface Protein Deposition

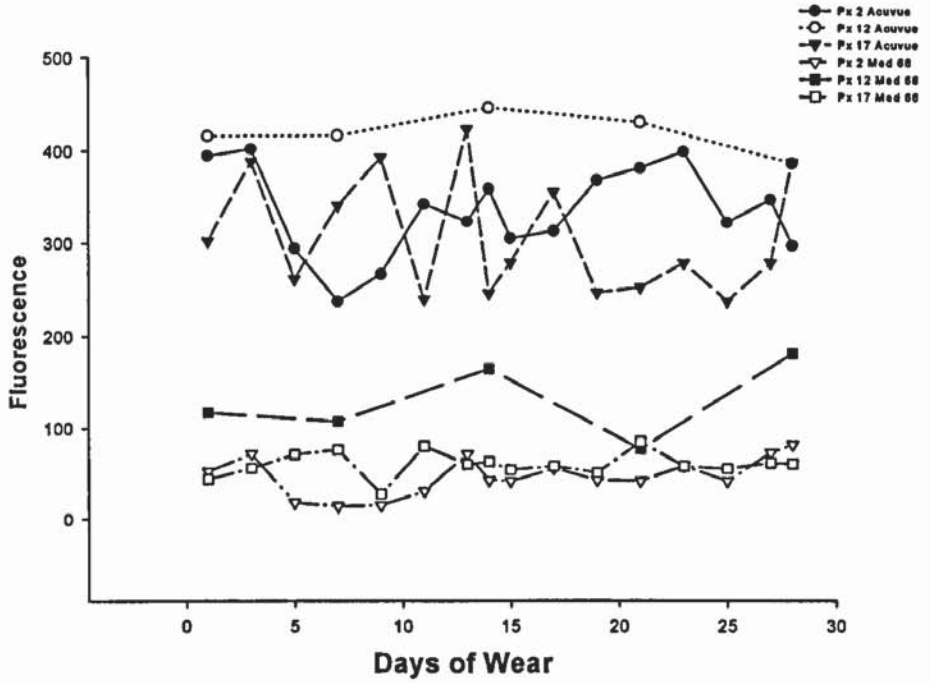


Figure 5.2.8 - Surface protein by fluorescence for 3 subjects right eyes for all measured days

c) Lipid By Fluorescence @ 360nm

This is summarised in Table 5.2.11, with the R eye being graphically illustrated in Figure 5.2.9. The dotted lines join the results for each lens type for all subjects.

Table 5.2.11 - Surface Lipid (Mean±sd) By Fluorescence (fluorescence units)

Acuvue	Medalist 66
12 + 8	40 + 19

Figure 5.2.9 - Mean Lipid Deposition By Fluorescence For All Subjects

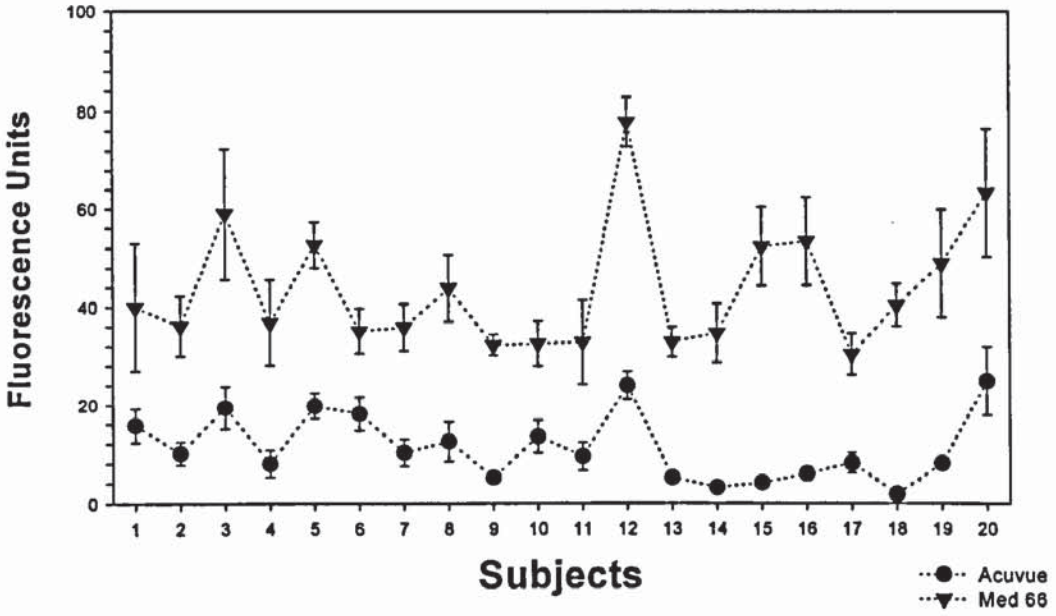


Figure 5.2.9 - Mean (±sd) lipid deposition by fluorescence for all subjects for all measured lenses from R eye

Inter-subject and lens differences were analysed by running a 3-way repeated measures ANOVA with visit as the repeat and lens, eye and subject as the factors, using the results from days 1, 7, 14, 21 and 28 as these were the only days that results were obtained for all subjects. The results are detailed in Table 5.2.12.

The results demonstrate that the group II lens (Medalist 66) deposited significantly more lipid than the group IV lens ($p<0.001$), there was no difference between eyes (p=NS) and that there were significant inter-subject differences ($p<0.001$). For Acuvue there was a significant difference ($p<0.05$) between subjects 5, 12 and 20 compared with 14, 15 and 18 and all other subjects were not significantly different (p=NS). For Medalist there was a significant difference ($p<0.05$) between subjects 5 and 12 compared with 10, 11 and 17 and all other subjects were not significantly different (p=NS).

Table 5.2.12 - 3-Way RM ANOVA - Lipid By Fluorescence @ 360nm

Source of Variation	DF	SS	MS	F	P
visit	80	20130.932	251.637		
lens	1	106282.520	106282.520	650.199	<0.001
subject	19	35313.657	1858.614	7.386	<0.001
eye	1	3.984	3.984	0.024	0.876
subject x lens	19	19008.359	1000.440	6.120	<0.001
subject x eye	19	3473.270	182.804	1.118	0.333
lens x eye	1	2.036	2.036	0.012	0.911
subject x lens x eye	19	3022.155	159.061	0.973	0.494
Residual	240	39230.752	163.461		
Total	399	226467.666	567.588		

The day-to-day deposition traces for three of the patients' R eyes (subjects 2, 12 and 17) are graphed in Figure 5.2.10.

Figure 5.2.10 - Day-to-Day Variation in Lipid Deposition

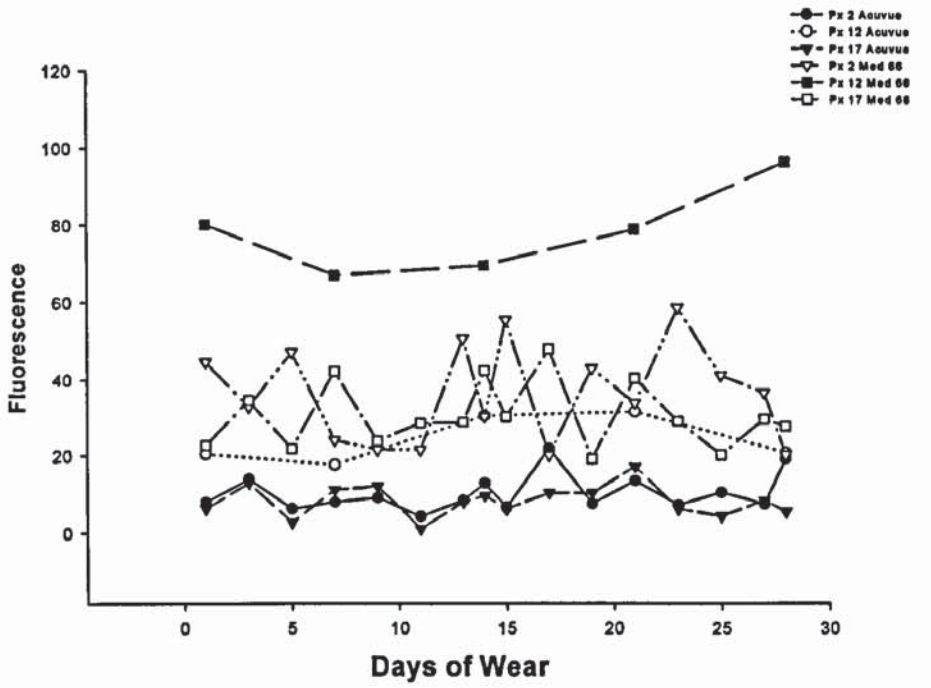


Figure 5.2.10 - Lipid deposition by fluorescence for 3 subjects' right eyes for all measured lenses

Inter-subject differences are clearly seen in Figures 5.2.9 and 5.2.10. The standard deviation bars in Figure 5.2.9 indicate the degree of intra-subject variation. The variation was ± 52% (range 24-62) for Acuvue and ±56% (range 18-100) for Medalist.

5.2.5.6 Correlations

a) Correlations Between Deposition Types

The inter-relationship between total protein, surface protein and lipid for both materials is summarised in Tables 5.2.13 and 5.2.14.

Table 5.2.13 - Correlation Between Deposition Types - Acuvue

	Total By UV	Surface @ 280	Surface @ 360
Total By UV	-	0.17	0.06
Surface @ 280	0.17	-	0.20
Surface @ 360	0.06	0.20	-

Table 5.2.14 - Correlation Between Deposition Types - Medalist 66

	Total By UV	Surface @ 280	Surface @ 360
Total By UV	-	0.49	0.23
Surface @ 280	0.49	-	0.35
Surface @ 360	0.23	0.35	-

These results suggest that there is no correlation between the deposition types for Acuvue and only poor correlations for Medalist, except total protein vs surface protein.

b) Correlations Between Eyes

The correlation between the R and L eyes is outlined in Table 5.2.15.

Table 5.2.15 - Correlations Of R v L Eyes For Deposition Types

	Acuvue		Medalist 66	
	Correlation	p value	Correlation	p value
Total Protein	0.592	<0.0001	0.841	<0.0001
Surface Protein	0.107	<0.18	0.369	<0.0001
Lipid	0.403	<0.0001	0.451	<0.0001

The correlation between eyes was highest for Medalist 66 for all deposit types. The results show that there is little correlation between eyes for surface deposition, but that there is a reasonable correlation for total protein.

c) Correlations Between Symptoms and Deposit Types

The inter-relationship between symptoms and deposition types is summarised in Tables 5.2.16 and 5.2.17.

Table 5.2.16 - Correlation Between Symptoms and Deposition Types - Acuvue

	Total By UV	Surface @ 280	Surface @ 360
Visual Quality	0.03	0.08	-0.02
Comfort	0.02	0.01	-0.10

Table 5.2.17 - Correlation Between Symptoms and Deposition Types - Medalist 66

	Total By UV	Surface @ 280	Surface @ 360
Visual Quality	0.12	-0.10	0.10
Comfort	-0.21	-0.19	-0.01

These results indicate that there is no correlation between subjective symptoms and gross deposition when these materials are worn for such short periods of time.

5.2.6 Discussion

For the first time to our knowledge this study investigates the relationship between the repeatability of deposition across time with one-day disposable lenses.

The results show clearly that subjects can discriminate between lenses concerning their comfort and visual acuity and that "poor" lenses will not be tolerated by subjects, despite their short period of wear. The results overwhelmingly conclude that subjects found the visual quality of the Acuvue lenses to be greater than that found with Medalist 66. Figures 5.2.1 - 5.2.4 indicate both the inter- and intra-subject differences that occur over the month in addition to the differences between lenses. Inspection of figures 5.2.1 and 5.2.2 shows that some subjects notice alarming differences in visual performance between the two lenses (for example subjects 2 and 17), whereas others notice virtually no difference at all (for example subjects 3 and 11). Upon initial collection with all lenses a careful over-refraction was conducted and so it is safe to assume that these differences relate to optical performance and not uncorrected refractive errors with the Medalist 66 lenses. Of all the subjects only one noted better visual performance with the Medalist lens (subject 19). Review of figures 5.2.3 and 5.2.4 reveal similar, although not statistically significant, results for comfort, with several subjects noting markedly different comfort scores (for example subjects 2, 4 and 17) and only two subjects recording significantly better comfort with the higher water content material (subjects 19 and 20). These differences may be attributed to the manufacturing techniques and overall lens design used in the two lenses.

Acuvue is a thin medium water content lens (58% EWC, 0.07mm Tc) which is fabricated from a patented process in which the lens is continually hydrated during the manufacturing process. Medalist 66 is a high water content thicker material (66% EWC, 0.10mm Tc) which is moulded in the dry-state and subsequently hydrated. The movement with the Medalist lens is typically a looser fit than that seen with thinner moulded lenses like Acuvue, as demonstrated in this study (40% vs 55% on a push-up test) and this increased lens "bulk" and movement may have contributed to variations in comfort and visual performance. In addition, the acuity achieved with the Acuvue lens is exceptional when compared with other frequent replacement lenses. [366]

The degree of staining seen with the two lenses indicates some differences, with Acuvue exhibiting more conjunctival staining than Medalist 66. This agrees with other studies [472] investigating this factor and is almost certainly related to the edge-design of the lens. The results were not considered clinically relevant. The incidence of recorded corneal staining (40-50%), with the majority being inferiorly placed, is also in accordance with other published studies. [391 392 453 454]

These results indicate that even after one day there is a significant difference in the type and quantity of deposition that occurs on group II and group IV materials. Significantly greater quantities of lipid were deposited on the group II material (Medalist 66) and more protein was deposited on the group IV material (Acuvue). These results are explained in the Chapter discussing material influences on deposition (Chapter 6) and are entirely in agreement with the findings alluded to in those studies (6.1 - 6.4).

The principal points of interest in this study related to the inter and intra-subject differences which occur with deposition. There were significant inter-subject differences in total protein, surface protein and lipid deposition, as evidenced by figures 5.2.5, 5.2.7 and 5.2.9. Broadly speaking 3-5 subjects could be classified as atypical depositors for each of the deposition types in question, reflecting some 15-25% of wearers for each category. In addition there was also significant intra-subject variability over the days of the study, as graphically demonstrated in figures 5.2.6, 5.2.8 and 5.2.10. The day-to-day variation differed for lens materials and factor under investigation. The variation was approximately 30% for total protein, 40% for surface protein and 50% for lipid, suggesting that there was a greater intra-subject variation for this latter factor. These values are slightly lower but in the same order as those found in study 5.1. As lipid deposition is mediated by the meibomian glands it is entirely plausible that lipid deposition should demonstrate greater variability. Several events could be envisaged which would influence the degree of lipoidal deposition, such as a poor lens removal, a foreign body which caused the subject to remove their lens or merely the subject rubbing their lid during the day and stimulating the meibomian glands to produce increased lipid.

As in the first study of this Chapter, no correlation could be sought between deposition types, indicating the lack of inter-play between these factors. The only correlation of any value was that between surface protein and total protein for the group II material, which will be discussed in Chapter 6.4 (pages 210-212). The correlation between eyes also mirrored that found in the first study in this Chapter, with the lowest correlation occurring for surface protein and the greatest for total protein. The correlation in this study for all types of deposition was greater for Medalist than Acuvue. These results confirm the conclusions of the earlier study

that results from contralateral eye studies must be interpreted with caution.

One point of note is that the inter-eye results and the intra-subject variability factors may be mildly influenced by the handling characteristics of the materials. The Acuvue material is much more flexible than the Medalist material and during insertion it is possible that the lens will have a greater contact area and time of contact with the subject's finger during lens insertion. As demonstrated in Chapter 4 (pages 118-121) handling does impact on deposition. This is the reason that subjects were provided with alcohol wipes to remove finger-contamination during lens removal. However, subjects did not use the wipes before insertion due to the potential for transferring alcohol to their eye, should they fail to rinse their fingers sufficiently in the morning. It is possible that this may have influenced the deposition results to a minor extent.

Finally, combining the results from the questionnaires with the deposition results indicates that subjective performance is not related solely to differences in deposition over such short wearing periods, with the lenses used in this study. Factors such as lens fit, handling, visual performance and wettability are more likely to determine clinical success over such short wearing periods.

5.3 Key Points

- Subjective satisfaction, visual acuity and PLNIBUT with Medalist 66 lenses remain repeatable across a year if the lenses are replaced regularly.

- Subjective satisfaction varies significantly between lens types even if only used on a daily disposable basis.

- Subjective satisfaction is not directly correlated with gross deposition with the lenses and care systems used in these studies.

- Palpebral hyperaemia with the combination of lenses and care systems used in study one increased during the study. This observation warrants further investigation as such systems (polyhexanide preserved) are relatively new to the UK market.

- Group IV materials preferentially deposit protein and group II materials principally deposit lipid.

- Inter- and intra-subject variation occurs with both bulk and surface deposition.

- Intra-subject variation is greatest for lipid deposition and least for total protein deposition.

- In this sample it would appear that 15-25% of subjects can be classified as atypically "heavy depositors", even after as little as one day of wear.

- With the materials examined in these projects no correlation between lipid and protein could be proved.

- Bulk deposition is more closely correlated between eyes than is surface deposition, particularly in group II materials. Care should be taken in extrapolating results from deposition studies using contralateral eye paradigms.

- PLNIBUT is not correlated with either deposition type or amount.

Chapter 6

The Influence of Material Composition on the Spoilation and Clinical Performance of Hydrogel Lenses

"Success is a journey, not a destination". [1]

The cornea is avascular and derives most of its oxygen supply from the atmosphere. To minimise corneal complications any contact lens must transmit sufficient oxygen to maintain as near normal corneal metabolism as possible. Gas transport in hydrogels is directly proportional to the equilibrium water content of the material, with higher water content materials transmitting more oxygen and carbon dioxide. [342 - 344] Currently available hydrogel contact lens materials have water contents ranging from 38% (polyHEMA) to 85%. Patients exhibit different corneal metabolic requirements [345 - 349] and some, notably those with higher prescriptions, may exhibit corneal swelling (oedema) and develop chronic hypoxic complications if only fitted with low water content materials. [349 - 351] Such patients require high water content hydrogels or high permeability (Dk) rigid gas permeable lenses to prevent such complications.

Two principal strategies are available to increase the water content of hydrogels above that of polyHEMA. Small quantities of charged groups such as methacrylic acid or larger amounts of more hydrophilic, neutral groups such as polyvinyl alcohol (PVA) or N-vinyl pyrrolidone (NVP) are added to polyHEMA or methyl methacrylate to raise their equilibrium water contents to 60% or greater. The Food and Drug Administration (FDA) in the USA currently classifies contact lens materials into four groups, depending upon their charge and water content (see Chapter 2, page 66). The degree of protein deposition encountered depends upon both the water content and ionic binding capacity of the lens material, [60 119 122 123] with deposits being detectable on ionic, high water content lenses (FDA group IV) almost immediately upon insertion. [7 11] Lipid also deposits on soft lenses, [8 72 80 83] and is similarly influenced by FDA grouping, with NVP-containing FDA group II lenses depositing more lipid than other FDA groups. [203]

Attempts to control contact lens deposition currently consist of two opposing methodologies. An attempt may be made to keep the lenses clean for longer periods of time or the lenses can be routinely discarded before they deposit to the extent where clinical problems occur. The latter of these options (via the use of frequent replacement or disposable systems) has been the subject of many studies and has been reviewed elsewhere.

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[1] Ben Sweetland

[456] The use of disposable and frequent replacement lenses is successful in preventing deposit-related complications. [5 457-459] Strategies to reduce deposition in non-disposable lenses include optimising the care regimen and manipulating the lens material such that the polymers can resist deposition to a greater extent. Cleaning and disinfecting systems are only partially effective at removing deposits, removing no more than 75% of deposited material. [62 64 65 255]

Current commercially available high water content, non-ionic hydrogel lenses (FDA group II materials), with water contents in the 60-80% range, are almost exclusively based on the cyclic lactam pyrrolidone hydrophile, which is introduced as N-vinyl pyrrolidone. More recently other hydrogel lenses with similar oxygen performance and mechanical strength have been introduced based on hydrogen bondable but non-ionic hydroxyl groups in the form of polyvinyl alcohol (PVA). [118 340 460 - 462] To date, all commercially available FDA group IV materials include ionic species based on methacrylic acid, with certain materials incorporating various amounts of other monomers to increase water content further.

The purpose of this chapter is to investigate the differences in clinical and deposition behaviour between and within FDA material groupings. This has important implications for all biomaterials applications, including contact lenses, but it is difficult to obtain appropriate *ex vivo* evidence within the general context of prosthetic appliances due to the difficulties of obtaining explants after short periods of use.

6.1 THE *IN VIVO* DEPOSITION PERFORMANCE OF N-VINYL PYRROLIDONE CONTAINING FDA GROUP II AND GROUP IV MATERIALS WHEN USED ON A FREQUENT REPLACEMENT BASIS

Many manufacturers now offer their hydrogel lenses on a four week frequent replacement system, with the most popular options being either FDA group II or group IV materials. [463] Monthly replacement is the most common replacement schedule. [464 465] The purpose of this study was to investigate if there is a difference between the spoilation profiles of such materials when they are replaced every four weeks. An attempt was made to avoid complications arising from differences in chemical composition by selecting N-vinyl pyrrolidone (NVP) containing materials from each of these groups.

6.1.1 Materials and Methods

Twenty subjects (eight male and twelve female) were entered onto the study, whose demographic details are described in Table 6.1.1.

Table 6.1.1 - Subject Demographics

	Mean ± SD	Range
Age (years)	32.7 ± 7.5	18 - 47
Flat Keratometry (mm)	7.90 ± 0.22	7.50 - 8.40
Steep Keratometry (mm)	7.85 ± 0.31	7.40 - 8.30
Sphere (D)	-3.52 ± 1.44	-6.00 to -1.00
Cyl (D)	-0.31 ± 0.32	-0.75 to 0.00

All subjects were fitted with Precision UV from Pilkington Barnes-Hind and Focus Visitint from CIBA-Vision, details of which are given in Table 6.1.2.

Table 6.1.2 - Lens Parameters

	Focus	Precision UV
Water Content (%)	55	74
Monomers	HEMA + PVP + MA	MMA + NVP
USAN	Vifilcon A	Vasurfilcon A
FDA Category	Group IV	Group II
ISO Category	Filcon 4b	Filcon 4a
Manufacture	Moulded	Moulded
Back Optic Zone Radius	8.60; 8.90	8.70
Total Diameter	14.00	14.40
Centre Thickness	0.10	0.14

Elemental (CHN) analysis of the two materials indicated the proportions of carbon, hydrogen and nitrogen present in the two materials and is detailed in Table 6.1.3.

Table 6.1.3 - Elemental (CHN) Analysis Results

	Vifilcon A	Vasurfilcon A
Carbon	53.20%	54.60%
Hydrogen	7.40%	7.25%
Nitrogen	1.85%	7.90%
Oxygen *	37.55%	30.25%

* The balance of the elemental analysis is taken to be oxygen, on the assumption that no other elements are present.

The lenses were worn as a randomised contralateral pair for three consecutive one month periods on a daily-wear basis while using a surfactant cleaner (CIBA-Vision "Miraflow") and two-step hydrogen-peroxide disinfection system (CIBA-Vision "10:10"). Subjects were reviewed in the clinic after 28 ± 3 days. The order of use of the different lenses was randomized by a clinical assistant and the investigation was double masked.

6.1.2 Analytical Protocol

The constraints imposed by the presence of a UV blocking agent in Precision UV limited the range of useful non-destructive analytical procedures. However, when this study began this was the only commercially available frequent replacement group II hydrogel. The lenses were analysed for lipid using fluorescence spectrophotofluorimetry @ 360nm and for protein deposition using a modified Lowry technique, as

previously described.

6.1.3 Data Analysis

Summary statistics were calculated for all variables. As the data proved non-parametric in nature a one-way repeated measures ANOVA on ranks was used to compare deposition across the wearing periods for each material and between subjects, Wilcoxon signed rank test was used to compare deposition between the two materials for each month and Mann-Whitney rank sum test for differences between sexes. Correlations were carried out using a Spearman rank order correlation. A "p" value of <0.05 was considered statistically significant.

6.1.4 Results

6.1.4.1 Protein Deposition

The mean aggregated extractable protein for all lenses for all subjects during all periods of wear is shown in Table 6.1.4, with the mean extractable protein for successive wear periods being shown in Figure 6.1.1. The amount of extractable protein for individual subjects across the three successive wear periods is indicated in Figure 6.1.2. There was a significant difference in the degree of protein deposition encountered with the two materials, with the group IV material depositing substantially more protein (mean 488µg v 28µg; p< 0.0001). There was no significant difference between the months of wear (p=NS) or between subjects (p=NS).

Table 6.1.4 - Aggregated extractable protein (µg/lens) for all lenses worn during the study

	Overall Mean ± SD	Range
Vifilcon A (G IV)	488 ± 40	415 - 580
Vasurfilcon A (G II)	28 ± 20	10 - 50
p	<0.0001	

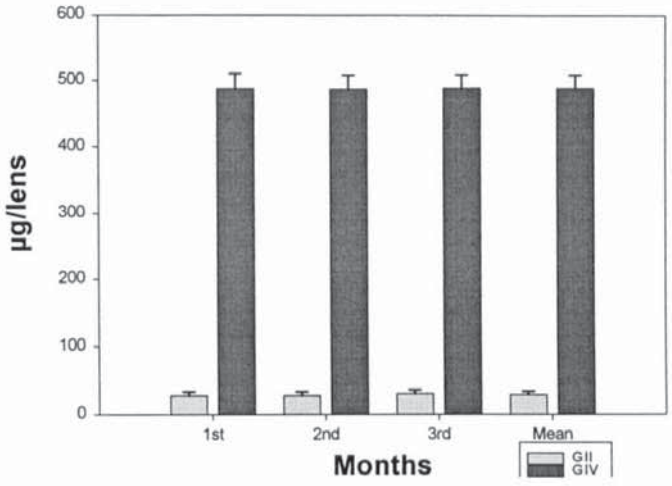


Figure 6.1.1 - Mean (±sd) extractable protein for all subjects across three successive months of wear and total aggregated protein deposition for all worn lenses

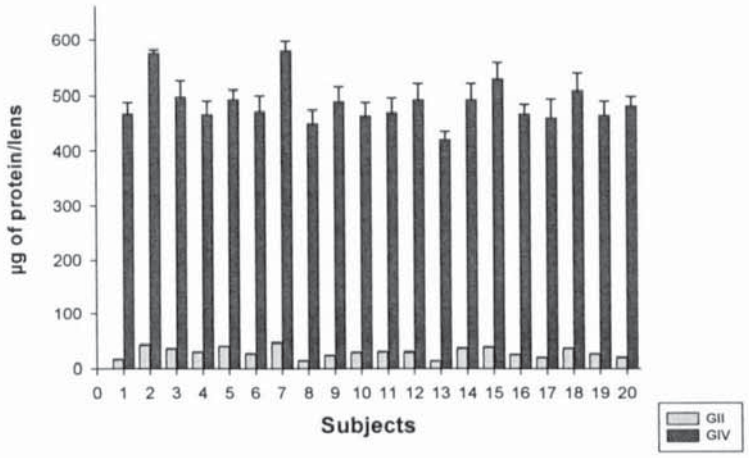


Figure 6.1.2 - Mean (±sd) extractable protein for individual subjects across three successive monthly wear periods.

6.1.4.2 Lipid Deposition

The mean aggregated amount of lipid deposited for all lenses for all subjects during all periods of wear is shown in Table 6.1.5, with the mean deposited lipid for successive wear periods being shown in Figure 6.1.3. Variability in the degree of lipoidal deposition between individual subjects for the three successive wear periods is indicated in Figure 6.1.4. The lipid results reveal that the group II material deposited more lipid (mean 73 v 38 fluorescence units; $p < 0.0001$). There was no statistically significant difference between the months of wear (p=NS), but there was between subjects ($p<0.02$), with subjects 2, 3, 10 and 18 depositing

greater amounts of lipid than the other subjects on their group II lenses ($p<0.05$) and subjects 2 and 3 depositing more lipid on their group IV lenses ($p<0.05$).

Table 6.1.5 - Aggregated lipid deposition (fluorescence units) for all lenses worn during the study. The arbitrary units reflect relative levels rather than specific quantities

	Overall Mean ± SD	Range
Vifilcon A (G IV)	38 ± 41	0 - 200
Vasurfilcon A (G II)	73 ± 45	10 - 275
p	<0.0001	

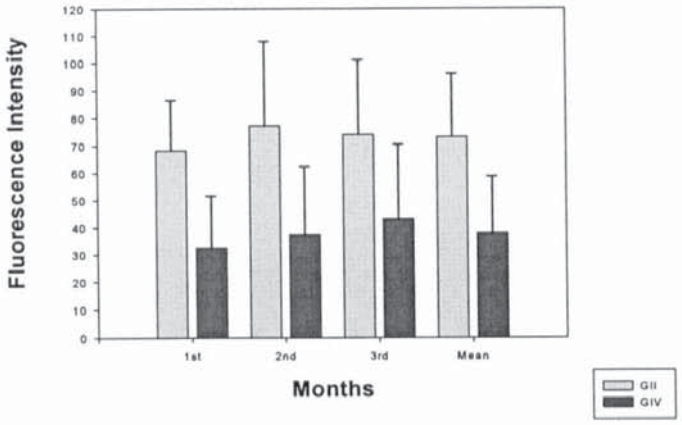


Figure 6.1.3 - Mean (±sd) surface lipid for all subjects across three successive months of wear and total aggregated lipid deposition for all worn lenses.

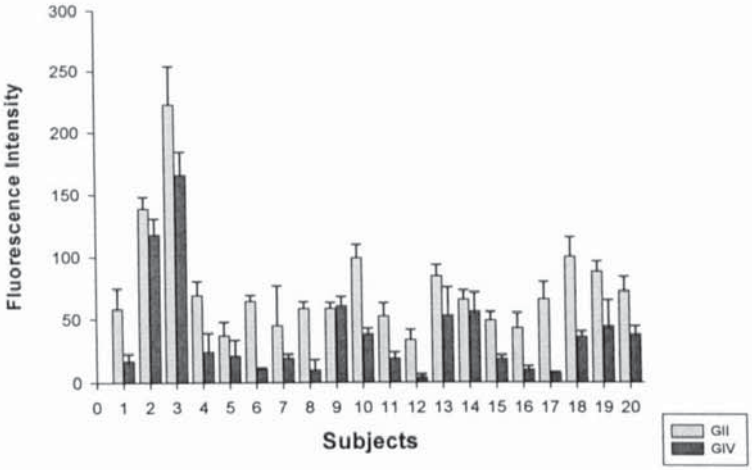


Figure 6.1.4 - Mean (± sd) surface lipoidal deposition for individual subjects across three successive monthly wear periods by fluorescence assay.

6.1.4.3 Correlations

The degree of correlation between the deposited lipid and protein for each lens worn is plotted in Figure 6.1.5. This shows that lipid deposition was predominantly controlled by subject variation and that protein deposition was dominated by material charge.

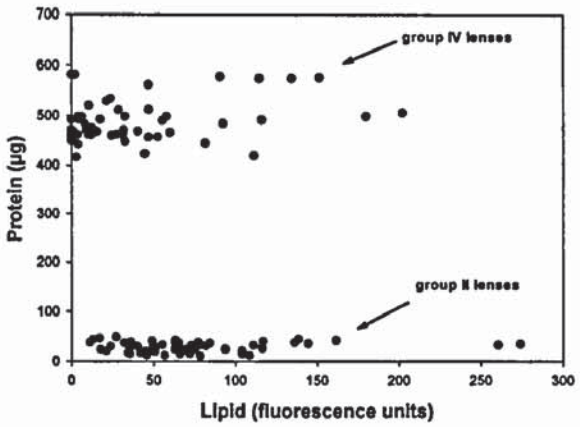


Figure 6.1.5 - Lipid versus protein deposition for individual lenses worn across all wear periods.

There was only a weak negative correlation between the protein and lipid deposits on the lenses ($r = -0.3$; $p=0.05$). Analysis of the results from the males and females showed no difference between the sexes for either type of deposition (p=NS).

6.1.5 Discussion

This study clearly shows that hydrogel lenses worn under identical situations but fabricated from differing materials deposit in different ways. Precision UV contains some four times the level of pyrrolidone-based hydrophilic groups than does Focus (Table 6.1.3). In contrast, Focus contains ionic carboxyl groups which are substantially absent in Precision UV. These two structural factors, taken together, provide a sound basis for understanding the deposition behaviour of the two materials.

The deposition of protein onto hydrogel lenses has been extensively studied. [7 9 11 54 60 63 115 116 118 119 122 123] The group IV material attracted substantial quantities of protein, which is in accordance with previously published work. [7 11 115 116 119 122 123 466] This is due to methacrylic acid imparting a negative charge to the material and thus thermodynamically favouring the deposition of positively charged species such as lysozyme, which may deposit within as little as one minute of wear. [7 11] Examination of Figures 6.1.1, 6.1.2, 6.1.5 and Table 6.1.4 confirms that protein deposition is principally related to polymeric charge and water content, with only a

small degree of inter- and intra-subject variation. Recent work has shown that there is no correlation between individual tear proteins and the degree of deposition encountered on hydrogel lenses [13] and that total protein deposition is not the sole factor in deciding lens comfort [29] and overall success.

The lipoidal deposition of hydrogel lenses has only received scant attention in the literature when compared with protein deposition. [8 70 - 72 83 203 206] The lipid deposition results found in this study are consistent with the *in vitro* work of Bontempo and Rapp, [203] which concluded that hydrogel lenses with nonionic polymer matrices (groups I and II) deposit more lipid than materials that have ionic matrices (groups III and IV) and that group II lenses attract more lipid than any other hydrogel group. The predominant reason that group II lenses are associated with high levels of lipid accumulation is that most group II materials are based on N-vinyl pyrrolidone. The reason for the high propensity to accumulate lipids relates to the known lipid solubility of pyrrolidone derivatives, which has led to considerable interest in their use as trans-dermal penetration enhancers. [467] Although both of the lens types studied here contain N-vinyl pyrrolidone, there is a four fold difference in the level of NVP and this is consistent with the differing levels of lipid absorbed. An additional factor, although of lesser significance, is that the anionic surface of the group IV material will exhibit some small level of repulsion to lipids, some of which are also anionic in nature. As might be anticipated from an extension of these arguments, group IV lenses that contain no NVP absorb less lipid than group IV lenses that are pyrrolidone-based. Recent studies in our laboratories (unpublished work) confirm that, as expected, group IV lenses that are substantially based on polyHEMA absorb less lipid than do uncharged polyHEMA lenses under identical wear conditions. The results of this study and that of Bontempo and Rapp [203] contradict those of Mirejovsky and co-workers, [117] who found that group IV lenses deposited more lipid than group II lenses. The most likely reason for this is the relative insensitivity of staining techniques compared with fluorescent spectrophotofluorimetry and high performance thin layer chromatography.

It is likely that lipid deposition, while being influenced to some extent by material characteristics, will also be significantly influenced by variations in tear film chemistry. Significant differences exist in the lipid composition of individuals with satisfactory tear film function [158] and the production of lens calculi (which are predominantly lipoidal in nature) [8 80 82 83] can be influenced by factors such as an elevated intake of alcohol and cholesterol. [83] Examination of Figures 6.1.4 and 6.1.5 confirms this fact. Significant inter- and intra-subject variation is seen, with some subjects (subjects 2 and 3) depositing almost five times the amount of lipid compared with other subjects (such as subjects 5, 7 and 12). The fact that *in vivo* lipid deposition is subject rather than material dependent is in agreement with other published studies. [206]

Using pre-lens non-invasive break-up time (PLNIBUT) as an indication of lens contamination Guillon and co-workers have shown that optimum replacement schedules are patient dependent. [363 373] This study lends further credence to this concept as it demonstrates the wide variation encountered in the deposition of contact lens materials by patients. Our results suggest that protein deposition is largely influenced by polymer charge and to a lesser extent by water content, with little subject variation occurring. While lipid deposition is influenced by material characteristics, patient factors also play a role. To ensure that the optimum performance of hydrogel contact lenses is maintained lenses must be replaced regularly. [363 366 373] While ionic materials are more likely to deposit increased amounts of protein, non-ionic polymers will deposit more lipid.

Regular replacement of both types will result in improved clinical performance. These results indicate that monthly replacement of these materials is quite sufficient for the majority of the subjects. However, it is worth commenting that these subjects were all initially asymptomatic wearers and that symptomatic subjects may require shorter replacement frequencies.

Further studies are required to elucidate if materials within identical FDA groups exhibit similar deposition behaviour. The next two studies address this issue, concentrating on the differences encountered within FDA group II and IV materials.

6.2 THE *IN VIVO* DEPOSITION PERFORMANCE OF FDA GROUP II HYDROGELS BASED ON NVP AND PVA HYDROPHILES

The purpose of this study was to provide an *in vivo* comparison of the relative effects of NVP and PVA as neutral hydrophilic groups in hydrogels, particularly in relation to their propensity to attract protein and lipid. This information is of considerable specific importance to the field of ocular biomaterials, where materials of this type are classified in the same group (FDA group II). It is also of relevance to other areas of biomaterials research, where detailed analysis of the progressive interaction of these hydrogels with the biological environment is not feasible because of the impracticability of retrieving explants from patients after identical and relatively short time periods.

6.2.1 Materials and Methods

Twelve subjects (six male and six female) were entered onto the study, whose demographic details are described in Table 6.2.1.

Table 6.2.1 - Subject Demographics

	Mean ± SD	Range
Age (years)	33.7 ± 8.9	20 - 45
Flat Keratometry reading (mm)	7.90 ± 0.34	7.50 - 8.30
Steep Keratometry reading (mm)	7.85 ± 0.33	7.20 - 8.30
Sphere (D)	-1.82 ± 3.93	-6.75 to +5.00
Cyl (D)	-0.41 ± 0.41	-1.00 to 0.00

Lens materials were chosen to ensure that one constituent in each material remained consistent (methyl methacrylate) and that equilibrium water contents were similar. All subjects were fitted with the two test lenses, which were "Lunelle ES70" from Essilor and "Excelens" from CIBA-Vision, details of which are given in Table 6.2.2.

Table 6.2.2 - Lens Parameters

	ES70	Excelens
Water Content (%)	70	64
Monomers	MMA + NVP	MMA + PVA
USAN	-	Atlafilcon A
FDA Category	Group II	Group II
ISO Category	Filcon 4a	Filcon 4a
Manufacture	Lathed	Moulded
Back Optic Zone Radius	8.30, 8.60, 8.90	8.40, 8.60, 8.80
Total Diameter	14.0	14.0
Centre Thickness	0.14	0.12

The subjects were divided into two equal groups, each of which wore one of the lens types on a daily wear basis for six months. Subjects were reviewed in the clinic on initial collection, after one month (28 ±3 days), three months (90 ± 7 days) and six months (180 ± 14 days). At the six month visit a cross-over took place, with all subjects repeating the above visit schedule with the other lens material. The study design used a randomised, double-masked paradigm, in which neither the practitioner nor the subject was aware of the lens type being used at the time of the clinical visits.

During the study lenses were cleaned at the end of every day with an isopropyl alcohol based surfactant cleaner (CIBA-Vision "Miraflow"), rinsed in non-preserved saline and subsequently disinfected overnight with a two-step 3% hydrogen peroxide system (CIBA-Vision "10:10"). In the morning the lenses were neutralised for at least twenty minutes with sodium pyruvate before insertion took place. No enzyme tablets or rewetting drops were used at any time during the study.

6.2.2 Clinical Protocol

At each visit subjective satisfaction, lens fit, visual acuity, PLNIBUT, visible wettability on the slit lamp, Rudko deposition and physiological performance were assessed.

6.2.3 Analytical Protocol

At the end of the six month wearing schedule each lens was removed with plastic-tipped tweezers and placed in a glass vial containing sterile non-preserved saline. The vials were then capped, labelled with the subject's initials and study number and refrigerated prior to spoilation analysis taking place. Surface deposition was evaluated using fluorescence spectrophotofluorimetry, exciting at 280nm to evaluate proteins and 360nm for lipid. Total protein was assessed using transmission UV at 280nm.

As lenses were subjected to cleaning by patients each day upon their removal it was important to gain some idea of the degree to which deposited material may be removed each day and thereby indirectly assess the amount of material which is firmly and irreversibly adherent. In order to assess this the lenses were subjected to one further surfactant clean following the non-destructive analysis detailed above. Lenses were cleaned with CIBA-Vision "Miraflow". The investigator wore sterile surgical gloves (to prevent the transfer of skin lipids) and the lens was digitally cleaned with the index finger for exactly 10 seconds and then copiously rinsed with saline. Following this the lenses were re-examined for residual total protein by transmission UV and residual lipid deposits by fluorescence spectrophotofluorimetry.

6.2.4 Data Analysis

Summary statistics were calculated for all variables. The grading scales, being ordinal measurements, were considered to be non-normal data. All other data was tested for normality of distribution. Wilcoxon signed rank test was used to compare sets of non-parametric data and Students paired t-Test was used to compare normally distributed data. Correlations which included non-parametric data were undertaken using Spearman Rank Correlations. In all cases a "p" value of <0.05 was taken as being statistically significant.

6.2.5 Results

6.2.5.1 Subjective Factors

a) Visual quality

The visual quality results are summarised in Table 6.2.3. There was no significant difference at any visit between the two lens types (p=NS). Reported visual quality reduced with both materials during the period of wear, although this reduction was not statistically significant (p=NS).

Table 6.2.3 - Subjective Visual Quality (Median and 25% quartile)

	NVP		PVA		p
After 1 month	9.0	8.0	9.0	9.0	NS
After 3 months	8.5	8.0	9.0	8.0	NS
After 6 months	8.0	8.0	8.0	7.0	NS
p	NS		NS		

b) Comfort

These results are summarised in Table 6.2.4. There was no significant difference between the lenses at any of the visits (p=NS) and whilst comfort reduced during the study the change was not statistically significant (p=NS).

Table 6.2.4 - Subjective Comfort (Median and 25% quartile)

	NVP		PVA		p
After 1 month	10.0	8.0	9.5	8.5	NS
After 3 months	9.0	8.0	9.0	8.0	NS
After 6 months	9.0	7.5	9.0	7.0	NS
p	NS		NS		

c) Overall satisfaction

Table 6.2.5 indicates that no significant difference was detected between the two lens types at any visit (p=NS) and that, while satisfaction reduced with each lens type across the wearing period, this was not statistically significant (p=NS).

Table 6.2.5 - Subjective Overall Satisfaction (Median and 25% quartile)

	NVP		PVA		p
After 1 month	9.0	9.0	9.0	8.0	NS
After 3 months	9.0	8.0	9.0	8.0	NS
After 6 months	8.0	7.5	8.0	7.0	NS
p	NS		NS		

6.2.5.2 Visual Acuity

There was no significant difference at high or low contrast between the lens types at any appointment or was there any detectable reduction in acuity during the period of the study with either lens type (p=NS). Mean high contrast visual acuities (VAR units) were 105 ± 6 for the NVP lenses and 107 ± 4 for the PVA lenses (approximately -0.12 logMAR; 6/4.5). Low contrast results were 96 ± 2 for the NVP lenses and 97 ± 4 for the PVA lenses. This correlates well with published reports. [369 468]

6.2.5.3 Visible Deposition

To investigate the change in visible deposition across time the results for the one month visit and the six month visit are compared in Figures 6.2.1-6.2.3.

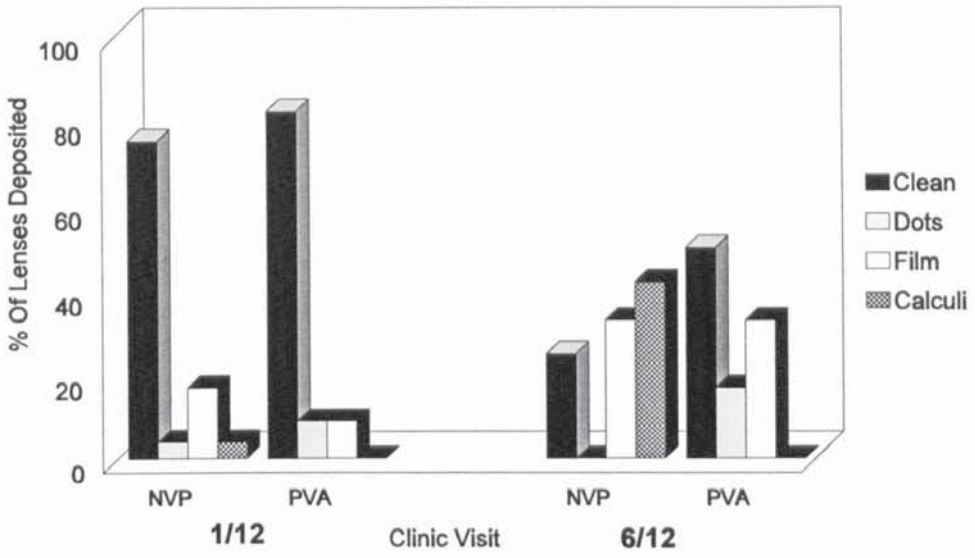


Figure 6.2.1 Visible deposit type as assessed and graded by a modified RUDKO procedure. The results present the percentage of lenses exhibiting each type of deposit at the one month and six month clinic visits.

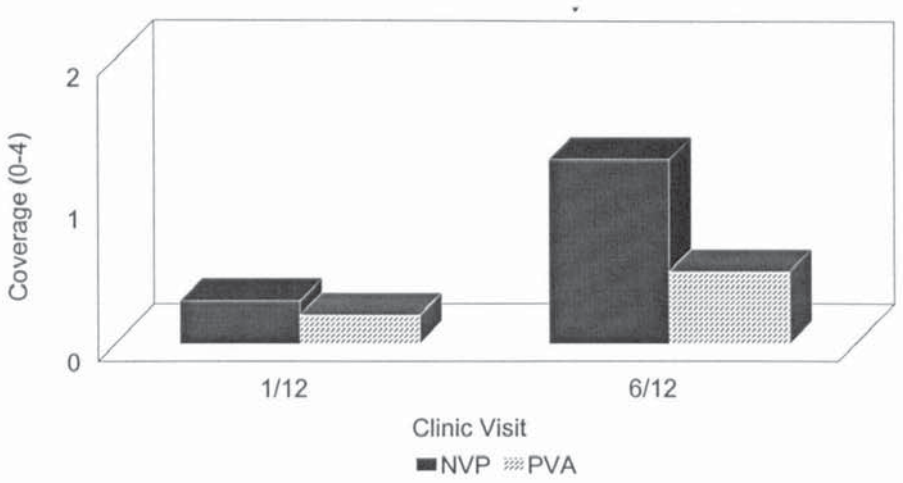


Figure 6.2.2 - Visible deposit coverage as assessed and graded by a modified RUDKO procedure. The results indicate the mean coverage (expressed on a 0-4 scale) for the two lens types at the one month and six month clinic visits.

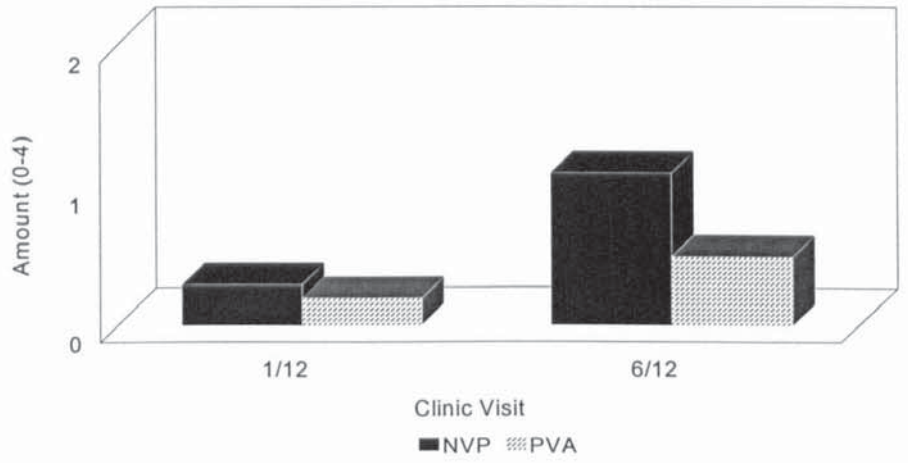


Figure 6.2.3 - Visible deposit amount as assessed and graded by a modified RUDKO procedure. The results indicate the mean amount (expressed on a 0-4 scale) for the two lens types at the one month and six month clinic visits.

These results indicate that a significant increase in visible deposits occur in the NVP-based high water content lens worn for six months when compared with one based on PVA. The differences were significant for type ($p<0.002$), coverage ($p<0.05$) and amount of deposition ($p<0.05$) at the six month review. The principal type of deposition observed on the NVP lenses were small, circumscribed, nodular deposits (calculi), whilst filmy deposits predominated on the PVA lenses.

6.2.5.4 Front Surface Wettability

Table 6.2.6 and Figure 6.2.4 detail the mean (±sd) PLNIBUT in seconds for each visit with each lens material.

Table 6.2.6 - Mean (±sd) front surface tear film break-up time (PLNIBUT) in seconds for all lenses for all clinic visits, as assessed by the Loveridge Grid

	NVP	Range	PVA	Range	p
On Collection	17.0 ± 4.9	6 - 30	15.9 ± 5.8	7 - 30	NS
After 1 month	16.2 ± 7.0	2 - 27	15.4 ± 4.1	7 - 26	NS
After 3 months	15.0 ± 7.1	2 - 30	13.9 ± 6.2	1 - 28	NS
After 6 months	13.5 ± 6.4	4 - 27	13.2 ± 6.8	5 - 30	NS
p	<0.04		<0.04		

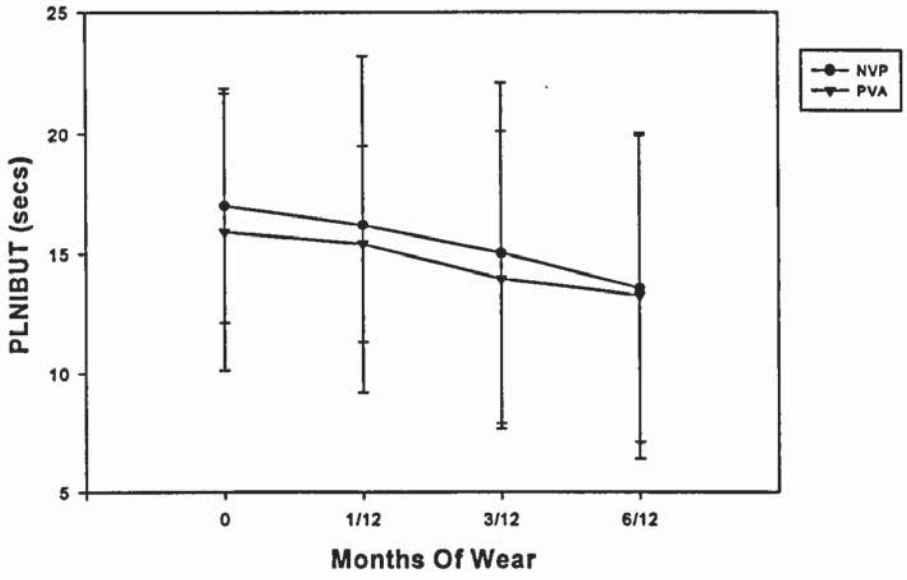


Figure 6.2.4 - Mean (±sd) front surface tear film break-up time (PLNIBUT) in seconds for all lenses for all clinic visits, as assessed by the Loveridge Grid.

Figure 6.2.4 clearly indicates the significant inter-subject variations present and that front surface lens wettability reduces with wearing time with both lens types ($p<0.04$). Post-hoc analysis by Tukey testing indicates that the significant change occurs at the six month visit. These results suggest that to provide maximum wettability both lens types should be replaced at the six month stage, irrespective of the degree of deposition or visible appearance at the slit lamp.

6.2.5.5 Slit-Lamp Examination

There was no significant difference between the lenses for corneal staining, bulbar conjunctival hyperaemia or palpebral conjunctival appearance. Conjunctival staining was greater with the tighter fitting PVA-based lens (0.6 v 1.4; $p<0.02$). Whilst this is statistically significant it was not considered clinically significant and was attributed to tighter fitting characteristics of the PVA lens.

6.2.5.6 Lens Deposition Analysis

The lipid and protein spoilation results for each lens type are summarised in Tables 6.2.7-6.2.9 and Figures 6.2.5-6.2.10.

a) Total Protein by UV

Figure 6.2.5 indicates the total protein deposited on a per subject basis, as assessed by transmission UV. These results indicate that there are very significant differences between the two materials ($p<0.0001$) and that inter-subject differences also exist, although statistical analysis of the results indicate that these inter-subject differences are not statistically significant ($p>0.05$). Figure 6.2.6 and Table 6.2.7 summarise these results for all worn lenses and present the mean aggregated total protein deposited on each material.

Table 6.2.7 - Mean (±sd) aggregated total protein deposition for all worn lenses (μg/lens) as assessed by transmission UV

	Mean ± SD	Range
NVP	70 ±15	50 - 95
PVA	10 ± 5	5 - 25
p	<0.0001	

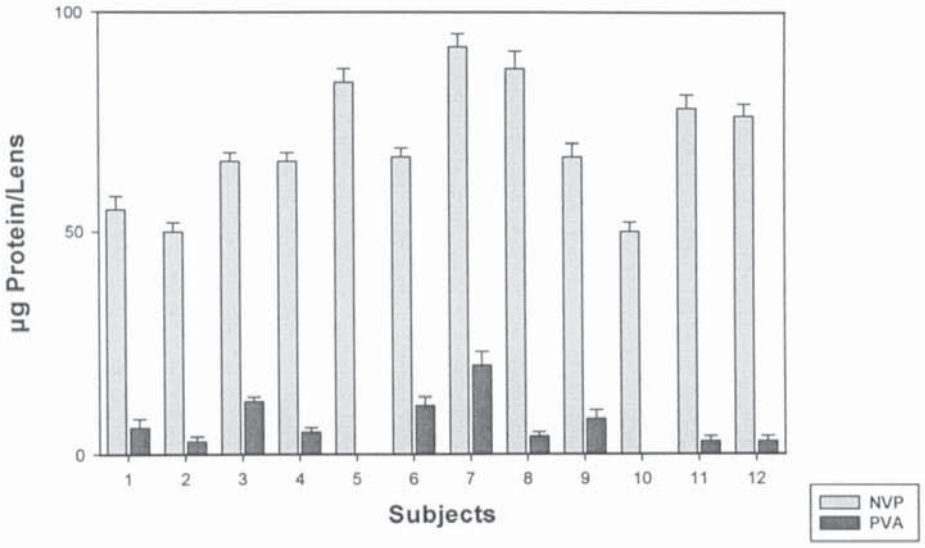


Figure 6.2.5 - Total protein deposition as assessed by transmission UV at 280nm. These results indicate the mean (±sd) of the right and left lenses for each subject.

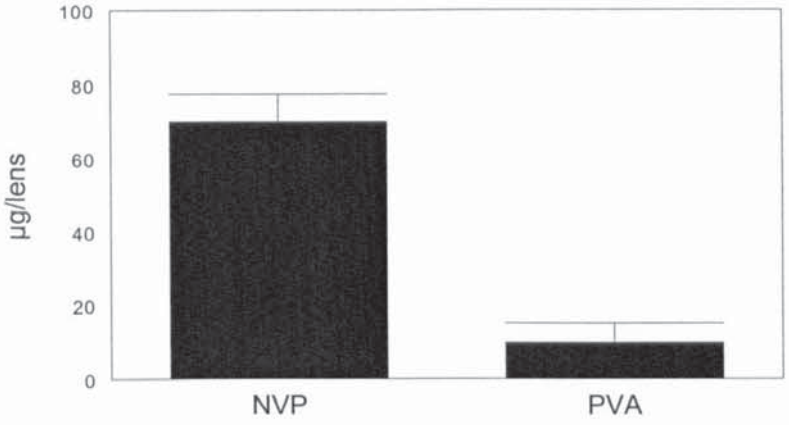


Figure 6.2.6 - Mean (±sd) aggregated total protein deposition for all worn lenses as assessed by transmission UV.

b) Surface Protein By Fluorescence @ 280nm

Figure 6.2.7 indicates the mean surface protein deposited by fluorescence (±sd) of the right and left lenses for each subject. The results indicate that, as with total protein, there are clear differences between lens materials and subjects. Whilst the difference between materials was statistically significant ($p<0.0001$), there was no significant difference between subjects ($p<0.05$). These results are summarised in Figure 6.2.8 and Table 6.2.8, which present the mean aggregated surface protein deposition for all lenses.

Table 6.2.8 - Mean (±sd) aggregated surface protein deposition for all worn lenses (fluorescence intensity units) as assessed by fluorescence spectrophotofluorimetry

	Mean ± SD	Range
NVP	144 ± 75	60 - 300
PVA	15 ± 26	0 - 100
p	<0.0001	

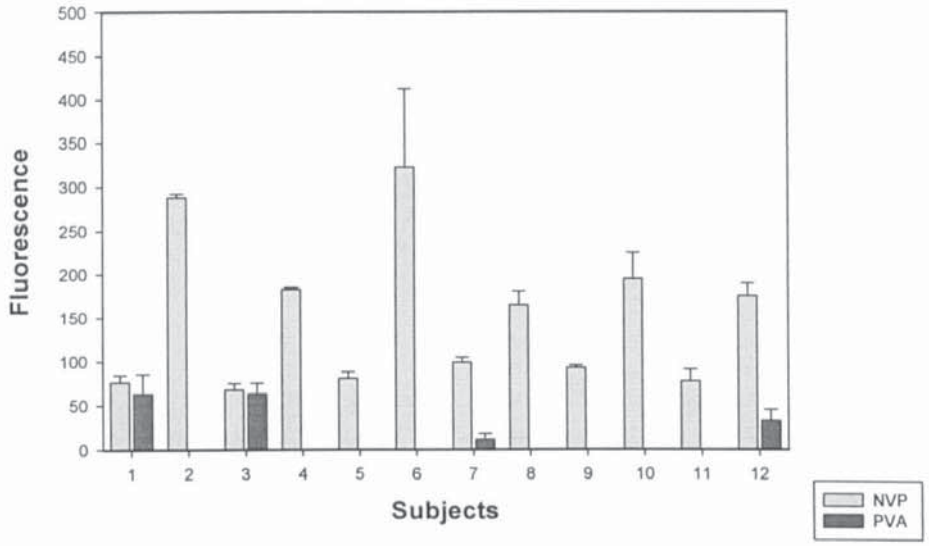


Figure 6.2.7 - Surface protein deposition as assessed by fluorescence spectrophotofluorimetry at 280nm. The results indicate the mean (±sd) of the right and left lenses for each subject.

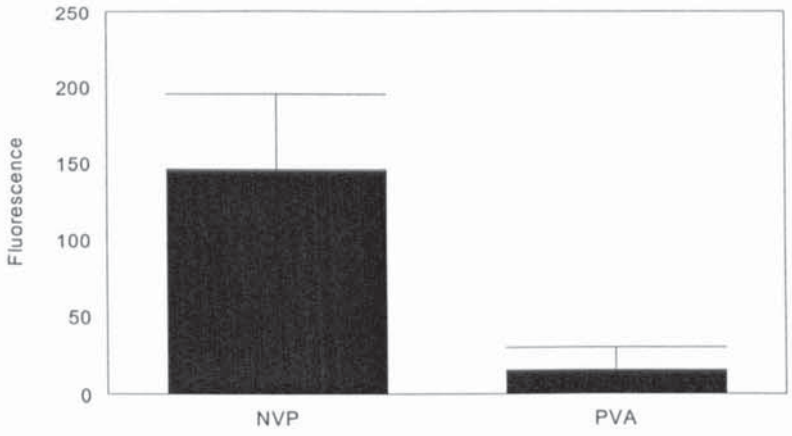


Figure 6.2.8 - Mean (±sd) aggregated surface protein deposition for all worn lenses as assessed by fluorescence at 280nm.

c) Lipid By Fluorescence

Figure 6.2.9 presents the mean (±sd) for lipid deposition as assessed by fluorescence for the subjects' right and left lenses. The results indicate that whilst large differences exist between the two materials ($p<0.0001$), significant differences also occur between subjects, with some (such as subject 12) depositing almost 10x more lipid that others (subject 11). Statistical analysis of the results indicate that the inter-subject differences are significant for both the NVP-based material ($p<0.02$) and the PVA-based material ($p<0.04$). The aggregated results for all lenses are presented in Table 6.2.9 and Figure 6.2.10.

Table 6.2.9 - Mean (±sd) aggregated surface lipid deposition for all worn lenses as assessed by fluorescence at 360nm

	Mean± SD	Range
NVP	1324 ±1089	205 - 4030
PVA	28 ±45	5 - 200
p	<0.0001	

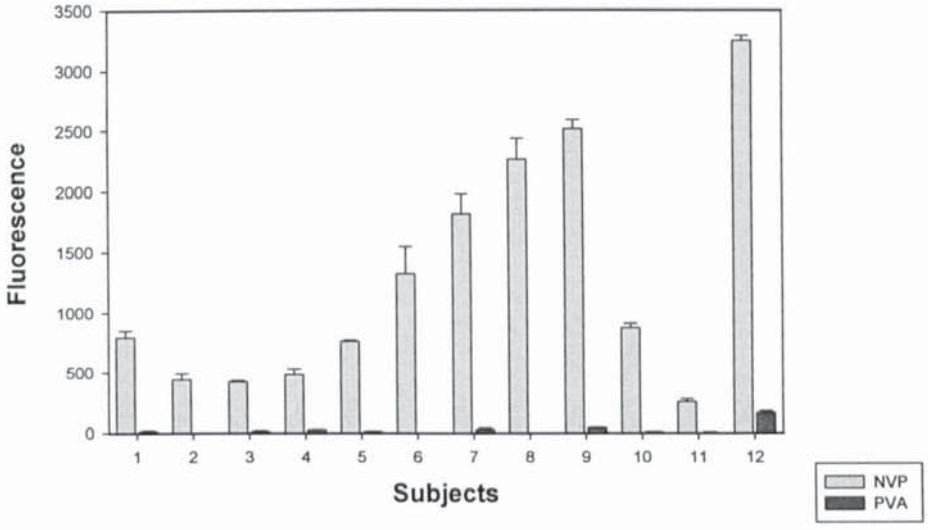


Figure 6.2.9 - Surface lipid deposition as assessed by fluorescence spectrophotofluorimetry at 360nm. These results indicate the mean (±sd) of the right and left lenses for each subject.

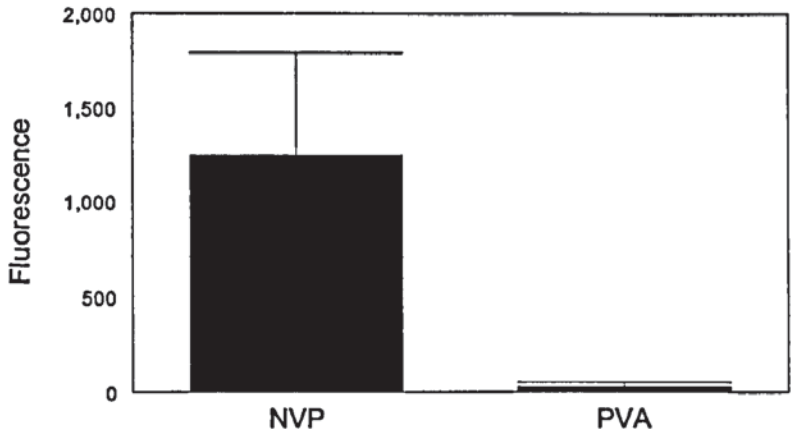


Figure 6.2.10 - Mean (±sd) aggregated surface lipid deposition for all worn lenses as assessed by fluorescence at 360nm.

Various measures of association were sought between the factors assessed. Deposition between right and left eyes for individual patients was well correlated for both protein and lipid with both materials ($r>0.7$; $p<0.02$). However, as deposition progressed for individual patients, no correlation was found between protein and lipid for either material ($r=0.12$ for NVP and 0.14 for PVA). That is to say, although individually, protein and lipid progressively increased with time, this increase was not correlated. There was no clear statistical correlation between the reduction in PLNIBUT and the degree of accumulated deposits with either material (r-0.5 to 0.1; p=NS), nor was there any statistical correlation between the amount of deposition and the change in subjective comfort or vision during the study (r 0.4 to 0.1; p=NS).

d) Cleaning Evaluation

The amount of removable total protein and surface lipid was assessed by transmission UV and fluorescence respectively, as previously described. Following the deposition analysis described above, lenses were cleaned once more with Miraflow, which was rinsed off with saline, prior to further analysis. The amount of deposit reduction is expressed as a percentage of that initially measured and is detailed in Table 6.2.10 and Figure 6.2.11. The results indicate that protein deposits are more easily removable than lipoidal deposits from both materials ($p<0.03$) and that both deposit types are more easily removed from the PVA-based material ($p<0.05$).

Table 6.2.10 - Mean (± sd) percentage of deposits removable by surfactant cleaning.

	NVP (%)	PVA (%)	p
Protein	44 ± 12	56 ± 14	<0.02
Lipid	24 ± 24	32 ± 25	<0.05
p	<0.03	<0.01	

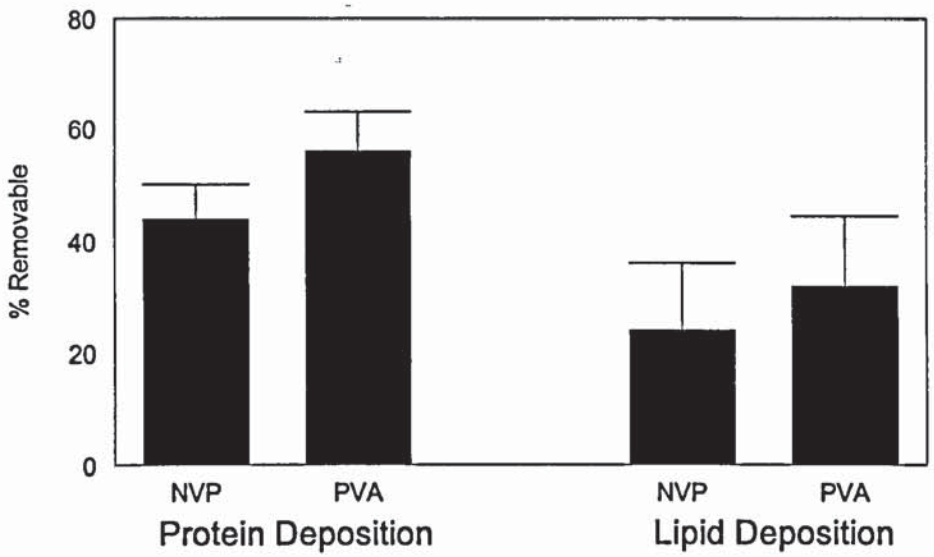


Figure 6.2.11 - Amount of removable deposits following surfactant cleaning. Protein deposition was assessed by transmission UV and lipid deposition by fluorescence at 360nm.

It is important to point out that this cleaning operation was carried out on lenses which had been subjected to a care regime which included a cleaning step and therefore reflects the ease of removal of residual levels of protein and lipid. The differences between general levels of protein and lipid removal at this stage reflects the fact that protein is entirely superficially deposited on group II materials, whereas lipoidal deposition partitions into, and thus penetrates, the lens matrix.

e) Visible vs Analytical Deposition

Intuitively it would appear that a good correlation should exist between the appearance of calculi on the NVP-based lenses and the degree of analytically evaluated lipid deposition, as calculi contain appreciable quantities of lipoidal material. [8 80 82 83] The lenses were divided into two groups, those which exhibited visible calculi at the six month visit and those which did not and the deposited lenses were then further subdivided into their grades of calculi. Figure 6.2.12 indicates the level of deposited lipid when correlated with their

presence or absence and Figure 6.2.13 the degree of visible calculi when plotted against the amount of deposited lipid. Tables 6.2.11 and 6.2.12 indicates the mean differences and their statistical significance.

Table 6.2.11 - Mean (± sd) degree of lipid deposition as ascertained by fluorescence excitation at 360nm compared with presence or absence of visible calculi at the six month visit

	Lipid Deposition
Present ("Yes")	1697 ± 578
Absent ("No")	578 ± 198
p	<0.02

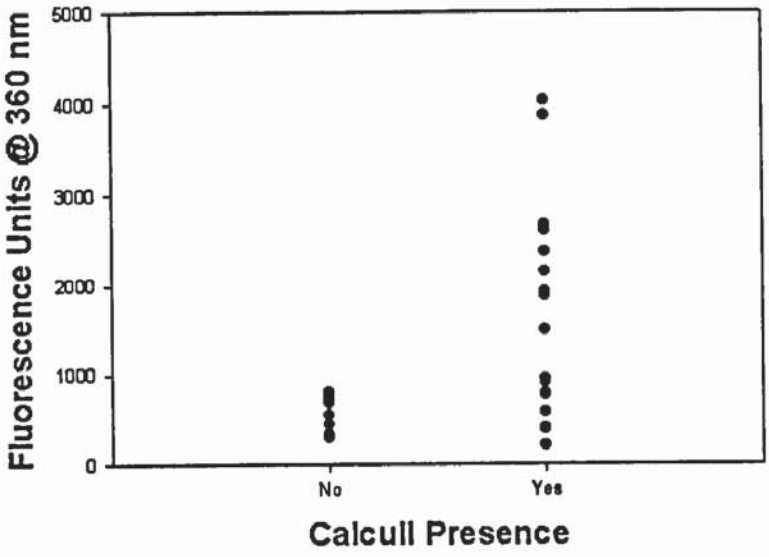


Figure 6.2.12 - Correlation of deposited lipid versus visible lens calculi at the six month visit for the NVP-based lenses.

Table 6.2.12 - Mean (± sd) degree of lipid deposition as ascertained by fluorescence excitation at 360nm compared with degree of visible calculi at the six month visit.

	Lipid Deposition
Grade 1	1536 ± 198
Grade 2	1733 ±668
Grade 3	1853 ± 1913
p	0.09

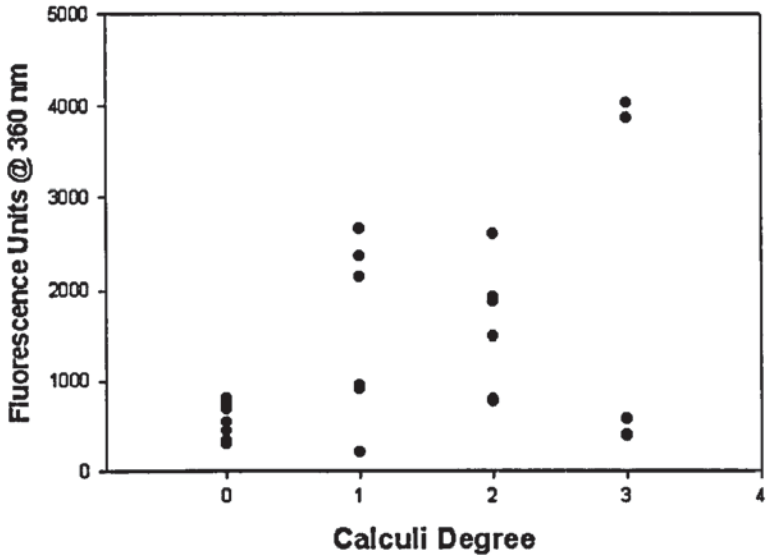


Figure 6.2.13 - Correlation of calculi degree at six month visit versus lipid deposition evaluated by 360 nm excitation fluorescence for the NVP-based lenses.

These results indicate that the presence of calculi are a good predictor of the presence of high levels of lipoidal deposition but that increasing levels of calculi are not necessarily good predictors that the level of lipid deposition is increasing.

6.2.6 Discussion

In this study two non-ionic materials of similar water content but significantly different chemical structure were compared. Clinical performance between the materials (comfort, visual quality, visual acuity and physiological performance) were virtually identical and indicate that such factors do not differ widely between lenses fabricated from materials within the same FDA group. The fact that clinical performance is

so similar within broad groups of hydrogels (both in this study and evidenced by other workers [469]) is almost certainly due to the fact that the "bulk" characteristics (mechanical properties, water content and lens design) are also broadly similar and are the principle determinants in clinical success. However, the differences in the materials' propensity to deposit protein and lipid is clearly far more dependent upon the materials detailed chemical structure.

A variety of factors are known to cause differences in protein deposition, including the charge and water content of the hydrogel material. [7 9 11 54 60 63 115 116 118 119 122 123] This controlled clinical study clearly shows that even materials with similar water contents and ionicity (and therefore FDA grouping) demonstrate significant differences in deposition characteristics. Deposition is predominantly related to the material chemistry, as evidenced by these results, which compare favourably with those from other published studies. [470] There are sound chemical reasons for the observed differences in deposition behaviour between these materials. The relative resistance of polyvinyl alcohol based hydrogels to the accumulation of tear proteins must be considered against the background of the behaviour of poly (2-hydroxyethyl methacrylate), more commonly known as polyHEMA. Although the neutral hydroxyl hydrophile interacts only weakly with the range of proteins in the eye, the well recognised presence of methacrylic acid within even the purest optical grades of HEMA monomer ensures that commercial available polyHEMA lenses have a low level of surface anionicity, which leads to the accumulation of the positively charged tear protein lysozyme. The polyvinyl alcohol/methyl methacrylate based Excelens polymer is the only current contact lens material combining the advantage of the hydroxyl hydrophile whilst avoiding the presence of acidic anionic impurities and the results from this study are similar to the levels of protein deposition reported for Atlafilcon by other workers. [118 470 471]

Although N-vinyl pyrrolidine carries no charge at physiological pH, the pendant pyrrolidone ring presents a relatively hydrophobic external surface, resulting in appreciable levels of albumin adsorption. The ampiphilic nature of the pyrrolidone is the underlying reason for its considerable susceptibility to lipid accumulation. This feature of the pyrrolidone ring has led to a considerable interest in the use of N-alkyl pyrrolidones as dermal penetration enhancers. [467 472] The fact that doubts exist in relation to the chronic toxicity of pyrrolidone derivatives [473] emphasises the well recognised need to remove residual N-vinyl pyrrolidone monomer from contact lenses following fabrication, prior to insertion. The potential adverse effects of significant quantities of a transmembrane penetration enhancer of uncertain toxicity in the eye would clearly be a matter of considerable concern if this were not done. Incorporation of N-vinyl pyrrolidone into the polymer network gives no such problems, but does provide a substrate with a marked propensity to partition lipid into the lens matrix. This study showed that NVP-based polymers deposit 45x more lipid and 7-9x more protein than polymers which are PVA-based ($p<0.0001$).

In this study a good correlation was found between the visible on-eye deposition performance of the materials and the extent and nature of the tear components deposited, as determined by laboratory analytical techniques. However, this is not always the case, with visible typing of deposits frequently resulting in an inaccurate estimation of the true level of deposition present. [115 116] This study indicates that gross deposits (such as lens calculi, which are predominantly lipoidal in nature [8 80 82 83]) can provide an indication of the

degree of lipoidal deposition present. As a general rule, FDA group II lenses attract more lipid than other FDA groups [203] and deposit higher levels of lens calculi. [8 48 80 83] Previous studies indicate that 6-20% of NVP-based Lunelle lenses require replacement due to deposition with films or calculi. [474 475] and the increase in deposition across time with Lunelle lenses compared with other materials of similar water content is also in agreement with other studies. [469]

This study further demonstrates that subjective comfort and visual quality, overall satisfaction and PLNIBUT all reduce with time, whilst deposition progressively increased. Whilst the subjective changes were not statistically significant, the downward trends were clearly visible. In an ideal model it would be possible to demonstrate that increasing deposition resulted in reduced PLNIBUT, which subsequently resulted in reduced lens comfort. However, this relationship could not be proved statistically as there was no direct correlation between the degree of deposition and the change in comfort across the wearing period, possibly as a result of the small sample size. This is in accordance with previously published work, [29] indicating that other factors aside from deposition are important in determining lens comfort. Other studies have postulated this close inter-relationship between increasing lens deposition and reducing comfort, [28 476] although not all studies found this to be the case. [30] Bruce and colleagues [30] found that as lenses aged they increased in visible deposition and that this resulted in a reduction in PLNIBUT, but that this could not be directly correlated with a reduction in comfort.

As time passes the repeated surfactant cleaning and disinfection of the lenses produces various chemical and conformational changes to contact lens deposits, resulting in tear film disruption and possibly a reduction in lens comfort. Front surface lens wettability, as measured by the pre-lens non-invasive break-up time (PLNIBUT), should therefore provide a clear indication of lens contamination. Using this technique Guillon and colleagues have clearly demonstrated that suitable replacement schedules are highly patient dependent, [363 373] a finding reiterated in this study and evidenced by the wide range and standard deviation encountered in the PLNIBUT measurements and deposition with both lipid and protein. Figure 6.2.4 clearly indicates that PLNIBUT reduces with wearing period, is patient dependent and is independent of material type.

Previous work in these laboratories has indicated that subjects wearing contact lenses show substantial contralateral eye differences in lens deposition and that these differences are more marked with short wearing periods. This study indicates that the differences in deposition between eyes becomes less marked with the passage of time and that after six months of wear there is a good correlation between the amount of deposition which occurs in each eye ($r>0.7$). This is an important finding in the context of the design of clinical studies, as contralateral eye studies concerning deposition analysis of group II lenses worn for less than six months should be viewed with some caution.

Currently available solution systems are not fully effective at removing deposited material.[62 123 255] This study indicates that immobilised deposition is greater on NVP-based polymers ($p<0.05$) and that neither protein nor lipid can be fully removed from hydrogel lenses once they have been worn for six months. Surfactant cleaning is more effective at removing protein deposits than lipid deposits ($p<0.03$) but at best can only remove 50-60% of deposited material, which is in accordance with other studies.[62 123 255]

In conclusion, this study clearly shows that careful choice of lens material can enable practitioners to reduce deposition without sacrificing material oxygen permeability. The wettability results would support the fact that even lenses which do not deposit heavily should be replaced in less than six months. These results should be considered in the context that all the subjects were asymptomatic and that symptomatic subjects may require even shorter replacement intervals. Whilst some degree of deposition control can be achieved by careful choice of lens material it is likely that the rate of deposition is merely altered and that the ultimate fate of significant deposition of all hydrogel materials is inevitable. Examination of Figures 6.2.5, 6.2.7 and 6.2.9 indicates that some 30-50% of patients are likely to deposit any material and for these patients the best choice will be a lens which is more frequently replaced.

6.3 THE INFLUENCE OF NVP ON THE *IN VIVO* DEPOSITION PERFORMANCE OF FDA GROUP IV HYDROGELS

The purpose of this study was to evaluate the *in vivo* clinical and deposition performance of two FDA group IV materials consisting of differing monomers, when replaced every four weeks. Previous studies have indicated that such materials attract considerable amounts of protein, [7 11 60 63 115 116 119 122 123] but no published study to date has evaluated the lipid and protein deposition on two materials within two FDA group IV materials fabricated from differing monomers.

6.3.1 Materials and Methods

Twenty-one subjects (six male and fifteen female) were entered onto the study, whose demographic details are described in Table 6.3.1.

Table 6.3.1 - Subject Demographics

	Mean ± SD	Range
Age (years)	40.6 ± 10.5	22 - 56
Flat Keratometry reading (mm)	7.90 ± 0.32	7.55 - 8.45
Steep Keratometry reading (mm)	7.80 ± 0.31	7.30 - 8.35
Sphere (D)	plano ± 3.82	-7.00 to +5.25
Cyl (D)	-0.42 ± 0.31	-1.00 to 0.00

All subjects were fitted with the two test lenses, which were Vistakon "Surevue" and CIBA-Vision "Focus", details of which are given in Table 6.3.2. Materials were carefully chosen to ensure that the compositions were different by only one monomer.

Table 6.3.2 - Lens Parameters

	SUREVUE	FOCUS
Water Content (%)	58	55
Monomers	HEMA+MA	HEMA+PVP+MA
USAN	Etafilcon A	Vifilcon A
FDA Category	Group IV	Group IV
ISO Category	Filcon 1b	Filcon 1b
Manufacture	Moulded	Moulded
Back Optic Zone Radius	8.4, 8.8,(-ve); 9.1 (+ve)	8.6, 8.9
Total Diameter	14.0 (-ve); 14.4 (+ve)	14.0
Centre Thickness	0.105	0.10

Elemental (CHN) analysis of the two materials indicated the proportions of carbon, hydrogen and nitrogen present in the two materials and is detailed in Table 6.3.3. The concentration of methacrylic acid in Surevue and Focus is 3.8% and <1% respectively and Etafilcon has a greater number of carboxyl groups that Vifilcon (2.4×10^{-4} vs 1.61×10^{-4} COO- mol g^{-1}). [477]

Table 6.3.3 - Elemental (CHN) Analysis Results

	Vifilcon	Etafilcon
Carbon	53.20%	50.10%
Hydrogen	7.40%	7.10%
Nitrogen	1.85%	0%
Oxygen	37.55%	42.80%

During the study lenses were cleaned and disinfected each day with Bausch & Lomb's "ReNu" disinfecting system, utilising a "rub and rinse" step prior to disinfection. The subjects were divided into two groups, each of which wore a pair of lenses on a daily wear basis for one month. At the end of the one month period a cross-over took place. Each test lens was exposed equally for each month. Subjects were reviewed in the clinic on collection, after two weeks (14 ± 2 days) and four weeks (28 ± 3 days). The study design utilised a randomised, double-masked paradigm.

6.3.2 Clinical Protocol

At each visit subjective satisfaction, lens fit, visual acuity, PLNIBUT, visible wettability on the slit lamp, Rudko deposition and physiological performance were assessed.

6.3.3 Analytical Protocol

At the end of the one month wearing schedule each lens was removed with plastic-tipped tweezers and placed in a glass vial containing sterile non-preserved saline. Surface deposition was evaluated by using fluorescence spectrophotofluorimetry, exciting at 280nm to evaluate proteins and 360nm for lipid. Total protein was assessed using transmission UV at 280nm.

6.3.4 Data Analysis

Summary statistics were calculated for all variables. The grading scales, being ordinal measurements, were considered to be non-normal data. All other data was tested for normality of distribution. Wilcoxon signed rank test was used to compare sets of non-parametric data and Students paired t-Test was used to compare normally distributed data. One-way RM ANOVA on ranks was used to compare differences between subjects. Correlations which included non-parametric data were undertaken using Spearman Rank Correlations. In all cases a "p" value of <0.05 was taken as being statistically significant.

6.3.5 Results

6.3.5.1 Subjective Results

a) Visual quality - These are summarised in Table 6.3.4.

Table 6.3.4 - Visual Quality (Median and 25% Quartile)

	Vifilcon		Etafilcon		p
After 2 weeks	9.0	8.0	9.0	7.75	NS
After 4 weeks	8.0	8.0	9.0	7.75	NS
p	NS		NS		

There was no significant difference at either follow-up visit between the two lens types or was there a significant change with either type between the 2 week and 4 week visit (p=NS).

b) Comfort - These results are summarised in Table 6.3.5.

Table 6.3.5 - Comfort (Median and 25% Quartile)

	Vifilcon		Etafilcon		p
After 2 weeks	9.0	7.00	9.0	7.00	NS
After 4 weeks	8.0	6.75	8.0	6.75	NS
p	NS		NS		

There was no significant difference between the lens types at either follow-up appointment or was there any change across time (p=NS).

c) Handling - These results are summarised in Table 6.3.6.

Table 6.3.6 - Handling (Median and 25% Quartile)

	Vifilcon		Etafilcon		p
After 2 weeks	8.0	7.00	6.0	5.0	0.03
After 4 weeks	8.0	6.75	7.0	5.0	0.05
p	NS		NS		

Whilst there was no change across time with either lens type, the Vifilcon lens was graded as easier to handle at both the 2 week (p=0.03) and 4 week visit (p=0.05).

d) Overall satisfaction - Table 6.3.7 indicates that no significant difference at any time was detected between the two lenses overall and that satisfaction remained high across the 4 week wearing period.

Table 6.3.7 - Overall Satisfaction (Median and 25% Quartile)

	Vifilcon		Etafilcon		p
After 2 weeks	8.0	6.0	8.0	6.0	NS
After 4 weeks	8.0	7.0	8.0	7.0	NS
p	NS		NS		

6.3.5.2 Visual Acuity

There was no significant difference at high or low contrast between the lens types at any appointment or was there any detectable reduction in acuity during the period of the study with either lens type. Mean high contrast visual acuities (VAR units) were 107 ± 3 for Focus and 108 ± 5 for Surevue (-0.12 logMAR; 6/4.5). Low contrast results were 96 ± 6 for Focus and 97 ± 4 for Surevue. This correlates well with published reports. [369 468]

6.3.5.3 Lens Fit

Table 6.3.8 indicates the average fitting characteristic of the lenses, as assessed by percentage tightness. No significant differences were found between the lens types or across visits.

Table 6.3.8 - Percentage Tightness (Mean±SD)

	Vifilcon	Etafilcon	p
Collection	52.2 ± 9.8	50.0 ± 7.2	NS
After 2 weeks	50.8 ± 8.2	48.6 ± 6.7	NS
After 4 weeks	51.2 ± 6.8	51.2 ± 7.2	NS
p	NS	NS	

6.3.5.4 Visible Deposition

Figures 6.3.1-6.3.3 detail the visible deposition encountered in the study.

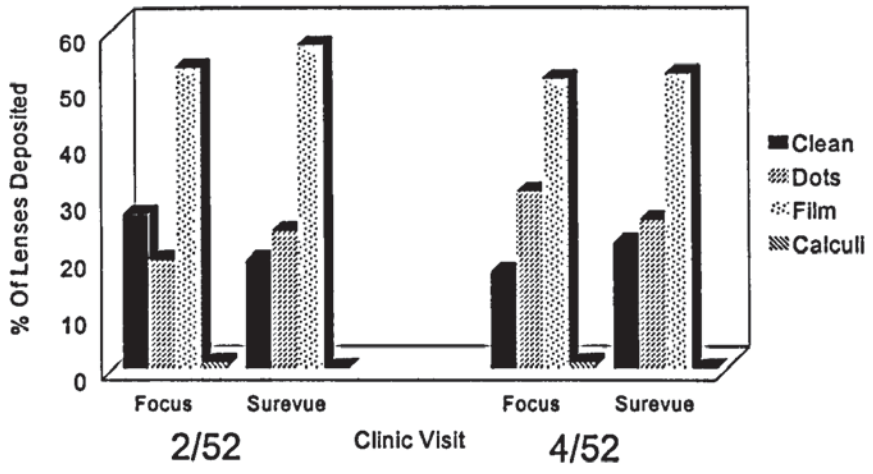


Figure 6.3.1 - Type of visible deposition by Rudko classification for 2 week and 4 week visit

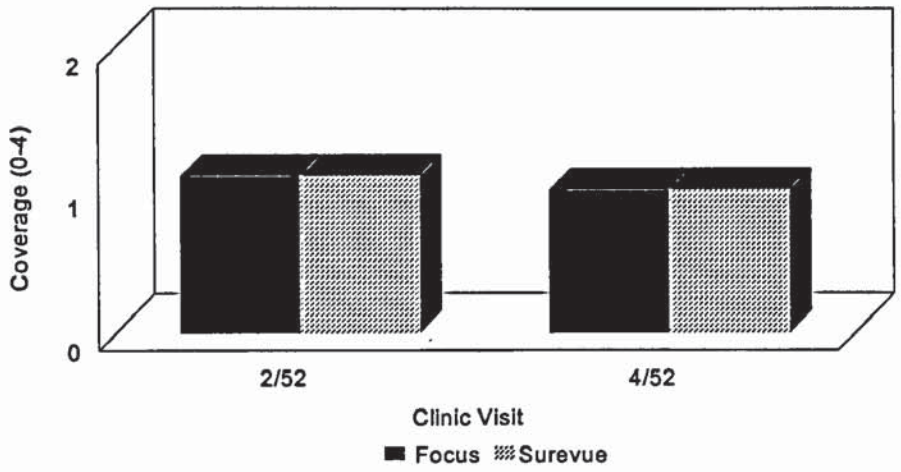


Figure 6.3.2 - Mean area of coverage by Rudko classification for 2 week and 4 week visit

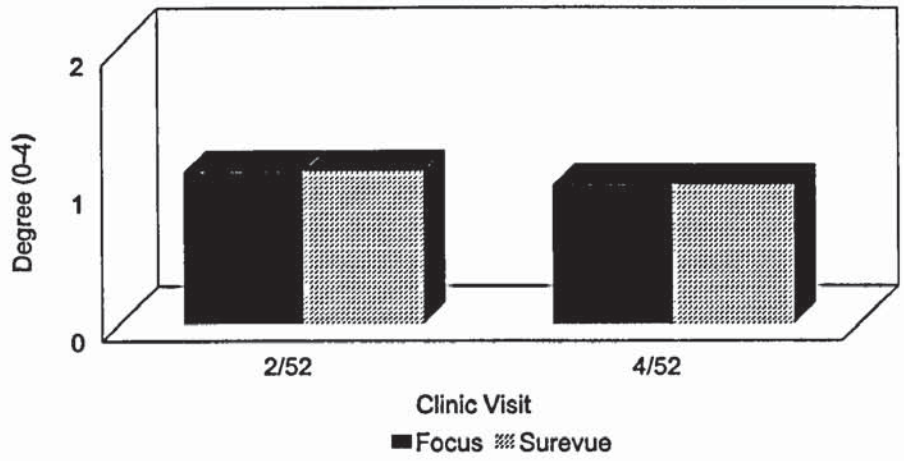


Figure 6.3.3 - Mean degree of deposition by Rudko classification for 2 week and 4 week visit

These results indicate that no significant difference in deposition occurred between the two materials (p=NS).

6.3.5.5 Front Surface Wettability

Table 6.3.9 and Figure 6.3.4 detail the mean (±sd) pre-lens non-invasive break-up time (PLNIBUT) in seconds for each visit with each wear schedule.

Table 6.3.9 - PLNIBUT (secs) Results (Mean ± SD)

	Vifilcon	Range	Etafilcon	Range	p
On Collection	12.9 ±4.9	3 - 21	13.0 ±5.0	6 - 15	NS
After 2 weeks	11.8 ±3.8	5 - 15	11.5 ±4.1	1 - 17	NS
After 4 weeks	10.2 ±4.5	6 - 13	10.1 ±4.2	3 - 14	NS
p	0.002		0.001		

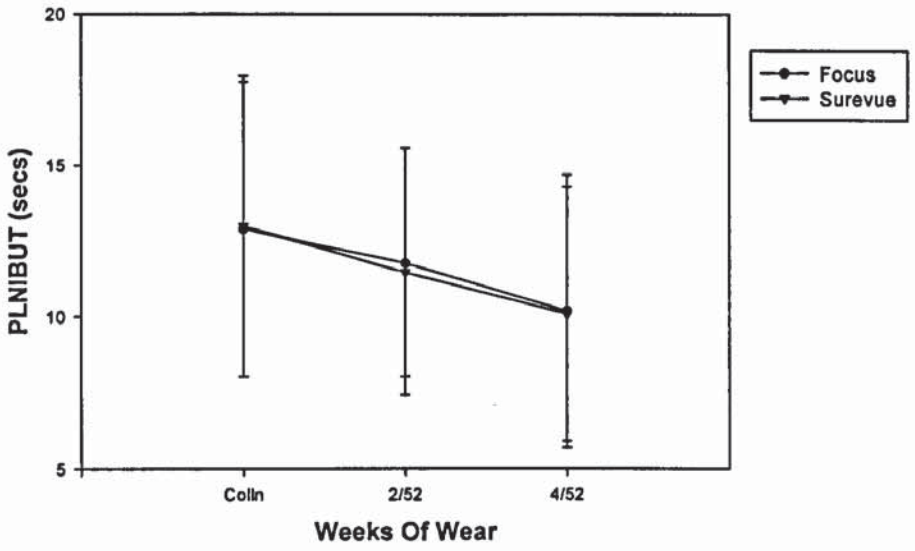


Figure 6.3.4 - Mean ± sd PLNIBUT by Loveridge Grid

There were significant inter-subject variations and a reduction in wettability over the period in which the lenses were worn with both lens types (p=0.002 for Vifilcon and p=0.001 for Etafilcon). Post-hoc testing using the Tukey test indicates that the reduction occurred between the collection and two-week appointment (p<0.05), with no significant reduction occurring between weeks two and four (p=NS). No significant differences were found between the lens types (p=NS).

6.3.5.6 Slit-Lamp Examination

There was no significant difference between the lens types for corneal staining, conjunctival staining, bulbar conjunctival hyperaemia or palpebral conjunctival appearance. The majority of the clinical findings were clinically insignificant. Mean corneal staining score was <grade 1, with the majority of the staining for each lens type (59% for Vifilcon and 64% for Etafilcon) being inferior "smile" type staining, invariably due to incomplete blinking [478] and dehydration, [479] which is a not uncommon finding in wearers of hydrogel content lenses [480] and is also the principle location for staining in non lens wearers. [453] [481] Of all possible occasions, the incidence of corneal staining was 44% with Vifilcon and 54% with Etafilcon, which correlates well with

other estimates of corneal staining in contact lens wearers. [392 454]

As with many moulded lenses [482 483] the incidence of recorded conjunctival/limbal staining was fairly high, with 68% of Vifilcon and 79% of Etafilcon lenses exhibiting some degree of conjunctival staining. However, this was clinically insignificant with a median score of "1" (mild) and mean of 1.6 for each lens type.

6.3.5.7 Deposition Analysis

The deposition results are outlined in Tables 6.3.10 - 6.3.12 and Figures 6.3.5 - 6.3.8.

a) Total Protein By UV

Figure 6.3.5, 6.3.6 and Table 6.3.10 indicates that Etafilcon deposited more protein than Vifilcon during the four weeks of wear ($p<0.0001$). Analysis of the results indicate that there was no significant difference between subjects with either lens material (p=NS).

Table 6.3.10 - Total Protein By UV (µg/lens)

	Mean ± SD	Range
Vifilcon	249 ± 141	95 - 737
Etafilcon	515 ± 130	205 - 715
p	<0.0001	

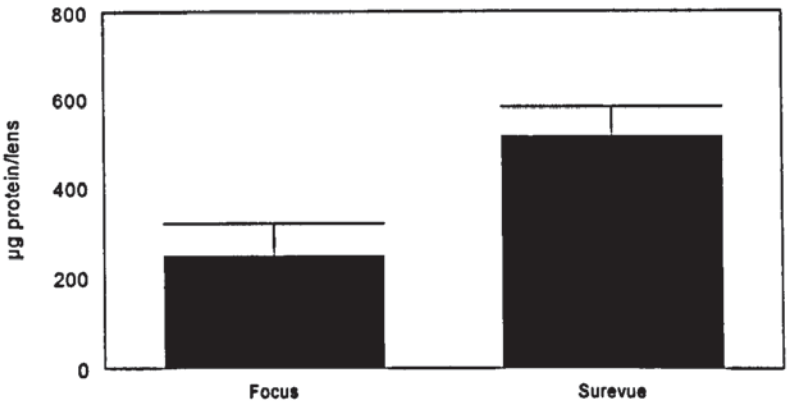


Figure 6.3.5 - Mean (±sd) total protein assessed by transmission UV

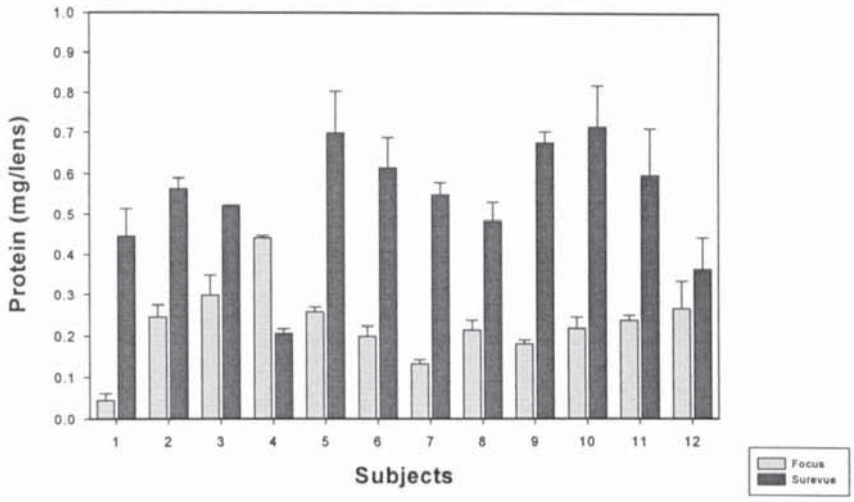


Figure 6.3.6 - Total protein assessed by UV for each subject.

b) Surface Protein By Fluorescence

Figures 6.3.7, 6.3.8 and Table 6.3.11 indicates that surface protein was greatest with the Etafilcon material ($p<0.0001$). Statistical analysis of the individual results indicates that there was no significant difference between subjects for either material (p=NS).

Table 6.3.11 - Surface Protein By Fluorescence (Fluorescence Units)

	Mean ± SD)	Range
Vifilcon	164 ± 64	64 - 302
Etafilcon	315 ± 98	168 - 495
p	<0.0001	

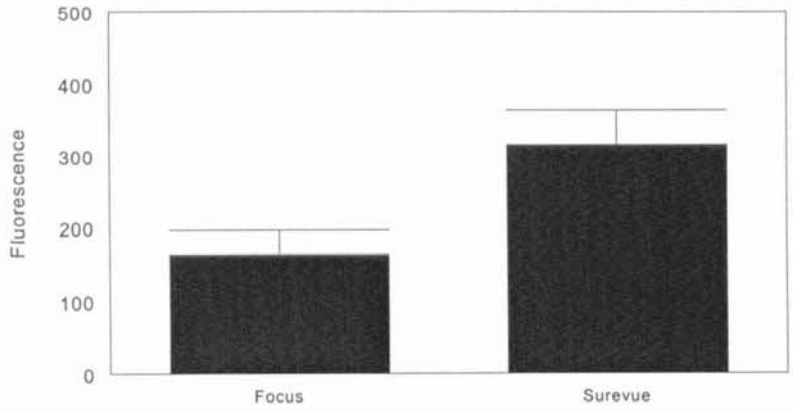


Figure 6.3.7 - Mean (±sd) surface protein by fluorescence @ 280nm

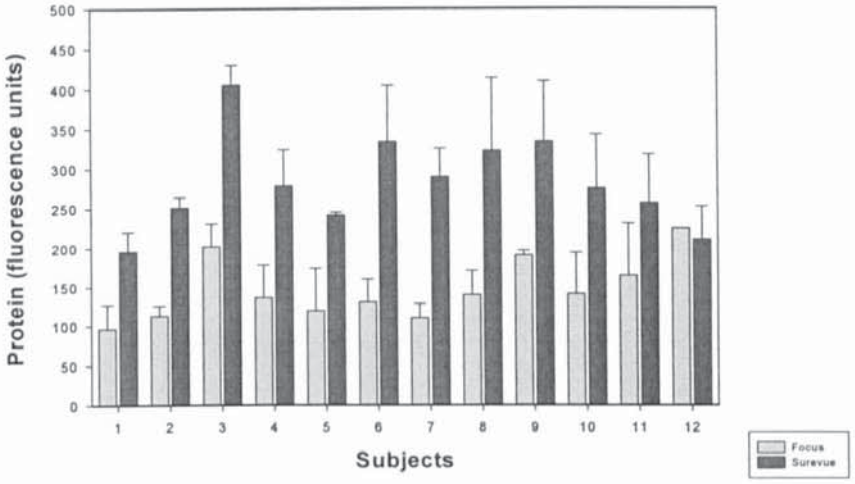


Figure 6.3.8 - Surface protein by fluorescence for each subject

c) Surface Lipid

Figures 6.3.9, 6.3.10 and Table 6.3.12 indicates that the Vifilcon material deposited more lipid than Etafilcon ($p<0.0001$). Statistical analysis of the results indicate that there is a significant difference between subjects for the Vifilcon material ($p<0.03$) but not for the Etafilcon material.

Table 6.3.12 - Surface Lipid By Fluorescence (Fluorescence Units)

	Mean ± SD	Range
Vifilcon	152 ± 86	35 - 390
Etafilcon	21 ± 11	8 - 65
p	<0.0001	

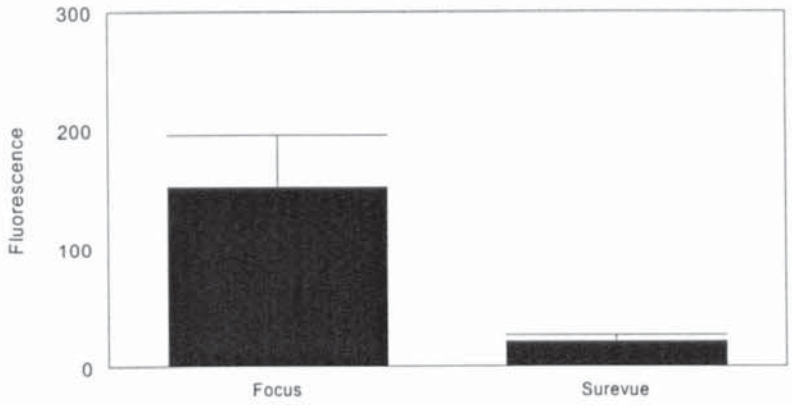


Figure 6.3.9 - Mean (±sd) lipid by fluorescence @ 360nm

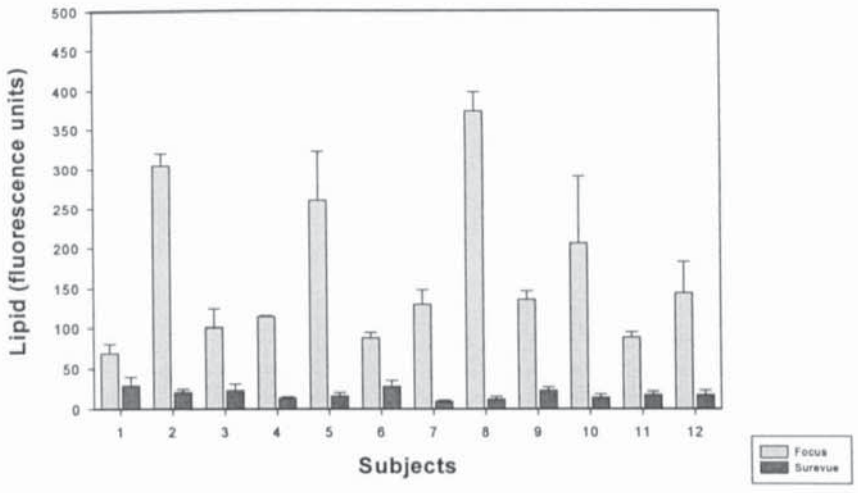


Figure 6.3.10 - Lipid deposition @ 360nm for each subject

6.3.5.8 Correlations

a) RE vs LE deposition

Spearman rank correlations for right eye vs left eye deposition for all three measured deposits are outlined in Table 6.3.13.

Table 6.3.13 - Deposition Correlations (RE vs LE)

	Vifilcon	Etafilcon
Total Protein (UV)	0.92	0.66
Surface Protein (Fluorescence)	0.61	0.54
Lipid (Fluorescence)	0.57	0.45

The results indicate that there is better correlation between eyes for Vifilcon than for Etafilcon and that there is better correlation for total protein than for either surface protein or lipid. This strongly suggests that caution should be exercised when interpreting the results from contralateral eye studies.

b) Protein vs lipid

Correlations of protein vs lipid for both materials are outlined in Table 6.3.14.

Table 6.3.14 - Protein vs Lipid

	Vifilcon	Etafilcon
UV vs lipid	0.22	-0.05
280 fluorescence vs lipid	0.17	0.24

These results clearly indicate that protein and lipid deposition occur irrespective of each other.

c) Visible vs Laboratory Deposition

No correlation was detected between these two variables for any of the measured deposition types ($r < 0.2$).

6.3.6 Discussion

In this study two ionic materials of similar EWC but significantly different chemical structure were compared. Clinical performance between the materials (comfort, visual quality, visual acuity and physiological performance) were virtually identical and indicate that such factors do not differ widely between lenses fabricated from materials within the same FDA group. Recently published work by Young and colleagues [484] indicated similar results, with no significant difference for visual quality, comfort and percentage tightness between the lenses. Their results report slightly lower visual quality and higher comfort than these results, but the order is identical to that reported in this study. Other studies comparing frequent replacement lenses demonstrate clearly that no single lens type will provide ideal clinical results for all patients and that practitioners must utilise more than one lens type if they are to provide maximum clinical success. [485 486]

As in the previous study, the differences in the materials' predisposition to deposit protein and lipid is clearly far more dependent upon the materials detailed chemical structure. The increased concentration of methacrylic acid in Etafilcon explains its propensity to deposit twice as much protein (a finding identical to that found by other workers [487]) and the increased amount of NVP in Vifilcon is the reason behind its ability to deposit more lipid. The explanations behind this have been explained in the previous study.

This study showed that there is a two-fold difference in protein and lipid deposition behaviour between materials classified in the same FDA group. No correlation was found between the visible on-eye deposition performance of the materials and the extent and nature of the tear components deposited, as determined by laboratory analytical techniques.

Single protein *in vitro* studies have clearly demonstrated that protein deposition is a complex factor, depending upon protein type, lens charge/water content and solvent characteristics. *In vitro,* Vifilcon lenses adsorb more albumin than Etafilcon, due to the high affinity of albumin for NVP, [477] whereas the reverse is true for lysozyme, due to the increased amount of methacrylic acid present. [487] *In vivo,* competition between proteins for access to the lens surface will further complicate matters. This study only investigated total protein, and further work to characterise the deposited protein types would prove a worthwhile exercise.

This study demonstrates that subjective comfort, visual quality, visual acuity and overall satisfaction remains relatively unaffected over a four week time period. However, PLNIBUT reduces over such a time frame. Examination of the deposition results indicate that there is greater variability between subjects for lipid deposition and less inter-subject variability for protein deposition and that deposition between eyes is only loosely correlated.

6.4. PROTEIN LOCATION

Useful information about the position of the deposited protein is obtained by combining information from all three material studies. Whilst such analysis is not ideal as the subjects in each study were different, combining the results provides an overview of the protein deposition which could not be achieved in any other way, from the studies conducted in this thesis.

Plots of fluorescence (surface protein) versus UV results (total protein) enable the position of the deposited protein to be located.

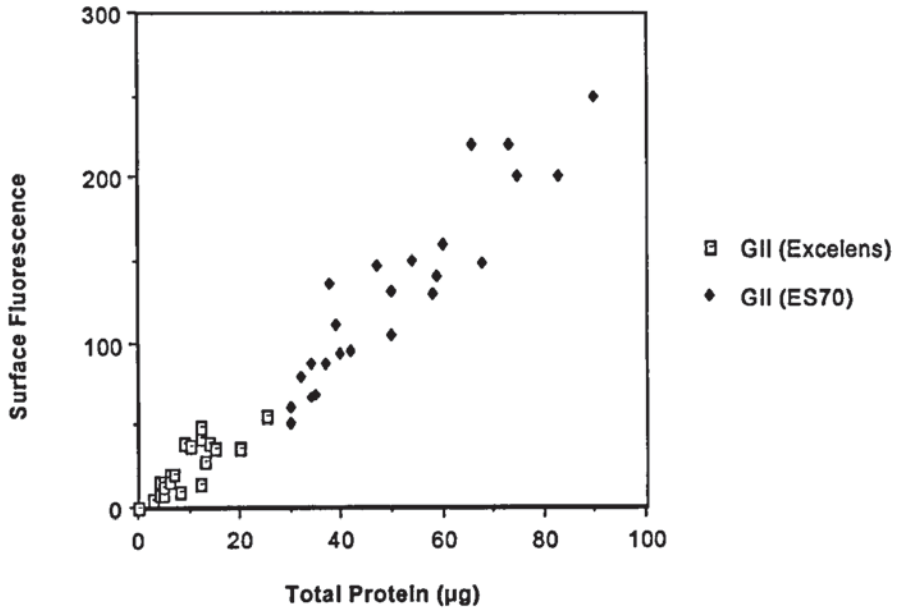


Figure 6.4.1 - Surface vs bulk protein for ES70 and Excelens materials (FDA group II)

Figure 6.4.1 clearly indicates that there is an excellent correlation between surface and bulk protein (r>0.9). Other studies conducted in-house (unpublished data) indicate that the protein on neutral group II materials is located at, or very near, the surface. The results from these materials and those seen with another NVP-based group II material (Medalist 66) worn for one month and reported earlier in this thesis (Chapter 5, page 149) appear at odds. In this earlier study only a poor correlation could be demonstrated between the surface and bulk deposited protein (r = 0.28). Previous *in vitro* studies in these laboratories [431 488] have also found that group II materials of all types (commercially available and novel in nature) all correspond to the general pattern found in Figure 6.4.1; it unlikely that Medalist 66 should not conform to this general trend. The likely reason for this apparent departure is found in Table 4.2 (page 124), in which the background fluorescence of materials is examined. This table clearly shows that Medalist 66 has a very high - and unpredictable - background fluorescence. It is therefore suggested that this non-linearity is caused mainly by the variability in the background fluorescence, which influences the fluorescent value obtained with this material. Indeed, if figure 5.1.7 (page 149) is closely scrutinised it is obvious that the majority of results are found around the value of 35 µg of total protein relating to a surface fluorescence of 130 units. Examination of figure 6.4.1 indicates that a value of 35 µg correlates with approximately 75 units with these neutral polymers. This would suggest that the surface fluorescence values with Medalist 66 were elevated, as proposed by the results in

table 4.2. Adding small amounts of methacrylic acid (such as occurs in Vifilcon) produces a different situation entirely. Figure 6.4.2 indicates that incorporating small amounts of methacrylic acid results in a shift of the deposited protein into the bulk and away from the surface.

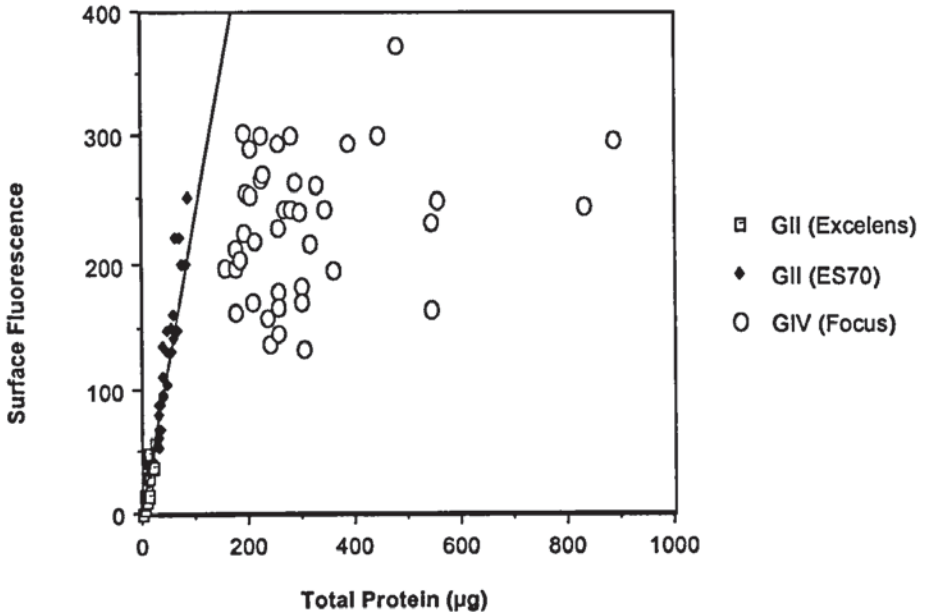


Figure 6.4.2 - Surface vs bulk protein for Excelens and ES70 (FDA group II) and Vifilcon (FDA group IV)

The reason behind this is that the methacrylic acid attracts substantial quantities of positively charged protein (in particular lysozyme), which is sufficiently small to penetrate the lens surface. Adding greater amounts of methacrylic acid (as occurs in Etafilcon) results in movement of the protein even further into the bulk, as shown in Figure 6.4.3

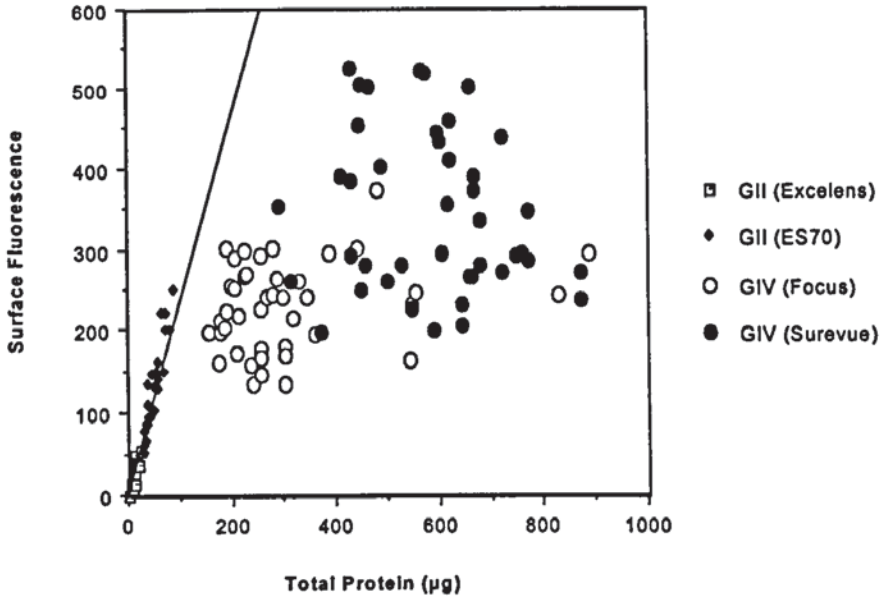


Figure 6.4.3 - Surface vs bulk protein for Excelens and ES70 (FDA group II) and Vifilcon and Etafilcon (FDA group IV)

This pattern of protein location in such materials has been confirmed in this laboratory by other workers using plasma etching techniques. [488] Work by Meadows and Paugh using confocal microscopy [489] indicated clearly that the amount of deposited protein does not directly correlate with wearing period, that inter-subject differences clearly exist, that group IV materials deposit more protein than group I materials and that group IV materials deposit protein predominantly in the bulk rather than on the surface. All of these results are entirely in accord with the results published in this Chapter.

6.5. KEY POINTS

- Inter- and intra-material differences exist in the deposition behaviour of FDA materials, which are closely related to the detailed chemistry of the materials in question.

  i. FDA group IV materials deposit predominantly protein, due principally to the ionic carboxylate groups.

  ii. FDA group II materials deposit predominantly lipid, due principally to the presence of N-vinyl pyrrolidone.

  iii. Protein deposition is principally related to charge and is therefore material dominated, with little inter-subject differences.

- Lipid deposition exhibits marked inter-subject differences.

- Protein location is determined by material charge. In neutral group II hydrogels the protein is almost exclusively located on the surface, whereas in ionic group IV materials the protein is progressively moved into the material, depending upon the degree of ionicity present.

- Over a six month wearing schedule subjective satisfaction with lenses reduces and this reduction is not necessarily directly correlated with deposition.

- PLNIBUT reduces with increasing wearing period, irrespective of material type.

- Visible deposition is only a poor indicator of deposition type, but the presence of considerable numbers of lens calculi indicates the presence of increased quantities of lipoidal deposition.

Chapter 7

The Influence Of Replacement Period On The Spoilation and Clinical Performance Of Hydrogel Lenses

"Knowledge is the intellectual manipulation of carefully verified observations". [1]

To date there is little, if any, available information concerning the optimum time of replacement of hydrogel lenses, with the life expectancy of polyHEMA lenses being approximately one year [490] and high water content lenses being 6-7 months. [491] *In vitro* studies looking at the influence of time on deposition have been conducted, [164 - 166] but these studies may not be directly transferable to the *in vivo* state. There is some evidence to suggest that deposit formation is in part due to the thinning and drying of the tear film, resulting in the lipid layer directly collapsing onto the lens surface and partitioning into the dry lens surface. [71 82 91] *In vitro* studies in which lenses are soaked in protein solutions cannot account for such phenomena. In addition, factors such as the shear force of the lids, environmental influences and differences in tear film composition further complicate the arguments.

The purpose of this chapter is to evaluate the degree of deposition control that may be achieved by replacing FDA group II and group IV lenses following wearing periods between three months and one day and to chart the kinetics of deposit build-up over this time. Such studies will provide useful information regarding the optimum time for replacement of differing contact lens materials.

[1] Sigmund Freud

7.1 THREE-MONTHLY VS ONE MONTHLY REPLACEMENT OF AN FDA GROUP II MATERIAL

7.1.1 Materials and Methods

Twelve subjects (six male and six female) were entered onto the study, whose demographic details are described in Table 7.1.1.

Table 7.1.1 - Subject Demographics

	Mean ± SD	Range
Age (years)	27.9 ± 6.7	20 - 44.5
Flat Keratometry Reading	7.90 ± 0.31	7.50 - 8.30
Steep Keratometry Reading	7.85 ± 0.32	7.30 - 8.20
Sphere (D)	-2.72 ± 1.32	-5.25 to -1.00
Cyl (D)	-0.32 ± 0.30	-0.75 to 0.00

All subjects were fitted with Precision UV from Pilkington Barnes-Hind, the relevant details of which are given in Table 7.1.2. All subjects used Bausch & Lomb's "ReNu" disinfecting system, using a "rub and rinse" step before disinfection. The subjects were divided into two equal groups, one of which wore the lenses for three months and the other for three consecutive one monthly periods, before replacement occurred. At the end of the three-month period a cross-over took place. Subjects were reviewed in the clinic on collection, after one week (7 ± 1 days), one month (28 ± 3 days) and three months (90 ± 7 days).

Table 7.1.2 - Lens Parameters

Water Content (%)	74%
Monomers	MMA/NVP
USAN	Vasurfilcon A
FDA Category	Group II
ISO Category	Filcon 4a
Manufacture	Moulded
Back Optic Zone Radius (mm)	8.70
Total Diameter (mm)	14.40
Centre Thickness (mm)	0.14

7.1.2 Clinical Protocol

At each visit subjective satisfaction, visual acuity, PLNIBUT, Rudko deposition and physiological performance were performed, as previously described.

7.1.3 Analytical Protocol

At the end of the wearing schedule (4 or 12 weeks) each lens was removed with plastic tipped tweezers and collected as previously described.

Lipid analysis via fluorescence was conducted, as previously described. The UV blocker in Precision UV prevented the use of fluorescence and standard, non-destruction transmission UV to analyse the protein deposition, although this was the only group II frequent replacement lens available upon commencement of the project. Protein deposition was evaluated using a modified Lowry technique on a Hitachi U2000 spectrophotometer. As with any destructive technique it is impossible to completely remove all of the deposited protein, with approximately 10±5 μg of protein remaining firmly adherent to these Group II lenses when protein is extracted using this technique. [431]

7.1.4 Data Analysis

Summary statistics were calculated for all variables. A one-way repeated measures ANOVA on ranks was used to compare changes across time and between subjects. Correlations were undertaken using a Spearman Rank test. A "p" value of <0.05 was considered statistically significant.

7.1.5 Results

7.1.5.1 Subjective Results

a) Visual quality

The visual quality results are summarised in Table 7.1.3. There was no significant difference at any visit between the two schedules or with either schedule between the one week (1/52), one month (1/12) and three month (3/12) visits (p=NS).

Table 7.1.3 - Visual Quality (Median and 25% Quartile)

	Monthly Replacement		3 Monthly Replacement		p
After 1/52	9.5	9.0	9.0	9.0	NS
After 1/12	9.0	9.0	9.0	9.0	NS
After 3/12	9.5	9.0	9.5	9.0	NS
p	NS		NS		

b) Comfort

These results are summarised in Table 7.1.4. There was no significant difference between the wear schedules at any of the visits and no change across time (p=NS).

Table 7.1.4 - Comfort (Median and 25% Quartile)

	Monthly Replacement		3 Monthly Replacement		p
After 1/52	9.0	9.0	9.5	9.0	NS
After 1/12	9.0	8.5	9.0	9.0	NS
After 3/12	9.0	8.0	9.0	8.25	NS
p	NS		NS		

c) Overall satisfaction

Table 7.1.5 indicates that no significant difference was detected with the two schedules at the 1/52 and 1/12 visit, but there was a significant reduction at the 3/12 review with the 3 month replaced lenses that was not present with the 1 month replacement lenses. Tukey testing indicates that the reduction occurred between the one-week and three-month visit ($p<0.05$).

Table 7.1.5 - Overall Satisfaction (Median and 25% Quartile)

	1 Month Replacement		3 Month Replacement		p
After 1/52	9.5	9.0	10.0	9.0	NS
After 1/12	9.0	9.0	9.0	9.0	NS
After 3/12	9.5	9.0	8.0	8.0	0.04
p	NS		0.04		

7.1.5.2 Visual Acuity

There was no significant difference at high or low contrast between the lens types at any appointment or was there any detectable reduction in acuity during the study with either lens type (p=NS). Mean high contrast visual acuities (VAR units) were 107 ± 6 for the one-month lenses and 106 ± 5 for the three-month lenses (-0.16 logMAR; 6/4.5). Low contrast results were 97 ± 6 for the one-month lenses and 96 ± 5 for the three-month lenses .

7.1.5.3 Visible Deposition

Figures 7.1.1 - 7.1.3 detail the visible deposition encountered in the study.

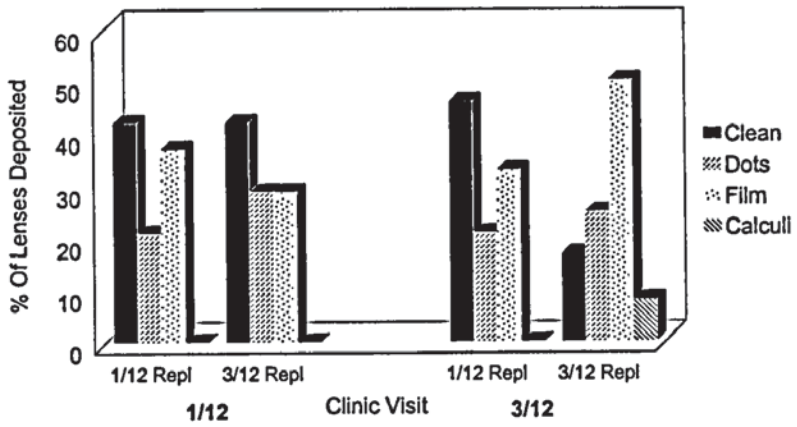


Figure 7.1.1 - Rudko Type - comparison of the visible type of deposition at the 1/12 and 3/12 visits for both replacement schedules.

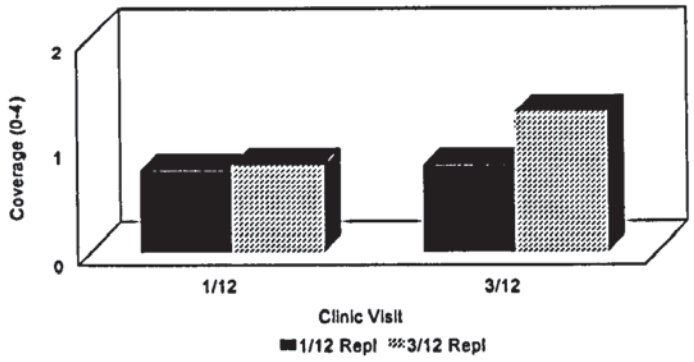


Figure 7.1.2 - Rudko Coverage - mean visible deposited area at the 1/12 and 3/12 visits, for both replacement schedules.

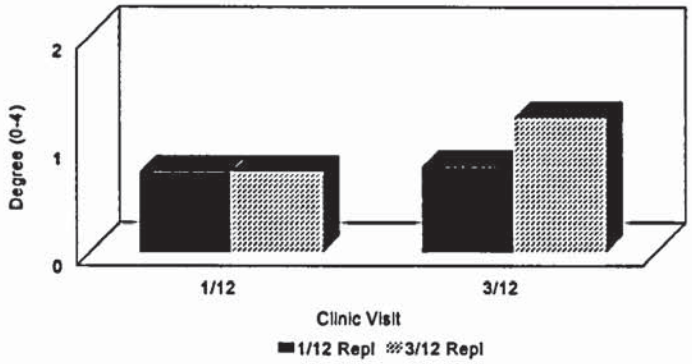


Figure 7.1.3 - Rudko Degree - mean visible degree of deposition at the 1/12 and 3/12 visits, for both replacement schedules

The differences were significant for type ($p<0.02$), coverage ($p<0.02$) and amount of deposition ($p<0.03$) at the three-month review.

These results show that a significant increase in visible deposits occurs in a high water content neutral material containing NVP worn for three months when compared with one worn for one month.

7.1.5.4 Front Surface Wettability

Table 7.1.6 and Figures 7.1.4 and 7.1.5 detail the mean (±sd) pre-lens non-invasive break-up time (PLNIBUT) in seconds for each visit with each wear schedule.

Table 7.1.6 - PLNIBUT Results (Mean ± SD)

	Monthly Repl	Range	3 Monthly Repl	Range	p
On Collection	12.4 ±2.7	7 - 20	12.0 ±1.9	8 - 18	NS
After 1/52	12.0 ±2.3	9 - 17	11.5 ±3.2	6 - 16	NS
After 1/12	11.6 ±1.7	7 - 19	11.2 ±2.1	7 - 16	NS
After 3/12	11.7 ±2.9	7 - 24	9.3 ± 1.7	5 - 15	0.003
p	NS		0.002		

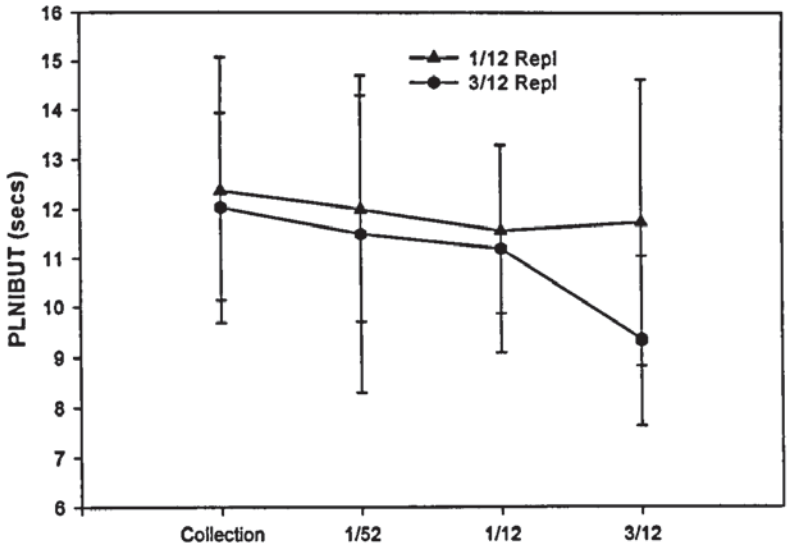


Figure 7.1.4 - Mean (±sd) PLNIBUT by Loveridge Grid for both replacement schedules for all subjects across all clinical visits.

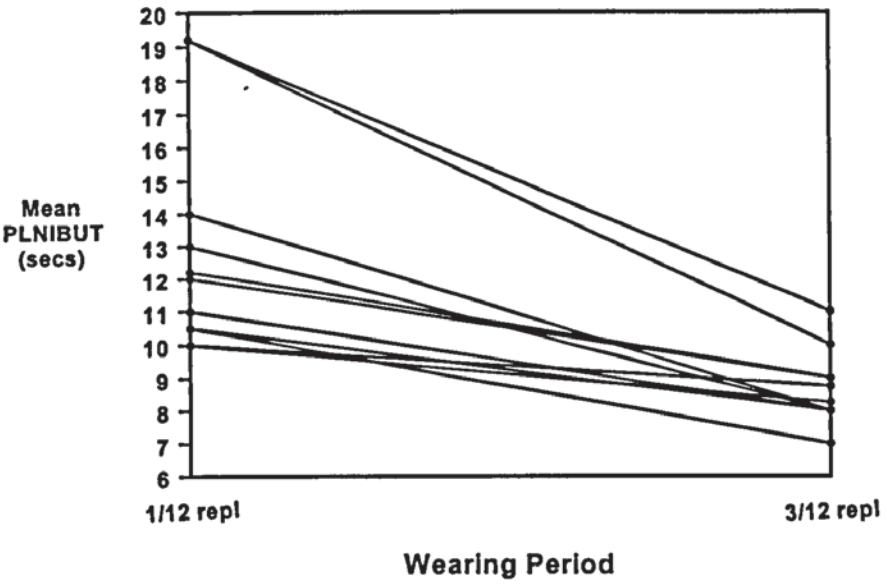


Figure 7.1.5 - PLNIBUT results by Loveridge Grid. The results show the change in mean PLNIBUT at the 3/12 visit for both replacement periods. Solid lines join the results for individual subjects and demonstrate that there is a reduction in PLNIBUT for all subjects if the 1/12 and 3/12 results are compared.

There are clearly large significant inter-subject variations and a reduction in wettability over the period in which the lenses were worn with the 3 month replacement lenses ($p<0.002$). Post-hoc testing using the Tukey test indicates that the differences occur between the one and three-month visits ($p<0.05$). No significant differences were found with the 1 month replacement lenses (p=NS).

7.1.5.5 Slit-Lamp Examination

There was no significant difference between the wearing periods for corneal staining, conjunctival staining, bulbar conjunctival hyperaemia or palpebral conjunctival appearance.

7.1.5.6 Spoilation Analysis

The lipid and protein spoilation results for each wearing schedule are summarised in Tables 7.1.7 and 7.1.8 and Figures 7.1.6 - 7.1.9. Figures 7.1.6 and 7.1.8 indicate the individual subject variation that occurs and Figures 7.1.7 and 7.1.9 the means (±SD) for each wearing period.

Table 7.1.7 - Protein Results (µg/lens)

	Mean ± SD	Range
3/12 Replacement	106 ±16	82 - 133
1/12 Replacement	42 ±7	31 - 54
p	<0.0002	

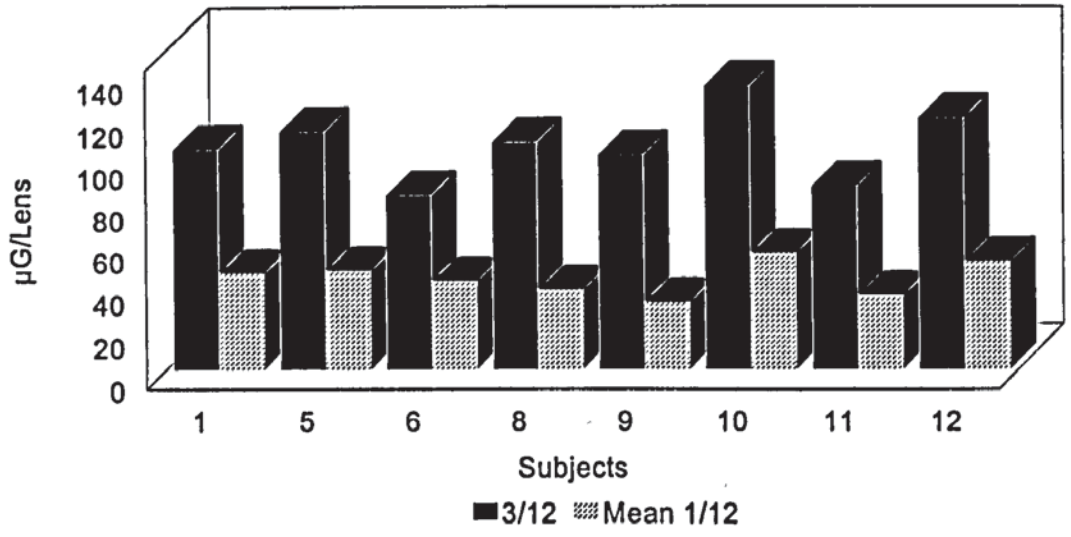


Figure 7.1.6 - Extracted protein for individual subjects for both replacement periods, indicating the differences between subjects compared with replacement periods.

The results indicate that there is no significant difference between subjects for either replacement frequency (p=NS).

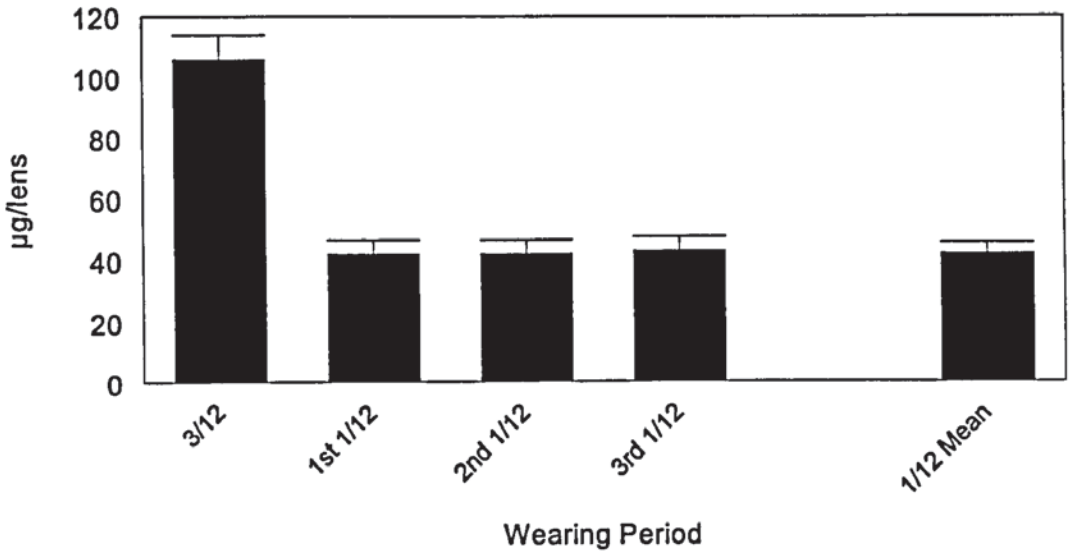


Figure 7.1.7 - Mean extracted deposited protein for all subjects.

Table 7.1.8 - Lipid Results (fluorescence)

	Mean± SD	Range
3/12 Replacement	97 ±52	29 - 180
1/12 Replacement	54 ±34	33 - 170
p	<0.0002	

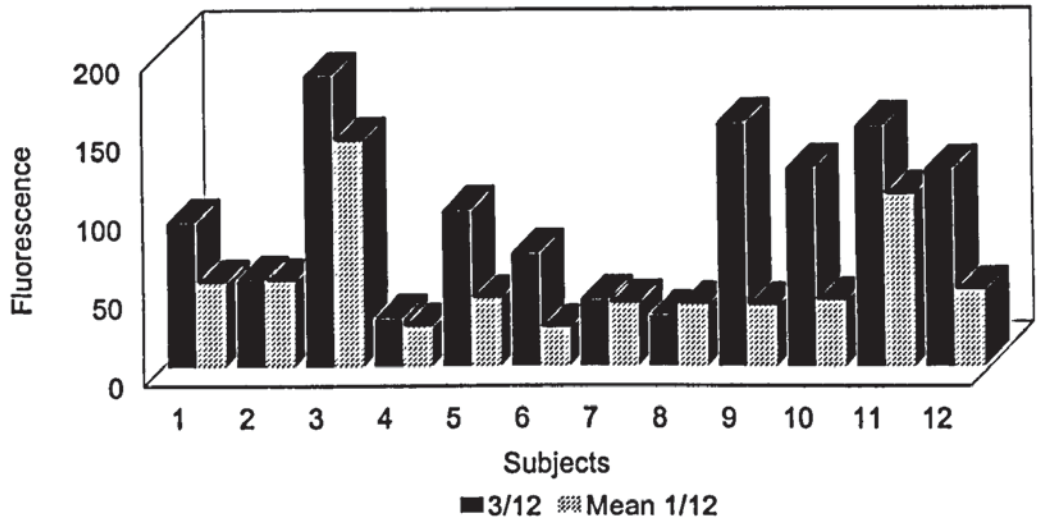


Figure 7.1.8 - Extracted lipid for individual subjects for both replacement periods, indicating the differences between subjects compared with replacement periods.

These results indicate that there is a significant difference between subjects ($p<0.004$), clearly showing that lipoidal deposition is subject dependent.

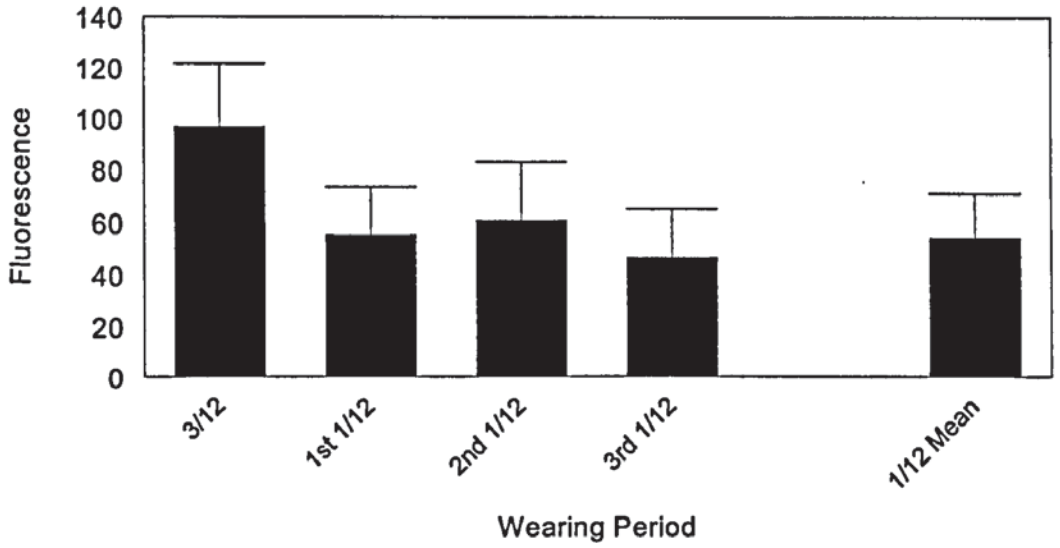


Figure 7.1.9 - Mean lipid deposited for all subjects.

The results indicate that protein and lipid spoilation are significantly worse with the 3 month replaced lenses.

7.1.5.7 Correlation of Wettability vs Deposition

No correlation existed between the change in wettability across appointments and the deposition evaluated in the laboratory for either protein or lipid ($r<0.2$). This shows that while deposition increased over the wearing period and wettability reduced, these were not directly inter-related.

7.1.6 Discussion

This study shows that high and low contrast visual acuity, physiological performance and subjective visual quality and comfort remain unaltered if Vasurfilcon lenses are replaced at least every three months. However, subjective satisfaction, lens front surface wettability, visible deposits and analytically evaluated deposits are all improved by replacing the lens regularly.

Visible typing of deposits results in an inaccurate estimate of the true level of deposition present, when compared with analytical evaluation of worn lenses, as demonstrated in Chapter 6 of this thesis and by other workers. [115 116] Gross deposits, such as lens calculi, can provide an indication of the degree of lipoidal deposition present, particularly in lenses worn for longer than one month, as demonstrated in Chapter 6 and this study. FDA group II lenses attract more lipid than other FDA groups [203] and deposit high levels of lens

calculi, as shown in this study. Figures 7.1.1 - 7.1.3 show that the level of deposition increases with wearing time and can be effectively controlled by replacing the lens more frequently.

As previously discussed, Guillon and co-workers have shown that suitable replacement schedules are highly patient dependent, [363 373] a finding reiterated in this study and evidenced by the wide range and standard deviation encountered in the PLNIBUT measurements. Figures 7.1.4 and 7.1.5 indicate that PLNIBUT reduces with wearing period and is significantly reduced if Vasurfilcon (FDA group II) lenses are replaced at a longer replacement frequency than one month. Suitable replacement schedules are also intimately related to material characteristics and other FDA group II materials may be suitable for longer replacement frequencies of up to three months. [373] The reason for this is almost certainly due to the absence of significant quantities of either methacrylic acid (MA) or N-vinyl pyrrolidone (NVP), both of which will enhance spoilation (as shown in Chapter 6) and ultimately reduce the biocompatability of the lens surface.

Examination of the individual extractable protein levels (Figure 7.1.6) indicates that protein deposition varied little among these individuals after identical wearing periods. This controlled clinical study clearly shows that using this FDA group II lens type with the combination of wearing times and solutions used here that deposition is cumulative and increases with wearing time, a finding in agreement with other studies that have examined group IV materials up to 7 days post-insertion. [7 11]

Examination of the lipoidal results (Fig 7.1.8) reveals much greater subjective variation, with some subjects (subjects 2, 4, 7 and 8) depositing similar amounts of lipid irrespective of the wear period and others (subjects 5, 6, 9, 10 and 12) depositing up to twice the level of lipid over the longer wearing time. Analysis of the mean results (Figs 7.1.7 and 7.1.9) shows that monthly replacement will reduce protein deposition by 60% and lipid deposition by 44% when compared with a three-month wearing period, supporting the premise behind the replacement of Vasurfilcon lenses on a monthly basis. Individual differences in deposition clearly exist and certain subjects may well benefit from more frequent replacement. However, from a practice management position it is far easier to have a "blanket" decision on replacement times rather than to try to maximise the replacement frequency for each individual patient.

Further evidence supporting the use of shorter replacement periods is found in the study by Pritchard et al. [5] This study reported reduced corneal staining, limbal and bulbar injection and visible deposits and enhanced overall satisfaction with frequent replacement lenses when compared with conventional lenses. Contradictory evidence is reported in a comparative study in which lenses were replaced every two weeks versus every three months, [492] in which it was shown that the longer replacement period was preferred. However, the study used different lens designs and materials and asked subjects to consider cost, handling and other issues such as convenience that were clearly not related to spoilation characteristics. To reduce such variables the study should have been conducted using the same material and design for both replacement periods, as in the study conducted in this thesis and in the study by Pritchard et al. [5]

This project investigated the differences between one and three month replacement schedules with a group II material. In the next study the deposition that occurs in both FDA group II and IV materials over shorter wearing periods is investigated.

7.2 ONE DAY VS ONE MONTH REPLACEMENT OF FDA GROUP II AND GROUP IV MATERIALS

The purpose of this study was to evaluate the spoilation characteristics of two different types of frequent replacement materials when worn for periods between 1 day and 28 days. To date the options for one-day disposable lenses in the UK cover both group II and group IV lenses. [456] [493] While several studies have demonstrated that such lenses produce increased patient comfort, vision and overall satisfaction, [330] [495 - 497] no study to date has investigated if such improvements are correlated with changes in the degree of deposition.

7.2.1 Materials & Methods

Twenty-two subjects (four male and eighteen female) were entered onto the study, whose demographic details are described in Table 7.2.1.

Table 7.2.1 - Subject Demographics

	Mean ± SD	Range
Age (years)	32.4 ± 10.6	18 - 48
Flat Keratometry Reading	7.70 ± 0.21	7.35 - 8.05
Steep Keratometry Reading	7.60 ± 0.22	7.20 - 8.00
Sphere (D)	-3.40 ± 1.2	-5.75 to -0.75
Cyl (D)	-0.41 ± 0.32	-1.00 to 0.00

All subjects were fitted with the two test lenses, which were Vistakon "Acuvue" and Bausch & Lomb "Medalist 66", details of which are given in Table 7.2.2. All subjects used Bausch & Lomb's "ReNu" disinfecting system, using a "rub and rinse" step before disinfection.

Table 7.2.2 - Lens Parameters

	Acuvue	Medalist 66
EWC	58%	66%
Monomers	HEMA/MA	HEMA/VP
USAN	Etafilcon A	Alphafilcon A
FDA Category	Group IV	Group II
ISO Category	Filcon 1b	Filcon 4a
Manufacture	Moulded	Moulded
Back Optic Zone Radius (mm)	8.40; 8.70; 9.00	"Medium"
Total Diameter (mm)	14.00	14.20
Centre Thickness (mm)	0.07	0.10

The subjects were divided into two equal groups, such that each test lens was exposed equally for each wearing schedule. Subjects wore lenses for 1, 7, 14, 21 and 28 days in a randomised fashion. Subjects were reviewed in the clinic on collection and at the end of the 71-day wearing period. After the first set of lenses were collected, a cross-over took place and the subjects repeated the wearing schedules with the second lens type.

7.2.2 Analytical Protocol

At the end of the designated wearing schedule each lens was collected by the subject for analysis. Previous work in these laboratories (Chapter 4, pages 118-121) has discussed the influence of lens handling on deposition. To prevent this subjects were provided with alcohol wipes (Chauvin Pharmaceuticals' "Medi-Wipes") to clean any residual lipid from their fingers. Lenses were collected and stored as previously described.

Surface deposition was evaluated using fluorescence spectrophotofluorimetry, exciting at 280nm to evaluate proteins and 360nm for lipid. Total protein was assessed using transmission UV at 280nm.

7.2.3 Data Analysis

Summary statistics were calculated for all variables. Comparisons between materials for each period of wear were undertaken using a Wilcoxon Signed Rank test, with comparisons over time being undertaken using a one way repeated measures ANOVA. In all cases a "p" value of <0.05 was considered significant.

7.2.4 Results

7.2.4.1 Total Protein

Total protein by transmission UV is detailed in Table 7.2.3 and Figure 7.2.1.

Table 7.2.3 - Total Protein (Mean±sd) By UV - µg protein/lens

	1 day	7 days	14 days	21 days	28 days	p
Etafilcon	140 ± 38	470 ± 68	482 ± 67	475 ± 88	493 ± 101	<0.0001
Alphafilcon	15 ± 4	20 ± 4	22 ± 7	32 ± 4	40 ± 7	<0.0001
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

As expected, the group IV material deposited substantially more protein after all wearing schedules than the group II material. The difference was significant at all appointments ($p<0.0001$). Figure 7.2.1 shows that the kinetics of the protein deposited in the bulk was markedly different for the two materials. In the group IV material the protein increased significantly between day 1 and day 7 of wear. The change in deposition across time was significant for the group IV material ($p<0.0001$), with SNK post-hoc testing indicating that the difference was significant ($p<0.05$) between one day and all other wearing schedules. There was no difference (p=NS) between all other wearing schedules.

There was also a significant increase in total protein deposition across time with the group II material ($p<0.0001$). SNK post-hoc testing showed that there was no difference between one day and one week (p=NS), but then the degree of deposition progressively increased across all appointments ($p<0.05$).

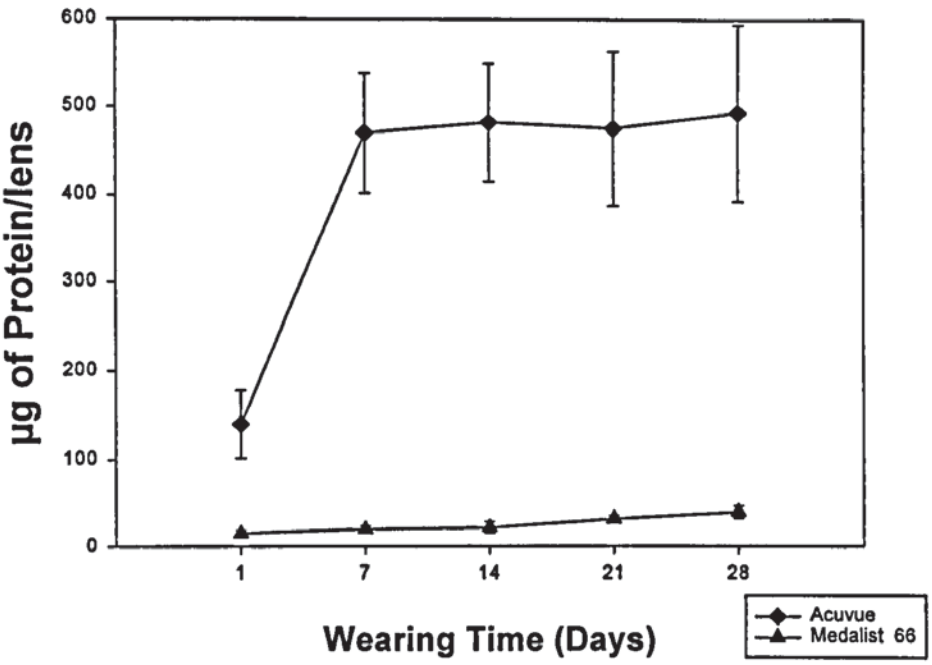


Figure 7.2.1 - Total protein (mean ±sd) for all lenses across all wearing schedules, as assessed by transmission UV.

7.2.4.2 Surface Protein

Surface protein by fluorescence is detailed in Table 7.2.4 and Figure 7.2.2.

Table 7.2.4 - Surface Protein (Mean ± SD) By Fluorescence - Fluorescence Units

	1 day	7 days	14 days	21 days	28 days	p
Etafilcon	342 ± 97	372 ± 98	357 ± 90	364 ± 100	380 ±101	NS
Alphafilcon	100 ± 31	112 ± 28	129 ± 38	122 ± 44	130 ± 58	NS
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

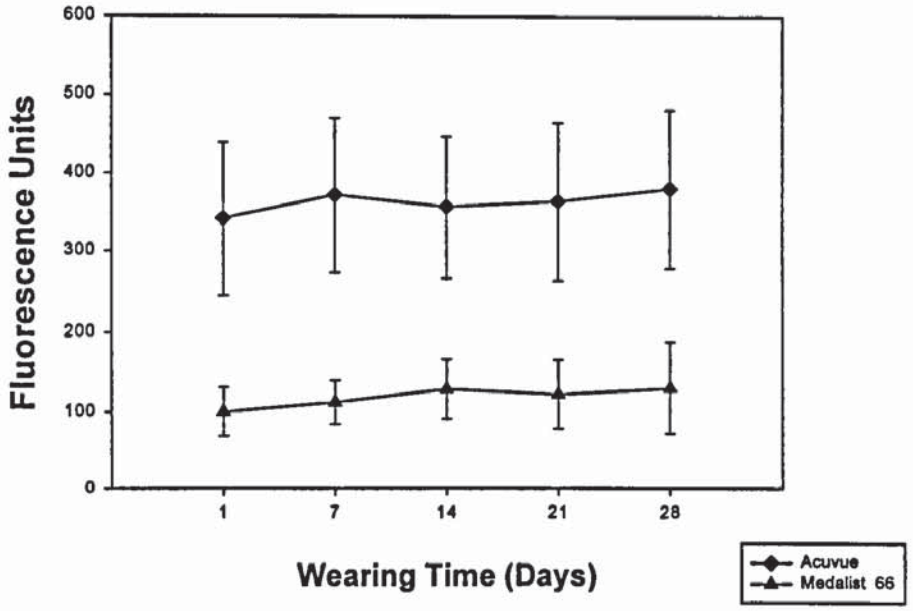


Figure 7.2.2 - Surface protein (mean ±sd) for all lenses across all wearing schedules, as assessed by fluorescence.

As expected, the group IV material deposited substantially more protein after all wearing schedules than the group II material. The difference was significant at all appointments ($p<0.0001$). Figure 7.2.2 indicates that the kinetics of the protein deposited on the surface was similar for the two materials, with both materials exhibiting little increase in surface deposition across the period of the study. The change in deposition across time was insignificant for both materials (p=NS).

7.2.4.3 Lipid

Lipid deposition by fluorescence is detailed in Table 7.2.5 and Figure 7.2.3.

Table 7.2.5 - Surface Lipid (Mean ± SD) By Fluorescence - Fluorescence Units

	1 day	7 days	14 days	21 days	28 days	p
Etafilcon	26 ± 13	28 ± 13	24 ± 8	28 ± 13	29 ± 12	NS
Alphafilcon	74 ± 47	193 ± 110	339 ± 164	380 ± 200	427 ± 223	<0.0001
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

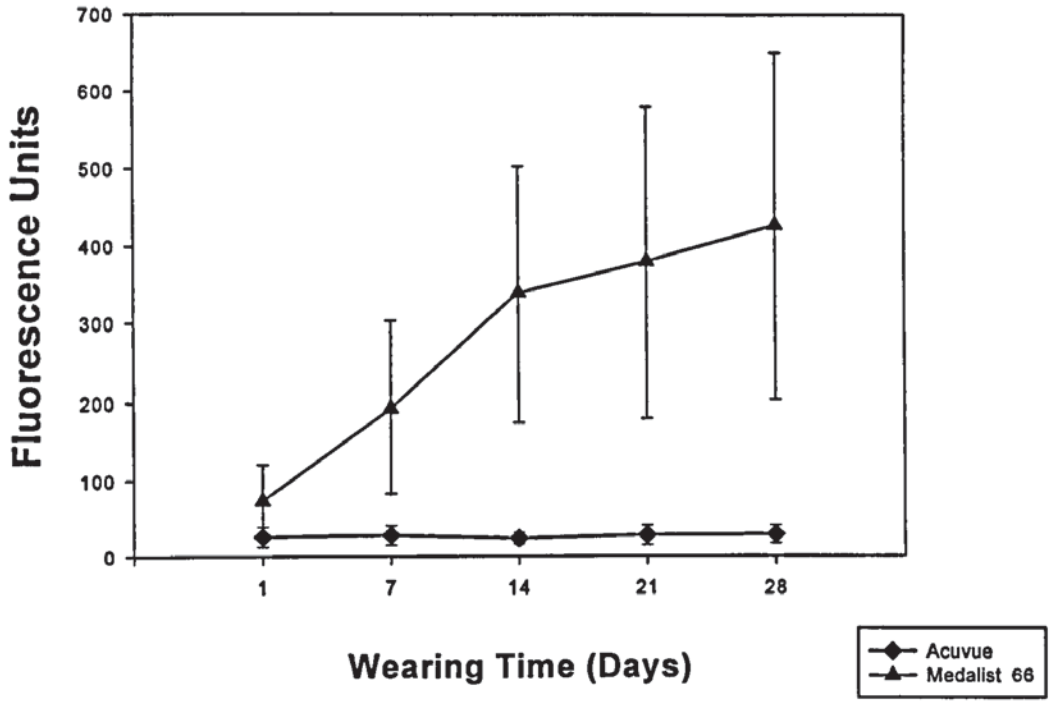


Figure 7.2.3 - Lipid (mean ±sd) for all lenses across all wearing schedules, as assessed by fluorescence.

The group II material deposited substantially more lipid after all wearing schedules than the group II material. The difference was significant at all appointments ($p<0.0001$). Figure 7.2.3 shows that the kinetics of the lipid deposited was markedly different for the two materials. In the group IV material the change in lipid deposition across time was insignificant (p=NS). There was a systematic, progressive increase in lipid deposition across time with the group II material ($p<0.0001$), with SNK post-hoc testing indicated that the difference between all appointments was significant ($p<0.05$).

7.2.5 Discussion

This study, for the first time to our knowledge, clearly defines the *in vivo* kinetics of both protein and lipid deposition in materials that are commonly used as frequent replacement lenses. As reported in previous studies described in this thesis, group IV materials attract large amounts of protein and NVP-containing group II hydrogels deposit substantial amounts of lipid.

These *in vivo* results indicate that bulk protein in group IV materials deposits rapidly but then plateaus, with a maximum being achieved somewhere between 1 and 7 days. In group II materials protein deposits over a slower time course, but no plateau is achieved. If the surface protein is considered then evidently both materials rapidly develop a surface protein "coating" and that this level of surface deposition remains largely unaltered in quantity. However, it is important to bear in mind that while the quantifiable amount of deposited protein may remain the same, the actual protein itself is different.

Previous work in these laboratories [431] has shown that the protein that deposits on group IV materials is virtually exclusively lysozyme, which, as previously discussed, is very mobile. Some 30% of the protein which deposits during the day will be passively released at night by the care system, in particular care systems with a pH close to neutral that contains a surfactant, such as ReNu. This can clearly be seen by studies conducted in our laboratory (unpublished), which suggest that care systems with a low pH (such as those based on hydrogen peroxide) and care systems without a surfactant (such as Opti-Free) result in a lower "passive" release of protein than others containing surfactants. We have shown that this passive release and then uptake of lysozyme from the tear film during the following days wear is responsible for the protein levels on group IV materials such as Etafilcon reaching a plateau and not depositing any more. It is likely that the increase over time with the group II material is due to the progressive increase in larger, more firmly adherent proteins such as lactoferrin and albumin. Further work [431] has shown that the protein types deposited on neutral group II materials are equally divided between lysozyme, lactoferrin and albumin. Of these only lysozyme can freely diffuse into and out of the matrix of the group II material, with lactoferrin and albumin progressively increasing in concentration as the wear period continues. Lysozyme does not denature immediately upon adsorption, but progressively denatures with time. [498] This increase in largely denatured, firmly adherent protein accounts for the increasing levels of protein on group II materials seen in both time dependency studies described in this Chapter.

Examination of the lipoidal results (Figure 7.2.3) indicates that, as previously shown in this thesis, NVP-containing hydrogels deposit large quantities of lipid, whereas non-NVP containing group IV materials deposit little if any lipid. The deposition of lipid in such group II materials becomes cumulative, and rapidly, monotonously increases. Comparison of the protein and lipid results also reveals much greater subjective variation in the degree of lipid deposition, with a substantially greater increase in the variation between subjects in lipid deposition with increasing length of wear.

Several studies [5 457 458 494] indicate that clinical complications are reduced if replacement intervals are shortened. This study clearly proves that such findings are not solely related to increases in deposition. In a study by Snyder and Hammack, [499] no difference in physiological or subjective factors was reported when polyHEMA (FDA group I) daily lenses were compared with two-weekly worn polyHEMA lenses. However, this was a six-week study only and a longer period of wear (at least 12 months) should be undertaken before such conclusions can logically be drawn.

7.3 Key Points

- NVP-containing group II materials deposit substantial quantities of lipid, this deposition is cumulative and does not reach a plateau in the lens materials examined in this section.

- Non NVP-containing group IV hydrogels deposit little lipid from the tear film and there is no increase across time in the lens materials examined in this section.

- NVP-containing group II materials deposit only small quantities of protein, but their spoilation with protein does not reach a plateau in the lens materials examined in this section.

- Non NVP-containing group IV hydrogels rapidly deposit large levels of protein from the tear film, but the deposition reaches a plateau between 1 and 7 days post-insertion.

- Protein deposition is unrelated to subjective differences, with minimal inter-subject differences being seen. Protein deposition is predominantly related to material charge and water content.

- Lipid deposition shows considerable inter-subject differences.

- Visual acuity, physiological performance and subjective visual quality and comfort remain unaltered if Vasurfilcon lenses are replaced at least every three months, if the lenses are disinfected with multipurpose care systems.

- Subjective satisfaction, lens front surface wettability, visible deposits and analytically evaluated deposits are all improved by replacing Vasurfilcon lenses monthly, if the lenses are disinfected with multipurpose care systems.

The previous three chapters have provided useful evidence and information concerning the deposition of tear-derived substances onto various materials, the differences between subjects and charted the change in deposition over time. A further factor of considerable interest is the influence that the care system may have on the deposition process. The next chapter will specifically address this issue.

Chapter 8

The Influence Of Care Systems On the Spoilation and Clinical Performance Of Hydrogel Lenses

"Far and away the best prize that life has to offer is the chance to work hard at work worth doing". [1]

The first disposable lens to achieve FDA licensing ("Acuvue" in 1987) was initially developed for use on an extended-wear basis, with several studies indicating its superior performance over conventional extended-wear lenses. [500 - 502] Reports of microbial keratitis with extended wear disposable contact lenses (EWDCL) in the early 1990's suggested that the premise that disposability would reduce the incidence of microbial keratitis compared with conventional extended-wear lenses was incorrect.

The relative risk of suppurative keratitis with extended wear soft lenses is 10-15 times greater than with daily wear soft lenses [503] and 21 times greater than with daily wear rigid gas permeable lens wearers. [504] EWDCL are as likely to induce microbial keratitis as conventional extended wear lenses. [505 - 507] However, the complications induced by disposable extended-wear lenses are less than those induced by conventional extended-wear lenses, [508] with one study [507] finding approximately 25% fewer overall complications, reported symptoms and unscheduled visits. Where microbial keratitis does occur it is smaller and more peripheral in location, resulting in a better prognosis for full visual recovery and a return to lens wear. [509] The published studies thus far concerning microbial keratitis would point to the conclusion that all soft lenses, whether frequent replacement or conventional, should be worn on a daily wear basis (necessitating their use with a care system), with extended-wear being reserved for specific medical and occupational uses only.

When a contact lens is placed on the eye, it becomes contaminated with a biofilm consisting of both tear contaminants [7 10 11] and bacteria. [37 38 44] On removal, and before re-insertion, lenses must be both cleaned and disinfected to reduce the risk of infection. Surfactant cleaning (or at least a "rub & rinse" step) before disinfection is vital to reduce the level of microbial contamination. Although the precise magnitude is unknown, current estimates vary from a 1-2 log reduction [293] to a 4 log unit decrease in the bacterial bioburden. [251 252] The omission of a surfactant cleaner [288 289] and/or a suitable disinfectant [510] has resulted in several cases of microbial keratitis in wearers of daily-wear frequent replacement lenses. A case control study [511] involving daily-wear frequent replacement lens wearers showed that patients who omit to use a surfactant have a 3.07 times higher risk of developing microbial keratitis, while those failing to adequately disinfect

[1] Theodore Roosevelt

them have a 4.65 times higher risk.

The philosophy with frequent replacement lenses is that the care system should be as simple and convenient for the patient as possible. In most of Europe and the USA preserved disinfection systems such as Opti-Free and ReNu are used for the routine maintenance of daily wear frequent replacement lenses. [512] The recent introduction of these products into the UK has resulted in their adoption as the principal method of choice for the disinfection of frequent replacement lenses. [465] Before the introduction of these systems practitioners recommended either hydrogen peroxide systems or chlorine based systems.

Three percent hydrogen peroxide based systems are extremely effective at disinfecting soft contact lenses. [290-292] Exposure to contaminated lenses, lens cases and solutions are causative factors of ocular infections in contact lens wearers. [337 513 514] Using this assumption, the efficient kill-rate of peroxide-based systems should provide subjects with a larger margin of error and increased safety in cases of poor compliance, which occurs in 40% [317] to 74% [318] of all contact lens subjects. However, the use of peroxide-based systems with group IV materials results in lens parameter changes [304 - 307] that might affect the fit of such lenses following disinfection and subsequent neutralisation. This, coupled with the increased cost and inconvenience of peroxide systems, led to many practitioners recommending the use of chlorine release systems, generally without the use of surfactant cleaners. [515] Several publications have questioned the use of chlorine systems, drawing attention to their reduced efficacy, [278] particularly in the presence of residual cleaner or organic material such as tear debris or mucous. [284] These publications, in combination with three papers [288 289 326] describing cases of microbial keratitis in patients using daily-wear frequent replacement lenses while disinfecting with chlorine-systems, have resulted in practitioners considering alternatives to chlorine for the disinfection of frequent replacement lenses.

The recent introduction into the UK of multi-purpose products has increased the variety of care systems that practitioners can recommend to patients. Many of these systems are specifically aimed at increased simplicity and are ideally suited to care for planned replacement lenses. Confusion among both practitioners and patients exists due to the overwhelming variety of such systems. Several comparative studies have shown greater patient preference for one product over another, although much of the published data is either anecdotal in nature or company sponsored, with few independent studies existing. Consequently, a greater scientific understanding of the interactive effect of solution/material combinations on clinical performance is required. This, coupled with an enhanced understanding of the influence of such combinations on the accumulation of deposits will provide hitherto unavailable evidence of the importance of such factors on changes in subjective performance.

The purpose of this chapter is to investigate whether differences exist between care systems in their ability to control deposition and to clarify if such deposition influences subjective performance.

8.1 THE INFLUENCE OF CARE SYSTEMS ON DEPOSITION & SUBJECTIVE PERFORMANCE

8.1.1 Materials and Methods

Ten male subjects were entered onto the study. All the subjects had previously used two-step hydrogen peroxide disinfection systems with prior surfactant cleaning and had no previous exposure to the test lenses. The demographic details are described in Table 8.1.1.

Table 8.1.1 - Subject Demographics

	Mean ± SD	Range
Age (years)	29.6 ± 6.7	20 - 40
Flat Keratometry reading (mm)	7.92 ± 0.31	7.40 - 8.30
Steep Keratometry reading (mm)	7.87 ± 0.42	7.40 - 8.30
Sphere (D)	-3.1 ± 1.40	-5.25 to -1.50
Cylinder (D)	-0.3 ± 0.21	-0.75 to 0.00

All subjects were fitted with both test lenses, which were chosen to represent the major classes of materials used for frequent replacement lenses in the UK, the relevant details of which are given in Table 8.1.2. Each subject wore a RE-LE combination of the two lens types on a daily-wear basis for 28 days (contralateral eye paradigm). The RE-LE arrangement of the lenses remained the same throughout the period of the study, but the RE-LE combination was randomised such that each lens was worn an equal number of times in each eye.

Table 8.1.2 - Lens Parameters

	SUREVUE	RYTHMIC
Water Content (%)	58	73
Monomers	HEMA + MA	MMA + VP
USAN	Etafilcon A	-
FDA Category	Group IV	Group II
ISO Category	Filcon 1b	Filcon 4a
Manufacture	Moulded	Moulded
Back Optic Zone Radius	8.40, 8.80	8.90
Total Diameter	14.00	14.20
Centre Thickness	0.105	0.15

During the study all subjects randomly and sequentially used one of five disinfection systems:

1. 0.001% Polyquad - Alcon "Optifree" with Alcon "Opticlean II". The system used initial surfactant cleaning with Opticlean followed by rinsing and disinfection with the single-dose unit vial version of Optifree.

2. 0.00005% Polyaminopropyl biguanide with poloxamine - B&L "ReNu". The system used a prior rub-and-rinse with "ReNu" before overnight disinfection in ReNu.

3. 3ppm free chlorine system - Alcon "Softab" with Alcon "Pliagel". The Softab tablet was initially dissolved in "Salette" (non-preserved saline) in the lens case. Lenses were then cleaned with Pliagel, rinsed with Salette and finally placed in the Softab solution overnight.

4. One-step 3% hydrogen peroxide system - Allergan "Oxysept 1-Step". Lenses were removed, "rubbed and rinsed" with the 3% peroxide and then placed into the 3% peroxide and neutralised overnight with the catalase-coated tablet. No prior surfactant cleaning was undertaken.

5. Two-step 3% hydrogen peroxide system - Allergan "Oxysept 2-Step". Lenses were removed, "rubbed and rinsed" with the 3% peroxide, placed directly into the 3% peroxide and left overnight. They were subsequently neutralised according to the manufacturers' instructions for 20 minutes, before insertion took place. No prior surfactant cleaning was undertaken.

An assistant randomly assigned the order for the solutions for each subject and which lens was worn in each eye, to ensure that each solution system was exposed equally at each stage of the trial. Each care system was only taken from a single lot. Verbal advice on the use of the solutions was backed-up with the issue of written instructions to ensure that compliance remained high during the study. A new case was given to the patients after each solution system was changed. Subjects were instructed to refrain from using enzyme

tablets or rewetting drops throughout the course of the study and were informed that they should attempt to wear their lenses for a minimum of ten hours and maximum of sixteen hours daily.

The information regarding the lenses was unknown to either subjects or investigator (double-masked) and the care regimen was unknown to the investigator (single-masked).

8.1.2 Clinical Protocol

During the study subjective satisfaction was assessed by the patient at the end of the first day of wear and every seven days thereafter. At the end of two weeks (14 days ± 2) the subjects returned to the test centre for evaluation of the lenses. Lens fit, visual acuity, PLNIBUT, visible wettability on the slit lamp, Rudko deposition and physiological performance were assessed, as previously described. The subjects were then dismissed for a further two weeks. The final check-up with each solution regime occurred 28 ± 3 days after starting with each regimen.

At this appointment the same protocol was followed as above, except that after the slit-lamp examination each lens was removed with sterile, plastic-tipped forceps, placed in saline in sterile glass vials, capped, labelled with the patient's initials and study number and refrigerated, before being taken for spoilation analysis. The subjects were then rinsed-out with single-dose sterile saline, the next set of lenses inserted by the clinical assistant, the preliminary measurements taken and the subjects finally dismissed with the next set of lenses, to return at four further intervals of two and four weeks from the initial fitting appointment.

8.1.3 Analytical Protocol

Surface deposition was evaluated using fluorescence spectrophotofluorimetry, exciting at 280nm to evaluate proteins and 360nm for lipid. Total protein was assessed using transmission UV at 280nm. In addition, lysozyme activity was assessed using the standard micrococcus assay.

8.1.4 Data Analysis

The grading scales, being ordinal measurements, were considered non-normal data. All other data were tested for normality of distribution. Statistical analysis was undertaken using either two-way repeated measures ANOVA on two factors or three-way RM ANOVA on three factors, using a balanced design. Where differences were found, post-hoc testing was undertaken using the Tukey method. Correlations were undertaken using Spearman Rank Correlations.

8.1.5 Results

8.1.5.1 Visual Acuity

There was no significant difference between lens, solution or visit (p=NS) for high or low contrast acuity. Previous work has shown that the visual acuity of frequent replacement lenses remains stable over short wearing periods. [366]

8.1.5.2. Lens Fit

a) Percentage Tightness

There was no significant difference between solutions or visits (p=NS), but there was a significant difference between lens types (p<0.001), as demonstrated in Table 8.1.3. These results mirror those of Young and co-workers, [473] who also showed a difference in fitting characteristics between these two lens types, although their smaller sample size possibly resulted in the difference being not statistically significant.

Table 8.1.3 - Percentage Tightness Assessed By Push-Up Test

	Group IV	Group II
Mean ± SD	54.5 ± 1.4	42.1 ± 2.2
Range	40-70	20 - 70

b) *Vertical Movement*

There was no significant difference between solutions or visits (p=NS), but there was a significant difference between lens types (p<0.001), as shown in Table 8.1.4. This is in accordance with other published work. [473]

Table 8.1.4 - Vertical Movement Assessed By Graticule

	GIV (graticule units)	GIV (mm)	GII (graticule units)	GII (mm)
Mean ± SD	4.1 ± 0.4	0.33	8.5 ± 0.8	0.68
Range	1-10		3-20	

8.1.5.2 Wettability

a) PLNIBUT by Loveridge Grid

Results are detailed in Figures 8.1.1 and 8.1.2.

Clearly the greatest variable in this factor is subjective tear film differences. There was no significant difference between solutions or lens types (p=NS), but there was a significant reduction in PLNIBUT across visits for both materials, which occurred irrespective of care system ($p<0.04$). Post-hoc Tukey analysis indicated that the significant reduction occurred for both materials between collection and the two-week visit ($p<0.05$), with no significant reduction occurring thereafter (p=NS).

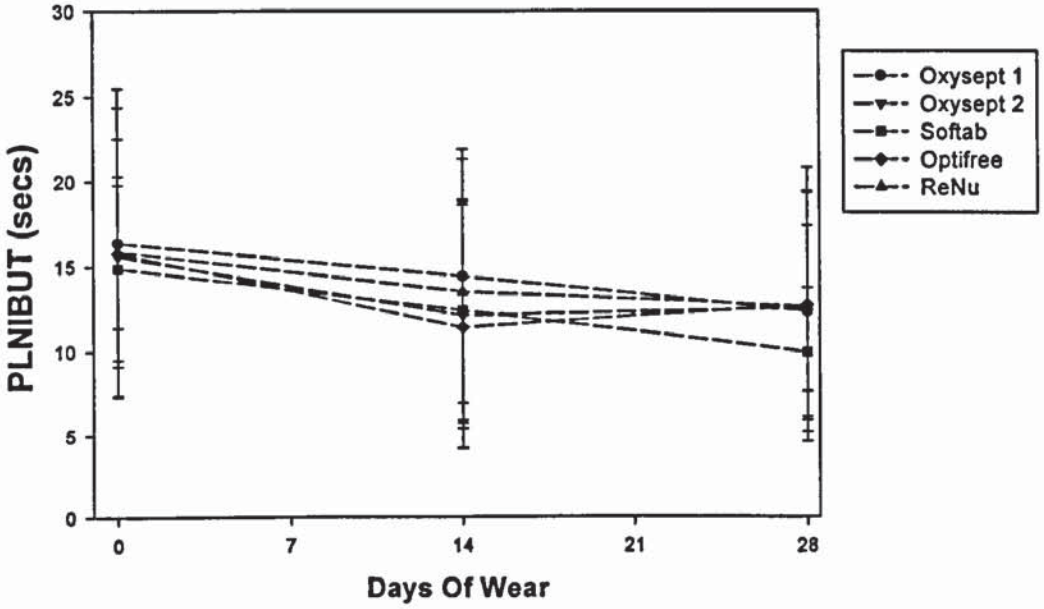


Figure 8.1.1 - Mean (±sd) PLNIBUT for group II material, as measured using the Loveridge Grid

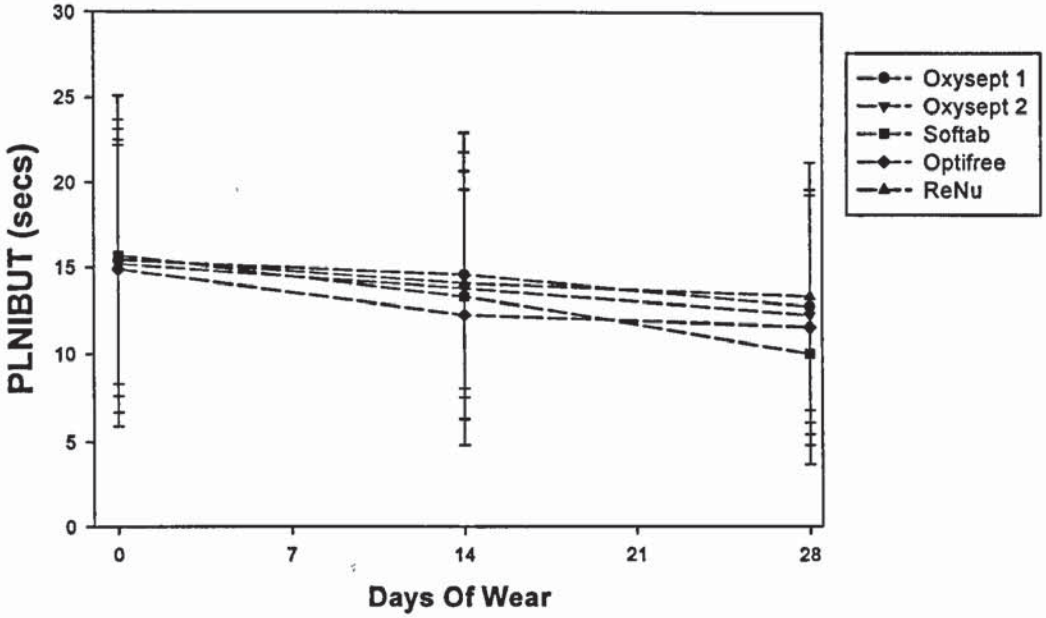


Figure 8.1.2 - Mean (±sd) PLNIBUT for group IV material, as measured using the Loveridge Grid

b) PLNIBUT by Slit Lamp

These results are detailed in Figure 8.1.3. There was no significant difference between solutions, lens types or across visits (p=NS). The differences in results between PLNIBUT by slit-lamp and by Loveridge Grid clearly displays the poor sensitivity of the slit lamp at detecting subtle changes in wettability.

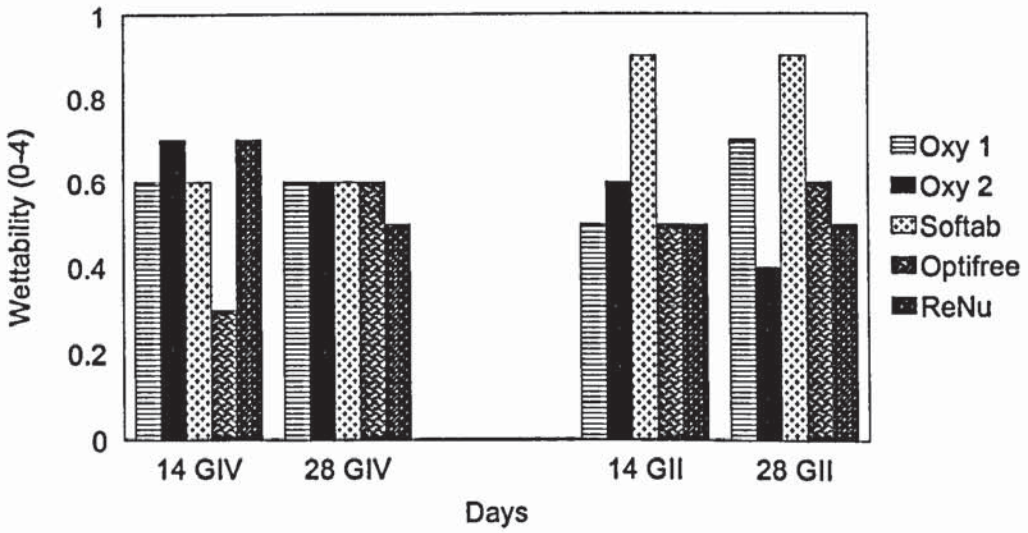


Figure 8.1.3 - Wettability by slit lamp assessment

8.1.5.3 Visible Deposition

a) RUDKO Degree

Results are shown in Figure 8.1.4. There was a specific interaction between the Group IV material and Oxysept 1-step. There were no significant differences across visits (p=NS), but a difference did exist between solutions ($p<0.005$) and lenses ($p<0.01$).

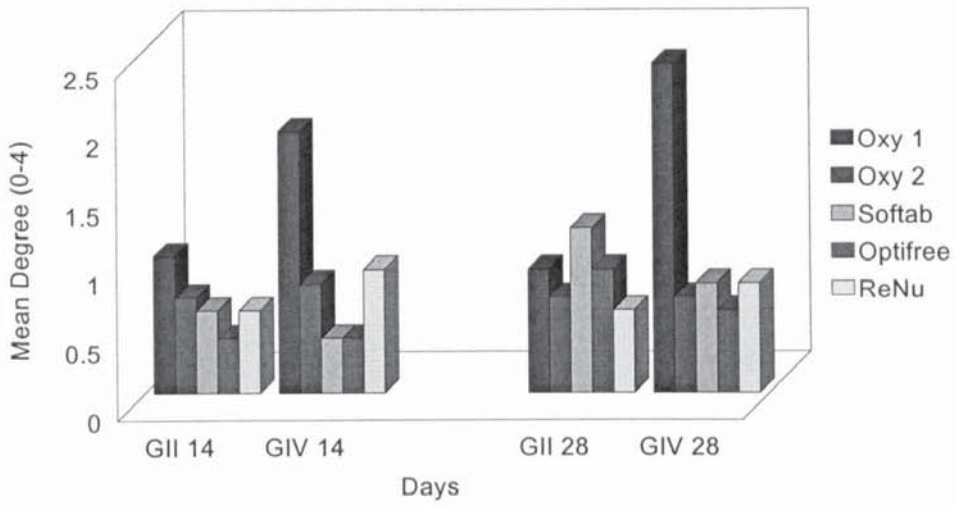


Figure 8.1.4 - Visible deposition by Rudko classification - Degree

b) RUDKO Coverage

Results are shown in Figure 8.1.5. Again there was a specific interaction between the Group IV material and Oxysept 1-step. While there was no statistically significant difference between lens types or across visits (p=NS) there was a difference between solutions ($p<0.005$).

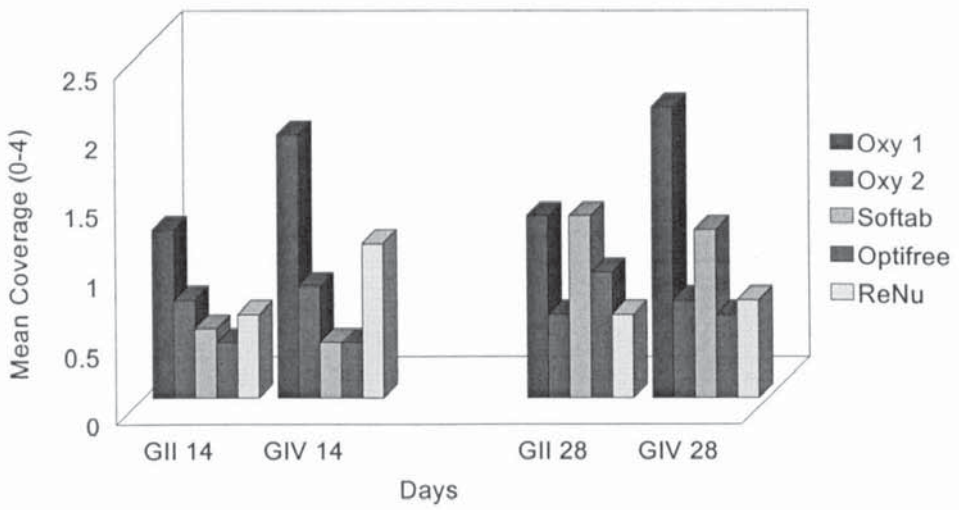


Figure 8.1.5 - Visible deposition by Rudko classification - Coverage

c) RUDKO Type

Results for day 28 are given in Figures 8.1.6 and 8.1.7. Of principal interest is the filming on the Group IV lens with Oxysept 1-step. It is also quite clear that the Group IV lens deposited no calculi while the Group II lens deposited calculi with all solutions. While there was no significant difference between lens types or visit (p=NS) there was a difference between solutions ($p<0.005$).

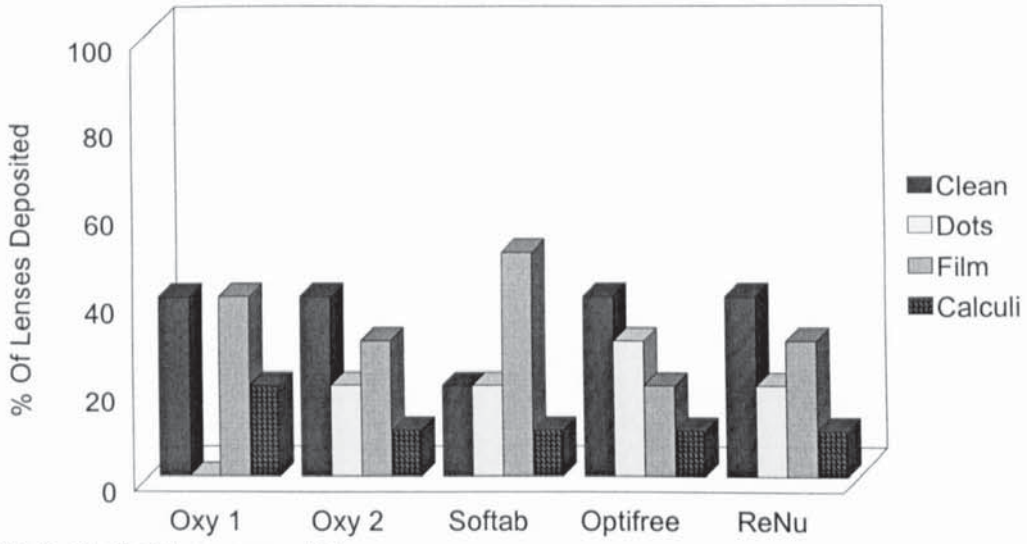


Figure 8.1.6 - Rudko Type - group II lens

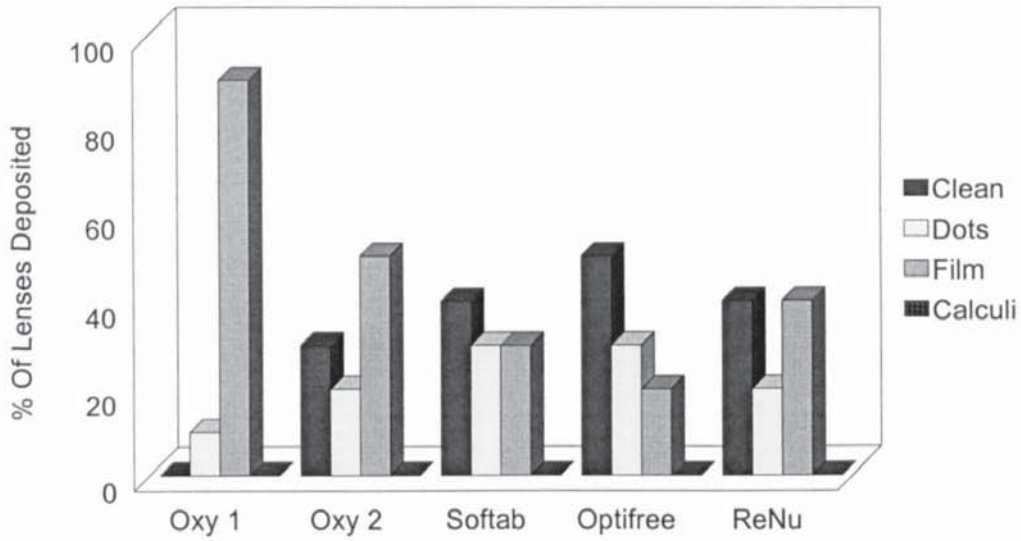


Figure 8.1.7 - Rudko Type - group IV lens

8.1.5.4. Subjective Factors

a) Solution

i. Comfort on Insertion

Results are shown in Figure 8.1.8 and Table 8.1.5.

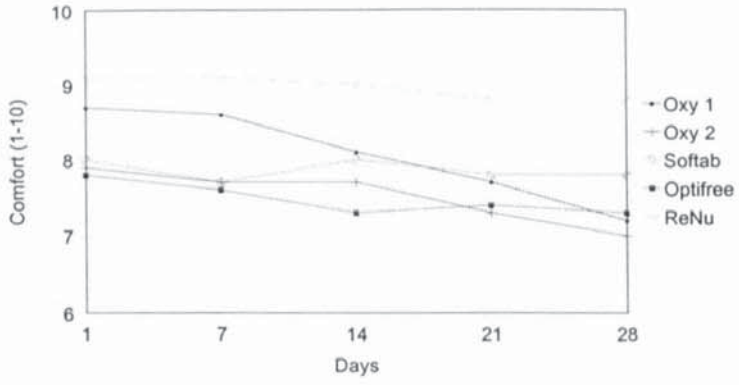


Figure 8.1.8 - Subjective comfort for solution

Table 8.1.5 - ANOVA Table For Subjective Comfort

Source of Variation	DF	SS	MS	F	P
subj	9	122.496	13.611		
soln	4	101.456	25.364	8.462	<0.001
soln x subj	36	107.904	2.997		
visit	4	9.336	2.334	3.815	0.011
visit x subj	36	22.024	0.612		
soln x visit	16	16.064	1.004	1.709	0.051
Residual	144	84.576	0.587		
Total	249	463.856	1.863		

Table 8.1.5 clearly shows that there were differences between solutions, which were significant ($p<0.001$) and there was also a difference across visits ($p<0.02$). Post-hoc testing showed that ReNu was significantly more comfortable than all the other solutions ($p<0.05$). The other products were not significantly different from each other (p=NS). Results also indicate that comfort reduced after day 7.

ii. Solution Convenience

Results are depicted in Figure 8.1.9 and Table 8.1.6.

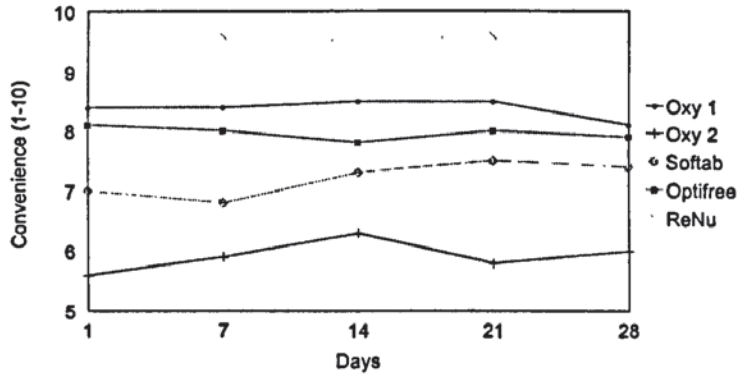


Figure 8.1.9 - Subjective convenience score for care system

Table 8.1.6 - ANOVA Table For Subjective Convenience Rating

Source of Variation	DF	SS	MS	F	P
subj	9	189.344	21.038		
soln	4	371.224	92.806	12.202	<0.001
soln x subj	36	273.816	7.606		
visit	4	1.144	0.286	0.837	0.511
visit x subj	36	12.296	0.342		
soln x visit	16	6.816	0.426	1.274	0.221
Residual	144	48.144	0.334		
Total	249	902.784	3.626		

Clearly there were differences between solutions, which were significant ($p<0.001$). There was no difference across visits (p=NS). The results were as expected, with simpler systems being graded as more convenient. Post-hoc testing indicated that all systems were significantly different from each other ($p<0.05$), except Oxysept 1-step and Optifree (p=NS).

iii. Overall Solution Performance

Results are shown in Figure 8.1.10 and Table 8.1.7.

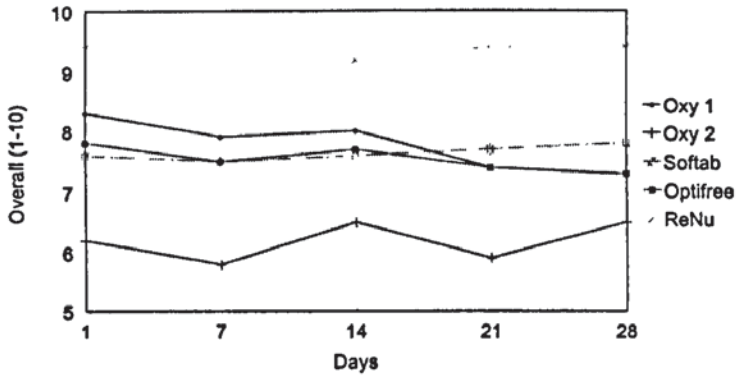


Figure 8.1.10 - Mean overall score for care systems

Clearly patients preferred using certain solutions. These differences were significantly different ($p<0.001$). There was no difference across time (p=NS). Post-hoc testing indicates that ReNu was significantly preferred to the other systems ($p<0.05$), Oxysept II was significantly worse than all the other systems ($p<0.05$) and the other three systems were not different (p=NS).

Table 8.1.7 - ANOVA Table For Subjective Ratings of Overall Score For Care System

Source of Variation	DF	SS	MS	F	P
subj	9	133.824	14.869		
soln	4	255.704	63.926	8.686	<0.001
soln x subj	36	264.936	7.359		
visit	4	4.224	1.056	1.583	0.200
visit x subj	36	24.016	0.667		
soln x visit	16	12.536	0.784	1.709	0.051
Residual	144	66.024	0.459		
Total	249	761.264	3.057		

b) Lenses

Differences between lenses were analysed using a 3-way RM ANOVA with subject as the repeat and lens, solution and visit as the factor terms.

i. Lens Comfort - Results are shown in Figures 8.1.11 and 8.1.12 and Table 8.1.8.

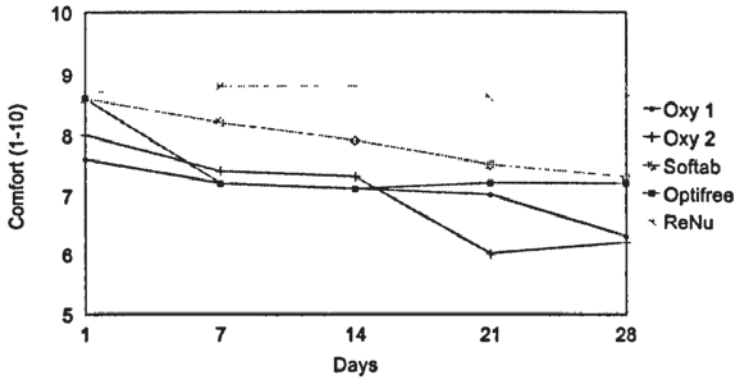


Figure 8.1.11 - Mean lens comfort - group II lens

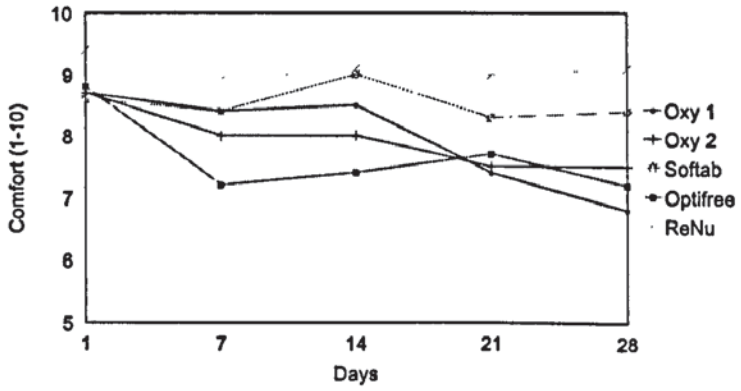


Figure 8.1.12 - Mean lens comfort - group IV lens

Table 8.1.8 - 3-WAY RM ANOVA Table For Lens Comfort

Source of Variation	DF	SS	MS	F	P
lens	1	45.000	45.000	1.59	0.223
soln	4	143.408	35.852	20.53	<0.001
visit	4	68.628	17.157	9.82	<0.001
lens x soln	4	11.200	2.800	1.603	0.1725
lens x visit	4	3.460	0.865	0.495	0.7392
soln x visit	16	43.532	2.721	1.558	0.0768
lens x soln x visit	16	18.540	1.159	0.663	0.8302
Residual	432	754.512	1.747		
Total	499	1597.768	3.202		

There were significant differences between solutions and visits ($p<0.001$), but not between lenses (p=NS). Post-hoc testing indicated that in order, ReNu was more comfortable than Softab ($p<0.05$), which was more comfortable than the other three products ($p<0.05$), which were all equally uncomfortable (p=NS). Lens comfort reduced from day 1 through to day 14 ($p<0.05$), but then comfort remained similar out to 28 days (p=NS).

ii. Visual Quality - Results are depicted in Figures 8.1.13 and 8.1.14 and Table 8.1.9.

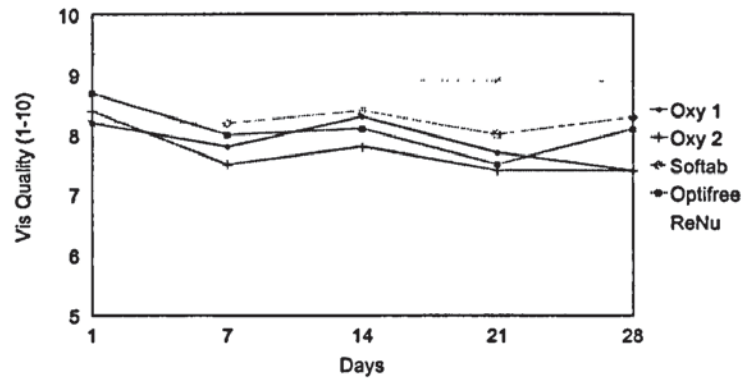


Figure 8.1.13 - Mean visual quality score - group II material

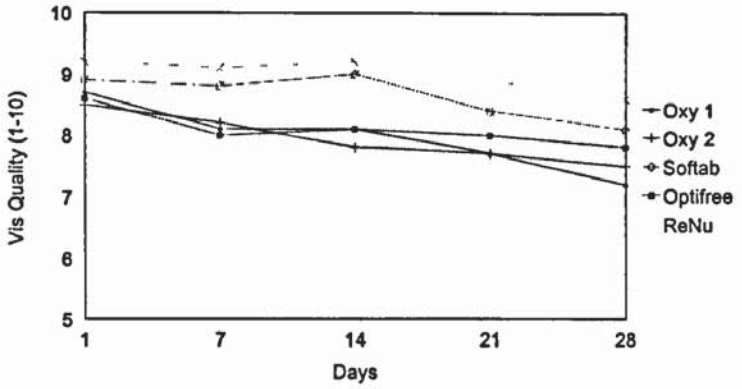


Figure 8.1.14 - Mean visual quality score - group IV material

Table 8.1.9 - 3-WAY RM ANOVA Table For Visual Quality

Source of Variation	DF	SS	MS	F	P
lens	1	1.682	1.682	0.072	0.791
soln	4	103.492	25.873	22.542	<0.001
visit	4	35.192	8.798	7.665	<0.001
lens x soln	4	4.988	1.247	1.086	0.3627
lens x visit	4	0.568	0.142	0.124	0.974
soln x visit	16	14.708	0.919	0.801	0.685
lens x soln x visit	16	6.412	0.401	0.349	0.992
Residual	432	495.84	1.148		
Total	499	1080.742	2.166		

There was no significant difference between lens types (p=NS), but there were clear differences between solutions and across visits (p<0.001). Post-hoc testing showed that in order, ReNu provide better visual quality than Softab (p<0.05), which gave better vision than the other three products (p<0.05), which were all the same (p=NS). Visual performance was stable for the first 14 days (p=NS), after which it reduced each visit (p<0.05). These results clearly indicate that solution choice is critical in maintaining optimal lens performance.

iii. Overall Lens Performance - Results are graphically detailed in Figures 8.1.15 and 8.1.16 and Table 8.1.10.

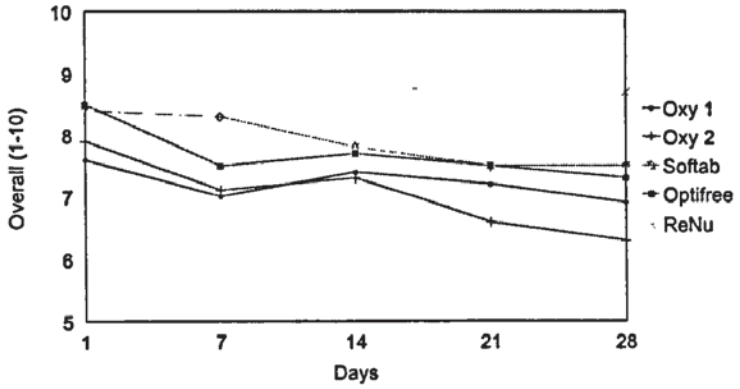


Figure 8.1.15 - Mean overall score for group II lens

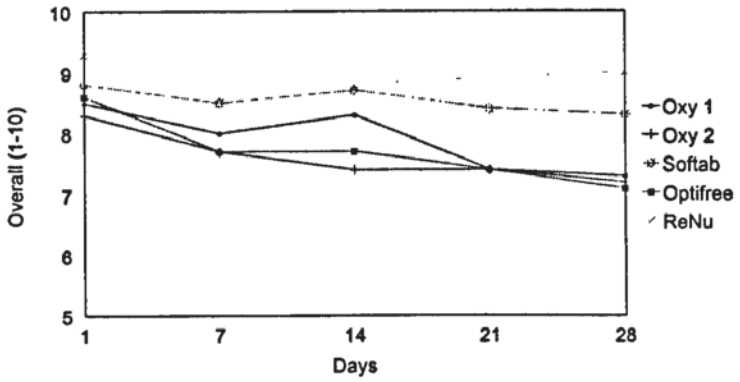


Figure 8.1.16 - Mean overall score for group IV lens

Table 8.1.10 - 3-WAY RM ANOVA Table For Overall Lens Satisfaction

Source of Variation	DF	SS	MS	F	P
lens	1	28.322	28.322	1.363	0.258
soln	4	155.988	38.997	21.897	<0.001
visit	4	37.888	9.472	5.319	<0.001
lens x soln	4	8.308	2.077	1.166	0.325
lens x visit	4	0.288	0.072	0.040	0.997
soln x visit	16	24.452	1.528	0.858	0.619
lens x soln x visit	16	10.532	0.658	0.370	0.988
Residual	432	769.344	1.781		
Total	499	1409.078	2.824		

There were significant differences between solutions and visits ($p<0.001$), but not between lenses (p=NS). Post-hoc testing indicated that in order, ReNu was preferred to Softab ($p<0.05$), which was better than the other three products ($p<0.05$), which were all rated as being equal (p=NS). Overall performance was stable for the first 7 days only (p=NS), after which it reduced each visit ($p<0.05$).

8.1.5.5 Physiological Performance

a) Corneal Staining

Results are shown in Figure 8.1.17. While the level of corneal staining did show a small increase with all solutions across time, this was not significant (p=0.07). There were no differences between solutions or lens types (p=NS).

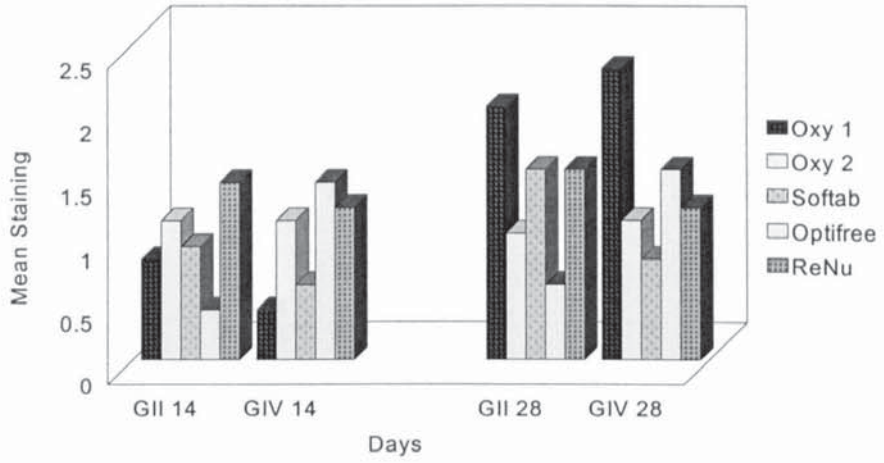


Figure 8.1.17 - Corneal staining score for both materials and all care systems

b) Conjunctival Staining

Results are shown in Figure 8.1.18. There was a significant difference between lens materials ($p<0.0001$), although this was not considered clinically significant. Previous studies have also demonstrated an increased level of conjunctival staining with moulded Etafilcon lenses. [483] There was no difference across time or between solutions (p=NS).

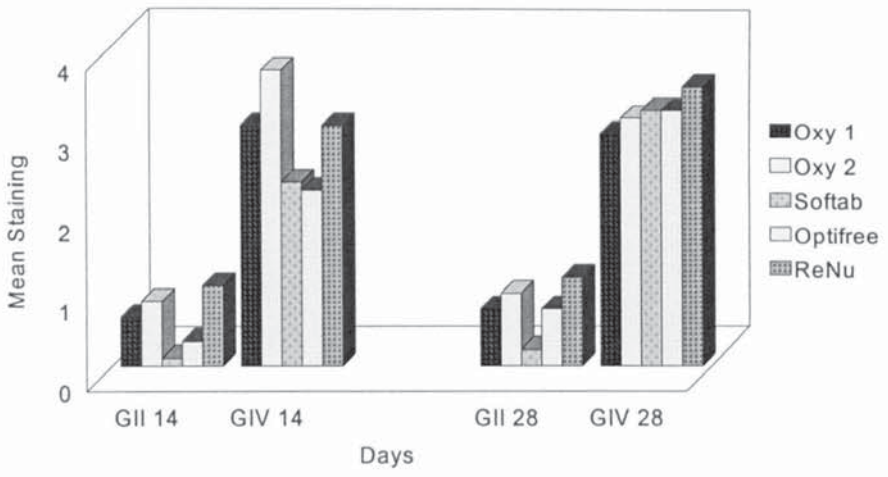


Figure 8.1.18 - Conjunctival staining score for both materials and all care systems

c) Conjunctival Hyperaemia

There was no change in conjunctival hyperaemia across time or between lens types or solutions (p=NS).

d) Lid Appearance

There was no change in either lid hyperaemia or papillae appearance with time, solution or material (p=NS).

8.1.5.6 Deposition Results

a) Total Protein By UV

Figures 8.1.19 and 8.1.20 shows the total protein deposited by the lenses depending upon solution. Figure 8.1.19 details the total protein on the lens only and Figure 8.1.20 outlines the total protein including that in the storage solution. It is important to consider this as a considerable quantity of the protein found deposited in and on Group IV lenses is lysozyme, which readily desorbs from the lens once placed in a neutral solution such as saline.

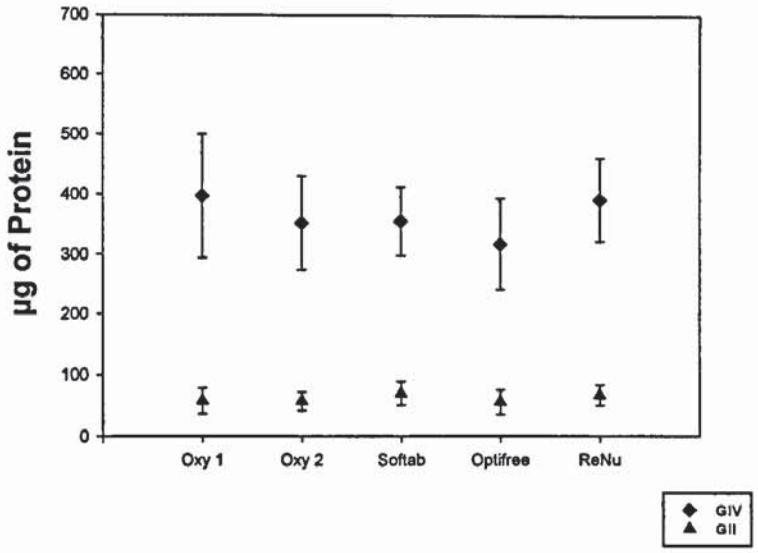


Figure 8.1.19 - Mean (±sd) total protein as measured by UV, lens only

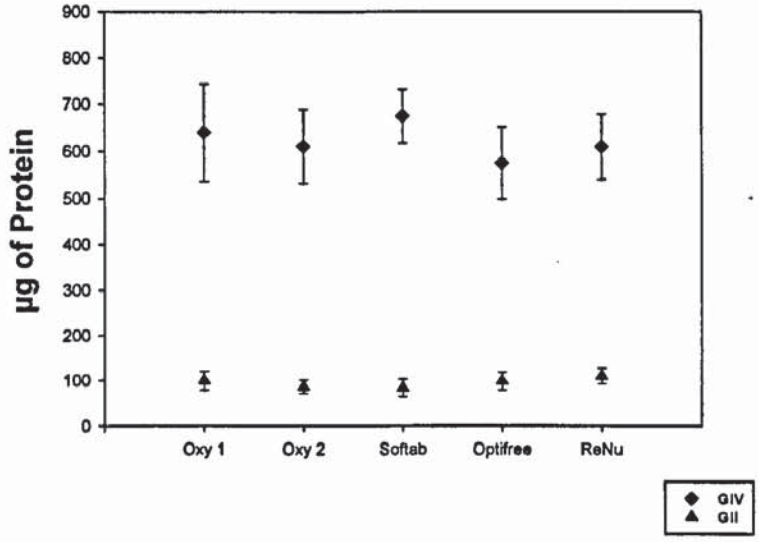


Figure 8.1.20 - Total protein as measured by UV, lens and storage solutions

There was a significant difference between lens types ($p<0.0001$) but not between solutions (p=NS).

Figures 8.1.21 and 8.1.22 outline the differences in protein deposition by subject for all lenses worn by each subject.

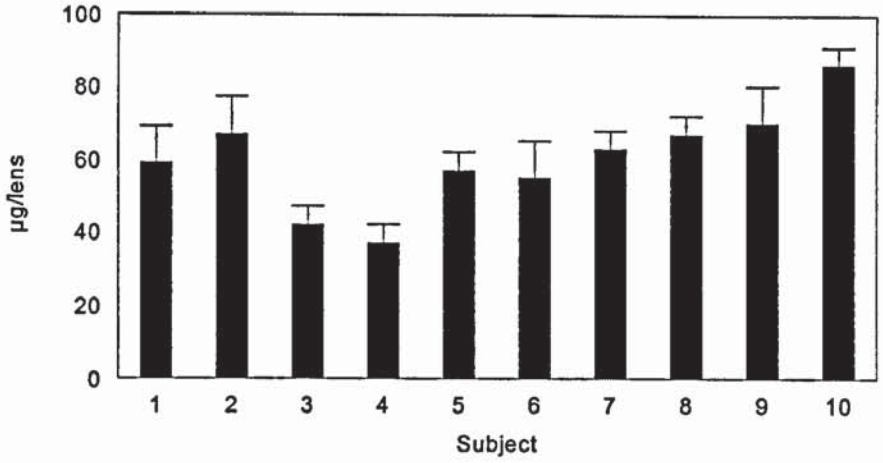


Figure 8.1.21 - Mean (±sd) total protein by subject for group II material. Results indicate differences between subjects for all worn lenses

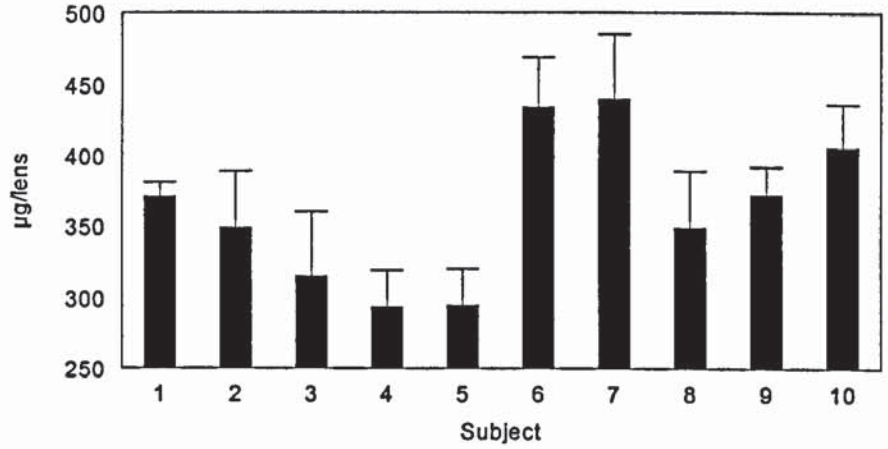


Figure 8.1.22 - Mean (±sd) total protein by subject for group IV material. Results indicate differences between subjects for all worn lenses

b) Surface Protein By Fluorescence

Figure 8.1.23 outlines the mean results.

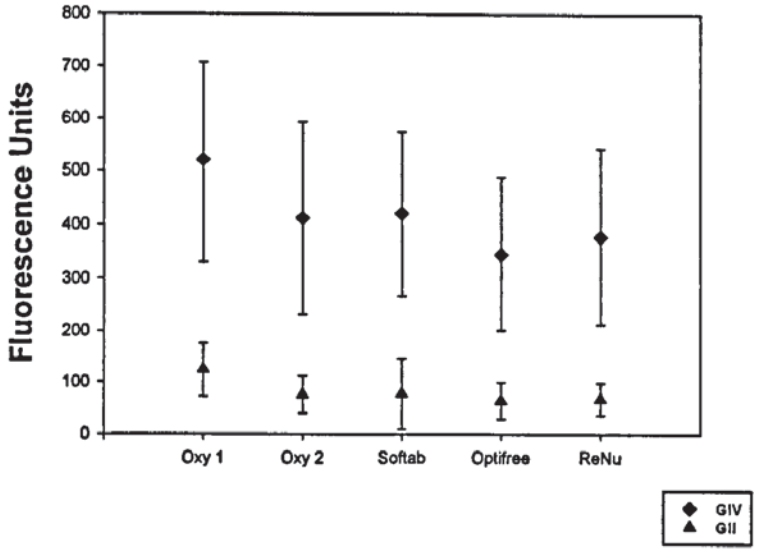


Figure 8.1.23 - Mean (±sd) surface protein as measured by fluorescence

There was a significant difference between lenses ($p<0.0001$) but not between solutions (p=NS). Figures 8.1.24 and 8.1.25 outlines the differences between subjects when all worn lenses are considered.

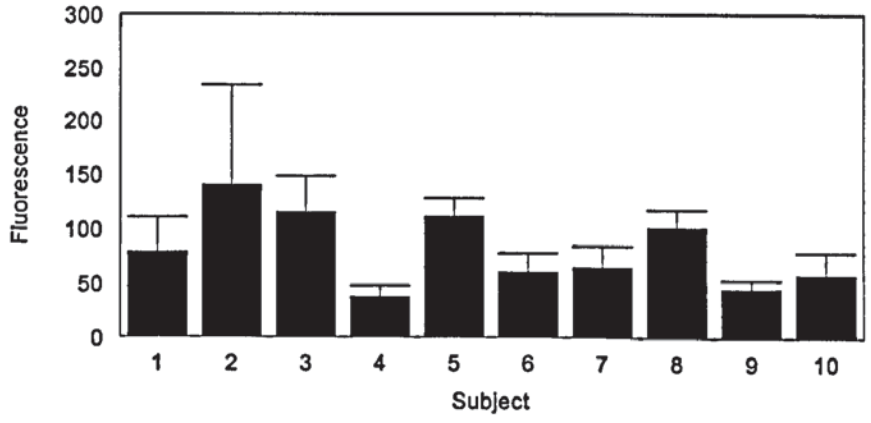


Figure 8.1.24 - Mean (±sd) surface protein by fluorescence for group II lens. Results indicate differences between subject for all worn lenses

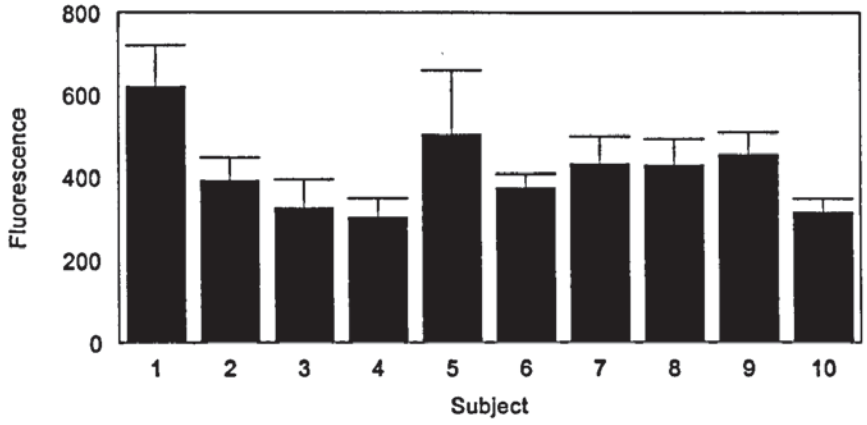


Figure 8.1.25 - Mean (±sd) surface protein by fluorescence for group IV lens. Results indicate differences between subjects for all worn lenses

c) Lipid By Fluorescence

Figure 8.1.26 describes the mean results.

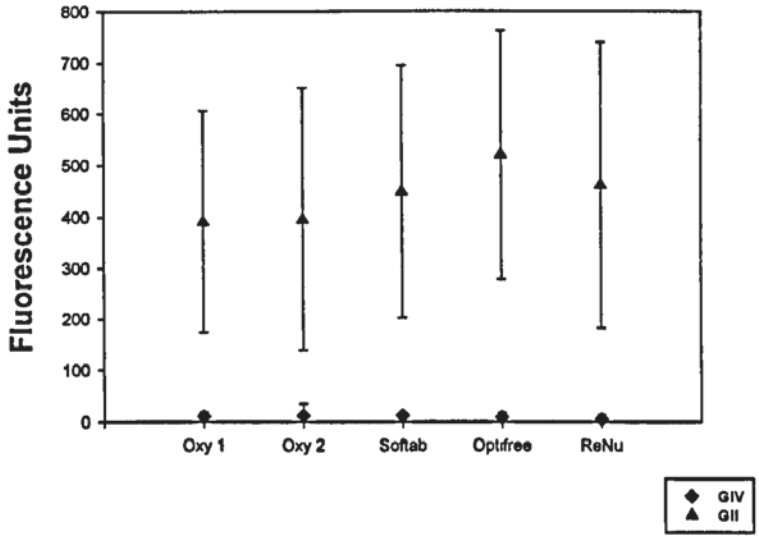


Figure 8.1.26 - Mean (±sd) lipid as measured by fluorescence

There was a significant difference between lenses ($p<0.0001$) but not between solutions (p=NS).

Figures 8.1.27 and 8.1.28 outlines the difference between subjects.

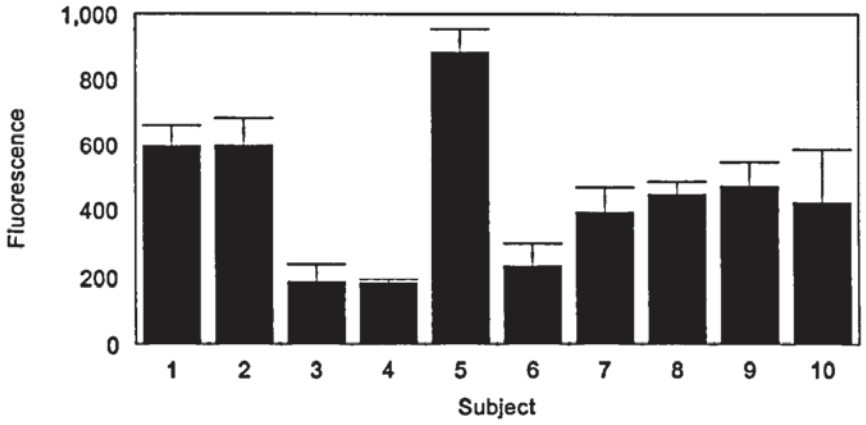


Figure 8.1.27 - Mean (±sd) surface lipid by fluorescence for group II lens for all subjects

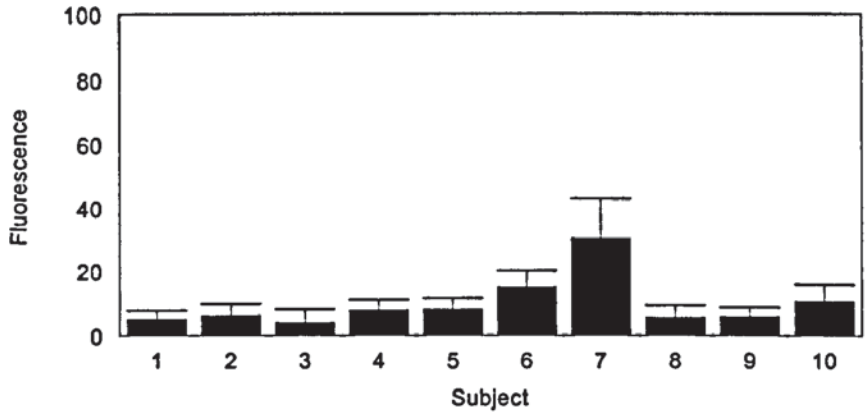


Figure 8.1.28 - Mean (±sd) surface lipid by fluorescence for group IV lens for all subjects

d) Protein Activity

Figures 8.1.29 and 8.1.30 indicate the activity of the protein deposited.

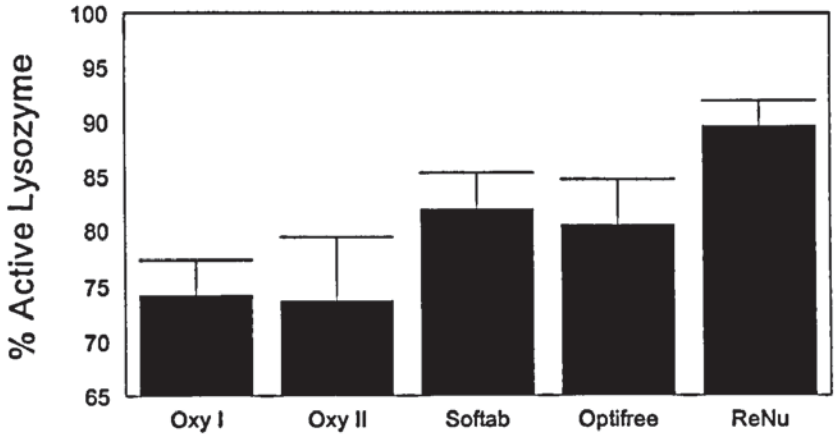


Figure 8.1.29 - Activity for group II

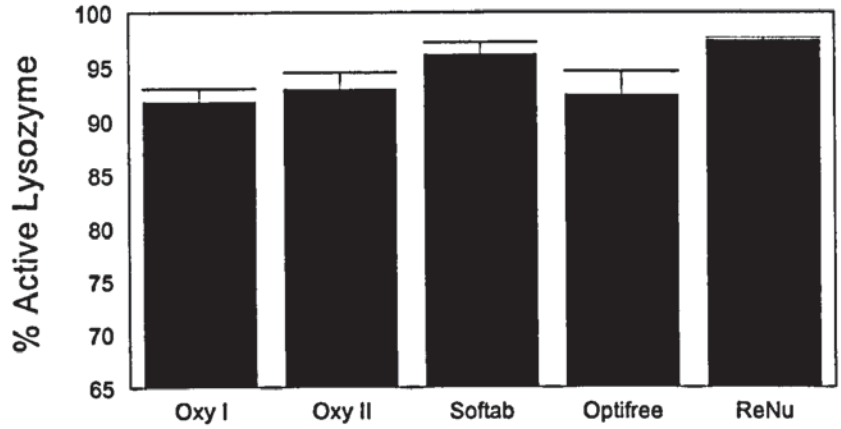


Figure 8.1.30 - Activity for group IV

There is clearly a difference between materials ($p<0.001$) and a difference between solutions ($p<0.0001$). There is also a significant interaction between lens and solution ($p<0.02$). ReNu and Softab were no different to each other (p=NS), but they were different from the other three solutions ($p<0.05$).

8.1.5.7 Correlations of Deposits With Symptoms

To discriminate which of the deposits were responsible for the changes in subjective performance across time, various correlations were conducted. The change in subjective performance between day 1 and day 28 was plotted against the mean deposition found at day 28.

Table 8.1.11 -

Correlation Coefficients: Change in Subjective Performance v Deposition - Group II

	Surface Lipid	Surface Protein	Total Protein	Active Protein
Comfort	0.20	-0.25	0.55	0.82
Vision	0.38	-0.29	0.88	0.90
Overall Satisfaction	0.02	-0.1	0.55	0.70

Table 8.1.12 -

Correlation Coefficients: Change in Subjective Performance v Deposition - Group IV

	Surface Lipid	Surface Protein	Total Protein	Active Protein
Comfort	-0.31	-0.37	0.15	0.97
Vision	-0.55	-0.83	-0.31	0.90
Overall Satisfaction	-0.38	-0.1	0.46	0.88

The hatched squares represent those correlations that are significant ($p<0.05$). It is clear from Tables 8.1.11 and 8.1.12 that the most significant factor influencing changes in subjective factors over the four-week wearing period is not the degree of deposition, but rather the nature of the deposited protein.

8.1.5.8. Final choice

Upon completion of the study subjects were asked a forced-choice question regarding their choice of solutions and lens combination. These are detailed in Table 8.1.13.

Table 8.1.13 - Final Choice

Group IV	8	Oxy I	0
Group II	2	Oxy II	0
		Optifree	0
		Softab	1
		ReNu	9

8.1.6 Discussion

The over-riding conclusion from this study is that solutions have a significant impact on the clinical performance of contact lenses, even those worn for short periods, and that solution choice is at least as important in the successful wear of lenses as the choice of lens. Comparisons between care systems such as those investigated in this study are well documented. Results have shown patient preference for Opti-Free, [516 - 519] ReNu [520 521] and peroxide-based systems. [522] However, the majority of these studies are company sponsored [516 - 519 521 522] and some bias in the interpretation of data may occur, however unintentional such inferences are. This is the first independent multi-solution study conducted in which patient preferences have been compared with laboratory detected deposition and the two factors inter-related.

The comfort differences reported between the lens materials may not be entirely related to deposition or wettability, but to differences in lens fit and bulk, [523] that were significantly different. In hindsight, the choice of the Rhythmic lens to represent the group II material was not ideal, as it is a flat-fitting, relatively mobile lens, as reported by Young and colleagues [484] and confirmed in this study. However, at the commencement of the study, apart from Precision UV (whose UV blocker poses great problems when trying to analyse spoilation non-destructively) it was the only group II frequent replacement lens commercially available. In addition, the choice was not necessarily flawed as the major points of interest in the study were not the differences between lenses but the deposition encountered with the test materials dependent upon the care system.

This study has shown for the first time that while performance with lenses reduces over time, the performance is not correlated with the quantity of deposition but is closely linked with the "quality" of deposition. This study was unable to show any statistical difference in quantity of deposition between care systems, a result similar to that reported in other studies. [519 524 525] Other comparative studies suggest that fewer protein deposits occur with peroxide-systems, [256] Opti-Free [516 518 526 527] or ReNu. [528] However, many of these studies used either visual [518 524] or destructive [516 518 526] means of assessing protein deposition, both of which have disadvantages previously discussed in Chapter 2 (page 42-43). In this study the Rudko technique demonstrated a specific interaction between the Group IV material and hydrogen peroxide. This "hazing" phenomenon was originally reported by Sack et al in 1989, [529] who conclusively proved that the hazing was caused by a specific interaction between lysozyme (which is deposited on group IV lenses to a large degree) and stannate anion derived from sodium stannate, a stabilizer in hydrogen peroxide systems. Denaturation of lysozyme was not responsible (as lenses doped with lysozyme and exposed to pure 3% hydrogen peroxide did not produce lens hazing) and suggestions that the hazing was related to calcium phosphate and/or carbonate were proved incorrect. Quinn reported increased visible deposition on group IV lenses with a peroxide-based system [530] and attempted to differentiate such deposition into "lipid deposition" and "protein deposition". Because of the number of studies that have clearly shown that such differentiation using visible means is impossible, [115 116] it is quite probable that such deposition was related to the hazing mechanism described above.

The impact of denatured protein is clearly proved in this study. Proteins are denatured by heat, [531] urea and

significant changes in pH. [532] Globular proteins such as lysozyme denature in acidic conditions (particularly between pH 2.0 - 4.5) at room temperature. [532] The pH of the disinfecting agent of Oxysept 1 and 2-Step (3% hydrogen peroxide) is approximately 3.7, which changes to a pH of 6.9 upon neutralization. [533] In addition, changes in pH will alter the degree of irreversibly bound protein, with a decrease in pH from 7.4 to 4.0 resulting in an increase in the amount of irreversibly bound albumin. [477] While changing subjects to a peroxide-based system will increase the bacteriocidal action of the disinfecting regime, its simultaneous effect on deposited protein will certainly need to be considered. Such denaturation may negate any possible benefits of changing to a system that is "stronger" in its disinfection capability.

Practitioners could monitor the reduction in lens surface quality and differences between care systems by monitoring lens surface wettability. The presence of a surfactant could theoretically reduce the PLNIBUT if it adhered to the lens surface and subsequently moved into the tear film, although this was not demonstrated in this study. These results confirm the work of Lakkis, [534] who showed that the incorporation of a surfactant into a solution has no influence on pre-lens NIBUT. The suggestion of Guillon and co-workers [363 373] that optimum replacement schedules are patient dependent is again borne out by these results, with a wide variation encountered in the wettability of the contact lens materials. Figures 8.2.1 and 8.2.2 indicates that PLNIBUT reduces with wearing period, is patient dependent and is independent of either material type or solution, which agrees with previous work investigating the performance of both rigid [535] and hydrogel materials. [455] Figures 8.2.5-8.2.10 also demonstrate that subjective satisfaction reduced with all combinations of lenses and solutions over the four-week period. The over-riding conclusion is that, with the lens materials and solutions tested in this study, optimum performance will only be achieved by replacing the lenses on a frequent replacement basis. Indeed, the changes in subjective response and the fact that there was a trend for corneal staining to increase with time strongly support the premise behind the use of shorter replacement periods. However, the ideal replacement period for lenses would appear to be related to a number of factors, including time of wear, individual patient characteristics, lens wettability and material deposition.

The fact that care systems using separate surfactant cleaners did not result in reduced deposition may appear a surprising finding. However, the effectiveness of the surfactants in multi-purpose solutions is supported by the work of Franklin [255] and by the fact that when compared with Opticlean daily cleaner, ReNu removed as many protein deposits as the dedicated cleaner. [519] In another comparative *in vivo* study comparing ReNu with polyquad and hydrogen peroxide, subjects using ReNu had reduced protein deposition, [528] although lipid deposition was not investigated.

In choosing a care system practitioners and patients must consider a number of variables, one of which is microbial efficacy. While hydrogen peroxide may denature protein to a greater extent than PHMB-preserved products, their efficacy against pathogenic organisms is well known. The mechanism for protein denaturation is closely aligned with the breakdown of microbial cell walls and this must be considered when choosing an appropriate care system.

This study clearly shows that polyhexanide (PHMB) preserved products are well received by patients and result in enhanced clinical performance. However, a variety of such systems exist and this study demonstrated a clear difference between two peroxide-based systems. A comparison of polyhexanide-based systems would be of value, indicating if differences exist between such systems and whether practitioners may interchange them with any degree of confidence.

8.2 A COMPARISON OF THE INFLUENCE OF THREE POLYHEXANIDE-PRESERVED MULTI-PURPOSE CONTACT LENS SOLUTIONS ON DEPOSITION & SUBJECTIVE PERFORMANCE

Although reportedly rare, [536] *Acanthamoeba* keratitis is a devastating eye disease, often resulting in substantial vision loss and recent studies have indicated that the incidence of such infections may be higher than initially thought. [537] Recent publicity in the UK, particularly on television and in the lay press, has resulted in many patients deciding to switch to care products perceived as more efficacious in their ability to kill pathogenic micro-organisms. While it is a widely accepted principle that patients should not switch care products without first consulting their practitioner, few studies have shown that such an action will result in any untoward consequences.

The purpose of this study was to investigate the clinical and analytical differences between three commercially available polyhexanide (polyhexamethylene biguanide - PHMB) preserved disinfection systems containing varying percentages of PHMB and in various formulations, when used with FDA group II and group IV frequent replacement contact lenses.

8.2.1 Materials and Methods

Ten male subjects were entered onto the study, whose demographic details are detailed in Table 8.2.1.

Table 8.2.1 - Subject Demographics

	Mean ± SD	Range
Age (years)	37 ± 12	22 - 55
Sphere (D)	-3.80 ± 1.12	-1.5 to -5.50
Cyl (D)	-0.23 ± 0.3	0 to -1.00
Flat keratometry reading (mm)	7.9 ± 0.3	8.35 - 7.50
Steep keratometry reading (mm)	7.83 ± 0.3	8.30 - 7.40

All subjects were fitted with the two test lenses, which were Bausch & Lomb "Medalist 66" and Johnson & Johnson "Surevue", details of which are given in Table 8.2.2.

Table 8.2.2 - Lens Parameters

	SUREVUE	MEDALIST 66
Water Content	58	66
Monomers	HEMA + MA	HEMA +VP
USAN	Etafilcon A	Alphafilcon A
FDA Category	Group IV	Group II
ISO Category	Filcon 1b	Filcon 4a
Manufacture	Moulded	Moulded
Back Optic Zone Radius	8.40, 8.80	8.70
Total Diameter	14.00	14.20
Centre Thickness	0.105	0.11

The care regimens evaluated were Sauflon "All-in-One", Allergan "Complete" and Bausch & Lomb "ReNu", whose details are given in Table 8.2.3.

Table 8.2.3 - Solution Details

	ALL-IN-ONE	COMPLETE	RENU
% polyhexanide	0.0005%	0.0001%	0.00005%
% EDTA	0.3%	0.05%	0.1%
Surfactant	poloxamine	tyloxapol	poloxamine

Lenses were rubbed, rinsed and stored in the solution overnight. The subjects used the instruction sheet and cases provided by the manufacturers. No saline solutions, enzyme tablets or rewetting drops were used at any stage during the study and each care system was only taken from a single lot. A clinical assistant randomly assigned the solutions and lenses for the study. Information regarding the lenses was unknown to either subjects or the investigator (double-masked paradigm) and the solution system being used at any time was unknown to the investigator (single-masked paradigm).

Each subject was issued with each of the three available disinfection systems in a randomised fashion. During the study the lenses were worn as a contralateral pair on a daily wear basis for one month periods, after which they were replaced. The right eye-left eye arrangement of the lenses remained the same throughout the period of the study, but the right eye-left eye combination was randomised such that each lens was worn an equal number of times in each eye.

8.2.2 Clinical Protocol

The protocol was exactly that adopted in 8.1.2.

8.2.3 Analytical Protocol

Surface deposition was evaluated using fluorescence spectrophotofluorimetry, exciting at 280nm to evaluate proteins and 360nm for lipid. Total protein was assessed using transmission UV at 280nm.

8.2.4 Data Analysis

Summary statistics were calculated for all variables. A one-way repeated measure ANOVA on ranks was used to compare results between solutions and Wilcoxon signed rank test to compare the two materials. If the ANOVA proved significant then multiple comparison testing was conducted using the Tukey test. A "p" value of <0.05 was considered statistically significant.

8.2.5 Results

8.2.5.1. Visual Acuity

Both high and low contrast visual acuity remained consistent across the four weeks of wear, with no significant reduction across the wearing period (p=NS). There was no significant difference between lens types or solutions (p=NS).

8.2.5.2. Wettability (PLNIBUT)

Figures 8.2.1 and 8.2.2 reveal that there was no significant difference between materials or solutions at any visit (p=NS) but that wettability reduced equally with all combinations across the four-week period and that this reduction was statistically significant ($p<0.05$). Tukey analysis indicated that the reduction occurred between the collection and four week visit ($p<0.05$), with no significant change between collection and two-weeks (p=NS) and the two-week to four week visit (p=NS).

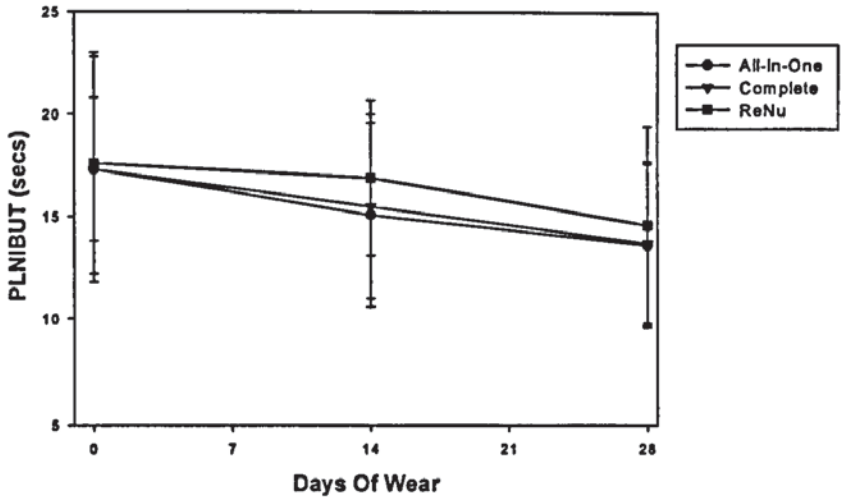


Figure 8.2.1 - Mean (±sd) PLNIBUT of group II material with all care systems

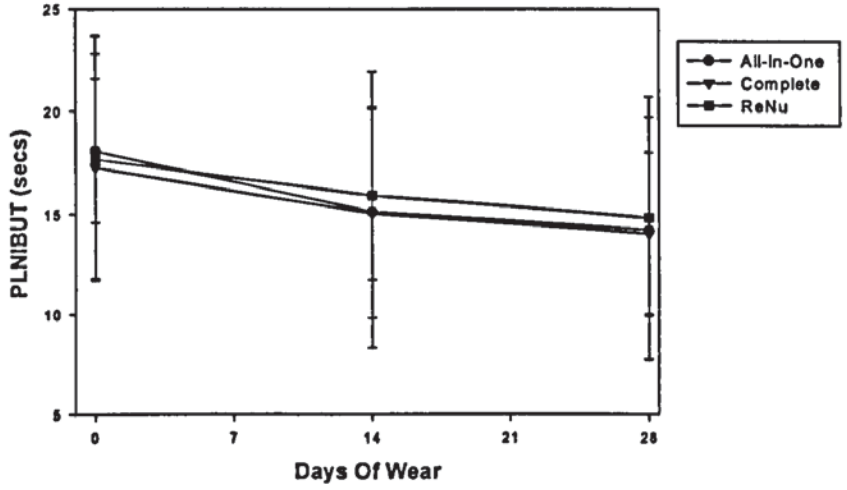


Figure 8.2.2 - Mean (±sd) PLNIBUT of group IV material with all care systems

8.2.5.3. Subjective Responses - Solution

a). Comfort - figure 8.2.3 indicates that comfort on insertion reduced as the lenses aged with all solutions. This reduction was significant with All-in-One ($p<0.03$) but not with the other two products (p=NS). The comfort scores were not significantly different between solutions at the initial visit (p=NS) but were for the final visit ($p<0.03$), with the comfort of All-in-One being less than the other two products ($p<0.05$).

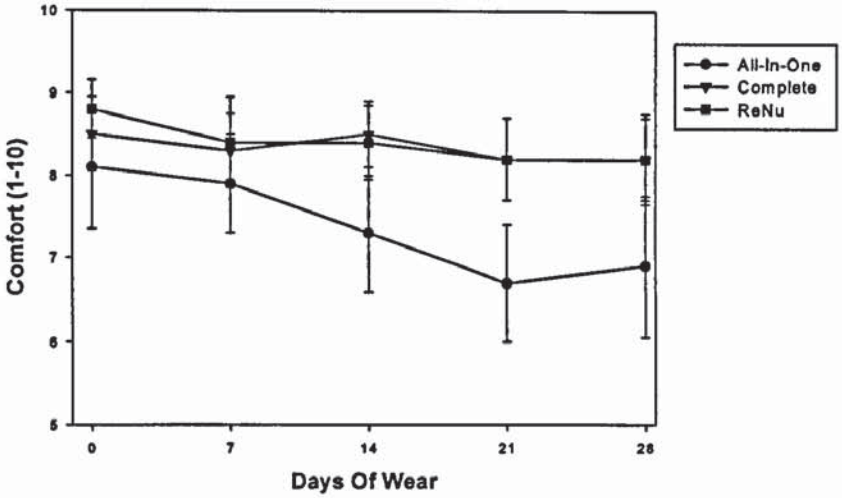


Figure 8.2.3 - Mean (±sd) comfort responses for solution comfort on insertion for all care systems

b). Overall satisfaction - figure 8.2.4 shows that subjects became increasingly dissatisfied with all solutions as the study period progressed. This reduction was significant only for All-in-One ($p<0.02$). While there was no significant difference between products at the beginning of the study (p=NS) there was at day 28 ($p<0.05$), with All-in-One resulting in lower satisfaction than the other two products ($p<0.05$).

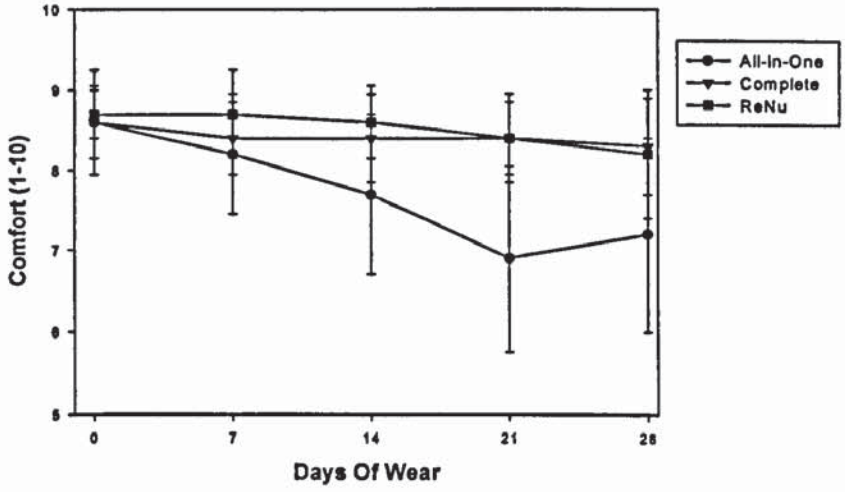


Figure 8.2.4 - Mean (±sd) subjective responses for overall satisfaction for all care systems

8.2.5.4. Subjective Responses - Lenses

a). Visual quality - figures 8.2.5 and 8.2.6 indicate that subjects felt that visual quality reduced during the study with all combinations of products. This reduction was only significant for All-in-One, for both the group II lens ($p<0.01$) and the group IV lens ($p<0.03$). There was no statistically significant difference between the three solutions at either collection or after 28 days (p=NS). There was no significant difference in subjective visual quality between the two lens types at any visit (p=NS), which agrees with the findings of Young et al. [473]

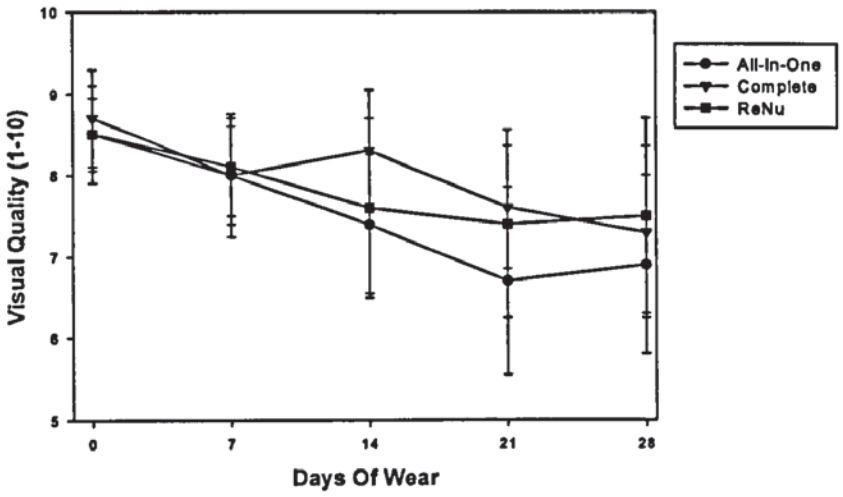


Figure 8.2.5 - Mean (±sd) visual quality for group II material

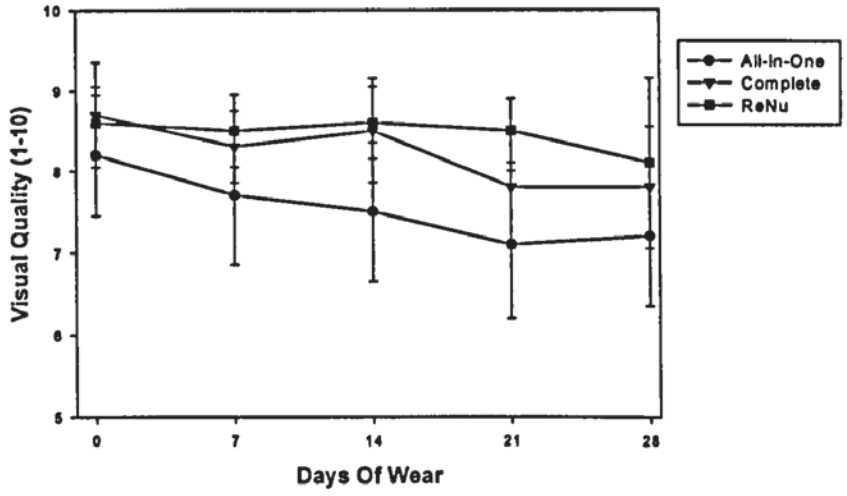


Figure 8.2.6 - Mean (±sd) visual quality for group IV material

b). Lens comfort - figures 8.2.7 and 8.2.8 indicate that at all visits lenses were less comfortable with All-in-One and that there was a significant reduction in comfort across time with the All-in-One/group II combination ($p<0.01$). At day 28 both lens types were less comfortable with All-in-One than the other solutions ($p<0.04$). There was no significant difference in subjective comfort between the two lens types at any visit (p=NS).

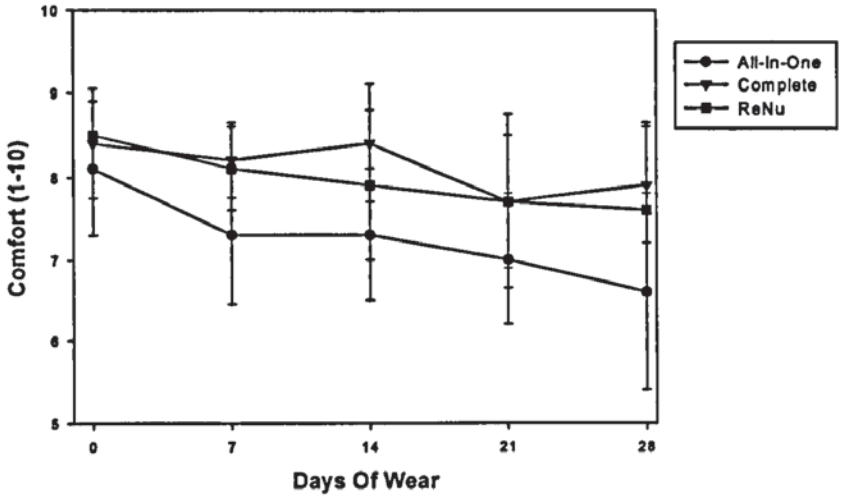


Figure 8.2.7 - Mean (±sd) lens comfort for group II material

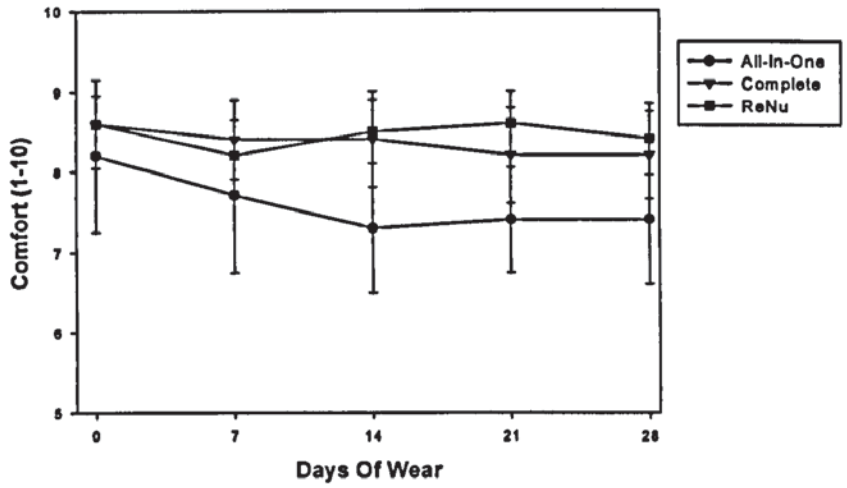


Figure 8.2.8 - Mean (±sd) lens comfort for group IV material

c). Overall satisfaction - figures 8.2.9 and 8.2.10 reveal that there was an obvious trend for subjects to be less happy with their lenses (regardless of type) when used with the All-in-One solution. While there was no difference in overall satisfaction upon collection, after 28 days both lenses displayed lower satisfaction scores when used with All-in-One ($p<0.05$). There was no significant difference in overall satisfaction between the two lens types at any visit (p=NS) and the reduction in satisfaction with all combinations was only significant for the All-in-One/group II combination ($p<0.04$).

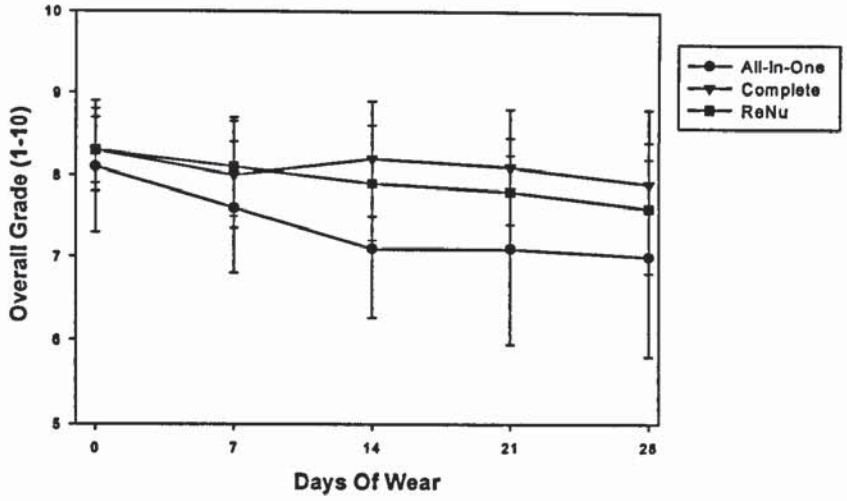


Figure 8.2.9 - Mean (±sd) overall satisfaction for group II material

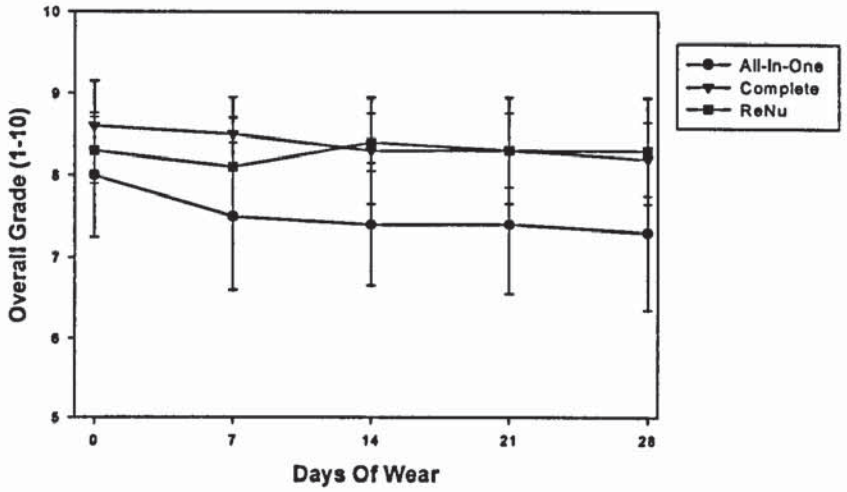


Figure 8.2.10 - Mean (±sd) overall satisfaction for group IV material

8.2.5.5. Physiological Response

a). Conjunctival staining - figure 8.2.11 indicates that while there was no difference between solutions (p=NS) or across time (p=NS), the group IV material produced more lens-edge staining on the conjunctiva than the group II material ($p<0.02$). None of the incidences of staining were considered clinically significant.

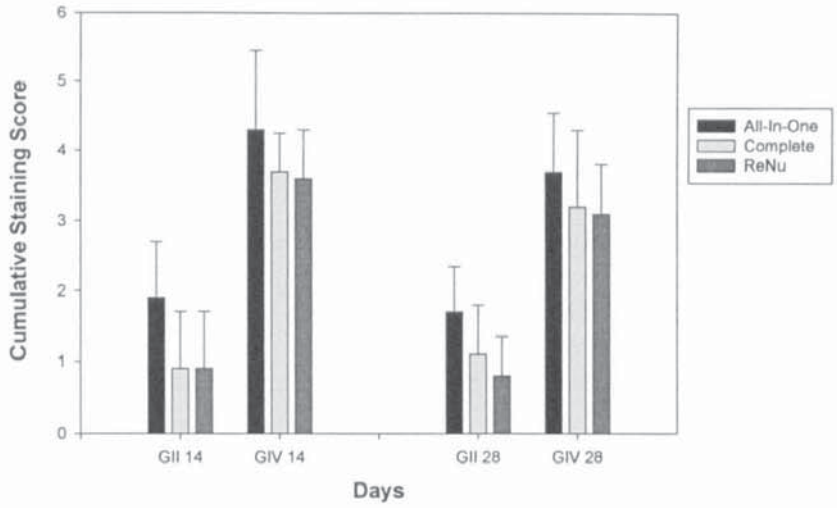


Figure 8.2.11 - Conjunctival staining score

b). Corneal staining - figure 8.2.12 indicates that while there was no difference across time (p=NS), corneal staining was significantly higher with All-in-One after both 14 days ($p<0.001$ with group II and $p<0.02$ with group IV) and 28 days ($p<0.00001$ with group II and $p<0.03$ with group IV). Six subjects (60%) exhibited clinically unacceptable staining in the eye wearing the group II lens when using All-in-One after 28 days, compared with one subject (10%) wearing the group IV lens. Figure 8.2.13 depicts the typical level of staining seen.

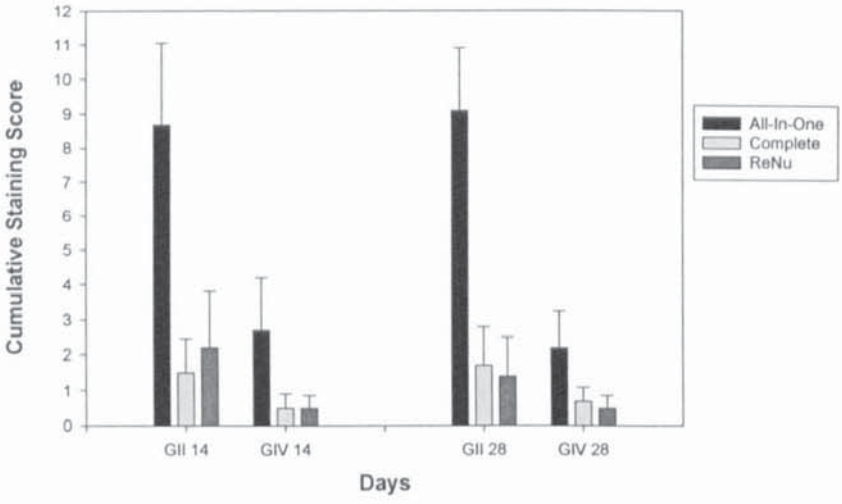


Figure 8.2.12 - Corneal staining score

Figure 8.2.13 - Slit-lamp picture of corneal staining encountered with All-in-One

c). Palpebral Hyperaemia - figure 8.2.14 indicates that there was no difference between material type or across time (p=NS), but that there was a tendency for increased palpebral hyperaemia with the All-in-One product with both materials. This difference was not statistically significant (p=NS).

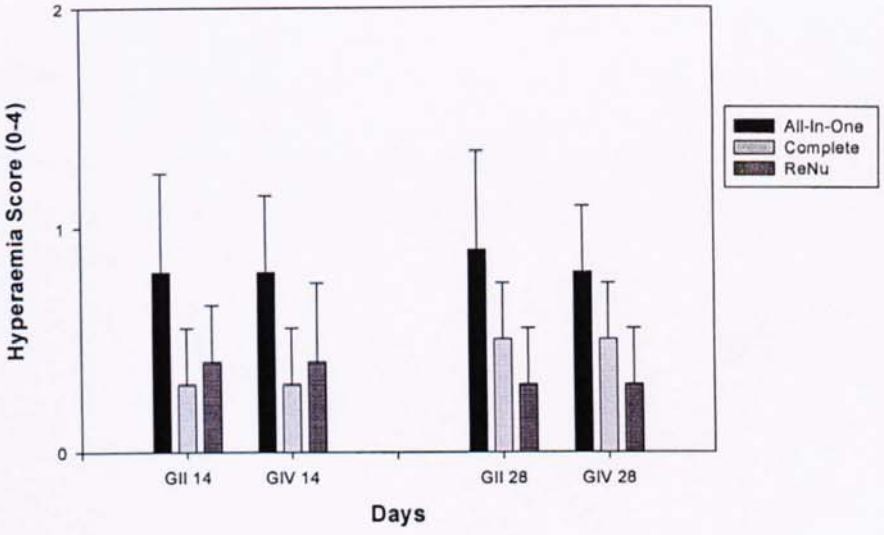


Figure 8.2.14 - palpebral hyperaemia score

8.2.5.6 Deposition Analysis

a) Total Protein

Figure 8.2.15 indicates that the group IV material deposited more protein than the group II material during the four weeks of wear ($p<0.0001$) and that the amount of deposited protein on either material was independent of the care system used (p=NS).

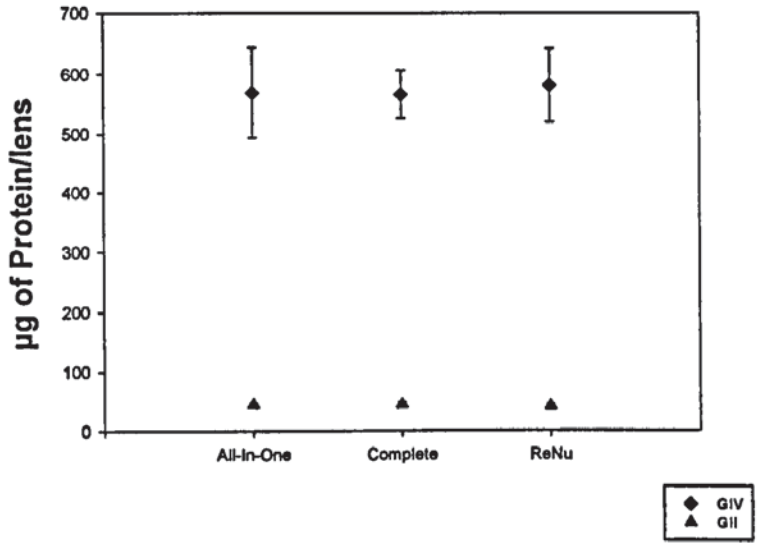


Figure 8.2.15 - Mean (±sd) total protein by UV for all care systems and materials

b) Surface Protein

Figure 8.2.16 indicates that the group IV material deposited more surface protein than the group II material during the four weeks of wear ($p<0.0001$) and that the degree of deposition was independent of the care system used (p=NS)

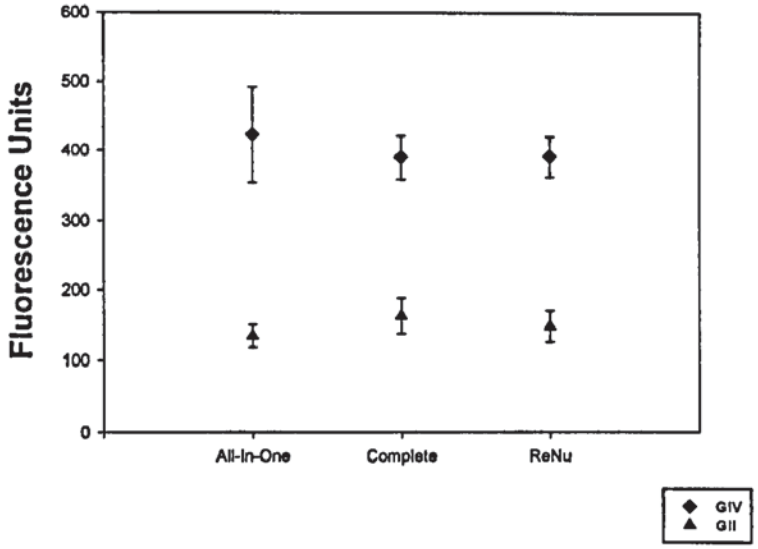


Figure 8.2.16 - Mean (±sd) surface protein by fluorescence for all care systems and materials

c) Surface Lipid

Figure 8.2.17 indicates that the group II material deposited more lipid than the group IV material during the four weeks of wear ($p<0.0001$) and that the degree of deposition was independent of the care system used (p=NS).

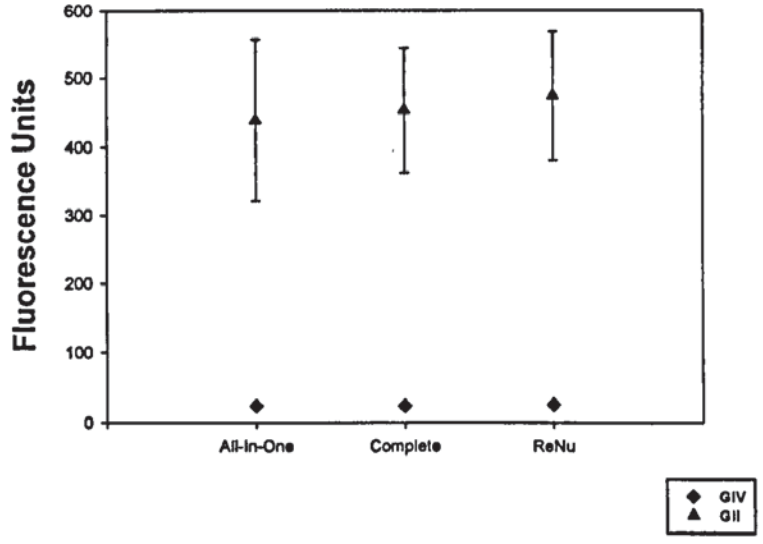


Figure 8.2.17 - Mean (±sd) surface lipid by fluorescence for all care systems and materials

8.2.5.7 Final choice

Upon completion of the study subjects were asked a forced-choice question regarding their choice of solutions and lens combination. These are detailed in Table 8.1.4.

Table 8.2.4 - Final Choice

Group IV	9	All-in-One	0
Group II	1	Complete	2
		ReNu	8

8.2.6 Discussion

Widespread concern has arisen due to recent reports concerning the increasing incidence of bacterial and *Acanthamoeba* keratitis, [538 539] particularly among wearers of frequent replacement lenses. [505 506] While several of these studies have merely confirmed previous reports that extended-wear of soft lenses is a major risk factor for developing microbial keratitis, [507 540] the increased incidence of such complications in the daily-wear population [288 289 326] has resulted in a call for practitioners to use care systems containing either hydrogen peroxide [541] or high concentrations of preservatives such as polyhexanide, [542 543] in the hope that such systems will provide greater margins of safety. However, several studies have shown that both low concentration polyhexanide-containing solutions and 3% hydrogen peroxide systems are equally as ineffective against *Acanthamoeba* cysts if left to soak for the recommended soaking time. [259 261] Other work has demonstrated that the mere action of rubbing and rinsing alone is sufficient to remove pathogenic organisms [251 252 544] and that, if used in the manner in which the systems are licensed, that peroxide and polyhexanide-based systems are equally effective in dealing with *Acanthameoba* attached to worn lenses. [259 261 545] It may therefore transpire that a higher concentration of preservatives in the soaking solution is unnecessary and could potentially result in unwanted toxic or hypersensitivity reactions. [263 265 266]

Polyhexanide (PHMB) was originally developed by ICI and belongs to the same pharmaceutical family as chlorhexidine, although it has a much higher molecular weight. It is a cationic (positively charged) disinfectant at physiological pH. In contrast to the antibiotics (that act by disrupting cell walls) PHMB binds to the phospholipids found in the bacterial cell membrane and the hydrophobic portions of the molecule become associated with the internal core of the bilayer membrane, disrupting the cytoplasmic membrane and producing precipitation of cell contents and subsequent cellular lysis. [275] Ethylenediamine tetra-acetic acid (EDTA) is a chelating agent and works synergistically to enhance the effect of preservatives.

Between 5-35% of patients exhibit toxic and/or hypersensitivity reactions to "older-technology" preserved contact lens solutions, based on thimerosal and/or chlorhexidine. [264 266] Newer products containing higher molecular weight disinfectants such as polyhexanide typically show a sensitivity reaction of less than 1%.

[546 - 548] While increasing the concentration of preservatives may impart enhanced microbial activity to a care product, such solutions may exhibit increased solution-related complications such as corneal staining and reduced comfort. Previous work by Begley et al [549] found that a solution of buffered saline containing the same concentration of PHMB as ReNu (0.00005%) did not cause high levels of epithelial toxicity, whereas increasing the concentration by 10-100 times resulted in significant epithelial damage. Further work [550] indicated that rigid gas permeable contact lens solutions preserved with 0.0015% PHMB resulted in significant damage to the corneal epithelium in a human model. Such findings are corroborated by this study, with the All-in-One solution producing inferior results, both in terms of subjective response and physiological appearance. Figures 8.2.3 and 8.2.4 indicate that solution comfort and overall satisfaction were clearly reduced by using a solution with a high concentration of preservatives. Furthermore, figures 8.2.5-8.2.10 show that subjective satisfaction with the lenses was also intimately linked with the solution type and resulted in reduced satisfaction with this solution. This has important implications when selecting an appropriate solution for a patient, particularly one who is new to lenses. Apparent discomfort in a new lens wearer may be incorrectly presumed to be due to the lens type, when it may actually be directly attributable to the chosen care system.

Figure 8.2.11 demonstrates that the group IV lens produced more conjunctival staining than the group II lens, which concurs with previous studies investigating the level of conjunctival staining with moulded Etafilcon lenses. [483] However, none of the incidences of staining were considered clinically significant. Of far greater concern are figures 8.2.12 and 8.2.13, which clearly demonstrate the effect of the All-in-One product on the ocular tissues over a four week period. Figure 8.2.13 indicates a clear trend, which, while not statistically significant, was considered clinically significant and indicates that palpebral hyperaemia was enhanced with the All-in-One product when compared with the other two multi-purpose products. This is surprising given the short period of the study. These results must be considered in the light of the lid changes found in an earlier study in this thesis (Table 5.1.4, page 142), in which hyperaemic changes were found with ReNu over a year period. These combined findings would suggest that PHMB does produce an increase in lid hyperaemia and that the effect is dose-related.

Figure 8.2.12 produces clear concerns regarding the degree of corneal staining experienced with the All-in-One product, particularly when used with the group II material. The enhanced staining seen was statistically and clinically worse when compared with ReNu and Complete, with six subjects (60%) exhibited clinically unacceptable staining in the eye wearing the group II lens when using All-in-One after 28 days and one subject (10%) when wearing the group IV lens. A patent relating to ReNu [551] demonstrates quite clearly that the degree of corneal staining increases with increasing concentrations of PHMB, with 5ppm producing 17% corneal staining and 55ppm producing 62%.

The subjective findings and slit lamp results suggest that there was a specific interaction between the group II material and the All-in-One solution, which resulted in one or more chemical constituents in All-in-One being concentrated in or on the group II lenses to a toxic level. Work reported in Chapter 7 (page 228) has shown that the accumulation of lipid on NVP containing group II materials progressively increases over a four week period, whereas lipid deposition plays only a minor role in non-NVP containing group IV lenses.

It appears likely that one or both of the constituents in All-in-One that is present at a high concentration (polyhexanide or EDTA) progressively bound to the increasing levels of deposited lipid on the group II material to form a toxic complex, which then interacted with the corneal epithelium to result in a marked superficial punctate keratitis. Further work is necessary to investigate if the staining seen was due to a particular interaction with Medalist 66 or whether this can be extrapolated to other group II materials and whether the study findings were directly attributable to the increased concentration of preservatives or if it related to the overall formulation of All- in-One. In addition, it could be conceivable that the concentration of PHMB resulted in greater protein denaturation than that encountered with the other two products and that this factor resulted in reduced comfort, as shown by the results of the earlier study in this Chapter (study 5.1, pages 257-258). Further work to investigate the denaturing effect of such high concentrations of PHMB are required to confirm this.

While this study demonstrated a clear preference against All-in-One, no difference could be found between the other two products. However, a study [552] comparing ReNu and Complete demonstrated a bias towards ReNu, with fewer slit lamp findings and greater patient preference. This further suggests that products of similar chemical formulation should only be interchanged with care. As in previous studies in this thesis, Figures 8.2.1 and 8.2.2 indicates that PLNIBUT reduces with wearing period, is patient dependent and is independent of either material type or solution, which agrees with previous work investigating the performance of both rigid [535] and hydrogel materials. [455] Figures 8.2.5-8.2.10 also demonstrate that subjective satisfaction reduced with all combinations of lenses and solutions over the four-week period. The over-riding conclusion is that different concentrations of the same compound impact subjective performance and that the impact is proportional to the age of the lens.

Whilst avoiding corneal infection remains a crucial factor in contact lens wear, there is a fine balance between efficacy and safety; avoidance of exposure to all pathogenic organisms remains the best approach to avoidance of keratitis. Failure to disinfect adequately is a significant factor in *Acanthamoeba* infection [287 289 541] and such organisms require bacteria to live on. [553 554] If patients use their solutions in the prescribed manner, always rub and rinse adequately, avoid tap water and replace their lens cases regularly [335 555] then the need for solutions with high concentrations of preservatives becomes unnecessary and toxicity reactions should remain at a low level. Finally, this study found no differences in deposition between the multi-purpose systems examined. In comparison, previous work indicated that borate buffered one-bottle systems (such as ReNu) resulted in greater protein deposition than other one-bottle systems [556] and that, following a passive soak, Complete removed twice as much protein from both worn and *in vitro* doped lenses than ReNu. [557] The differences between such results are explained by the use of differing methodologies, single protein types being used in doping studies and different analytical methods and extraction techniques.

8.3 Key Points

- Subjective vision, comfort and satisfaction with frequent replacement lenses reduces over a 4 week period.
- Significant subjective differences exist between solution/lens combinations worn over a 4 week period.
- High and low contrast visual acuity remain stable across a four week wearing schedule, irrespective of lens material or care regimen.
- Front surface wettability reduces over a four week wearing schedule, irrespective of care regimen and lens material. Large variations in wettability are seen within even small groups of subjects, indicating the influence of subject on this factor.
- The type of visible deposition encountered after 4 weeks of wear depends upon both solution system and lens material.
- Group II lenses deposit more lipid than Group IV and Group IV lenses deposit more protein than Group II.
- Bulk and surface deposition is little altered by solution type over a 4 week wearing period.
- Solution systems denature lysozyme to different extents.
- Lysozyme activity is strongly correlated with changes in visual quality, comfort and overall satisfaction
- The quality of the deposited material, rather than its quantity, influences subjective response.
- There is a trend for corneal staining and conjunctival hyperaemia to increase with lens age, irrespective of care regime.

- Whilst products with increased concentrations of PHMB and EDTA will impart enhanced antimicrobial activity to multi-purpose solutions, some subjects will notice reduced comfort and enhanced corneal staining may be seen, in particular with certain contact lens materials. Specifically, unacceptable levels of corneal staining may occur with multi-purpose solutions preserved with 0.0005% polyhexanide (PHMB) and containing 0.3% EDTA, in particular when used with FDA group II materials.

- Minimal staining is seen with multi-purpose products containing lower concentrations of polyhexanide and EDTA.

- Conjunctival staining may vary between lens types.

- Practitioners and patients alike should be advised that products with similar chemical constituents but differing formulations are not necessarily similar in action and should be interchanged with caution.

Chapter 9

The Influence of Biomimesis On the Spoilation and Clinical Performance of Hydrogel Lenses

"Knowledge advances by steps and not by leaps". [1]

Since the first discovery of polyHEMA as a material for biomedical applications [558] research has focused on the factors which govern the interactions of a synthetic material with natural tissue, particularly as the use of synthetic polymers for implantation materials in various body sites has increased. [559-565] Understanding of the basic principles behind these interactions will help in the development of a material that is readily accepted by the environment and does not undergo adverse reactions that, ultimately, result in rejection or failure of the artificial material for its application. Examples of the interactions which result in the rejection of a synthetic material include the formation of deposits on contact lenses (commonly termed "deposition"), the formation of dental plaque on dentures and thrombus formation on artificial blood vessels. [566] [567] One stage common to all processes of this type is deposition of protein onto the synthetic substrate.

Strategies to overcome deposition include frequent lens replacement, optimisation of the care system and the use of synthetic "biocompatible" materials. This Chapter will explore the latter of these options and investigate the ability of such materials to resist spoilation.

[1] TB Macaulay

9.1 Background

The development of materials that mimic a biological system (for example the external ocular environment) are of considerable interest to the contact lens field. Such a process is termed "biomimesis" and relates to the development of artificial materials that mimic physiological systems without necessarily resorting to the same detailed chemistry. The complexity of tears and the accessibility of the eye is ideal for examining potential new implantation materials, as materials are easily removed for examination and novel materials may be easily compared with existing types. To date only two contact lens materials are commercially available which claim to fulfill this role - "Proclear" and "Vistagel PLUS".

Proclear is a 60% water content lens available from Biocompatibles. The material is a spin-off from haemocompatibility studies initiated by Chapman and colleagues in the early 1980's. [561] The material is based on phosphorylcholine (PC). This is a key component of phosphatidylcholine, a natural phospholipid found in all human cell membranes and the major phospholipid headgroup component found on the extracellular surface of biological cell membranes. Biocompatibles have created a synthetic analogue of PC and, by combining it with polyHEMA, developed a new hydrogel contact lens material. [568] PC coatings have been used for several years to coat polyvinyl chloride tubing used in thoracic drainage catheters, cannulae and tubing sets for extracorporeal procedures, produce enhanced haemocompatibility, reduced bacterial adhesion and reduced protein deposition. [569 - 572]

Vistagel PLUS is a 40% water content material available from Vista-Optics, based upon polyethylene glycol derivatives. It uses the principles of controlled sequence distribution and enhanced water binding and was developed within the Biomaterials Research Unit at Aston University. Polymers containing long repeat units or long sequences of individual monomer units produce greater protein adsorption than those polymers that mimic the molecular architecture of naturally occurring polymers, which possess regular, short sequences of monomer units. [573 574] Protein adhesion requires areas of surface chemistry which are large enough to achieve stable physicochemical interactions between the protein and the material surface. [575 - 577] Biological cells can form adhesive focal or contact points on a surface but require a specific chemistry and area to adhere permanently. If the zones of contact are too small and/or too far apart from another equivalent zone then the cells are unable to spread and unable to attain permanent, firm adhesion to the surface. Further work [578] has investigated the adsorption of adhesion proteins and platelets onto copolymer surfaces. This work showed that preferential adsorption is affected by the surface distribution of chemical groups and that surface domains as small as nanometres are critical in determining adsorption to the surface.

When designing a polymeric material that is resistant to the adsorption of biological species it is necessary to consider the sequence distribution and the length of the monomer sequences formed in the copolymerization of the fabricated material. A computer program that simulates free radical copolymerization - developed at Aston University - can simulate the addition of successive monomer sequences for copolymer or terpolymer reactions. [573] Use of the computer program enables existing copolymers to be studied - and new ones designed - which possess alternating units in short sequence lengths of one monomer type. Ideally, a biomimetic polymer should possess alternating domains of hydrophobicity and hydrophilicity that avoid

clustering together of one molecular type, thereby preventing preferential adsorption of physiological molecules.

Figure 9.1.1 diagrammatically describes a typical NVP/HEMA polymer found in current clinical practice. Evaluation of the diagram and Table 9.1.1 indicates that, due to the reactivity ratios of the monomers, large blocks of NVP remain at the end of the reaction and attach to form large blocks. These areas within so-called "blocky" polymers act as foci for the attachment of biological species such as proteins and lipids. Figure 9.1.2 describes the situation that occurs if the sequence distribution is controlled. By careful selection of components with similar reactivity ratios a polymer is produced which possesses only short domains of one molecular type, as indicated in Table 9.1.2.

Table 9.1.1 - Sequence Lengths In "Blocky" Polymer

The simulated copolymer contains 1400 HEMA units and 600 NVP units

Sequence Length	Number of Sequences		Sequence Length	Number of Sequences	
	HEMA	NVP		HEMA	NVP
1	67	255	15	2	0
2	49	0	16	2	0
3	27	0	17	5	0
4	14	0	18	3	0
5	18	0	19	1	0
6	15	0	20	2	0
7	11	0	22	1	0
8	6	0	24	2	0
9	6	0	25	1	0
10	5	0	26	1	0
11	4	0	29	2	0
12	3	0	30	1	0
13	4	0	46	1	0
14	3	0	345	0	1

To produce a polymer with improved sequence distribution the polymer must exhibit a narrow distribution of sequence lengths, principally consisting of monomer or dimer units. The polymer backbone is not dominated by domains of one chemical type and is more characteristic of an alternating copolymer, as is commonly encountered in natural polymers. Figure 9.1.2 simulates a 70 mole% of HPMA copolymerised with 30 mole% ACMO continued to 100% conversion. In the simulated copolymer HPMA is represented by O and ACMO is represented by X.

Figure 9.1.1. Diagrammatic Representation of a "Blocky" Polymer

The simulation below represents 70 mole% of HEMA copolymerised with 30 mole% NVP continued to 100% conversion. In the simulated copolymer HEMA is represented by O and NVP is represented by X. This represents a typical polyHEMA - NVP copolymer.

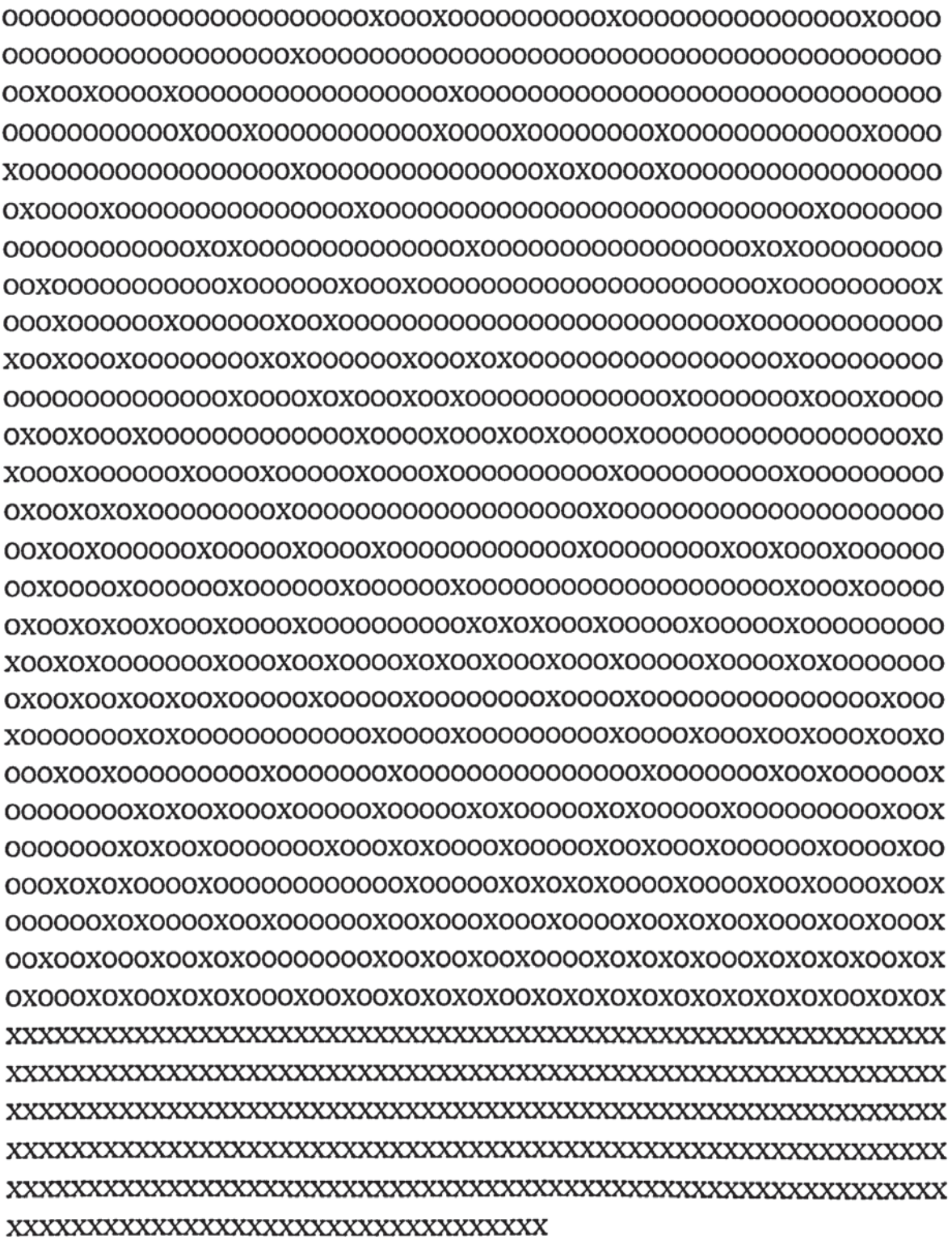


Figure 9.1.2. Diagrammatic Representation of a Typical Controlled Sequence Distribution Polymer

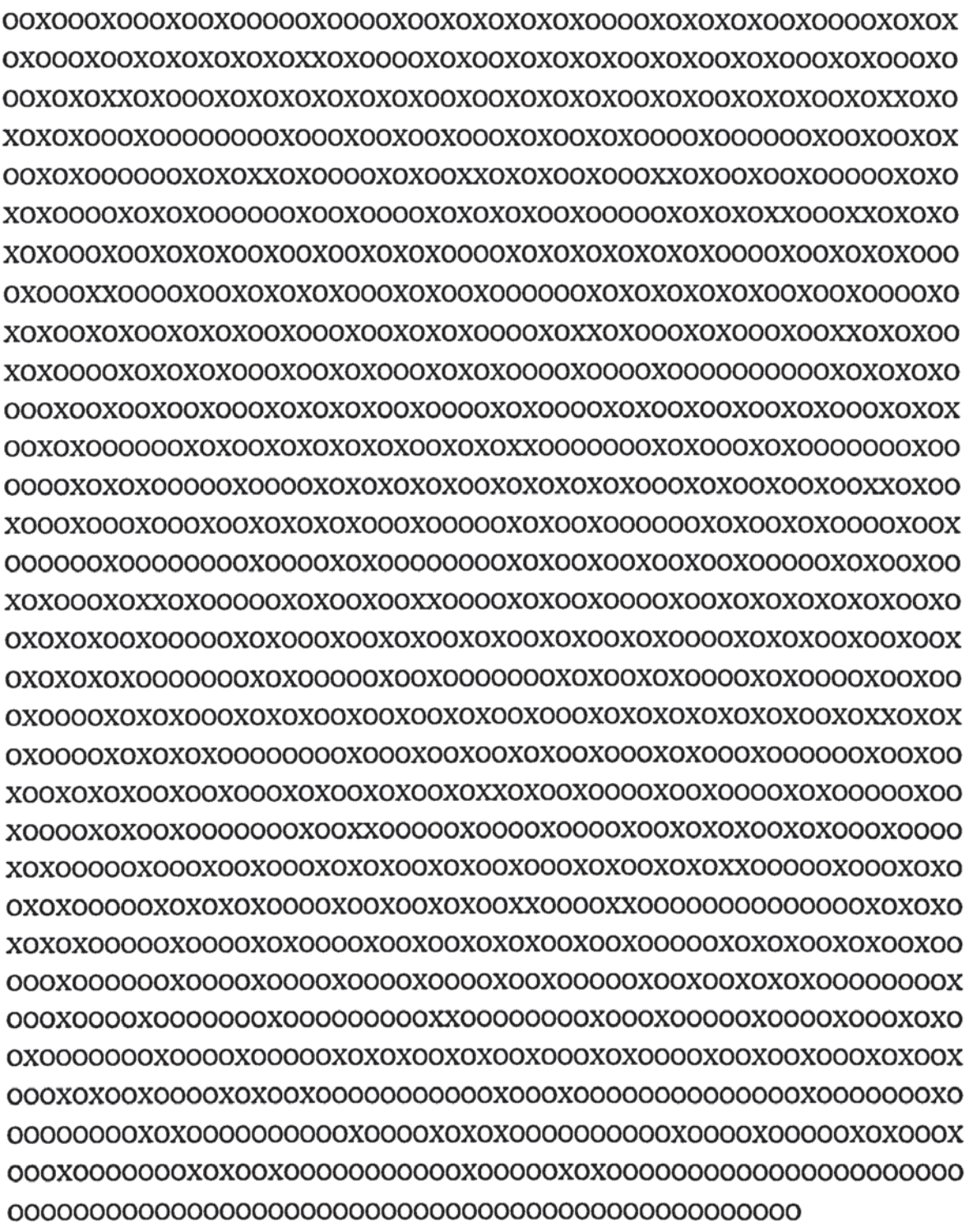


Table 9.1.2 - Sequence Lengths In "Controlled Sequence" Polymer

The simulated copolymer contains 1400 HPMA units and 600 ACMO units

Sequence Length	HPMA	ACMO
1	247	545
2	130	24
3	89	1
4	48	1
5	26	0
6	11	0
7	5	0
8	5	0
9	6	0
11	1	0
12	1	0
16	2	0
54	1	0

The diagram clearly indicates that no large "blocks" of monomer are found, reducing the adhesion properties of proteins and other depositing species.

The significance of the water binding characteristics of monomers used in hydrogel synthesis on the physicochemical interactions between the hydrogel surface and biological proteins is well known. [579] This work proved that it is not only the chemical groups at a surface which affect the amount and conformation of adsorbed adhesion proteins, but also the reorganisation of the structural arrangement of water molecules at the interface. Poly (ethylene oxides) [PEO's] or poly (ethylene glycols) [PEG's] are materials that contain multiple $-CH_2CH_2O-$ units. They are non-ionic materials that exhibit complex solubility behaviour and have a regular backbone structure with few side chains. They are used to produce hydrogels or are incorporated with other monomers to produce copolymers or terpolymers by a variety of techniques. [580] The importance of PEO in hydrogel chemistry is recognised due to its reduced protein adsorption and cell adhesion [581] and its effectiveness in reducing the adsorption of biological species is widely recognised. [582] This is in part due to the hydrophilicity of PEO and its unique solubility and water structuring properties, which produces surfaces in a liquid-like state due to the large degree of flexibility and mobility of the polymer chains [583 - 585] and their lack of ionic charge. [586] The thermal mobility of poly (ethylene oxide) chains is greater than that of other hydrophilic polymers such as poly (2-hydroxyethyl methacrylate) [polyHEMA] or poly (N-vinyl pyrrolidone) [NVP]. It is believed that this mobility, combined with the water structuring characteristics of the poly (ethylene oxide) chains, prevents adhesion of proteins [585] due to the rapid movement of the PEO chains, which prevent stagnation of the blood components on the hydrogel surface. Since one of the requirements for irreversible adhesion is a certain amount of contact time, protein adsorption is decreased. Figures 9.1.3 and 9.1.4 diagrammatically display the similarity between a typical cell membrane and that of the surface of PEG-containing polymers.

Figure 9.1.3 - Diagrammatic Representation of a Cell Membrane

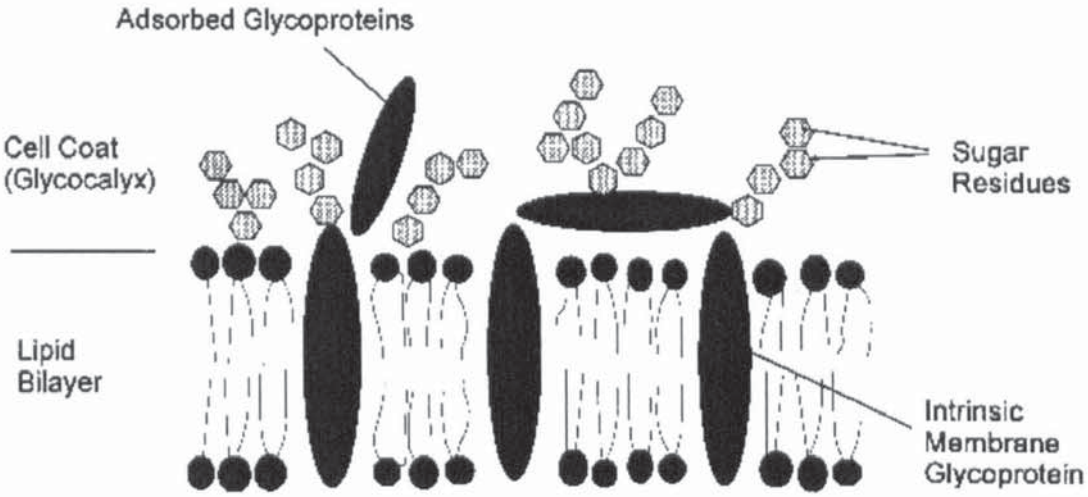
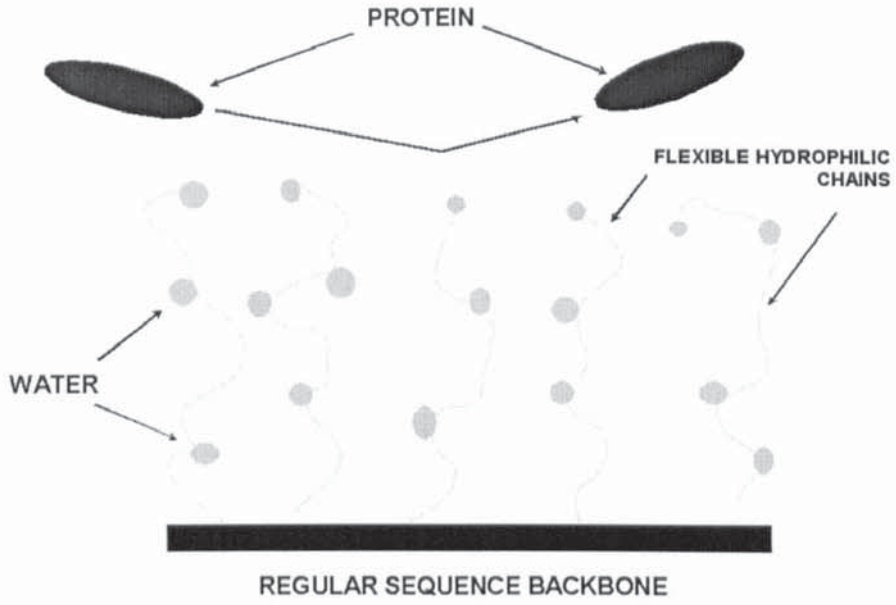


Figure 9.1.4 - Diagrammatic Representation of PEG-Containing Hydrogel Surface



The incorporation of long poly (ethylene oxide) chains with a degree of polymerisation of approximately 100 onto the surface of hydrogels results in long-lasting antithrombogenicity. [587] The percentage of poly (ethylene oxide) groups at the surface is independent of the poly (ethylene oxide) chain length, but protein and platelet adsorption are decreased with increasing chain length and chain separation or surface coverage is also important. [588] More recent work has shown that degrees of polymerisation of 400 and above may be required to provide more effective protein resistance [589] and that a threshold concentration of poly (ethylene oxide) is required.

In conclusion, an ideal biomimetic surface should model the corneal glycocalyx. A synthetic model should consist of a surface where mobile hydrophilic chains are evenly distributed throughout the polymeric material and are expressed at the surface. This will provide a protective "umbrella" to shield the underlying surface from the biological species. Additionally, the bulk material should possess a controlled sequence distribution to minimise bonding interactions of biological material to exposed chemical domains.

The purpose of this chapter is to evaluate the performance of polymers based on these principles.

9.2 THE SHORT-TERM SPOILATION AND CLINICAL PERFORMANCE OF HYDROGEL MATERIALS BASED ON BIOMIMETIC PRINCIPLES

The purpose of this study was to provide short-term *in vivo* and *in vitro* information on the relative performance of a biomimetic hydrogel compared with two commercially available hydrogel materials.

9.2.1 Materials

The lens types for this study included a new biomimetic polymer based on the principles of controlled sequence distribution, PEO's and complex water structuring groups (Vistagel PLUS) and two commercially available polymers (Tetrafilcon A and Atlafilcon A), both of which display reduced deposition when compared with more conventional polymers. [118 359 590] The details of the lens types are listed in Table 9.2.1.

Table 9.2.1 - Lens Parameters

	Vistagel PLUS (DR40)	Classic	Excelens
	Vista Optics	Wesley-Jessen/PBH	CIBA-Vision
Water Content (%)	40	42	64
Principal Monomers	HEMA+MMA+NVP+PEO	MMA+VP+HEMA	PVA
USAN	-	Tetrafilcon A	Atlafilcon A
FDA Category	I	I	II
ISO Category	Filcon 1a	Filcon 1a	Filcon 4a
BOZR (mm)	8.30; 8.70; 9.10	8.30; 8.60; 8.90	8.40; 8.60; 8.80
Total Diameter (mm)	14.00	14.00	14.00
Centre Thickness (mm)	0.07	0.06	0.12

9.2.2 METHODS

9.2.2.1 In Vitro Wettability Analysis

The wetting of a contact lens is an important factor in determining its physiological compatibility as the pre-corneal tear film must be maintained in the form of a thin capillary layer. The main factor in determining the wettability of a material is the chemical structure of the polymer at the air-solid-liquid interface. Polymer surfaces are mobile and the molecular orientation is modified in response to the environment. When a liquid contacts a solid surface the liquid will spread spontaneously over the surface by a variable amount, as described in Chapter 3 (pages 107-109). When the sample is in air the hydrophobic groups are orientated towards the solid-air interface. As the liquid wets the sample the hydrophilic groups reorientate themselves towards the liquid-solid interface. The wettability of the solid is measured in terms of a contact angle, θ, [591] [592] that is determined *in vitro* by several methods. The technique used in this study to determine θ involved measuring the dynamic contact angle, as described on pages 109-110. Samples were suspended on a microbalance attached to a computer, and the test solution was raised and lowered over the sample by means of a motorised jack, enabling the advancing and receding contact angles to be recorded. The advancing contact angle (θ_a) was recorded as the "dry" sample was lowered into the test solution, and the receding contact angle (θ_r) obtained as the "wet" sample was raised from the solution. The difference between θ_a and θ_r is defined as the contact lens hysteresis and provides an indication of the mobility of the functional groups at the surface between the dry and wet states. A low hysteresis indicates that the material has less movement of the functional groups. The dynamic wettability of three lenses of each type was determined in both saline and ReNu.

9.2.2.2 In Vitro Deposition Study

To rule out the influence of extrinsic factors and inter-patient differences an *in vitro* study was conducted to compare the deposition of the three materials with each other and with a polyHEMA control. In this investigation the lenses under test are spoiled in models that mimic the tear/contact lens interaction, as previously described in Chapter 3 (page 107). Three lenses of each type were examined. All lenses were run for a total of 28 days, with levels of deposition being measured every 3 days. This produces an accelerated spoilation equivalent to several times this period in normal wear. The degree of *in vitro* spoilation was subsequently monitored using fluorescence.

9.2.2.3 In Vivo Clinical Study

Twelve subjects (six male and six female) were entered onto an *in vivo* daily wear clinical study. The demographic details are described in Table 9.2.2.

Table 9.2.2 - Subject Demographics

	Mean ± SD	Range
Age (years)	33 ± 8	22 - 48
Horizontal K (mm)	7.85 ± 0.23	7.40 - 8.40
Vertical K (mm)	7.73 ± 0.22	7.30 - 8.20
Sphere (D)	-3.24 ± 1.85	-6.75 to -1.25
Cylinder (D)	-0.44 ± 0.33	-1.00 to 0.00

All subjects were test fitted with all three lens types. During the study all subjects used a two-step hydrogen peroxide system (Allergan "Oxysept") with 20 minute disinfection and overnight neutralisation. Prior to disinfection lenses were cleaned with a daily surfactant cleaner (Allergan "LC65"), which was subsequently rinsed off with sterile non-preserved saline. A new case was given to the patients after each lens type was changed.

Each subject wore each lens type for 28 days in a randomised, cross-over fashion such that each lens type was equally exposed at all stages of the trial. A double-masked paradigm was used in which neither subject nor investigator were aware of the lens type under investigation. Subjects were reviewed in the clinic on collection and after 28 ± 3 days.

a) Clinical Protocol

At each visit subjective satisfaction, lens fit, visual acuity, PLNIBUT, visible wettability on the slit lamp, Rudko deposition and physiological performance were assessed, as previously described.

b) Analytical Protocol

At the end of the one month wearing schedule each lens was removed and stored as previously described. Surface deposition was evaluated using fluorescence spectrophotofluorimetry, exciting at 280nm to evaluate proteins and 360nm for lipid. The non-destructive nature of these techniques enables further analysis of deposition to occur once the primary analysis has taken place. If the water structuring groups were successful in preventing deposition then the deposited species should be more easily removable following surfactant cleaning. To investigate this, 10 worn lenses from each type were randomly selected and subjected to a 10-second digital clean with LC65, which was copiously rinsed off with saline. This procedure was carried out by a sole investigator who was unaware of the lens type being cleaned. The lenses were finally re-examined to assess the degree of material that could be removed from the lenses.

c) Data Analysis

Summary statistics were calculated for all variables. The grading scales, being ordinal measurements, were considered non-normal data. All other data were tested for normality of distribution. Statistical analysis was undertaken using a one-way repeated measures ANOVA or one-way repeated measures ANOVA on ranks. Post-hoc testing was undertaken using the Student-Newman-Keuls (SNK) test. A "p" value of <0.05 was considered statistically significant.

9.2.3 RESULTS

9.2.3.1 IN VITRO WETTABILITY

Table 9.2.3 outlines the results from the dynamic wettability assessment.

Table 9.2.3 - *In Vitro* Wettability (Mean ± sd)

	Advancing Angle		Receding Angle		Hysteresis	
	Saline	ReNu	Saline	ReNu	Saline	ReNu
Atlafilcon	58.7 ± 10.4	56.3 ± 10.8	44.3 ± 6.7	51.3 ± 9.3	14.50	5.04
Tetrafilcon	51.1 ± 10.3	77.4 ± 5.0	31.4 ± 1.0	55.6 ± 7.2	28.80	21.82
Vistagel	40.8 ± 13.4	41.2 ± 3.5	24.4 ± 3.1	29.5 ± 9.4	16.40	11.59
p	NS	<0.05	<0.05	NS	NS	NS

Results show that Vistagel plus exhibits lower advancing and receding contact angles in both solutions compared with the other two materials. These differences were not statistically significant for the advancing angles in saline and receding angles in ReNu (p=NS), but it is entirely possible that mean responses of this level may be clinically significant. Post-hoc testing of the results indicate that the advancing angles in ReNu for Vistagel were lower than the other two materials (p<0.05) [which were not different from each other, p=NS] and that the receding angles in saline for Vistagel were lower than for Tetrafilcon (p<0.05), which was lower than Atlafilcon (p<0.05).

One final point to note is that Vistagel plus and Atlafilcon demonstrated minimal hysteresis compared with Tetrafilcon, although the results were not statistically different (p=NS).

9.2.3.2 IN VITRO DEPOSITION ANALYSIS

Figure 9.2.1 indicates the degree of deposition that occurs in the *in vitro* model. It is clearly seen that deposition becomes cumulative with the passage of time, that all three materials have reduced deposition compared with polyHEMA and that the biocompatible material has the least deposition of all.

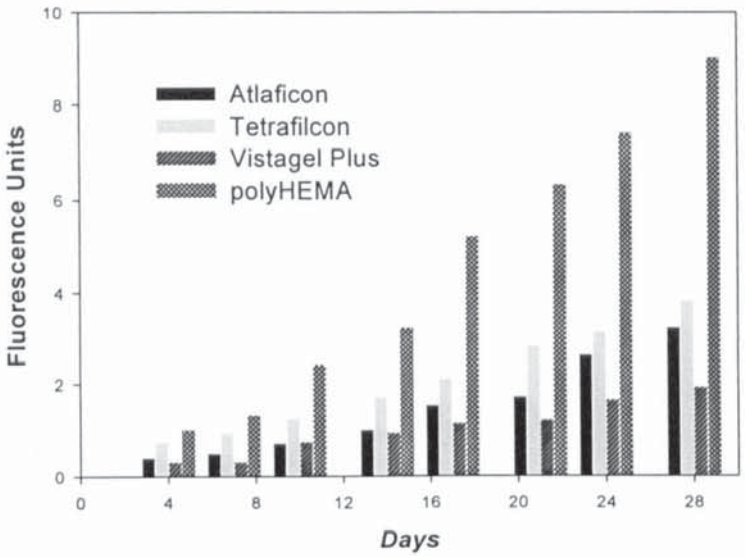


Figure 9.2.1 - *in vitro* model spoilation results for all materials and control material

9.2.3.3 IN VIVO CLINICAL STUDY

a) Front Surface Wettability (PLNIBUT)

The results are detailed in Figure 9.2.2, which clearly demonstrate that a large inter-subject variation exists in lens wettability and that PLNIBUT reduces during the month of wear. A two-way repeated measures ANOVA indicates that there was no significant difference between lenses (p=NS) but that the reduction across time with all lenses was significant ($p<0.02$).

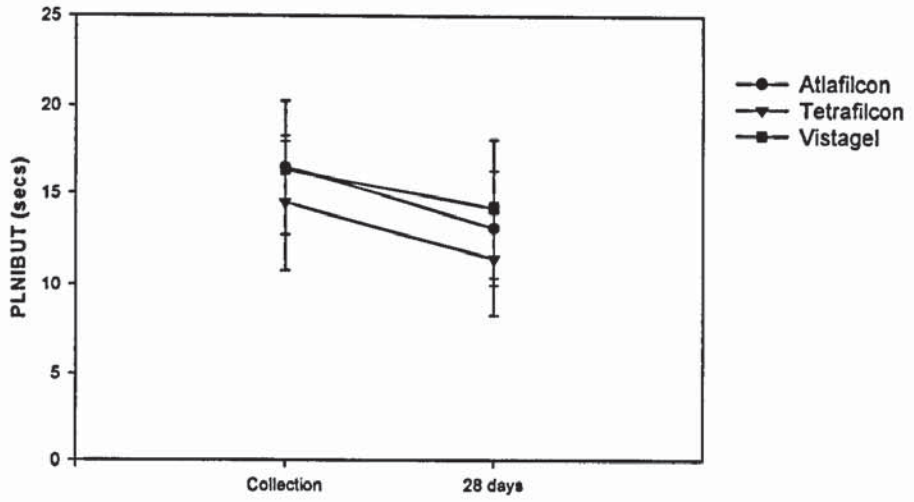


Figure 9.2.2 - PLNIBUT (mean ± sd) in seconds for all materials at the collection and one month appointment

b) Deposition

i) Protein Results

These are detailed in Table 9.2.4 and Figures 9.2.3 and 9.2.4. The results clearly show that subjects deposited lenses by different extents. The overall results were not significantly different (p=NS).

Table 9.2.4 - Protein By Fluorescence @ 280nm

	TETRAFILCON	VISTAGEL PLUS	ATLAFILCON	p
Mean ± SD	73 ± 23	72 ± 18	91 ± 32	NS
Range	136 - 16	195 - 34	235 - 17	

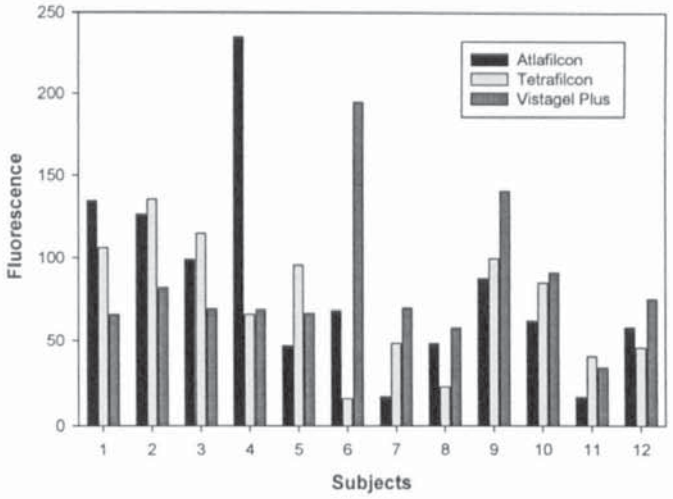


Figure 9.2.3 - protein results by fluorescence for all subjects

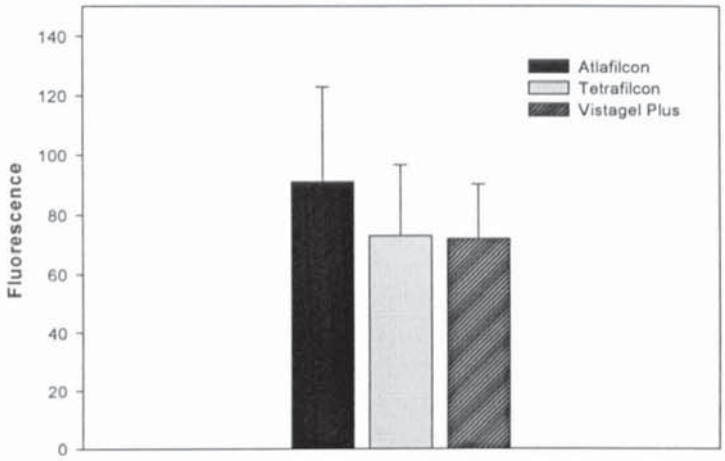


Figure 9.2.4 - mean ± sd protein results by fluorescence at 280 nm excitation for all subjects

ii) Lipid Results

These are detailed in Table 9.2.5 and Figures 9.2.5 and 9.2.6. The overall results were significantly different between materials ($p<0.001$). Post-hoc testing showed that this difference existed between Tetrafilcon and each of the other two materials ($p<0.05$), but that there was no significant difference between Vistagel PLUS and Atlafilcon (p=NS).

Table 9.2.5 - Lipid By Fluorescence @ 360nm

	TETRAFILCON	VISTAGEL PLUS	ATLAFILCON	p
Mean ± SD	149 ± 49	56 ± 12	52 ± 21	<0.001
Range	373 - 15	106 - 21	174 - 22	

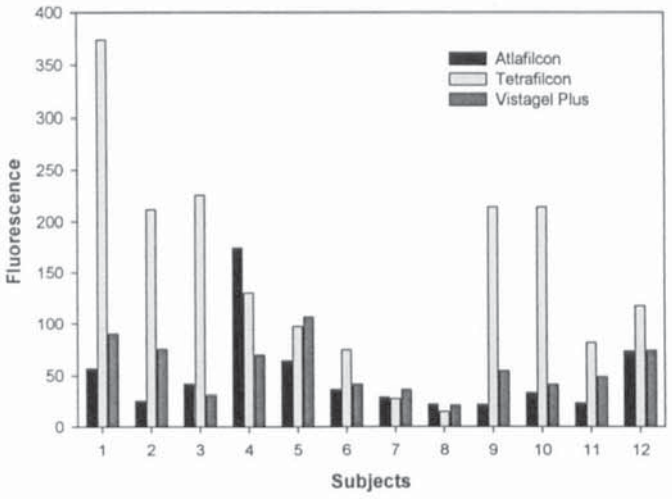


Figure 9.2.5 - Lipid by fluorescence @ 360nm for all subjects and all materials

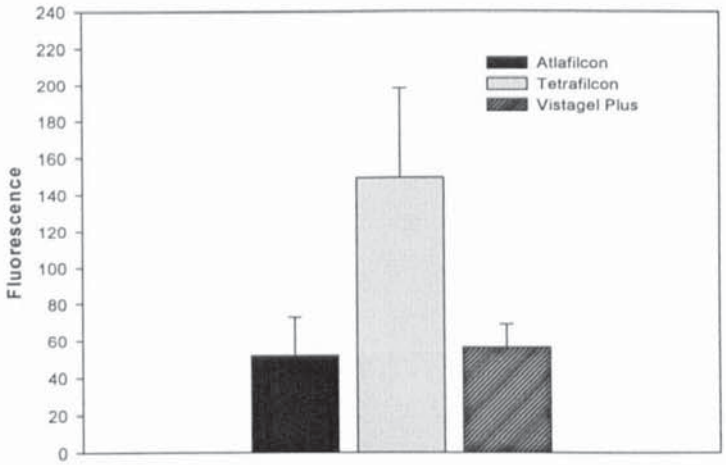


Figure 9.2.6 - mean ± sd fluorescence @ 360nm for all subjects and materials

iii) Overall deposition.

The lipid and protein results may be summed to gain an indication of the overall level of deposition present on the lenses. This "spoilation index" acts as a useful comparator between lens materials. These results are detailed in Figure 9.2.7. The results indicate that overall deposition was greatest with Tetrafilcon and that this difference was significant ($p<0.04$), with Tetrafilcon depositing more that the other two materials. There was no significant difference between Vistagel PLUS and Atlafilcon (p=NS).

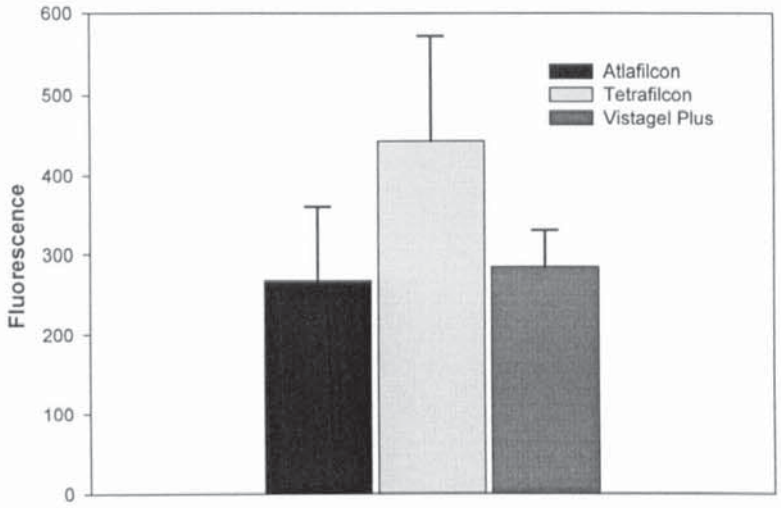


Figure 9.2.7 - "spoilation index" results. These results are achieved by summing the mean ± sd fluorescence results at both 280nm and 360nm excitation for all materials

iv) Cleaning Efficiency

Evaluation of the firmly adherent deposition is possible by evaluating the spoilation index (summed protein and lipid levels) of the materials in question following a final surfactant clean. The degree of firmly adherent deposition is outlined in Figures 9.2.8 and 9.2.9. These results indicate that the biomimetic material is more easily returned to its original condition than either of the other two materials. Figure 9.2.10 demonstrates that following cleaning the biomimetic material has a reduced level of deposition associated with the lens surface. These results are significant (p=0.0001) between Tetrafilcon and the other two materials, which are not different from each other (p=NS).

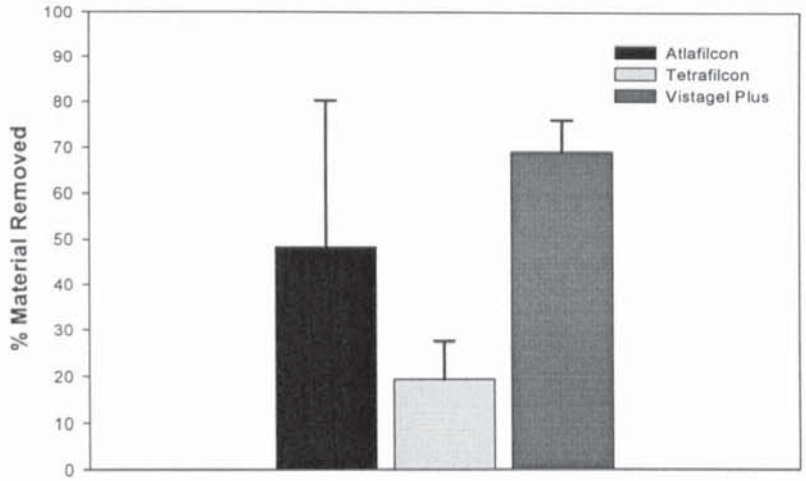


Figure 9.2.8 - degree of removable deposition at 280nm fluorescence

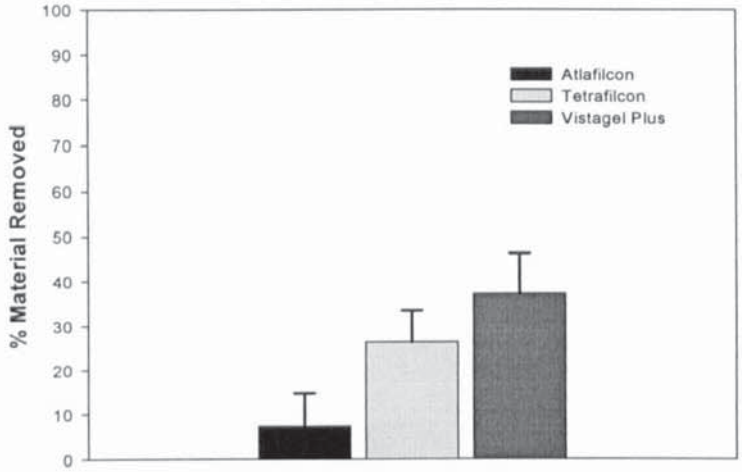


Figure 9.2.9 - degree of removable deposition at 360nm fluorescence excitation

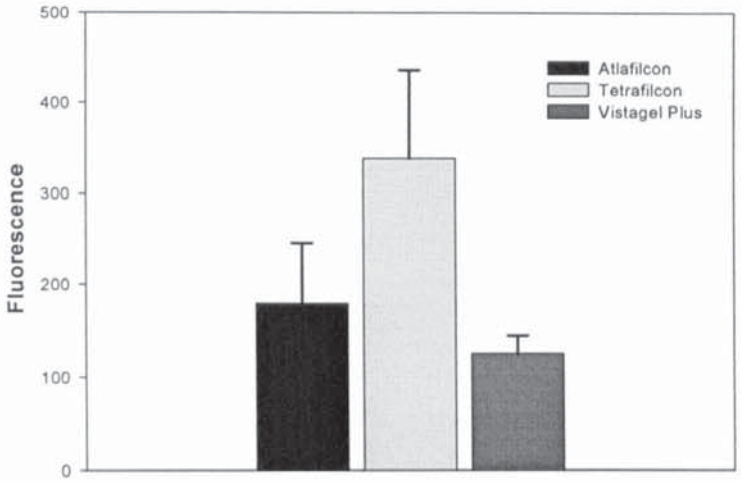


Figure 9.2.10 - cleaned spoilation index

v) Wettability vs Deposition

The results indicate that wettability reduced with the passage of time. It is possible that the PLNIBUT results were influenced by increasing levels of deposition. To confirm this a correlation was sought between PLNIBUT changes and deposition values. The results indicate that no significant correlation was seen between PLNIBUT changes and either 280 nm fluorescence ($r<0.2$) or 360 nm fluorescence ($r<0.4$). This indicates that while deposition increases and wettability reduces, in this group of subjects these factors were not directly inter-related.

9.2.4 DISCUSSION

Examination of the *in vitro* wettability (Table 9.2.3) results suggests advantages for the Vistagel PLUS material. The results are relatively simply explained by consideration of the chemical nature of the three materials. Atlafilcon is a PVA homopolymer which exhibits only minimal hysteresis when exposed to an alternating hydrophobic/hydrophilic environment. In a hydrophilic environment hydroxyl (OH) groups are expressed at the surface and in a hydrophobic environment hydrogen (H) atoms are exposed. Both surfaces exhibit some degree of hydrophilicity to the external environment, resulting in a minimal change in wettability (hysteresis) when moving from one environment to the next. In comparison, Tetrafilcon (a terpolymer), exposes hydroxyl and pyrrolidone groups in a hydrophilic environment, compared with methyl (CH_3) and methacrylate ($COOCH_3$) moieties upon exposure to air, both of which are relatively hydrophobic. This change in surface characterisation produces increased hysteresis, as evidenced by the results. In Vistagel PLUS, polyethylene oxide (PEO) is expressed at the surface in an extended chain conformation in a hydrophilic environment. When exposed to a hydrophobic environment the PEO chains are still expressed, but in a collapsed state. These remaining hydrophilic groups result in minimal hysteresis being exhibited. This reduced advancing angle and minimal hysteresis should result in minimal changes in wettability across time, which may influence both comfort and long-term deposition. Of final note is that ReNu is a surface active solution and becomes associated with the polymeric surface, restricting it's mobility and therefore reducing θ_a and the contact angle hysteresis. The hysteresis results also clearly demonstrate the influence of surface active molecules on the materials, with a lower hysteresis being found with ReNu for all materials.

Examination of the PLNIBUT results (Figure 9.2.2) demonstrates that *in vivo* performance is not equivalent to *in vitro* wettability. The lens surface is clearly modified by the subjects' tear film and indicates that *in vitro* performance may be a poor predictor of *in vivo* performance. One possible reason for this could be that increasing lipid deposition resulted in reduced wettability. However, there was no direct correlation seen in this study. The PLNIBUT results also indicate that wettability with all materials reduces with wearing time equally for all materials and that inter-subject differences clearly exist, as evidenced by the wide standard deviation measured. The differences between materials indicate that Atlafilcon and Vistagel PLUS are, on average, more wettable than Tetrafilcon.

The *in vitro* spoilation data shows clear advantages for the biomimetic material. Figure 9.2.1 indicates that Vistagel PLUS has the lowest initial spoilation and that it also has the lowest rate of increase in spoilation. This is regardless of cleaning function, as the spoilation model is a progressive model that mimics the situation which occurs in an extended-wear modality, where no cleaning occurs. The results support the theoretical model, which proposes that deposition should be limited by the biomimetic surface. Of particular note is that all materials show a clear advantage when compared with polyHEMA

The *in vivo* spoilation data is more equivocal concerning the advantages for the biomimetic material. Figures 9.2.3 and 9.2.5 clearly indicate that subjects deposit different materials to different extents and that significant inter-subject differences occur, particularly with regards to lipoidal deposition. There is clearly minimal difference in protein deposition on the lens surfaces after short periods of wear with all three non-charged

materials (Figure 9.2.4). However, it would appear that Tetrafilcon is more prone to lipoidal deposition than the other two materials (Figure 9.2.6), over the short wearing period examined in this study. Using a summation of the degree of deposition of both lipid and protein to give a "spoilation index" (Figure 9.2.7) indicates that the "older" technology material (Tetrafilcon) deposits to the greatest extent of the three materials.

A further factor of interest is to investigate the ease with which the deposited species is removable from the lens material. A material that is truly "biomimetic" should produce a deposited layer that is not firmly adherent to the underlying material. Such materials should be more easy to "clean" and result in significantly less deposition over the life-span of the lens. Figures 9.2.8 - 9.2.10 indicates that there are significant differences between the materials under investigation, with the materials based on newer technology exhibiting clear *in-vivo* trends after only 4 weeks of lens wear. Finally, examination of both wettability and deposition results indicate that certain subjects exhibit reduced wettability and/or increased deposition, regardless of the material used. Clearly such subjects would be best managed by using materials on a frequent replacement basis.

Whilst these results suggest that materials based on biocompatible technology may provide benefits for certain subjects, longer-term evaluations of such materials are required to clarify this point. The remaining studies in this Chapter investigate this point.

9.3 THE SPOILATION AND CLINICAL PERFORMANCE OF DAILY-WEAR "BIOMIMETIC" HYDROGEL LENSES

The purpose of this study was to investigate the degree of deposition control achieved by fitting patients with lens materials which attempt to mimic the principle of biomimesis.

9.3.1 Materials and Methods

Twenty subjects (seven male and thirteen female) were entered onto the study, whose demographic details are described in Table 9.3.1.

Table 9.3.1 - Subject Demographics

	Mean ± SD	Range
Age (years)	36.5 ± 12.1	19 - 59
Flat keratometry (mm)	7.86 ± 0.24	7.45 - 8.30
Steep Keratometry (mm)	7.79 ± 0.29	7.30 - 8.35
Sphere (D)	-2.02 ± 2.54	-7.00 to + 4.25
Cylinder (D)	-0.28 ± 0.31	-1.00 to 0.00

The subjects were fitted with Proclear, Excelens, Vistagel PLUS (DR40) and Vistagel HI-PLUS (DR50), details of which are given in Table 9.3.2. Three materials (Proclear, Excelens and Vistagel PLUS) have demonstrated their ability to resist spoilation in previous studies and the fourth (Vistagel HI-PLUS) is an investigative material based on similar principles to that described for Vistagel PLUS (pages 277-283).

Table 9.3.2 - Lens Parameters

	Proclear	Excelens	Vistagel PLUS	Vistagel HI-PLUS
Manufacturer	Biocompatibles	CIBA-Vision	Vista Optics	Vista Optics
EWC	59%	64%	40%	50%
Monomers	PC + HEMA	PVA	HEMA+MMA+ NVP+PEO	HEMA+MMA+ NVP+PEO
USAN	Omafilcon A	Atlafilcon A	-	-
FDA Group	Group II	Group II	Group I	Group II
Manufacture	Lathed	Lathed	Lathed	Lathed
BOZR	8.2, 8.5, 8.8	8.4, 8.6, 8.8	8.4, 8.7, 9.0	8.4, 8.7, 9.0
Diameter	14.20	14.00	14.20	14.20

The subjects were randomly divided into two equal groups. All subjects wore the Proclear and Excelens materials, while one group were only allocated Vistagel PLUS and the other group Vistagel HI-PLUS. Subjects were reviewed in the clinic on initial collection, after one month (28 ±3 days) and three months (90 ± 7 days) with each material. At the three-month visit a cross-over took place, with all subjects repeating the above visit schedule with the other two lens materials. The study design used a randomised, double-masked paradigm, in which neither the practitioner nor the subject was aware of the lens type in use at the time of the clinical visits.

Lenses were fitted to achieve adequate centration, corneal coverage and movement, in accordance with conventional fitting criteria. If an adequate fit with any lens type proved impossible then the subject was not enrolled onto the study. During the study patients cleaned their lenses following removal with a surfactant

cleaner (Allergan "LC65"), before placing them overnight in a two-step 3% hydrogen peroxide system (Allergan "Oxysept 2-Step"). In the morning they were subsequently neutralised according to the manufacturer's instructions for 20 minutes, before insertion took place. A new case was given to the patients with each lens type. Subjects refrained from using enzyme tablets or rewetting drops throughout the course of the study and wore their lenses for a minimum of ten hours and maximum of sixteen hours daily.

9.3.2 Clinical Protocol

At each visit subjective satisfaction, lens fit, visual acuity, PLNIBUT, visible wettability on the slit lamp, Rudko deposition and physiological performance were assessed, as previously described.

9.3.3 Analytical Protocol

At the end of the three-month wearing schedule each lens was removed with plastic-tipped tweezers and placed in a glass vial containing sterile non-preserved saline. The vials were capped, labelled with the subject's initials and study number and refrigerated prior to spoilation analysis taking place. Surface deposition was evaluated by using fluorescence spectrophotofluorimetry, exciting at 280nm to evaluate proteins and 360nm for lipid. Total protein was assessed using transmission UV at 280nm.

9.3.4 Data Analysis

Summary statistics were calculated for all variables. The grading scales, being ordinal measurements, were considered non-normal data. All other data were tested for normality of distribution. Statistical analysis was undertaken using a one-way repeated measures ANOVA or one-way repeated measures ANOVA on ranks. Where differences were found, post-hoc testing was undertaken using the SNK method. A "p" value of <0.05 was considered statistically significant.

9.3.5 Results

9.3.5.1 Subjective Factors

a) Comfort

Results are given in Table 9.3.3. Excelens and Proclear proved to be more comfortable than the Vista materials, although the differences were not statistically significant (p=NS). There were no significant changes in comfort across the wearing period with any lens type (p=NS), although a reduction across time with all materials is clearly visible.

Table 9.3.3 - Subjective Comfort (Mean ± sd)

	1 month review	3 month review	p
Atlafilcon	8.15 ± 0.54	7.85 ± 0.60	NS
Omafilcon	7.90 ± 0.78	7.55 ± 0.90	NS
Vistagel PLUS	6.90 ± 1.10	6.70 ± 1.00	NS
Vistagel HI-PLUS	7.10 ± 0.85	6.90 ± 0.61	NS
p	NS	NS	

b) Visual Quality

Results are detailed in Table 9.3.4. Subjects reported that the visual quality with the Vista materials was slightly lower than the other two lens types, although the differences were not statistically significant (p=NS). There were no significant changes in visual quality across the wearing period with any lens type (p=NS), although a reduction across time with all materials is clearly seen.

Table 9.3.4 - Subjective Visual Quality (Mean ± sd)

	1 month review	3 month review	p
Atlafilcon	8.00 ± 0.70	7.85 ± 0.80	NS
Omafilcon	8.30 ± 0.63	8.05 ± 0.72	NS
Vistagel PLUS	7.10 ± 0.85	6.90 ± 1.01	NS
Vistagel HI-PLUS	7.00 ± 0.74	6.70 ± 0.75	NS
p	NS	NS	

c) Overall Lens Performance

Results are detailed in Table 9.3.5. Subjects felt that the overall performance of the Vista materials was slightly lower than the other two lens types, although the differences were not statistically significant (p=NS). There were no statistically significant changes in performance across the wearing period with any lens type (p=NS), although a reduction across time with all materials was seen.

Table 9.3.5 - Subjective Overall Performance (Mean ± sd)

	1 month review	3 month review	p
Atlafilcon	7.95 ± 0.58	7.75 ± 0.56	NS
Omafilcon	7.95 ± 0.62	7.70 ± 0.78	NS
Vistagel PLUS	6.90 ± 0.87	6.80 ± 1.01	NS
Vistagel HI-PLUS	7.10 ± 0.75	7.00 ± 0.58	NS
p	NS	NS	

9.3.5.2 Visual Acuity

a) High Contrast Visual Acuity

There was no significant difference between lens types (p=NS) and the acuity with all lens types remained unaltered across the wearing period (p=NS).

b) Low Contrast Visual Acuity

There was no significant difference between lens types (p=NS) and the acuity with all lens types remained unaltered across the wearing period (p=NS).

9.3.5.3 Wettability

a) PLNIBUT by Loveridge Grid

Results are detailed in Figure 9.3.1. Clearly the greatest variable in this factor is differences between subjects. There was no significant difference between lens types (p=NS), but there was a significant reduction in wettability across the three-month period with all lens types ($p<0.005$).

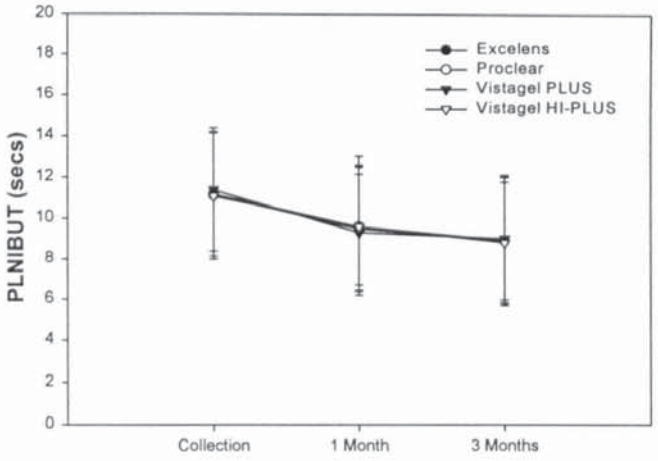


Figure 9.3.1 - PLNIBUT (mean ± sd) in seconds for all three materials across a three month wearing period

9.3.5.4 Visible Deposition

a) RUDKO Type

Results are given in Figure 9.3.2. No lenses demonstrated any calculi, which is unusual after 3 months of wear with group II materials. There was no significant difference between lens types or across visits (p=NS).

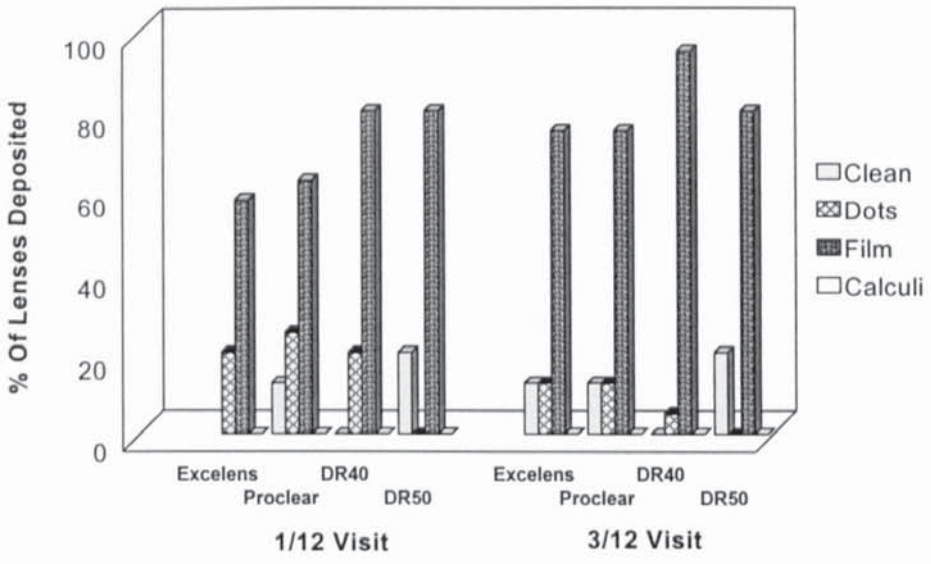


Figure 9.3.2 - Rudko type for all materials at one and three month reviews

b) RUDKO Degree

Results are shown in Figure 9.3.3. Clearly the degree of visible deposition increases with the passage of time with all materials, although this was not statistically significant (p=NS). There was no significant difference between materials (p=NS).

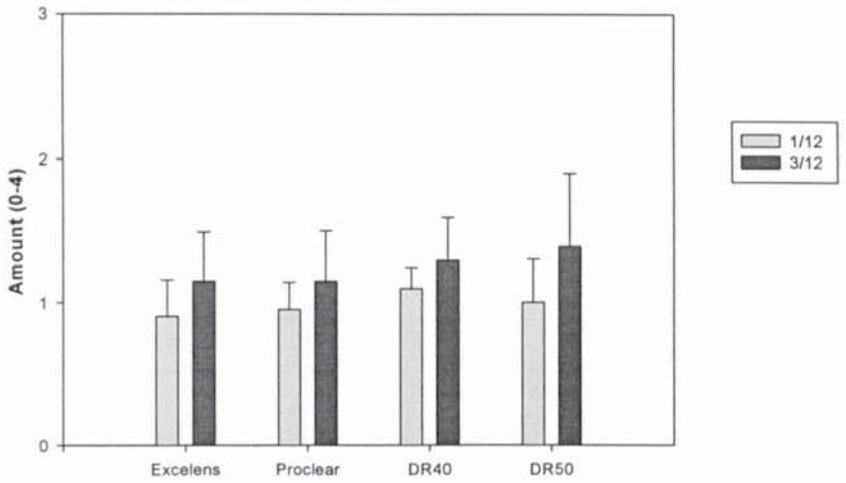


Figure 9.3.3 - Rudko degree for all materials (mean ± sd) at the one month and three month reviews

c) RUDKO Coverage

Results are shown in Figure 9.3.4. These results are similar to that for degree of deposition. Again, the amount of visible deposit coverage increases with the passage of time, although this was not statistically significant (p=NS). There was no significant difference between materials (p=NS).

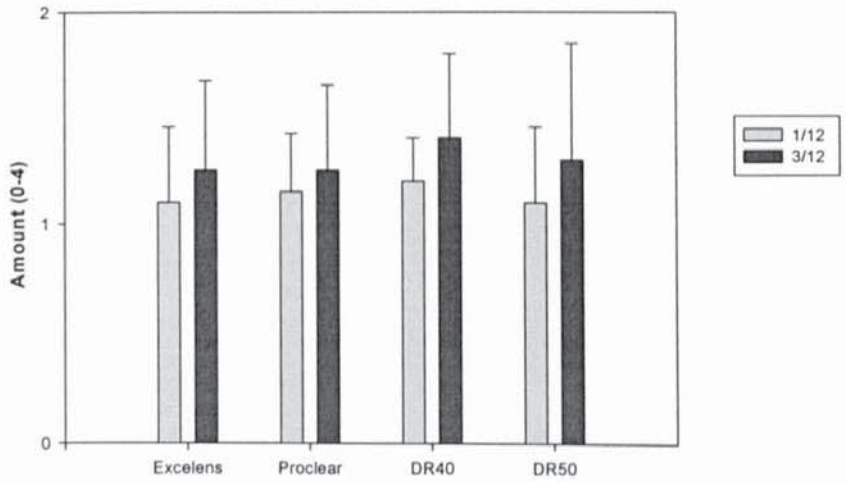


Figure 9.3.4 - Rudko coverage for all materials (mean ± sd) at the one month and three month reviews

9.3.5.5. Physiological Performance

a) Corneal Staining

The mean staining scores were 0.50 ± 0.25 for Atlafilcon, 0.35 ± 0.28 for Omafilcon and 0.30 ± 0.23 for both Vista materials. The differences were not significant between lens types or across time (p=NS).

b) Conjunctival Staining

The mean staining scores were 1.90 ± 0.80 for Atlafilcon, 1.95 ± 0.90 for Omafilcon, 2.0 ± 1.10 for Vistagel PLUS and 1.50 ± 0.92 for Vistagel HI-PLUS. The differences were not significant between lens types or across time (p=NS).

9.3.5.6. Deposition Results

As the deposition results could clearly be influenced by subject group, the results are displayed separately for the two groups of subjects.

a) Total Protein By UV - Vistagel PLUS Group

Figure 9.3.5 details the total protein deposited by the Vistagel PLUS group. There was a significant difference between lenses ($p<0.005$). Post-hoc testing shows that there was no significant difference between Omafilcon and the other two materials (p=NS) but that Atlafilcon deposited less total protein than Vistagel PLUS ($p<0.05$).

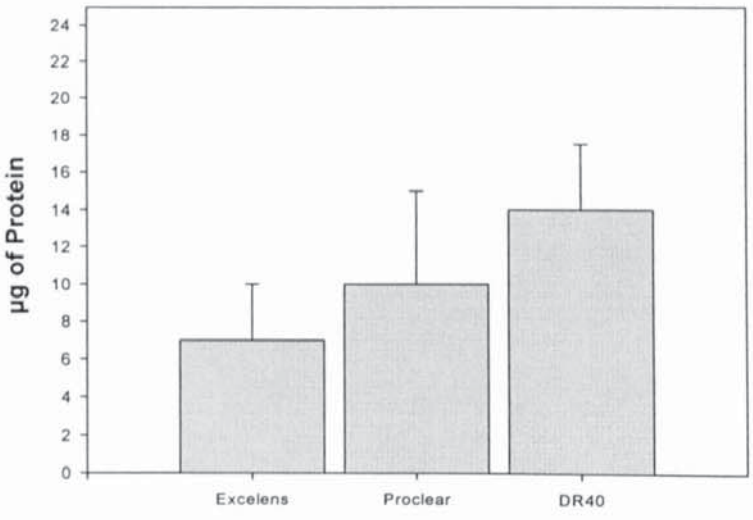


Figure 9.3.5 - total protein by UV (mean ± sd) for Vistagel PLUS study group

b) Total Protein By UV - Vistagel HI-PLUS Group

Figure 9.3.6 details the total protein deposited by the Vistagel HI-PLUS group. There was a significant difference between lenses ($p<0.002$). Post-hoc testing indicates that there was no significant difference between Omafilcon and Vistagel HI-PLUS (p=NS) but that Atlafilcon deposited less protein than either of the other two materials ($p<0.05$).

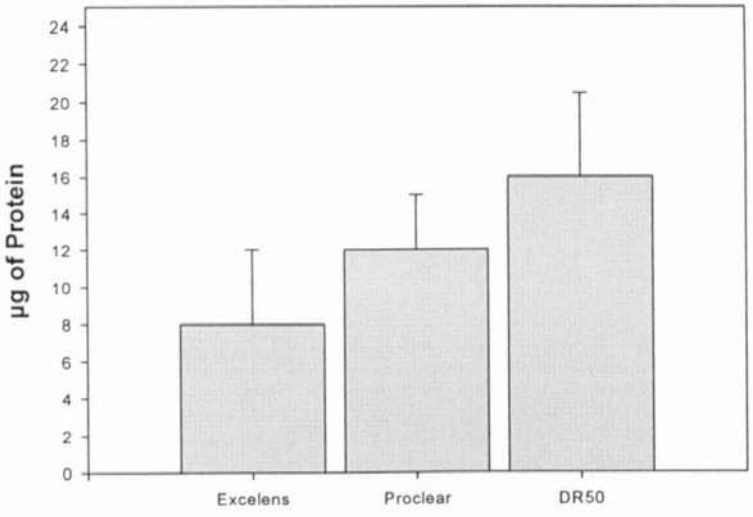


Figure 9.3.6 - total protein by UV (mean ± sd) for Vistagel HI-PLUS study group

c) Surface Protein By Fluorescence - Vistagel PLUS Group

Figure 9.3.7 outlines the results for the Vistagel PLUS group. There was no significant difference between lenses (p=NS).

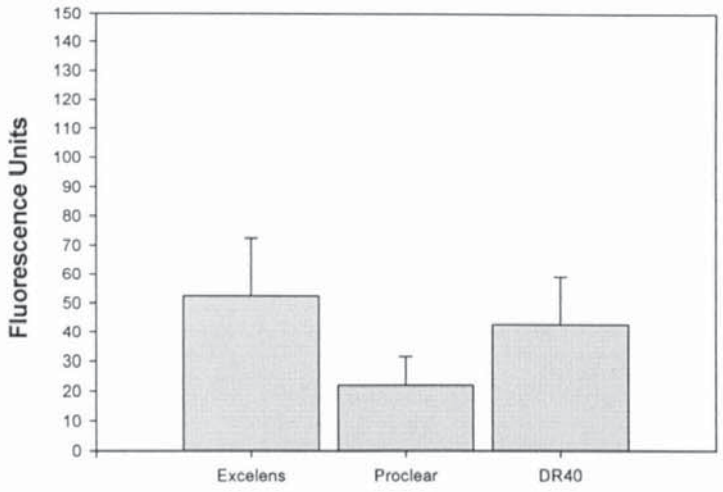


Figure 9.3.7 - surface protein by fluorescence (mean ± sd) for Vistagel PLUS study group

d) *Surface Protein By Fluorescence - Vistagel HI-PLUS Group*

Figure 9.3.8 outlines the results for the Vistagel HI-PLUS group. There was a significant difference between lenses ($p<0.0001$). Post-hoc testing indicates that there was no significant difference between Atlafilcon and Vistagel HI-PLUS (p=NS) but that Omafilcon deposited less surface protein than either of the other two materials ($p<0.05$).

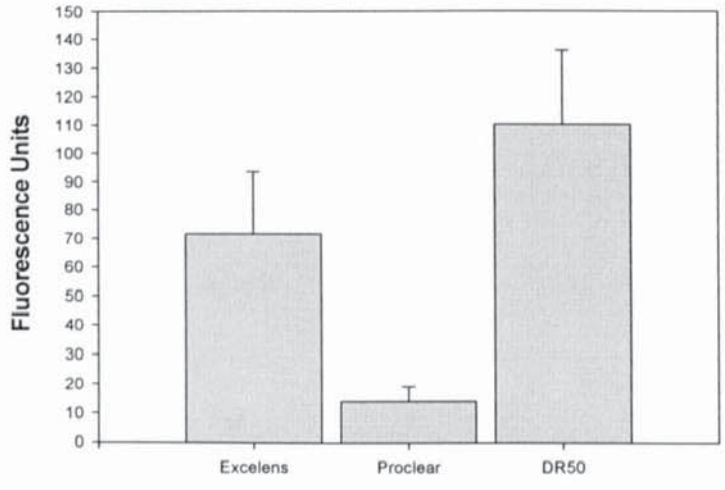


Figure 9.3.8 - surface protein by fluorescence (mean ± sd) for Vistagel HI-PLUS study group

e) Lipid By Fluorescence - Vistagel PLUS group

Figure 9.3.9 describes the results for the Vistagel PLUS group. There was a significant difference between lenses ($p<0.00001$). Post-hoc testing indicates that there was no significant difference between Atlafilcon and Vistagel PLUS (p=NS) but that Omafilcon deposited less lipid than either of the other two materials ($p<0.05$).

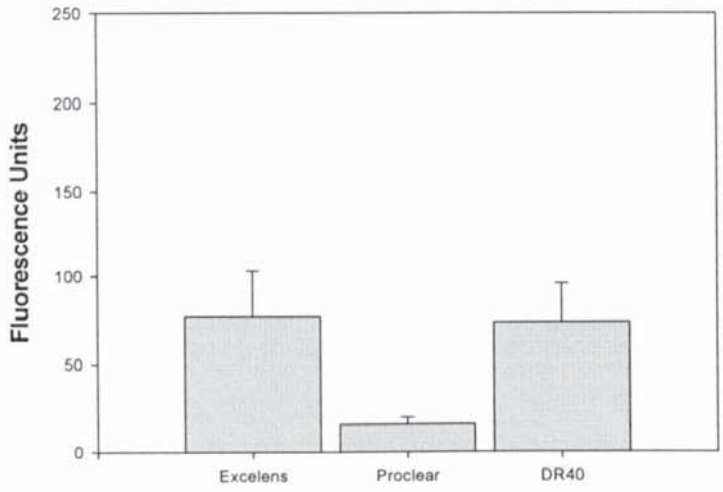


Figure 9.3.9 - lipid deposition by fluorescence (mean ± sd) for Vistagel PLUS study group

f) Lipid By Fluorescence - Vistagel HI-PLUS group

Figure 9.3.10 describes the results for the Vistagel HI-PLUS group. There was a significant difference between lenses ($p<0.00001$). Post-hoc testing indicates that there was a significant difference between all materials ($p<0.05$), with Omafilcon depositing the least and Vistagel HI-PLUS the most.

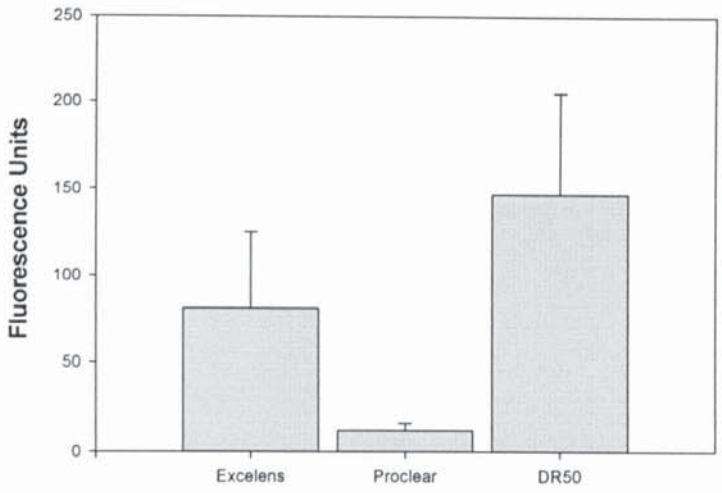


Figure 9.3.10 - lipid deposition by fluorescence (mean ± sd) for Vistagel HI-PLUS study group

9.3.5.7 Removable Deposition

As discussed in the first study of this Chapter (pages 291-292), the deposited species should be more easily removable with biocompatible lenses than with conventional lens materials. To investigate this, 10 worn lenses from each type were randomly selected and subjected to a 10-second digital clean with LC65, which was copiously rinsed off with saline. This procedure was carried out by a sole investigator who was unaware of the lens type being cleaned. The lenses were finally re-examined to assess the degree of material that could be removed from the lenses.

Figures 9.3.11 - 9.3.13 graphically demonstrate the degree of removable deposition seen with each lens material.

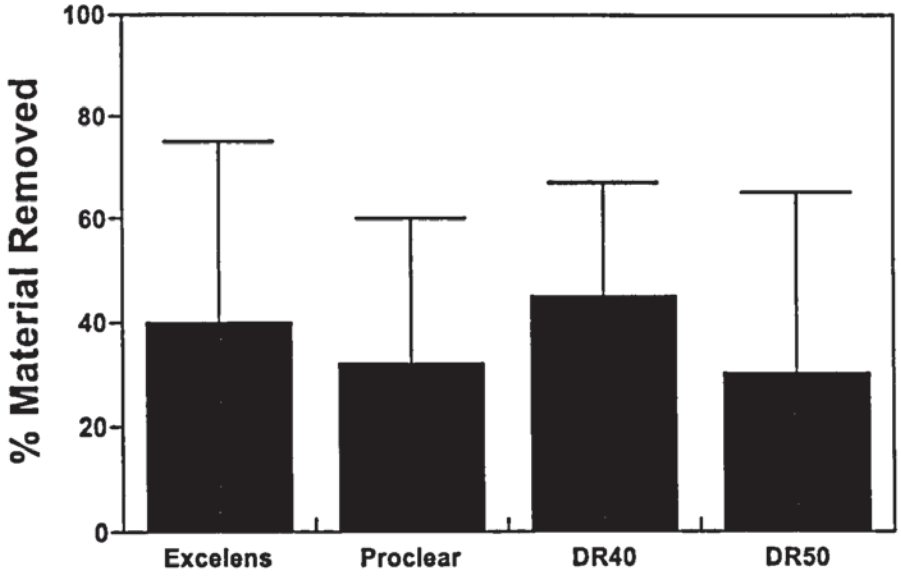


Figure 9.3.11 - Percentage of removal total protein (mean ±sd) from all three materials assessed using transmission UV

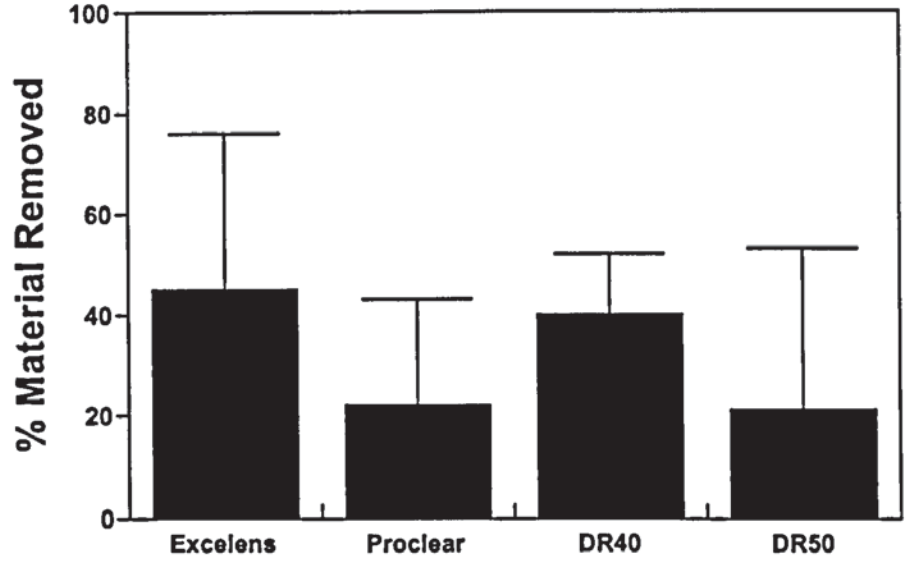


Figure 9.3.12 - Percentage of removal surface protein (mean ±sd) from all three materials assessed using fluorescence @ 280nm excitation

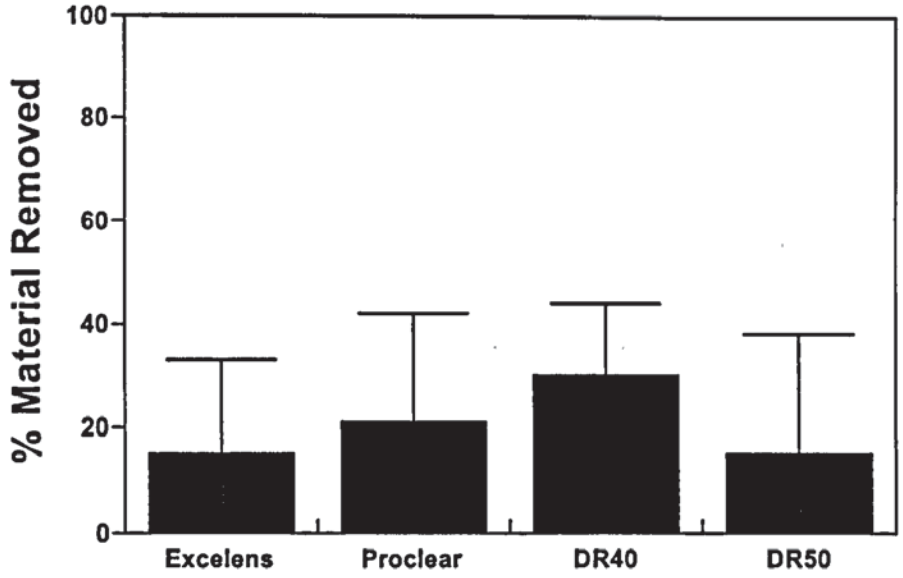


Figure 9.3.13 - Percentage of removal lipid (mean ±sd) from all three materials assessed using fluorescence @ 360nm excitation

These results indicate that the degree of removable deposition differed between the materials and also between the deposit type under investigation. Overall, protein was easier to remove than lipid, although this difference was not statistically significant (p=NS). For protein (both surface and bulk) DR40 and Excelens demonstrated the least tenaciously bound deposition, but statistical analysis indicated that the differences between materials were not significant (p=NS). For lipid, DR40 once again demonstrated that it was able to withstand immobilisation better than the other materials, particularly when compared with Excelens and DR50. However, again these differences were not significant (p=NS).

9.3.5.8 Split Lenses

During the study several Omafilcon, Vistagel PLUS and Vistagel HI-PLUS lenses were replaced due to breakage or discomfort. These are summarised below:

Table 9.3.6 - Replacement Lenses - Vistagel PLUS Study Group

	Omafilcon	Vistagel PLUS	Atlafilcon
Lost	2	0	2
Split	9	8	1
Faulty/Discomfort	0	11	0
Total	11	19	3

Table 9.3.7 - Replacement Lenses - Vistagel HI-PLUS Study Group

	Omafilcon	Vistagel HI-PLUS	Atlafilcon
Lost	3	0	0
Split	7	3	2
Faulty/Discomfort	1	3	0
Total	11	6	2

These numbers have a direct impact on the deposition results, as lenses replaced before the end of the three-month period had obviously not been worn for three months before analysis took place.

Obviously, while lost lenses cannot be avoided during any study, split and faulty lenses do not reflect well on the lens material, particularly as "biocompatible" materials should last longer than disposable lens materials. It is clear from the tables above that Atlafilcon is well produced and rarely split. However, the results are a cause of some concern when the numbers of split Omafilcon and faulty/split Vista lenses are considered. All of the Vista lenses that were faulty were replaced within the first 2-3 weeks, whereas the Omafilcon lenses were replaced at any stage throughout the study. The ultimate result of this is that, on average, the lenses analysed upon completion of the study had been worn longest for Atlafilcon and that few of the Omafilcon lenses lasted three months. This will inevitably reduce the level of deposition seen with these lenses. The average age of the lenses (in months) at the time of collection for deposition examination were 2.14 ± 0.89 for Omafilcon, 2.82 ± 0.56 for Atlafilcon and 2.39 ± 0.81 for the Vista materials.

9.3.5.8 Final Choice

Table 9.3.8 - Final Choice Vistagel PLUS Study Group

	Omafilcon	Vistagel PLUS	Atlafilcon
Final Choice	1	3	6

Table 9.3.9 - Final Choice Vistagel HI-PLUS Study Group

	Omafilcon	Vistagel HI-PLUS	Atlafilcon
Final Choice	2	2	6

9.3.6 Discussion

Consideration of the factors related to "bulk" material characteristics (such as vision, overall satisfaction, lens fit etc) suggest that biocompatibility has little, if any, influence on such factors. Subjective comfort, vision and satisfaction with all lens types reduced over the 12-week period, while high and low contrast visual acuity remained stable, regardless of lens material. However, the chemistry of the lens material clearly influences more subtle factors such as the degree of lens deposition and the extent to which this deposition becomes immobilised on the lens surface.

The protein results found in this study were similar to those reported in other studies for both Atlafilcon [470] and Omafilcon. [568] Omafilcon has been the subject of much recent publicity, particularly since its recent release as a monthly frequent replacement lens. [593] Consideration of the number of split lenses seen in this study shows that this is a sensible move as many lenses were not reaching the three-month stage. The improved comfort of the Omafilcon material reported by other workers [594 - 596] when compared with other hydrogels is not borne out in this study, but does agree with the conclusions of Situ et al, [597] that the comfort claims of Omafilcon are somewhat exaggerated. The suggestion that Omafilcon should exhibit excellent resistance to surface deposition of both lipids and protein [595 596] is supported by these studies. This is postulated to be due to the zwitterionic PC headgroup within the lens material. [595 596] Atlafilcon exhibits excellent resistance to protein deposition in all studies conducted in this Chapter. These results can be usefully compared with those seen with another CIBA-Vision biocompatible material designed for 30-day extended-wear use. [598] In this study, Group I lenses deposited approximately 20 μg protein after 6 nights extended wear and the experimental material approximately 5 μg after 30 nights use. These results compare favourably with the results seen with Atlafilcon and Vistagel HI-PLUS worn in this study for 90 days on a daily wear basis.

In this study the Vistagel PLUS and HI-PLUS materials exhibited similar performance to the Atlafilcon and Omafilcon materials in their resistance to bulk protein deposition, as evidenced by Figures 9.3.5 and 9.3.6. However, the performance of Vistagel HI-PLUS in its resistance to surface protein and lipid deposition was disappointing when compared with the other two materials (Figures 9.3.8 and 9.3.10). This may be due to the purity of the polyHEMA used to produce the base material. The presence of low levels of methacrylic acid (which occurs as a contaminant during the production of polyHEMA) would obviously attract appreciable quantities of lysozyme. [431] In addition, the Vistagel HI-PLUS material attracted a substantial amount of lipid, almost certainly due to the presence of NVP, which was incorporated to increase the water content and improve the oxygen transmissibility. Constant monitoring of the purity of the polyHEMA and the incorporation of a monomer without the lipophilic properties of NVP should result in considerable improvements in the deposition performance of these materials. The difference between the protein deposition results seen with the Vistagel PLUS material in this study and those seen in Study 9.2 suggests that the polyHEMA from which the earlier lenses were fabricated was almost certainly contaminated with methacrylic acid.

Examination of the ease with which deposited material can be removed also produced some interesting results and confirmed earlier findings (pages 192 and 294) that protein is more easily removable from hydrogels than lipid. This suggests that more attention should be paid to the lipophilic properties of surfactant cleaners than their protein cleaning function and that enzyme tablets may benefit from the incorporation of lipases.

Front surface wettability clearly reduced over the three-month wearing schedule, irrespective of lens material or design, in accordance with all other studies conducted during this thesis. Again, large variations in wettability were seen within the subject groups, indicating the influence of subject tear-films on this factor. The fact that such changes in wettability occurred irrespective of the differences in deposition suggests that there is no direct correlation between deposition and wettability. This confirms the fact that while biocompatibility affects the "rate" of deposition, the ultimate "fate" of deposition of any material cannot be prevented and every material, whatever its composition, will ultimately deposit to the point where clinical performance suffers.

9.4 KEY POINTS

- Subjective factors such as lens comfort, vision and satisfaction with all lens types reduce over a 12 week period, regardless of lens material. High and low contrast visual acuity remains stable over a similar period.

- Materials based on the principle of biocompatibility result in enhanced wettability compared with conventional materials, when tested in an *in vitro* model.

- Materials based on the principle of biocompatibility result in reduced deposition compared with conventional materials, when tested in an *in vitro* model.

- Visible deposition with all lens materials increases over time, regardless of the material composition.

- All materials, whatever their composition or design principle, progressively reduce in PLNIBUT with increasing wear.

- There are significant inter-subject differences in the deposition of tear-film derived species onto and into lens materials, particularly concerning the deposition of lipoidal material. These differences act to nullify to some extent the advantages of biocompatible materials seen with *in vitro* models.

- The firm adherence and immobilisation of deposited material onto the surface of biocompatible materials is less than that seen with conventional materials.

- Protein is more easily removed than lipids from the surface of hydrogel materials.

Chapter 10

Conclusions and Recommendations For Further Work

"The only man who never makes mistakes is the man who never does anything". [1]

This is the first time that such an extensive series of controlled *in vivo* studies have looked at the interaction between spoilation and clinical performance. Much of the previously published work investigated these factors independently, using inadequate analytical techniques and/or lenses sourced from an uncontrolled group of subjects. The material collected in this thesis is from a well-defined subset of people, namely successful contact lens wearers. This could be viewed as a weakness, in that the results can only be truly extrapolated to this subset and useful data may have been gathered from unsuccessful wearers. However, this would have introduced one further variable into an already very complex set of experiments. This choice of subjects is considered entirely defendable in that any project of this type and magnitude must "start somewhere". Further work looking at either neophyte lens wearers or previously unsuccessful wearers would be valuable and almost certainly extend our knowledge of the reason or reasons why certain subjects are unsuccessful with lens wear.

The studies within this thesis used well known clinical techniques to chart clinical performance both over time and between materials and used a novel set of analytical techniques to monitor the type, quantity and quality of deposition that occurred on several available and novel lens materials. The clinical techniques were carefully chosen to reduce the variation that occurs in clinical monitoring and the analytical techniques were selectively used to investigate various factors of interest. Due to the size and complexity of the studies undertaken it did not prove possible due to both time and cost constraints to undertake every conceivable analysis on all lenses harvested post-wear. This could also be conceived as a weakness as certain factors of interest may be missed adopting such a procedure. In most studies a more selective approach to a particular problem is taken, with a large population of subjects chosen and fewer variables investigated to explore a particular area. However, before this study little knowledge existed of the type of deposition that occurred on currently available materials and almost no knowledge existed of their impact on clinical performance. Consequently a "shotgun" approach was adopted in which a number of the most important factors were investigated using small groups of subjects in well controlled clinical studies. In addition, sample size calculations can only be undertaken if preliminary data is available to indicate the sort of differences that can be expected. The data from this thesis will be valuable to use as preliminary data in future sample size calculations.

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[1] Theodore Roosevelt

Using the results previously outlined, Table 10.1 details the sample-size calculations obtained (conducted using SigmaStat) for the three types of measured deposition for each of the studies conducted. The table outlines the minimum sample required to obtain the differences in means and standard deviations obtained during this thesis. In conducting the analysis, a power of 0.8 and alpha of 0.05 was chosen.

Table 10.1 - Sample Size Calculations

	Total Protein by UV	Surface Protein By Fluorescence @ 280nm	Surface Lipid By Fluorescence @ 360nm
MA/NVP containing GIV (Focus) vs NVP containing GII (Precision UV)	2	-	18
PVA containing GII (Excelens) vs NVP containing GII (ES70)	2	4	8
Low MA containing GIV (Vifilcon) vs high MA containing GIV (Etafilcon)	6	4	6
NVP containing GII (Precision UV), comparing 3/12 vs 1/12 wear	2	-	10
NVP containing GII (Medalist 66) vs MA containing GIV (Surevue), comparing 1 day vs 1 month	2	2	4
Care system variations with NVP containing GII (Medalist 66) vs MA containing GIV (Surevue)	27	20	34

As deposition of protein and lipid is so clearly related to chemical composition then it is apparent from the results within the thesis that large differences in deposition behaviour are seen. As a result, the sample sizes required to prove that such differences are statistically significant are quite small. This vindicates, to some extent, the method chosen within this thesis of taking a small number of subjects in a large number of studies. Materials of a similar composition will obviously produce a smaller difference in deposition and will obviously require larger sample sizes.

One point worthy of comment is that clearly the ten subjects chosen for the care system studies (Chapter 8) was insufficient to detect a difference between the care systems, although it was sufficient to detect differences between materials. In future such studies should be run with 40-50 subjects if differences between care systems is the principal factor of interest.

Of all the possible factors relating to deposition the most important were considered the patient, the lens material, the length of lens wear and the care system. The studies investigating these factors revealed the following general conclusions:

Symptomatology:

- Measured visual performance (at both high and low contrast) remains unchanged with lenses replaced up to three months.

- Visual quality is a better discriminator of subjective performance than visual acuity and these two factors are poorly correlated

- Subjective satisfaction with lenses reduces over a four week period with all combinations of lens materials and care systems, regardless of lens material composition. Up to two weeks post-insertion satisfaction remains relatively stable.

- Subjective symptoms are influenced by both the lens type and care system in use.

- Symptoms are correlated with protein denaturation but not with gross deposition of either protein or lipid.

Physiology:

- Approximately 50% of asymptomatic subjects exhibit corneal staining of some degree.

- There is a trend for corneal staining and conjunctival hyperaemia to increase with lens age, irrespective of care regime.

Inter/Intra-Subject Variability:

- The intra-subject variation in deposition across time is dependent upon the type of deposition being measured. There is approximately 30% variation in total protein, 40% in surface protein and 60% in lipid variation.

- Inter-subject variability in deposition is clearly seen with approximately 20% of subjects exhibiting atypical spoilation outside that normally encountered with any one lens material. These subjects deposit atypically whatever the lens material.

- There is a poor correlation in deposition between eyes in all subjects. The greatest variation is in lipid and the least in total protein and the variation reduces with increasing lens wear period. This has a significant influence on the development of studies that use a contralateral eye design.

- Protein deposition is principally related to material charge and is therefore material dominated, with minimal inter-subject differences occurring.

- Lipid deposition exhibits marked inter-subject differences. This is probably due to differences in tear-film composition but may also be affected to a lesser extent by lens handling.

Lens Wettability:

- Lens front surface wettability as assessed by measuring the PLNIBUT reduces over time, irrespective of the lens material or care system. There are significant inter-subject variations present within even small groups of subjects, with certain subjects reducing the wettability significantly in a very short period. These results are consistent across the year, being unaffected by seasonal changes and do not appear to be correlated with gross lipid or protein deposition as measured in this thesis.

- Materials based on the principle of biocompatibility result in enhanced wettability compared with conventional materials, when tested in an *in vitro* model. These advantages are not transferred to the *in vivo* situation.

- Reductions in wettability cannot be directly correlated with changes in subjective performance, although both reduce over time in any subject group.

Care System Influences:

- Polyhexanide-preserved care systems are preferred by subjects for their ease of use and result in greater subjective satisfaction than other systems, but may incur palpebral lid changes in certain individuals with increasing period of use.

- Solution systems denature lysozyme to different extents.

- While products with increased concentrations of PHMB and EDTA will impart enhanced antimicrobial activity to multi-purpose solutions, some subjects will notice reduced comfort and enhanced corneal staining, particularly when used with certain contact lens materials. As a result,

practitioners and patients alike should be advised that products with similar chemical constituents but differing formulations are not necessarily similar in action and should be interchanged with caution.

Material Influences:

- There are significant inter- and intra- FDA group variability in the deposition of tear-film derived species onto contact lens materials.

- FDA group IV materials deposit predominantly protein, due principally to the ionic carboxylate groups. The amount of deposited protein is directly correlated with the amount of ionic groups present.

- FDA group II materials deposit predominantly lipid, due principally to the presence of N-vinyl pyrrolidone.

- Visible deposition is only a poor indicator of deposition type, but the presence of numerous lens calculi indicates the presence of increased quantities of lipoidal deposition. These are almost exclusively seen in group II hydrogels.

- Protein location is determined by material charge. In neutral group II hydrogels the protein is almost exclusively located on the surface, whereas in ionic group IV materials the protein is progressively moved into the material, depending upon the degree of ionicity present.

- Materials based on the principle of biocompatibility result in reduced deposition compared with conventional materials, when tested in an *in vitro* model.

- The immobilisation of deposited material onto the surface of biocompatible materials is less than that seen with conventional materials.

- The immobilisation of lipoidal deposition is greater than that seen with protein.

- Neutral materials denature lysozyme to a greater extent than charged materials.

- There is a poor correlation between the deposition of lipid and protein with all materials.

Replacement Frequency Impact:

- NVP-containing group II materials deposit substantial quantities of lipid. This deposition is cumulative and does not reach a plateau up to three months post-insertion.

- Non NVP-containing group IV hydrogels deposit little lipid from the tear film and there is no increase across time up to three months post-insertion.
- NVP-containing group II materials deposit only small quantities of protein. This deposition is cumulative and does not reach a plateau up to three months post-insertion.

- Non NVP-containing group IV hydrogels rapidly deposit large quantities of protein from the tear film, but the deposition reaches a plateau between 1 and 7 days post-insertion.

- Visible deposition increases with time and depends on both the care system and material in use.

- Subjective satisfaction, lens front surface wettability, visible deposits and analytically evaluated deposits are all improved by replacing NVP-containing group II lenses on a frequent replacement basis.

The studies conducted during the completion of this thesis provide many answers to the complex questions that arise concerning the interaction of hydrogel materials with the tear film. However, on reviewing the conclusions a further series of questions arises, which shows that much work remains to enhance our understanding of this complex issue.

Future studies should use the conclusions from this thesis to specifically target certain factors of interest. One weakness of this study related to the measurement of symptomatology. A more diverse scale (for example 0-50) devised on a visual analogue basis [599] would provide better discriminability and enhance this measurement. In addition, visual grading scales based either on photographic measures [393] or using morphing software [394] would enhance the experimenters' ability to grade subtle changes in physiological performance.

One recurring theme that occurred throughout this thesis was that with the passage of time subjective satisfaction reduced while deposition increased and wettability reduced, whatever the type of material, care system, material or study design. However, no direct correlation between these factors was ever found. This is an area that is certainly worth further exploration.

Particular areas worthy of further investigation include:

- The relationship between subjects' tear film and the deposited species. Collected tear films could be analysed before commencing wear and during wear and a correlation sought between the deposited species and the tear film composition. The value of this, particularly if employed in a neophyte group, would be the possibility of producing a predictive factor for those atypical subjects

who would be best managed with planned replacement or who would benefit from a particular solution/material combination. The difficulties of this project would be obtaining sufficient subjects, the length of follow-up required, the difficulty in reducing variability in tear-film collection and the costs of such a large study.

- The relationship between lens wettability and the deposited species. Since the commencement of this thesis the Tearscope has become an accepted method of investigating not only tear film thickness and presence but also structure. Using this technique much better measures of the PLNIBUT and front surface tear film quality could be taken. Such information could then be compared with not only the type of deposition that occurs on worn lenses but also of the specific type of proteins and lipids deposited on the materials. Destructive analytical techniques such as HPLC and SDS-PAGE would provide information on the exact composition of the deposited protein and lipid film, which would augment the results from non-destructive analysis. Such information may suggest why certain subjects deposit to a greater extent than others and suggest why such large differences in inter-subject wettability are seen. This, coupled with careful measures of symptomatology, will provide useful information on the inter-relationship between satisfaction, wettability and lens deposition.

- Immunological techniques outside the realm of those used in this thesis could be used to probe the lens surface to investigate the impact of proteins such as the immunoglobulins, fibronectin, vitronectin and complement on lens deposition. It is quite possible that these proteins, which play an important part in regulating the inflammatory response, may influence lens wearing success by invoking a micro-inflammatory environment and converting a "successful" patient into a "symptomatic" one. This work would also be of great significance in monitoring the likely success of the soon-to-be-released continuous wear lenses.

- Larger scale studies investigating the influence of care systems on protein denaturation would be valuable, particularly concerning the denaturation of lactoferrin and albumin in addition to lysozyme. Additional work concerning the period over which such denaturation occurs is also worthy of investigation. Particular emphasis should be placed on the differences in denaturation between peroxide and non-peroxide based systems.

- The thickness of the surface deposition film that occurs is of interest in consideration of a material's biocompatibility. This could be achieved using either plasma etching, confocal microscopy, atomic force microscopy [600] or a combination. This information, combined with other destructive and non-destructive information would provide useful data.

In conclusion, this research has provided many answers to a complex phenomenon, but much work remains to follow-up on the preliminary findings of this thesis.

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Chapter 12

APPENDIX 1

RECRUITMENT ADVERTISEMENT

This is an example of a typical advert placed in the local newspaper to recruit subjects. Following a reply the subjects were screened to ensure that they were suitable candidates, prior to placement on the pertinent study occurring.

CONTACT LENS RESEARCH:

Patients Urgently Required

Collett & Jones Opticians
Sidcup & Eltham

Our Optometrists regularly act as clinical investigators for a number of contact lens companies and have a proven track record of conducting research, much of which is presented at International Conferences.

We are currently seeking 30 subjects to assist us in a study investigating the effect of the tear film on a new type of contact lens. The study lasts for 4 months, during which subjects will be provided with free visits and lenses. Upon completion of the study participants will be free to return to their previous practitioners.

If you are already wearing soft lenses, are available for occasional weekday appointments and would like to assist us in these studies then please contact our Eltham practice on 0181 850 3415 and speak to Diane, who will provide you with more details.

APPENDIX 2

INCLUSION AND EXCLUSION CRITERIA

During the studies all subjects were screened using the following "Inclusion" and "Exclusion" criteria.

Inclusion Criterion - The subject will be eligible for entry into the study if the subject:

1. Is willing and able to comply with the protocol and has given their written informed consent.
2. Has less than or equal to 1.00 dioptres of astigmatism.
3. Is between the ages of 18 and 60.
4. Has worn daily-wear hydrogel lenses for at least three months prior to starting the study.
5. Is a problem free wearer.
6. Has clear corneas.

Exclusion Criterion - Subjects will be ineligible to take part if:

1. They have eye disease, pre-existing ocular infections/disease or any history of problems with lens wear.
2. They have any history of allergies, asthma, hay-fever, psoriasis or current topical or systemic medication.
3. They are intermittent wearers.
4. They have current lens problems.
5. They have insufficient lacrimal secretions.
6. They have any lid or conjunctival abnormalities, corneal scars or opacities, or neovascularisation.
7. Is participating in any other clinical or research study.

APPENDIX 3

PRELIMINARY SCREENING FORM

Surname..... First Names.....

Title.....

Occupation..... DOB..... Age.....

Please answer the following questions concerning your personal and family medical history:

1. How would you describe you general health?

01 Excellent ( ) 02 Good ( ) 03 Fair ( ) 04 Poor ( )

2. Do you take any medication, daily or routinely?

01 Yes ( ) 02 No ( ) IfYES,what?.....

3. Have you ever had any medical problems with your eyes?

01 YES ( ) 02 NO ( ) If YES, what?.....

Please answer the following questions concerning your current contact lenses:

1. What type of CL's do you currently wear?

01 SCL ( ) 02 PMMA ( ) 03 RGP ( ) 04 DCL ( )

2. Are you happy with your lenses?

01 Yes ( ) 02 No ( )

If not, have you had problems such as dryness and/or scratchiness?

01 Yes ( ) 02 No ( )

3. Current contact lens specifications:

R..... L.....

Brand.....

4. How long have you worn contact lenses IN TOTAL?.....

5. What care regimen do you currently use?

Surfactant cleaner.....

Disinfectant agent.....

6. Keratometry readings (note axes):

R:..... al .... al .....

L:.....al .... al .....

7. Refraction today:

R..... VA = ... Binoc VA = ...

L..... VA = ...

SUBJECT MEETS SCREENING CRITERIA YES..... NO.....

ENROLMENT ONTO STUDY YES..... NO.....

Investigators signature..... Date.....

APPENDIX 4

TYPICAL INFORMED CONSENT FORM

1. Explanation Of The Study

The purpose of this study is to investigate the effect of various combinations of contact lens solutions and materials on the deposits which form on contact lenses. The lenses and solutions used are all commercially available and are not investigational products. This document informs you of the purpose of the study, what is expected of you during the study, the expected benefits and possible risks. You are encouraged to ask any questions you may have regarding either the products or the study itself. Your participation is voluntary and you will be free to withdraw at any time. Please be sure that you have clearly understood the requirements expected of you to complete the study before signing the Informed Consent.

2. Benefits of the Study

The use of contact lenses offers clarity of vision and the convenience of not wearing spectacles. There are alternatives to the products used in the study, as well as spectacles.

3. Duration of the Study

The period of the study is approximately 10 months, during which time you will be required to wear the lenses as directed. You will be one of 10 subjects taking part in the study. During this time you will be required to attend for 10 visits at approximately one monthly intervals. If you discontinue prior to this time then all investigational products must be returned to the investigator. During the period of the study you will receive free solutions, lenses and after-care visits.

4. Possible Risks

The following problems have been reported with contact lenses and/or contact lens solutions : blurring, unusual eye discharge, tearing, redness, light sensitivity and general eye irritation. In very rare cases corneal scarring and visual loss can occur. The possible complications induced in this study are no more likely to occur than in wearing any other type of high water content contact lenses on a daily wear basis whilst using currently available contact lens care systems.

5. Investigating Practitioner

Your practitioner is Lyndon Jones, who may be contacted on 0181 300 3222 during office hours and 0181 265 1396 after hours.

6. Confidentiality

The aims and objectives of this study and your participation remain confidential and should not be discussed with anyone except the investigating practitioner or members of staff of Collett & Jones.

7. Informed Consent

I, the undersigned, understand fully the requirements expected of me throughout this study. The aims and objectives of the work have been fully explained to me and any questions I have asked have been answered to my satisfaction.

Basic procedures of lens care, alternative vision correction and cleaning and disinfection procedures have been explained to me by my Research Optometrist. I have been provided with information concerning the study and understand the importance of adhering to the instructions given to me. I have also been provided with an out of hours contact number in case of emergencies.

I hereby register my intent to enrol onto the study and will, to the best of my ability, fulfill my obligations to the end of the study. However, I understand that I am free to withdraw participation in the study at any time by notifying the investigator in charge and returning ALL investigational supplies. I also understand that the Research Optometrist may discontinue me if I fail to attend for the required visits or fail to participate in the study as is required of me.

Withdrawals will not prejudice my future care, no sanctions will be taken against me for doing so and my rights are not compromised in law. Any non returned supplies will be fully charged for.

I agree that all personal details of mine (name and results etc) will be stored on a database retrieval system.

NAME (capitals) SIGNATURE

INVESTIGATOR DATE

APPENDIX 5

TYPICAL SATISFACTION SCALES

Please grade your current lenses in terms of the following factors, on a 1-10 scale, where 1 represents the worst case and 10 the best:-

VISUAL QUALITY

1. Very unstable, blurred vision. RE.....

10. Clear, crisp stable vision. LE.....

COMFORT

1. Unwearable, very painful. RE.....

10. Unable to feel the lens. LE.....

HANDLING

1. Impossible, never maintains shape RE.....

10. Very simple, maintains shape at all times. LE.....

OVERALL

1. Not at all satisfied. RE.....

10. Extremely satisfied. LE.....

FINAL VISIT ONLY

Which lens have you preferred during the study? RE..... LE.....

Please grade your current solutions in terms of the following factors, on a 1-10 scale, where 1 represents the worst case and 10 the best:-

CONVENIENCE

1. Very inconvenient, complicated .....

10. Very convenient, simple

COMFORT ON INSERTION

1. Unwearable, very painful .....

10. Unable to feel the lens

CLEANING EFFICIENCY

1. Excellent .....

10. Poor

OVERALL

1. Not at all satisfied, would not use .....

10. Extremely satisfied, happy to use

FINAL VISIT ONLY

Which soln have you preferred during the study? .....

APPENDIX 6

EXAMPLES OF FLUORESCENCE TRACES

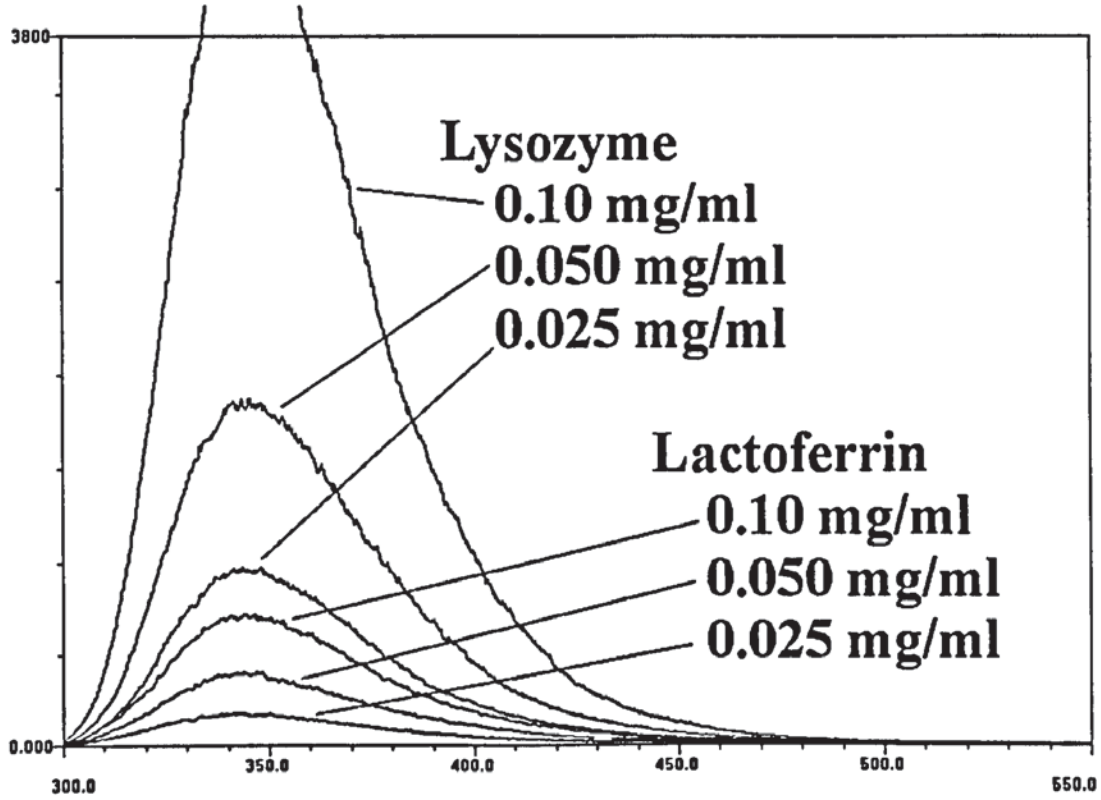

Figure A.1 - 280nm excitation traces for various concentration solutions of lysozyme and lactoferrin. The increased fluorescence of lysozyme compared with lactoferrin is clearly visible

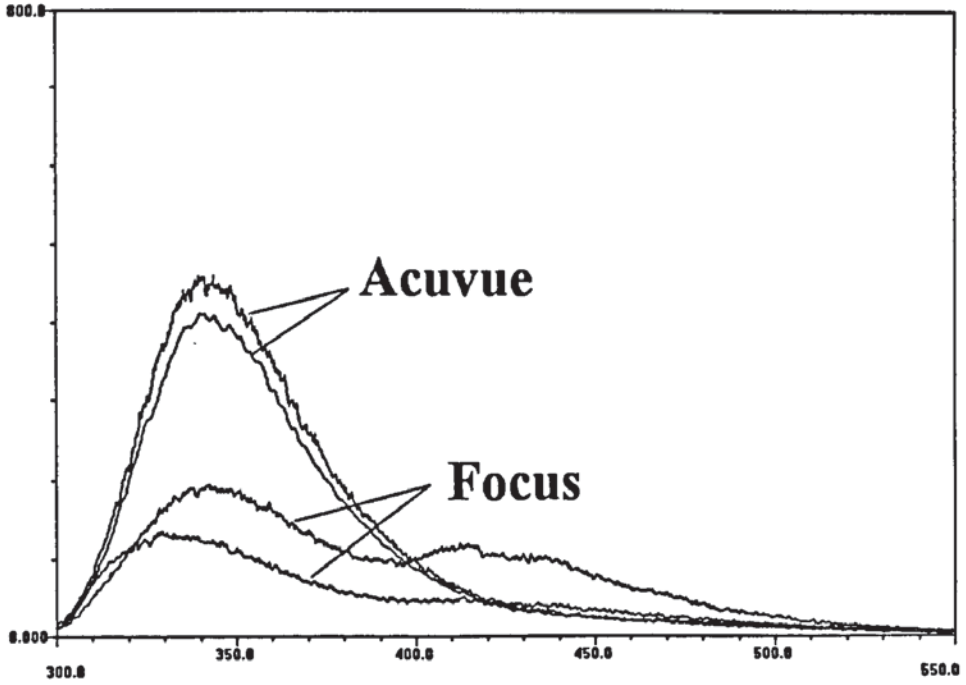

Figure A.2 - 280 nm excitation traces from the right eyes of subjects CL and DT after wearing Acuvue and Focus for four weeks. The increased protein deposition of Etafilcon in the 340-360nm emission area compared with Vifilcon is clearly visible

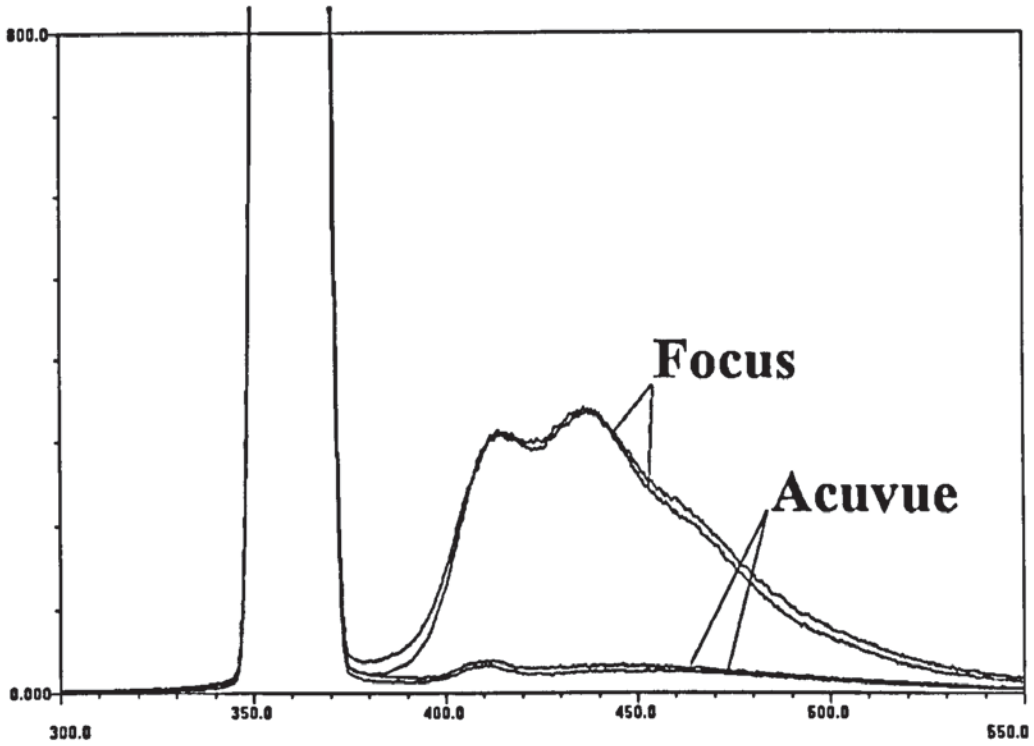

Figure A.3 - 360 nm excitation traces from the right eyes of subjects CL and DT after wearing Acuvue and Focus for four weeks. The increased lipid deposition of Vifilcon in the 430-450 nm region compared with Etafilcon is clearly visible

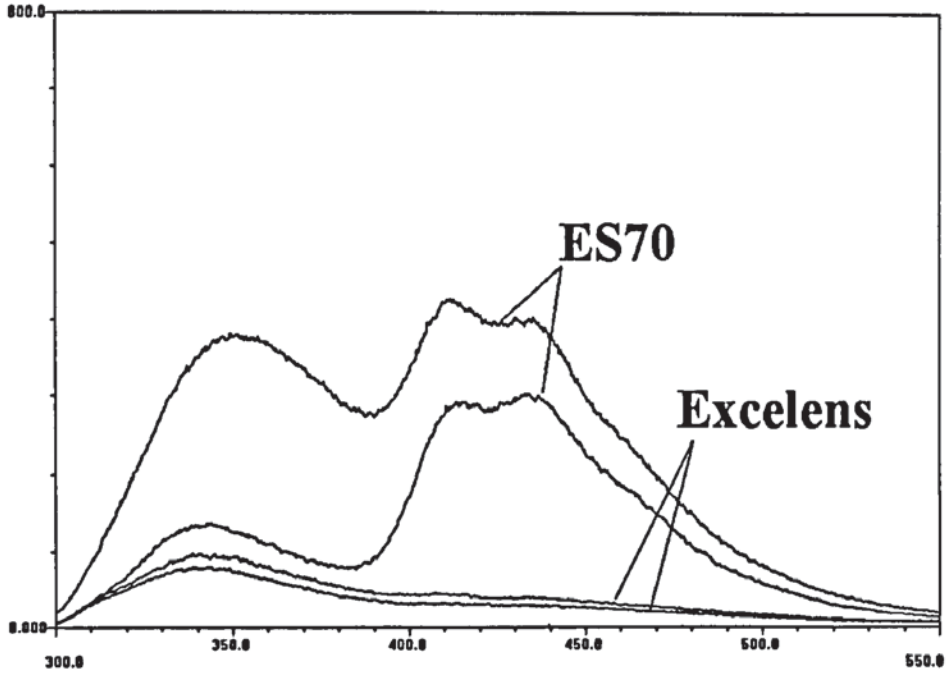

Figure A.4 - 280 nm excitation traces from the right eyes of subjects AG and SA after wearing ES70 and Excelens for six months. The increased protein deposition of ES70 in the 340-360 nm region and the secondary lipid deposition peaks in the 440 nm region compared with Excelens are clearly visible

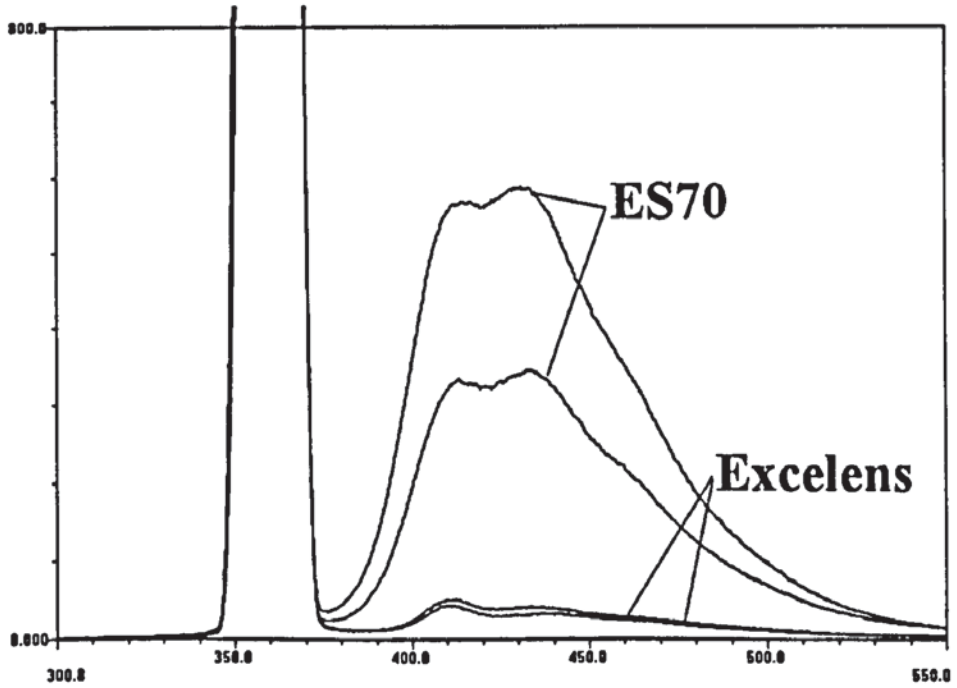

Figure A.5 - 360 nm excitation traces from the right eyes of subjects AG and SA after wearing ES70 and Excelens for six months. The increased lipid deposition of ES70 in the 440 nm area compared with Excelens is clearly visible

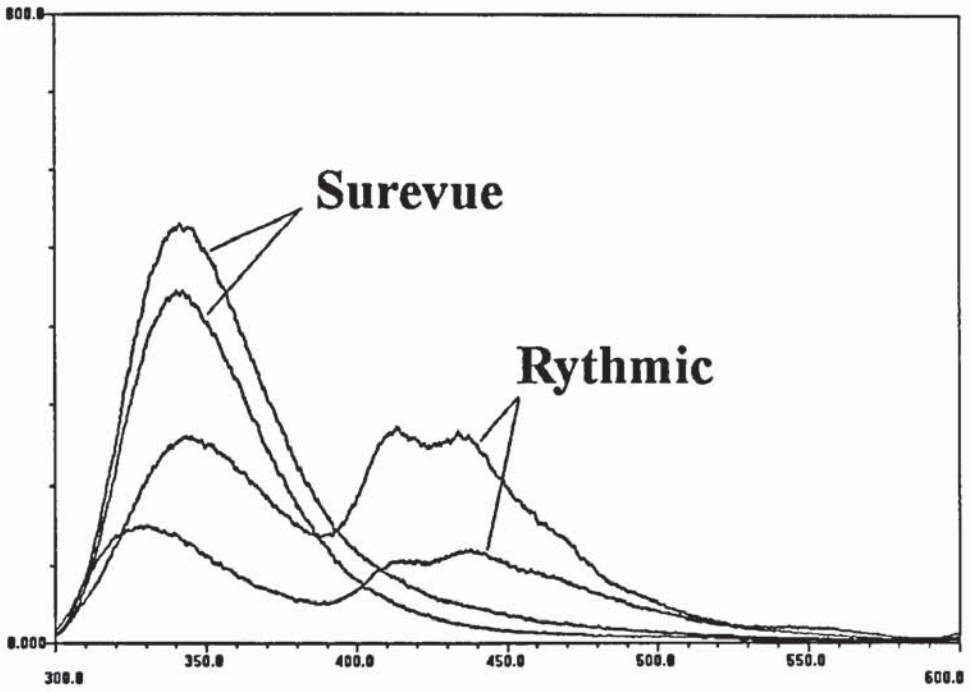

Figure A.6 - 280 nm excitation traces from the right eyes of subjects BB and IP after wearing Surevue and Rythmic lenses for one month. The increased protein deposition in the 340nm region of the Group IV material compared with the Group II material is clearly visible. Additionally, the secondary lipid peak of the Group II material in the 440nm area is also visible

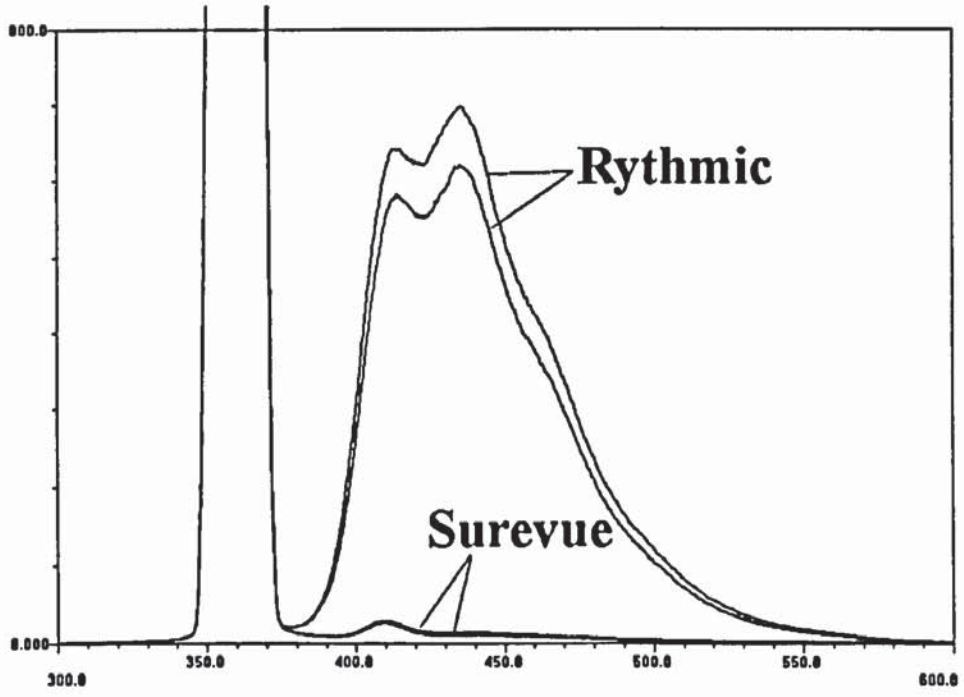

Figure A.7 - 360 nm excitation traces from the right eyes of subjects BB and IP after wearing Surevue and Rythmic lenses for one month. The increased lipid deposition in the 440nm region of the Group II material compared with the Group IV material is clearly visible.

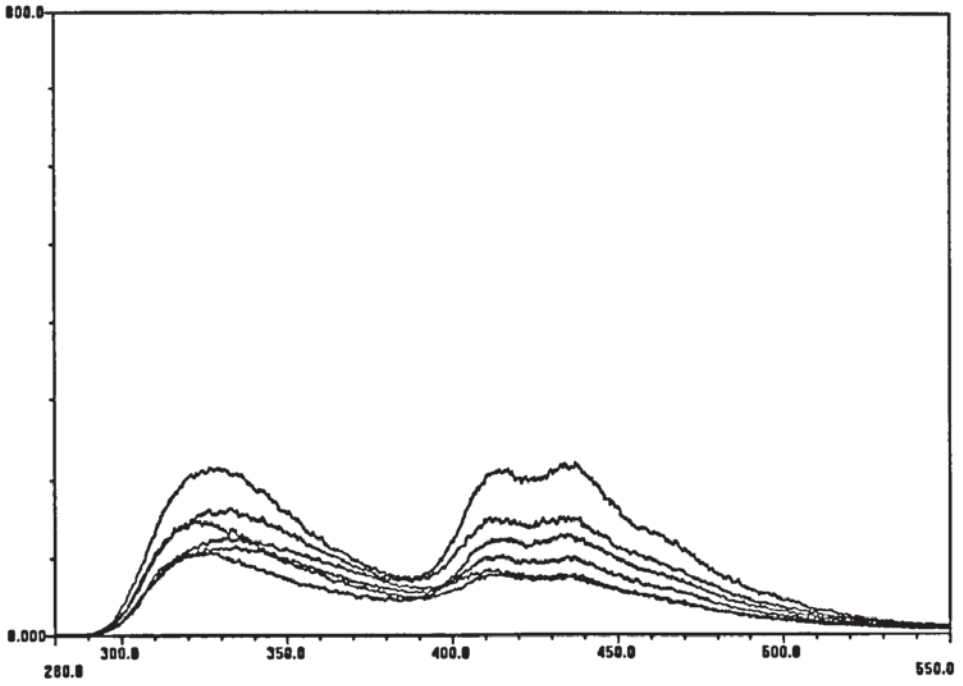

Figure A.8 - 280 nm excitation traces from the right eye of subject MH of Medalist 66 lenses worn for six consecutive monthly periods. These results indicate the degree of variability in protein deposition which occurs in a single subject with monthly frequent replacement lenses. Examination of the 440nm area also indicates the variability in lipid deposition

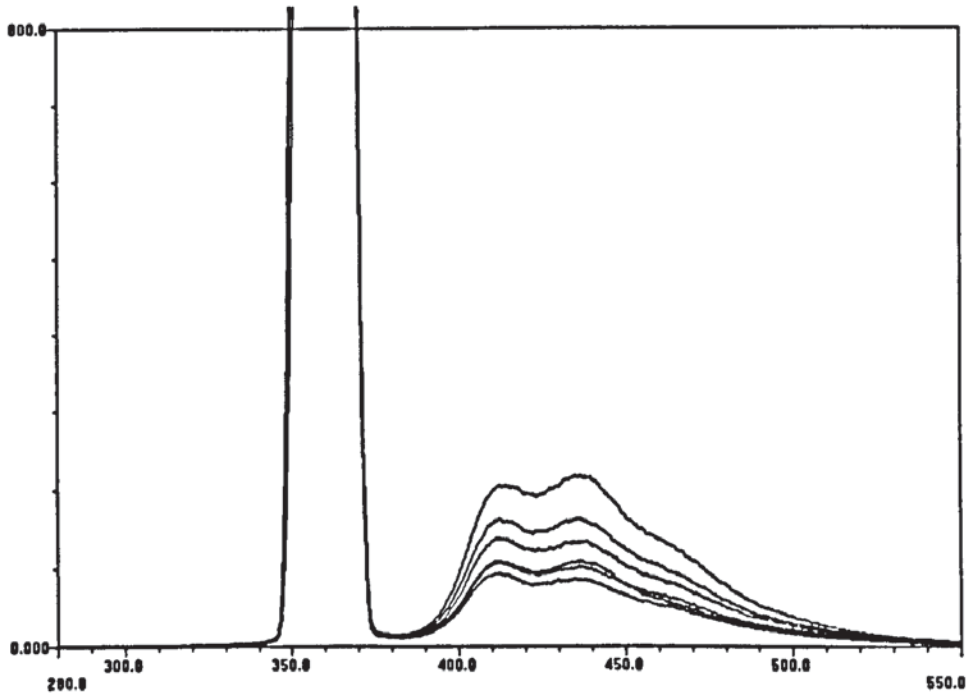

Figure A.9 - 360 nm excitation traces from the right eye of subject MH of Medalist 66 lenses worn for six consecutive monthly periods. These results indicate the degree of variability in lipid deposition which occurs in a single subject with monthly frequent replacement lenses.