DEVELOPMENT OF A NON-DESTRUCTIVE FLUORESCENCE TECHNIQUE FOR ANALYSIS OF CONTACT LENS DEPOSITION LEVELS.

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Development of a non-destructive fluorescence technique for analysis of contact lens deposition levels.

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The primary objective of this research has been to determine the potential of fluorescence spectroscopy as a method for analysis of surface deposition on contact lenses. In order to achieve this it was first necessary to ascertain whether fluorescence analysis would be able to detect and distinguish between protein and lipid deposited on a lens surface. In conjunction with this it was important to determine the specific excitation wavelengths at which these deposited species were detected with the greatest sensitivity. Experimental observations showed that an excitation wavelength of 360nm would detect lipid deposited on a lens surface, and an excitation wavelength of 280nm would detect and distinguish between protein and lipid deposited on a contact lens. It was also very important to determine whether clean unspoilt lenses showed significant levels of fluorescence themselves. Fluorescence spectra recorded from a variety of unworn contact lenses at excitation wavelengths of 360nm and 280nm indicated that most contact lens materials do not fluoresce themselves to any great extent.

Following these initial experiments various clinically and laboratory based studies were performed using fluorescence spectroscopy as a method of analysing contact lens deposition levels. The clinically based studies enabled analysis of contact lenses with known wear backgrounds to be rapidly and individually analysed following discontinuation of wear. Deposition levels in the early stages of lens wear were determined for various lens materials. The effect of surfactant cleaning on deposition levels was also investigated. The laboratory based studies involved comparing some of the *in vivo* results with those of identical lenses that had been spoilt using an *in vitro* method. Finally, an examination of lysosyme migration into and out of stored ionic high water contact lenses was made.

Key words.

Fluorescence spectroscopy.
Contact lens.
Protein.
Lipid.
Deposition.

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1. Introduction.

This study set out to assess the potential of a non-destructive fluorescence technique used as a method to analyse contact lens spoilation. As an introduction to the project a brief overview of the uses of biomaterials (with particular emphasis on opthalmic applications), and the problems encountered with their use will be given. Following this is a summary of the tear fluid composition and the manifestations of contact lens spoilation. Final sections in this introduction involve descriptions of techniques available for analysis of contact lens deposition, and a list of the aims of the project.

Polymers have a wide variety of biomedical applications. These include cardiovascular devices, burn dressings, joint replacements, drug delivery systems and contact lenses.[1-4] These materials are termed biomaterials. In such situations interaction will occur between the biomaterial and it's tissue environment. For this interaction to be successful the biomaterial must be biocompatible with it's particular environment.

A group of polymers that are quite different from conventional hydrophobic materials are those that contain water in their structure. These polymers, often referred to as hydrogels (water swollen polymer networks) were first documented by Wichterle and Lim in the 1960's.[5] The simplest hydrogel is poly(2-hydroxyethyl methacrylate) known as polyHEMA. It's basic structure is very

similar to poly(methyl methacrylate) (PMMA). The most significant difference is that every unit of the polyHEMA chain contains a hydroxyl group. The majority of opthalmic research stems from these two carbon backbone polymers and an elastomer, silicone rubber, which is based on the poly(dimethyl siloxane) heterochain structure.

Without water polyHEMA and PMMA are both hard, clear and glassy. However, when in contact with water they behave quite differently. PMMA remains hard and glassy, but the hydroxyl groups in polyHEMA take up water, converting it to a clear, flexible, elastomeric gel. The properties of the hydrogel are influenced both by the structure of the polymer network and by the water. The water will also perform two other very important functions. It acts as a bridge between the natural and synthetic systems giving greater biocompatibility, and it confers membrane properties on the hydrogel, allowing transport of oxygen and water soluble metabolites through the polymer matrix.

1.1 Biocompatibility problems.

Problems always arise in the biomedical use of synthetic polymers as ultimately a layer will be deposited from the biological system onto the biomaterial. This adsorbed layer will influence any further interaction with the surrounding environment.[6] This phenomenon is common to all biomaterial applications but is manifested in different ways. For example; deposition of tear components onto contact lenses, or the clotting of blood at foreign surfaces.[7]

The study of these initial reactions *in vivo* to biomaterials is problematical as

they are difficult to observe and monitor.

However, one successful *in vivo* model which can be used to study the early stages of the interaction of biological components with synthetic materials is the eye. The problem of deposit formation (spoilation) on contact lenses is a well known phenomenon. The manifestations of spoilation will be discussed in more detail later.[8,9] However the underlying processes that govern this interaction are common to many areas where materials come into contact with biological solutions.

This phenomenon frequently begins with the adsorption of protein at an interface followed by competitive adsorption of other biochemicals. The initiating processes in all these interactions can be easily studied in the eye because contact lenses can be removed and analysed after very short periods of wear. Thus, the eye presents a real opportunity for *in vivo* studies of some aspects of biocompatibility. This is especially true for hydrogels as they have considerable potential in various opthalmic applications.

1.1.1. Scleral buckling agents.

A detached retina can be re-attached to the underlying choria using a procedure known as scleral buckling. This is normally done by using an implant that causes indentation of the sclera. Traditionally the implant material has been made of silicone rubber but the potential advantages of a poly(methyl acrylate-*co*-hydroxyethyl acrylate) hydrogel implant are now recognised. For example, the hydrogel implant can be impregnated with a hydrophilic antibiotic which is then

locally released. This can help prevent infection during the immediate post-operative period. The swelling characteristics of the polymer can also be exploited in a number of ways. The implant can be pre-conditioned in a hypertonic saline before insertion, so that on absorbing body fluids it increases in size. Alternatively, by conditioning in a physiologically isotonic saline solution, it can remain a constant size. Finally the implant can be inserted into the eye in a swollen state, higher than it's equilibrium value in the body so that it gradually decreases in size.

1.1.2. Intraocular lenses.

For many years intraocular lenses (IOLs) were made from PMMA, with polypropylene or poly(ethylene terephthalate) haptics (fixation loops). However these lenses can damage the corneal epithelium if they come into contact with it during surgery. To overcome this problem PMMA lenses have been surface modified with a radiation grafted hydrogel coating of poly(vinyl pyrrolidone) which appears to improve biocompatibility and reduces endothelium damage.

The flexibility of hydrogels means that the lenses can be folded and inserted through a smaller incision than a conventional "hard" lens, or even inserted while dehydrated and allowed to hydrate in the eye. However, hydrogels do have some disadvantages. Their mechanical strength is low which may cause problems during manipulation for implantation and their relatively low refractive index may mean using thicker lenses to achieve a given optical power.

1.1.3. Ocular drug delivery.

Another area of interest is that of using synthetic polymers for sustained ocular drug delivery. When an eye drop is instilled into the eye it will have a half-time of residence ranging from 10 seconds to 4 minutes depending on the size of the drop. This is because of the reactions caused by instillation of the drop i.e. reflex tearing, blinking and lacrymal drainage. One method of increasing the residence of the instilled drug is to mimic the interactions that normally occur between the mucoid component of the tear film and the corneal surface. Wetting solutions and artificial tear solutions are both examples of solutions that aim to do this. The ideal tear substitute should promote the formation of a functional tear film which would preserve visual sharpness and restore and maintain the health of the cornea. These artificial tear solutions are based on viscous polymers, most commonly containing methyl cellulose, hydroxypropylmethyl cellulose or polyvinyl alcohol. The high viscosity of these solutions will keep them in the eye longer, while the polymer itself is intended to mimic the ocular mucins. The use of polymers such as hydroxypropylmethyl cellulose and polyvinyl alcohol as tear replacements and as vehicles for instilled substances increases their residence time in the tear film.

Another approach to ocular drug delivery is to insert a solid polymer device beneath the lid. For example hydrogel contact lenses can be used for the controlled release of antibiotics to patients waiting for cataract surgery or, to deliver medication such as pilocarpine used to reduce the intraocular pressure in glaucoma.

1.1.4. Contact lenses and the ocular environment.

It was in the late 19th century that the clinical implications of contact lenses were envisaged, but only relatively recently that their use became routine. As with synthetic polymers used for joint replacements, heart valves and haeomodialysis membranes the general biomedical principle of designing the material to give a balance of properties appropriate to the particular environment is equally important in the design of contact lenses. The situation is less critical in lenses used for daily wear only, than in extended wear lenses. However, in both cases similar properties to those in other biomedical applications are required.

Originally glass was used to make contact lenses and the lenses were mainly individually ground. PMMA began to replace glass in the 1940s because of it's toughness, optical properties and it's physiological inactivity. It was some years before the potential of hydrogels as contact lens materials was realised.

The cornea governs the permeability requirements of the material used in contact lenses. This is because the cornea is avascular, so needs to respire directly from the atmosphere. If the corneal oxygen supply is curtailed corneal oedema will arise. In hydrogels the transport properties are governed by water content. This must be sufficient to allow passage of oxygen, carbon dioxide and other water soluble metabolites to and from the cornea at levels that will maintain a healthy corneal surface. A polymer containing over 70% water is required to maintain adequate oxygenation of the cornea when wearing a lens whilst sleeping. Most commercial contact lens materials in this category are simple crosslinked copolymers of vinyl pyrrolidone with 2-hydroxyethyl methacrylate or methyl

methacrylate.

The presence of this amount of water in a hydrogel will affect it's mechanical properties, and the balance of properties will be governed by the eyelid. Soft materials are generally more comfortable, however, the eyelid exerts a fairly considerable deforming force during blinking so a contact lens material cannot be too soft. Thus an ideal contact lens material must have a high enough water content to allow efficient corneal respiration to occur, whilst maintaining sufficient mechanical properties to prevent deformation of the lens during blinking. The polymer must also be sufficiently wettable to sustain a coherent layer of tear fluid. This problem is not automatically overcome by the presence of water in the hydrogel as it's surface chemistry will govern the complex series of events that lead to a variety of possible deposits on the surface when the lenses are worn. This may alter the wettability of a contact lens.[10-12] Here, as in blood contact devices, protein adsorption is an important first step. Subsequent deposits are complex and contain calcium, mucopolysaccharides and lipids, in addition to the major tear fluid proteins.

1.2. Tear fluid composition.[13]

Human tears are formed by a group of glands collectively known as the Lacrimal system. The secretory apparatus consists of the main and accessory lacrimal glands (source of the aqueous tear components), the meibomian glands (which secrete lipids), and the conjunctival goblet cells (which secrete mucous glycoproteins). The accessory lacrimal glands provide minute-to-minute supply

of tears, while the main lacrimal gland acts as a back-up reflex system by flooding the eye in response to injury, irritation or emotions (reflex tearing). Normal tear volume is approximately 7.0-10.0 ml,[14] and there is a continual non-reflex secretion rate of about 1ml/min from the accessory lacrimal glands. The various secretions contribute to a complex mixture that contains proteins, enzymes, lipids, metabolites, electrolytes, hydrogen ions and excreted drugs. The location and distribution of the glands responsible for tear formation are shown in Figure 1.

Fig. 1 Cross section through the anterior of the eye to show the location of the main tear forming glands.

Tears serve a number of functions.[15,16]

1. They form and maintain a smooth refracting surface over the cornea.

2. They transport metabolic products i.e. oxygen and carbon dioxide to and from epithelial cells and the cornea.

3. They flush away noxious substances from the eye.

4. They provide a pathway for white blood cells in the case of injury.

5. They contain antibacterial substances and antibodies.

6. They provide lubrication between the eyelids and the ocular surface.

The composition of the tear fluid is of a very complicated and varied nature. For instance, composition will vary widely between individuals and according to the method used to collect the tears.

The tear film was thought to be "split" into three layers. This structure was suggested by Wolff in 1954.[17] It is generally believed that this structure, as illustrated in Figure 2, consists essentially of the superficial lipid layer, the aqueous layer below this and an absorbed mucin layer.[18]

1.2.1 The superficial lipid layer.

This is the outermost layer of the tear film. It is about 0.1υm thick and is believed to form about 1% of the total thickness. The superficial oily layer is secreted by the meibomian glands[19,20] and the glands of Moll and Zeis which are situated at the edge of the eyelids.

Fig. 2 The tear film in cross-section.

Analysis of the meibomian lipids demonstrates the presence of all possible lipid classes, mainly hydrocarbons, wax esters, cholesterol esters, triglycerides, and in lesser quantities diglycerides, monoglycerides, free fatty acids, free cholesterol and phospholipid.[21-23] The composition will however vary considerably between individuals.[24]

1.2.2 The aqueous layer.

This middle layer is 7υm thick and represents over 98% of the tear film. It originates from the main lacrimal gland located in the superior temporal aspect of

the orbit, and from accessory lacrimal glands (glands of Krause and of Wolfring, the infraorbital gland, and the glands of the plica and the caruncle) lining the surface of the conjunctiva. It contains numerous electrolytes,[25] vitamins, proteins, metabolites, enzymes, various organic acids and antiproteinases.[26]

Electrolytes. The major electrolytes found in this layer are sodium, potassium, chloride, bicarbonate,[27,28] calcium[29] and magnesium.[30]

Proteins. The protein composition of tears will vary according to the method of collection. The total concentration of proteinaceous substances is estimated to be 0.3-7.0% (800-2200υg/100ml),[31] but varies depending on whether the tears are unstimulated, emotional or irritant induced. Using electrophoretic techniques, more than 60 protein components can be detected in normal tear fluid, of which more than 20 have been found to be secreted by the lacrimal gland. The major proteins include tear albumin, specific tear pre-albumin, globulins, lysosyme, lactoferrin, transferrin, caeruloplasmin, immunoglobulins A, G and E, glycoproteins and complement.[19,31,32,33]

Metabolites. These include pyruvate, glucose, lactate, urea, catecholamines, histamine, endorphins and prostaglandins.

Enzymes. A number of enzymes are found in the aqueous layer including glycolytic enzymes, tricarboxylic acid cycle enzymes, amylase, lactate

dehydrogenase, lysosomal enzymes, peroxidase, plasminogen activator and collagenase.

Antiproteinases. These include α_1-antitrypsin, α_1-antichymotrypsin, inter-α-trypsin inhibitor and α_2-macroglobulin.

Vitamins. The vitamins present in the aqueous layer include vitamins A and C.

1.2.3. Mucoid layer.

This layer is most commonly quoted as comprising less than 0.5% of the film, being 0.02-0.05υm thick.[32] More recent observations have shown however, that mucins become diluted towards the aqueous layer and interact with, and mask, the hydrophobicity of the lipids.[34] The inner mucoid layer is secreted by specialized conjunctival cells in the crypts of Henle and the glands of Manz.[35] The mucus layer, which is the innermost layer of the tear film consists of salts (1%), free protein (0.5-1.0%), glycoprotein (0.5-1.0%) and water (>95%). The electrolyte content resembles that of serum or bile, and other constituents include immunoglobulins, salts, urea, glucose, leucocytes, tissue debris, and enzymes such as β-lysin, peroxidase and lysosyme. The glycoproteins or mucins, are 50-80% carbohydrate with the oligosaccharides attached to a protein backbone of molecular weight 2.0-15.0 X 10^6Da, which is composed of about 800 amino acid residues. Mucins play an important part in corneal wettability and tear stability,

although the precise mechanisms of interaction with the corneal surface are a subject of current discussion.

It can therefore be seen that the tear fluid is of a very complicated nature. A contact lens, when placed in the ocular environment will be directly in contact with the anterior corneal surface. The outer lens surface will also interact with the eyelids, particularly during blinking.[36] The contact lens therefore will be completely surrounded by tear fluid, and is rapidly coated with tear film derived material upon insertion into the eye. This interaction will result in the process known as spoilation of the contact lens.

1.3 Manifestations of spoilation.

Spoilation encompasses the physical and chemical changes in the hydrogel contact lenses, and the various extraneous deposits which may impair the optical properties of the lens or produce symptoms of discomfort and often intolerance to the wearer. Spoilation phenomena will vary with lens types and from person to person. The majority of patients will show spoilage after 3-6 months of daily wear. However, small amounts of deposition can be detected on a lens surface after very short periods i.e. after simply inserting and removing a lens.

A variety of organic, inorganic and mixed deposits will adhere to the lens surface. The observed types of spoilation can be divided into different classes.[37] However, there is overlap between classes, so classification is best regarded as a grouping of various related types of clinically observed phenomena.

Complex deposits. Discrete elevated deposits (white spots), particles.

Lens coatings. Proteinaceous films, specific calcium deposits, granular deposits, inorganic films.

Microbial deposits. Fungal and bacterial deposits.

Extrinsic factors. Cosmetics, dust, metallic particles from the atmosphere.

The white spot deposits are the most chemically complex manifestations of ocular incompatibility. They form on the surface of the lens with no specific site. Their morphology appears to be formed by success i.e laying down of globular structures to form a complex multi-layered structure.[38] The chemical composition of the deposits is variable and includes proteins, amino acids, mucins, glycoproteins, glucose, lipids, calcium, potassium, chloride, bicarbonate, phosphate and urea. A high proportion of the deposits is believed to be lipid including cholesterol, cholesterol esters, triglycerides, fatty acids and fatty alcohols.[39-42] These lipids are naturally occurring in tears.

Another major class of spoilation involves surface films, coatings and plaques. Proteinaceous films appear as thin semi-opaque white superficial layers of denatured protein.[43] The composition of these films has been attributed to mucoproteins, albumin, globulins, glycoproteins, lysosyme and mucin.[44-47] General accumulation of protein films on soft lenses leads to an increase in surface haziness and surface rugosity (roughness), resulting in a decrease in visual acuity due both to lens opacity and to poor lens movement on the eye.

Inorganic films are similar in gross appearance to protein films, although their incidence is lower. They are mainly formed from calcium phosphate which is co-

precipitated with protein.[48] Tear chemistry of the wearer, tear production and poor blinking all contribute to this type of deposit. Heavy inorganic films often cause damage to the lens surface, since the material may penetrate into the lens matrix.

Granular deposits are elevated white translucent crystalline formations of variable size. They too are dependent on patient's tear chemistry.

Inorganic calcium deposits also occur. These can penetrate the lens matrix and be covered by a protein film. The rough underlying inorganic material is composed mainly of hydroxyapatite which is the thermodynamically stable form of calcium phosphate.

Microbial spoilation can arise due to lens handling and inappropriate lens care regimes. Many species of fungi and yeasts have been identified on hydrogel contact lenses including *Candida tropicales*, *fusanum* and *albicans*, *Asperigillus fumigeltus, niger* and *penicillium* species. These species are rarely present in copious amounts on the lens surface due to the antimicrobial activity of tears.[49] Colonisation can occur however, i.e. the presence of deposits on the lens surface will provide the nutrients required for microbial growth.[50]

Discolouration of lenses also arises. This can be caused by a variety of factors including melanin formation, topical adrenaline, topical vasoconstrictors and tear components. Chlorhexidine, which is found in preserved saline can cause yellow, or yellow-green discolouration. A mercury containing compound, thiomerosal which is found in many hydrogel lens care systems can result in grey discolouration.[51] The possible nature of discolouration is described in various

papers, of which the most noteworthy is that of Kleist.[52]

Extrinsic factors including cosmetics, poor lens handling, airborne foreign bodies, dust, toxic fumes and metal particles can also contribute to the spoilation of soft contact lenses.[8]

It can therefore be seen that the chemical composition of lens deposits is extremely complex and nearly all chemical components found to be present in lens deposits are contained within the tear film, which raises analytical problems requiring detailed study. It has been noted by several workers that differing hydrogel polymeric compositions appear to highlight relatively specific deposit types. Another variable is the composition of the tear film. Great differences in component concentrations have been noted from one individual to another. Hydrophilic soft lens spoilation results primarily from the interaction of the polymer with the microenvironment of the eye. Differing degrees of spoilation from one polymeric composition to another result not only from differences in surface and bulk chemistry of the material, but also from differences in the patient tear film, together with the design and fit of the lens. For this reason the mechanism of formation is extremely instructive in understanding the underlying problem of hydrogel interaction with tears.

1.4 Studies of deposit formation.

Many trials and studies have been performed with the aim of trying to gain a greater understanding of contact lens spoilation, as it is only once the processes

involved are fully understood that deposit resistant materials can be designed. Spoilage of soft contact lenses is not a new or uncommon phenomenon, but it is only really in the last 10-15 years that attempts have been made to scientifically analyse the problem.

All worn soft contact lenses become deposited or coated and this coating becomes more complex with time.[53] The composition of these deposits has been inadequately studied; most of the attention has been directed at the more "spectacular" deposits. Early research into the nature of lens deposits showed them to be of varying aetoligies including; proteinaceous,[44,54-60] mucoidal,[61,62] lipoidal[63,64] and ionic.[65,66] The texture, location and geometry of these deposited areas was found to vary greatly from person to person.[67] The extent of the situation may be appreciated by considering the reported incidence of lens spoilation which ranges, according to various reports, from 7-82% of the extended wear lenses being worn.[8,68-70] Tripathi, Tripathi and Ruben, 1980[8] when looking at 300 spoiled lenses found that virtually all lens materials in use for daily or extended wear are subject to deterioration due to extraneous deposits. Their analysis indicated that the main causes of spoilage include mucoprotein-lipid deposits, inorganic calcareous deposits, microbial invasion, various extrinsic factors and manufacturing defects, lens ageing and decay. High water content lenses seemed more susceptible to spoilage, and although proper lens cleaning, disinfection, patient instruction and ocular hygiene are helpful in preventing lens spoilage, in a given patient some lenses will deteriorate earlier than others, and some patients form deposits more profusely than others. Bilbaut, Gachon and Dastugue[71] used microscopic examination and scanning electron microscopy

(SEM) on worn contact lenses. These techniques permitted classification of deposits into globular deposits, "Mulberry-like" growths, opaque deposits, protein films and red spots. They also used an electron probe to identify the specific composition of these deposit types. The use of electrophoresis demonstrated the presence of lysosyme in all the deposit types, with other unidentified proteins or degradation peptides. In another study Wedler[47] performed electrophoretic analyses using samples from a number of lenses, and qualitative fluorescent antibody probes with single lenses. These studies indicated that the proteins found in lens deposits were derived from the tear fluid, not from other sources. Proteinaceous deposits are one of the most common problems associated with hydrophilic contact lens wear.[72]

These are just a few examples of the many studies that have been performed in an attempt to gain more information about the processes involved in contact lens spoilation. However, in general these studies were carried out with lenses that were heavily spoilt i.e. at the end of their useful lives. There is a parallel here with studies performed with biomaterials that are inserted into the body. As with contact lenses these are generally only examined when problems occur, or when the patient dies. Another disadvantage with contact lenses that have been worn for long periods is that the clinical histories of the lenses is often poor, and they may have been stored for long periods after wear was stopped, but before any research was performed. During this storage period the deposition profile of the lens may alter quite substantially.

Problems can also arise with the techniques used for analysis. These usually are destructive, they rely on taking material off the lens to examine it. This gives rise to the question is all the material off the lens or not? Therefore, at the outset of this study it was felt that some attempt should be made to develop a technique for use in parallel with a controlled clinically supervised wearing programme.

1.5 Techniques available for contact lens analysis.

At the outset of this study it was necessary to review the techniques available for contact lens analysis. The main problem in analysing early lens spoilation is the fact that minute quantities of deposited species are involved. Techniques available for analysing spoilation can be divided into optical methods used for looking at the surfaces of the lens and chemical methods used for analysing the extracts from these surfaces. Techniques for analysing the surfaces include optical microscopy, histological and histochemical analysis, fluorescence spectroscopy, Fourier-transform infra-red spectroscopy (FTIR), scanning confocal laser microscopy and electron microscopy. FTIR is a very useful technique for looking at small areas of deposits but this method is not at present capable of quantifying low levels of deposition in the early stages of wear. Work is being performed on this technique but it is not appropriate for this project. Techniques for analysing the extracts include high performance liquid chromatography (HPLC), fluorescence spectroscopy, electrophoresis and thin-layer chromatography.

Another technique that could be used is Ultra-Violet (UV) spectroscopy.

However, there are several disadvantages associated with this method of analysis. Firstly UV spectroscopy is a transmission technique. This means that it can only be used to detect relatively large amounts and is therefore too insensitive to detect the very low levels of deposition found in the early stages of wear. Secondly, UV spectroscopy relies on the fact that compounds absorb light in the ultra-violet wavelength range. Since a vast number of compounds will absorb light in this region, UV spectroscopy has low specificity as an analytical technique.

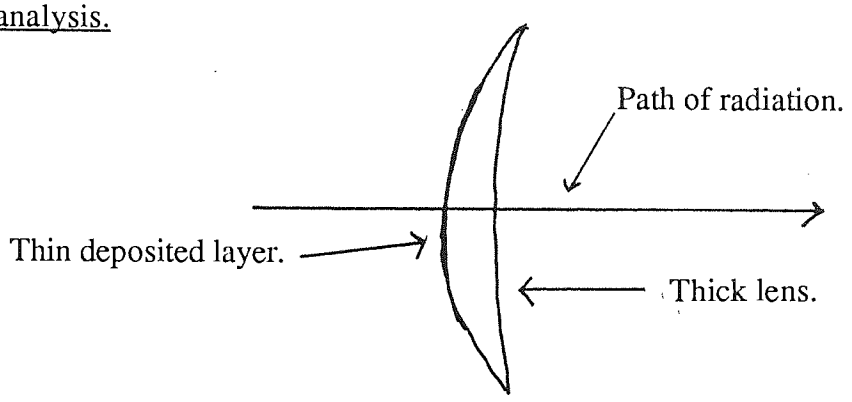
This project hinges on fluorescence spectroscopy used as an analytical technique in a way that has not previously been possible. Fluorescence spectroscopy can be used to non-destructively assess the levels of deposition on a contact lens both quantitatively and qualitatively, even after only very short wear periods. For the purpose of this project only qualitative analysis was performed. Fluorescence spectroscopy will be described in more detail in Chapter 3. However, from Figure 3 it can be seen that it has many advantages over UV spectroscopy.

1.6 Analysis of the early stages of deposition.

The processes involved in the analysis of deposition in the early stages of contact lens wear can most easily be shown in a flow diagram (Figure 4). In this project the schematic illustrates the way in which analysis has been used and in principle, the effect of care systems and materials can be looked at individually.

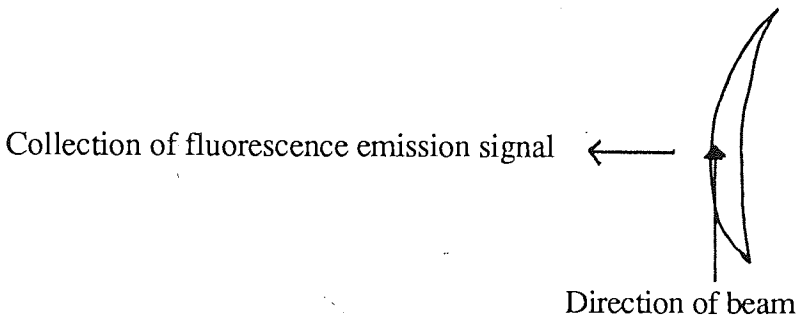
Fig. 3 Comparing UV versus fluorescence analysis of contact lenses.

1. UV analysis.



Here, absorption of the deposited layer is small in relation to the background absorption of the lens, which is one thousand or more times thicker.

2. Fluorescence analysis.



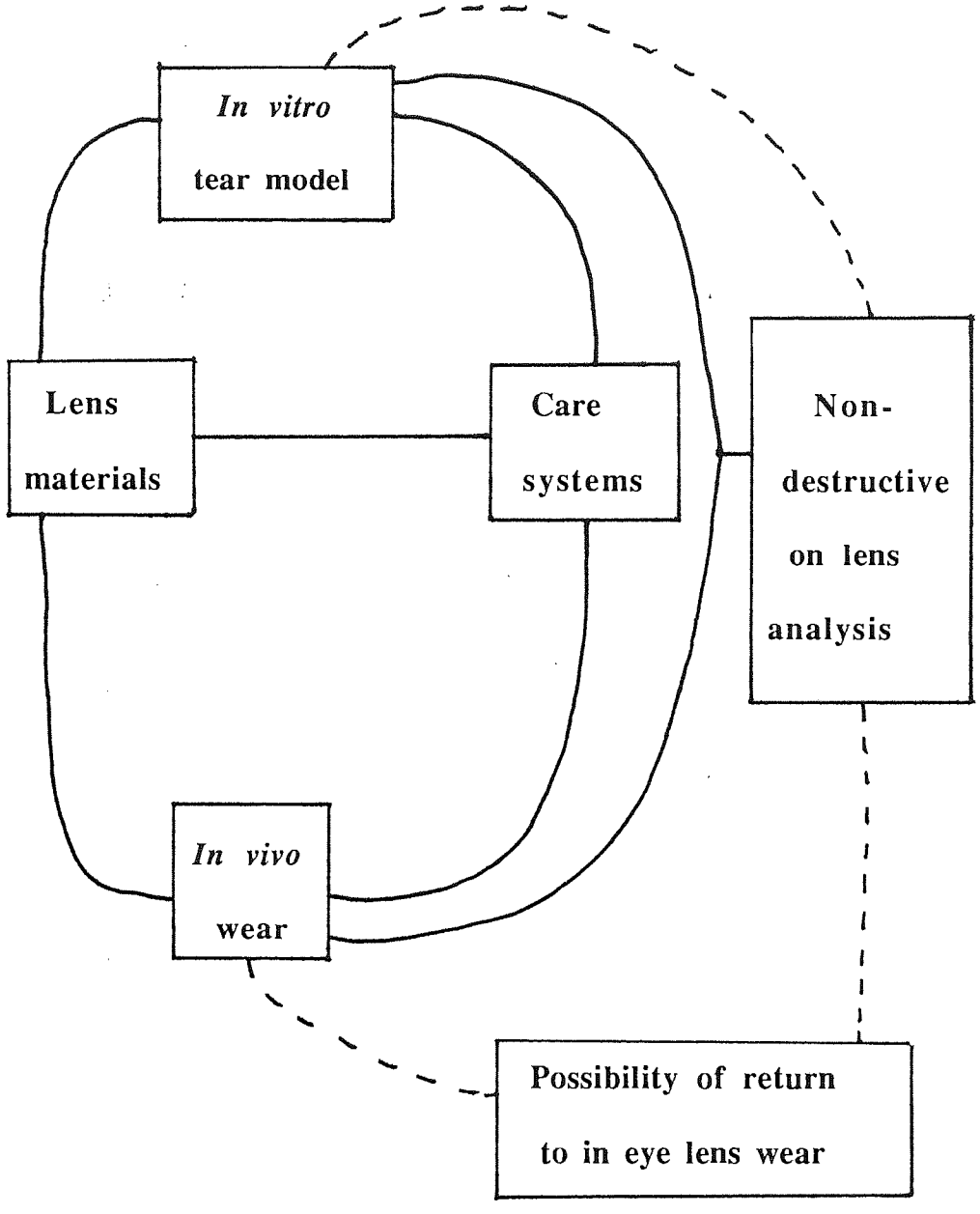
Here the fluorescence is (relatively) unaffected by the structure of the lens provided that the lens does not physically reflect or scatter light. The fluorescence signal is solely a function of the deposited layer. Most lenses will absorb UV in this type of analysis, the majority will not fluoresce. Therefore as a technique for analysis of contact lens deposition fluorescence spectroscopy has a great many advantages over UV spectroscopy.

The *in vitro* model allows you to observe accelerated spoilation in comparison to conventional wear times, or remove one tear component.[73] Non-destructive analysis allows a lens to be returned to the wearer after disinfection to see how longer wear times will effect the deposition profile of the contact lens. This has not been done in this project but could be a useful extension to this type of research in the future.

The advantages of the approach shown in the flow diagram (Figure 4) are that the specific physical properties of care systems, e.g. pH, viscosities etc. can be related to their performance. For example measurements of the surface tension and viscosity of a particular surfactant cleaner can be compared to it's cleaning ability (this is assessed using *in vitro* spoilt lenses and fluorescence spectroscopy). This yields information so that attempts can then be made to achieve these favourable physical properties in new cleaning products. Alternatively, an estimation of the probable cleaning efficacy of a new cleaning solution could be made purely from it's physical properties.

So it can be seen that optical techniques are advantageous. No chemical extraction of material is required from the contact lens surface before analysis can be performed.

Fig. 4 Schematic illustrating variables involved in contact lens analysis.



Of the optical techniques available UV and infra-red (IR) spectroscopy are not particularly useful, although UV spectroscopy does become valuable if a build up of a deposited substance occurs in the lens matrix e.g. the migration of lysosyme into the matrix of Etafilcon A lenses. UV spectroscopy will detect this in contrast to fluorescence spectroscopy which will only detect the deposited material in the surface "layers" of the contact lens. However, the much greater sensitivity and specificity of fluorescence spectroscopy make it a more valuable analytical technique.

1.7 Aims of the project.

The aim of this project was to assess the technique of fluorescence spectroscopy in various experimental situations to determine the potential of this analytical method.

The work proposed for this project can be summarised as follows:-

1. Using fluorescence spectroscopy as a method of analysing contact lens deposition levels it was hoped to be able to highlight differences in the susceptibility of lens materials to deposition. This could potentially yield information about the effects of water content and ionicity on deposition levels. In addition, the expected ability of this technique to distinguish between protein and lipid deposition should provide information about preferential deposition of a particular species by an individual lens material.

2. Another area of interest was the variations seen between patient deposition levels. It is known that different contact lens wearers will deposit tear components to different extents depending on a variety of factors e.g. variations in tear chemistry or care regimes. By using a combination of controlled wear and cleaning schedules it should be possible to obtain information about the differing chemistry of deposition between patients.

3. Similarly, the use of controlled wear and cleaning regimes in combination with fluorescence analysis should enable information about left eye/right eye differences to be highlighted.

4. Finally, by using a combination of *in vitro* and *in vivo* techniques it was hoped that it would be possible to study the ability of care systems to reduce deposition levels. This could be achieved by several experimental methods. *In vitro* spoilt lenses, could potentially have their fluorescence spectra analysed before and after the use of a particular lens care product. In this way the comparative efficacy of various cleaning products could be determined. In addition, *in vivo* studies could be performed which would give information about various factors i.e. the effect of lens cleaning on cumulative deposition levels, and how quickly the deposited layer is re-established after cleaning.

2. Experimental.

2.1 Hydrogel contact lenses and lens care products/solutions used.

2.1.1 Hydrogel polymers used in the manufacture of contact lenses.

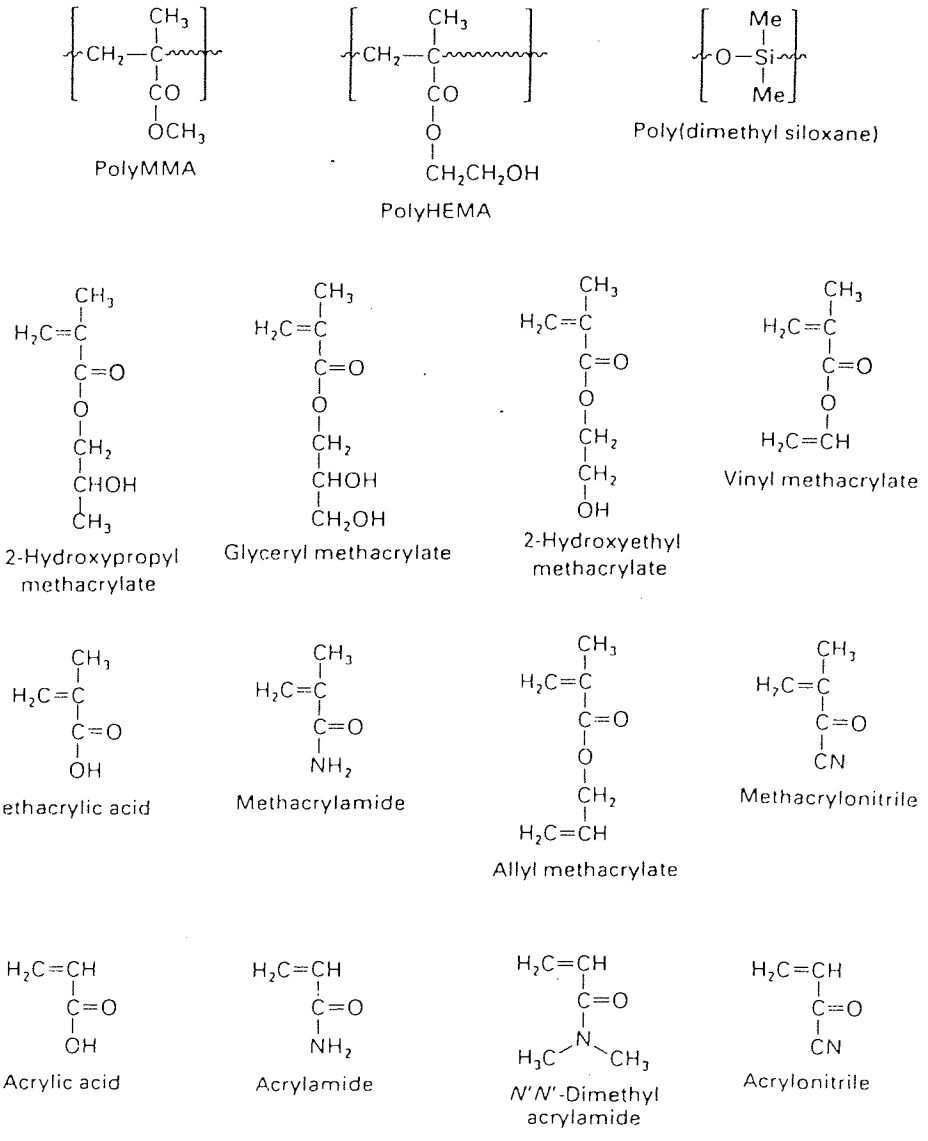
The use of polymers for contact lenses represents an example of the biomedical application of synthetic materials. For many years poly(methyl methacrylate) (PMMA) was used as the standard "hard" contact lens material. It's properties as a contact lens material were far from ideal though. It was not until hydrogel polymers (water swollen networks based on hydrophilic monomers) appeared on the scene that any serious competitor emerged.

A well known technique for converting glassy polymers into flexible materials is to incorporate a "plasticiser". This is a mobile component, often a high boiling point organic liquid that will act as an "internal lubricant". This principle has been used in an ingenious way in hydrogels. The simplest polymer is poly(2-hydroxyethyl methacrylate) known as polyHEMA. It's basic structure is very similar to PMMA. The most significant difference is that every unit of the polyHEMA chain contains a hydroxyl group. These groups take up water, forming a flexible elastomerc gel.

There are many different hydrogel contact lens materials currently available, containing 30-80% by weight of water. They are based on lightly crosslinked

(~0.5-1.0%) combinations of various monomers including HEMA, vinyl pyrrolidone, glycidyl methacrylate, glyceryl methacrylate, methoxyethyl metthacrylate, cyclohexyl methacrylate, methyl methacrylate, methacrylic acid and substituted acrylamides. Structures of some biomedically useful polymers and structures of typical monomers used in hydrogel synthesis are give in Figure 5.

Fig. 5 The structures of some biomedically useful polymers, and structures of typical monomers used in hydrogel synthesis.



Once a particular hydrogel has obtained the necessary regulatory (Food and Drug Administration, FDA) approval and a generic (US Adopted Name Council, USAN) name in the USA, it will often be sold by different manufacturers under different trade names. Table 1 shows the compositions, trade names and generic (USAN) names of the hydrogel lens materials used in this project.

Table 1 Showing contact lenses and lens materials used in this study.

Name	Manufacturer /supplier	Principal components	Water content	Group	USAN nomenclature
Eurothin	Pilkington Barnes-Hind	HEMA	38%	1	Polymacon
Seequence	Bausch+Lomb	HEMA	38%	1	Polymacon
Acuvue	Vistakon	HEMA,MA	58%	4	Etafilcon-A
B&L58	Bausch+Lomb	HEMA,MA	58%	4	Etafilcon-A
Surevue	Vistakon	HEMA,MA	58%	4	Etafilcon-A
Classic	Pilkington Barnes-hind	HEMA,VP,MMA	42.5%	1	Tetrafilcon-A
Softmate	Pilkington Barnes-Hind	HEMA,DAA,MA	45%	3	Bufilcon A

Abbreviations: DAA, diacetone acrylamide; HEMA, 2-hydroxyethyl methacrylate; MA, methacrylic acid; VP, *N*-vinyl pyrrolidone; MMA, methyl methacrylate; Group 1, low water content non-ionic; Group 2, high water content non-ionic; Group 3, low water content ionic; Group 4, high water content ionic.

2.1.2 Use of lens care products and solutions.

All contact lenses, regardless of lens material or lens wear schedules require care products and solutions to be used with them. Lenses must be cleaned to help prevent deposits building up on the lens surface. The contact lenses must be disinfected regularly to prevent bacterial contamination occurring, and solutions must be available for rinsing and storing lenses when they are not in use. A great many solutions are available; the care products most commonly used in Soft contact lens wear (and the products most commonly encountered in this project) are listed below:-

Saline.

Soaking solutions.

Disinfection solutions/tablets (and neutralising solutions).

Surfactant cleaners.

Enzyme tablets.

Salines. These can be both preserved and unpreserved. The majority of salines for use with Soft contact lenses are unpreserved. Unpreserved salines can be Buffered or Unbuffered. Generally saline is used for rinsing and storing purposes i.e. to rinse lenses after surfactant cleaning or to dissolve some some types of disinfection tablets Saline solutions are also used to rinse lenses after overnight disinfection (or neutralising), before insertion into the eye.

Soaking solutions. These solutions are usually used in a similar manner to saline solutions i.e. for storing and rinsing lenses.

Disinfection tablets /solutions and neutralising solutions. The most commonly used products in this category are oxidising disinfectants. Disinfection is achieved by a variety of methods (see Table 2). Some disinfection systems require a neutralisation step whereas others only require the lens to be thoroughly rinsed before re-insertion into the eye. Disinfection systems aim to prevent any bacterial contamination of the contact lens occurring. To achieve this the lens must remain in the disinfection (and/or neutralising) solution for a specific length of time.

Surfactant cleaners. Surfactant cleaners are used, generally on a daily basis to reduce deposition levels on the lens surface and to help prevent the formation of large deposits. Their main purpose is to remove lipoidal deposition, but they also have some ability to remove proteinaceous deposits as well. However, this is probably more as a result of the mechanical effect of digital rubbing of the surfactant cleaner on the lens surface. There are many different surfactant cleaners available, and some are more effective than others. In general they all have a fairly similar composition, most have a viscous polymeric base containing a surfactant. It is this surfactant, in combination with the mechanical effects from the digital rubbing of the lens surface which are responsible for reducing lipoidal deposition levels on the contact lens. Surfactant cleaning will not completely remove all the deposition from a lens surface, but is useful in the long term as a method of preventing large deposit formation and thus prolonging the life of a contact lens.

Enzyme tablets. Enzyme tablets are used to remove proteinaceous deposits from the lens surface. Generally they are used about once a month. The enzyme in the tablets is supposed to denature the protein from the lens surface, thus removing it. However, it is likely that the processes involved in enzymatic cleaning may in fact cause some of the denatured protein to irreversibly bind to the lens surface. Therefore enzyme tablets should be used with care and infrequently.

All these care products must be used under the Manufacturer's instructions to properly maintain contact lenses. Generally separate surfactant cleaners, disinfection systems and rinsing/storing solutions are required.

However, Multi-purpose solutions will probably become widely available in the future. One such solution was used in the later stages of this project for storage of worn contact lenses between analyses. This was Bausch and Lomb Renu, a combined disinfectant, daily cleaner and rinsing solution for soft lenses.

This product is based on a quarternary ammonium compound. It's disinfectant properties enabled lenses to be stored in the fridge for several weeks between analyses with no noticeable bacterial contamination occurring, thus resolving a problem which had previously arisen when the lenses were stored in saline between analyses.

Table 2 Examples of some oxidising disinfectant solutions and their neutralising solutions.

Name	Active ingredient	Neutralising solution	Active ingredient	Manufacturer
10.10 cleaning & disinfecting	Hydrogen peroxide	10.10 rinsing & neutralising	Sodium pyruvate	Ciba
Aerotab	Halozone	Self neutralising	N/A	Sauflon
Lensept	Hydrogen peroxide	Lensrins	With platinum catalyst	Ciba
Oxysept 1 disinfecting	Hydrogen peroxide	Oxysept 2 rinsing/neutralising	Catalyse	Allergan
Softab	Sodium Dichloroisocyanurate	Self neutralising	N/A	Alcon

Table 3 Contact lens solutions used in this project.

Name	Manufacturer	Type of solution
Softab	Alcon	Disinfection, free chlorine based.
Perform	Pilkington Barnes-Hind	Disinfection, 3% hydrogen peroxide based.
Miraflow	Ciba-Vision	Surfactant cleaner.
LC-65	Allergan	Surfactant cleaner.
Renu	Bausch and Lomb	Disinfection/cleaning Multi purpose solution-quarternary ammonium based.
Pliagel	Alcon	Surfactant cleaner.
Optimeyes	Bausch and Lomb	Disinfection, chlorhexidine based.
Daily Cleaner	Bausch and Lomb	Surfactant cleaner.

2.2 Method of *in vitro* lens spoilation.

2.2.1 Artificial tear solutions.

In vitro lens spoilation was performed using an artificial tear solution in a spoilation method developed at Aston University. The artificial tear solution is based on an animal serum which can be spiked with various compounds in appropriate quantities to closely mimic the composition of the tear fluid

With the exception of the of the the animal serum which is used as a base for the tear solution (this is Foetal calf serum, obtained from Gibco) all the other components are bought from Sigma Chemical Company.

The components which are added to the foetal calf serum are listed below:-

Phosphate buffered saline (pH 7.4),

Human albumin,

Sheep globulins,

Lysosyme,

Lactoferrin

Cholesteryl palmitate,

Arachidonic acid,

Oleic acid,

Linoleic acid,

Trilinolenin.

The artificial tear solution can be formulated in two ways:- Spiked and Unspiked. The unspiked version consists simply of foetal calf serum diluted with pH 7.4 phosphate buffered saline. The spiked artificial tear solution also consists of foetal calf serum diluted with phosphate buffered saline. However, in this case proteins and lipids (listed above) are added in appropriate quantities to closely mimic the composition of the tear fluid. The composition of the spiked tear solution can be varied by removing one or more components or, by increasing the concentration of a particular ingredient. In this way it is possible to determine the effect of one or more protein or lipid species on lens deposition levels.

These two different forms of the artificial tear solution give rise to two spoilations models; the Spiked and the Unspiked methods of *in vitro* spoilation. The unspiked spoilation method can be used as a preliminary screening to assess the ability of prospective contact lens materials to resist deposition. The spiked spoilation method will give more detailed information about a novel lens material's deposit resistance, and can also be used to spoil existing contact lens materials in a laboratory based situation.

2.2.2 Experimental methods for *in vitro* lens spoilation.

The *in vitro* lens spoilation model developed at Aston consists of two different methods; the Drop and Dry method and the Shaker method. Contact lenses can be spoilt by either method using either spiked or unspiked foetal calf serum.

1. Drop and dry method of *in vitro* lens spoilation.

This method of *in vitro* spoilation enables rapid and heavy spoilation of contact lenses to be achieved easily. Unspoilt lenses are placed concave side up on a stand. Over a period of 24 hours artificial tear solution is carefully dropped from a pipette onto the surface of the contact lens. The lens is then left to dry before addition of another drop of tear solution. This process is repeated over 24 hours. Lens spoilation may be speeded up by drying the lens surface after each addition of tear solution. This can be done by placing the lens and lens stand in an incubator, or by gently drying the surface of the lens with a hairdryer.

This method of *in vitro* lens spoilation gives rapid results but does not accurately mimic the conditions experienced by a contact lens during in eye lens wear. Obviously it would be extremely difficult, if not impossible to exactly reproduce the conditions of *in vivo* lens wear in a laboratory based experiment. The second method of *in vitro* spoilation used at Aston (the Shaker method) attempts to come closer to conditions found in in eye lens wear, but again cannot be said to exactly mimic *in vivo* contact lens wear.

2. Shaker method of *in vitro* lens spoilation.

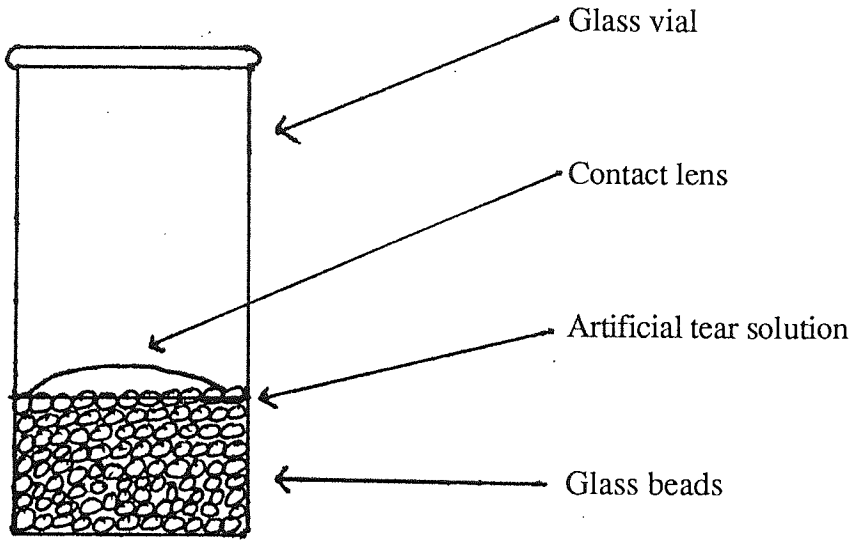
This method of *in vitro* lens spoilation is more time consuming than the drop and dry method, but comes closer to reproducing the conditions seen in *in vivo* contact lens wear. It enables contact lenses to be gradually spoilt over a period of 28 days. Deposition levels can be monitored by fluorescence spectroscopy

throughout the spoilation period. Contact lenses can be spoilt in the shaker model using either spiked or unspiked artificial tear solution.

In the Shaker method of *in vitro* spoilation clean unspoilt lenses are placed in numbered glass vials which are filled with clean glass beads to a depth of about 1.5cm. Both the beads and the glass vials are treated before use with a Siliconising solution. This helps to prevent deposition of components from the Artificial Tear solution onto the glass. The vials are filled with the tear solution to a level just below the surface of the glass beads. The contact lens is then placed over the surface of the beads as shown in Figure 5. The vials containing the lenses are then placed on a shaker. Every 24 hours the vials are removed from the shaker, to enable the tear solution to be changed. The vials are then replaced on the shaker. As stated the lenses are spoilt in the shaker model for 28 days. Throughout this period the tear solution will be changed daily. Fluorescence spectra are usually recorded twice a week throughout the spoilation period.

This method of *in vitro* lens spoilation enables the deposition profile of a contact lens to be determined over a specific time period. The intense nature of spoilation by the Shaker method will give accelerated spoilation levels in comparison to conventional wear times. The deposition levels seen on a contact lens after 28 days *in vitro* spoilation are equivalent to about 6 months *in vivo* daily wear.

Fig. 6 *In vitro* spoilation using the Aston Tear Model:- Shaker method.



2.3 Instrumentation.

Fluorescence Spectroscopy.

Fluorescence spectroscopy was performed using a specially modified Aminco-Bowman SPF 125 spectrophotofluorometer. A 1.3mm silica glass round cell was used to hold contact lenses or solutions for analysis. All contact lenses analysed were placed in distilled water in the cell. More specific details of lens mounting are given in Chapter 3.

3. Development of a non-destructive technique for assessing protein and lipid spoilation levels on contact lenses.

3.1 Introduction.

Fluorescence spectroscopy is undoubtedly the most important technique referred to in this thesis. This technique has been developed into a highly sensitive and reliable method for the analysis of contact lens deposits.

With some molecules, the absorption of a photon is followed by the emission of light from an electronically excited state. This light is of a longer wavelength (i.e. lower energy) than that of the absorbed photon. Fluorescence emission results from the return to the lower orbital of a paired electron. In a singlet excited state, the electron in the higher energy orbital has the opposite spin orientation to the second electron in the lower orbital. These electrons are paired.[73] Substances which display significant fluorescence generally possess delocalised electrons present in conjugated double bonds. There are two types of fluorophore; intrinsic and extrinsic. Intrinsic fluorophores are contained within the macromolecules themselves e.g. proteins and lipids. Extrinsic fluorophores are added to the system, usually binding to one of the components e.g. fluorescein, fluorescamine.[74-77]

As stated, substances which contain conjugated double bonds will display fluorescence. Many of the fatty acids and lipids found in the tear film contain these conjugated (alternating) double bonds, so would be expected to fluoresce. It

is also a well known fact that proteins and peptides will display fluorescence[74] as a result of the natural fluorophores found in almost all proteins; these being the amino acids tyrosine and tryptophan. Therefore, it can be seen that the various protein species found in the tear film and on lens deposits should also display fluorescence. However, although many of the protein and lipid species in the tear film can be expected to fluoresce, this will be at a very low level. Therefore a very sensitive instrument is required to detect the very small amounts deposited on a contact lens surface, particularly in the early stages of wear.

Further indications that fluorescence spectroscopy would be a valuable and sensitive analytical technique arose from fluorescence microscopy studies.[39] Here, white spot deposits on hydrogel contact lenses were being examined. On inspection of unstained deposits with fluorescence microscopy a distinctive and characteristic autofluorescence was observed. It was also known that generally the hydrogels used in contact lens manufacturing do not themselves possess any significant autofluorescence. This therefore demonstrated the fact that deposited species on contact lenses can be detected by fluorescence techniques. However, although this method can be used to show that fluorescent species i.e. proteins and lipids are deposited on a worn lens surface it cannot be used to give an accurate indication of the amount of protein and lipid (the degree of spoilation) on a contact lens. The level of observable fluorescence seen with a fluorescence microscope will of course vary with the amount of fluorescent material deposited on a contact lens, but this variation will only give rise to a subjective and not particularly sensitive estimation of the level of deposition.

There are other techniques available which enable detailed analysis of the

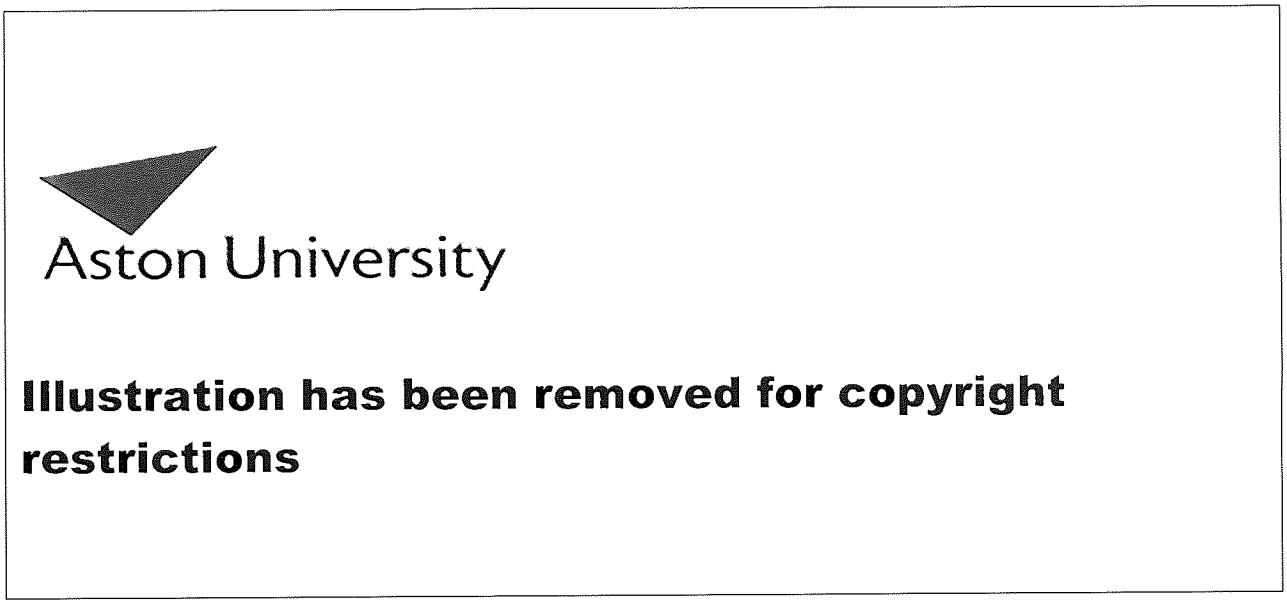
amount and composition of deposits to be performed. For example HPLC is an excellent technique for identifying the many members of the lipid family found in contact lens deposits. However, it can only measure material that has been extracted from the lens surface. The same is true of electrophoresis in protein analysis. Both these methods can also be quite costly and time consuming to perform.

It can therefore be seen that an analytical technique was required that was non-destructive (i.e. one that could be used to look at deposits actually on the lens surface), sensitive enough to detect even very low levels of deposition, relatively quickly and easily performed and that gave reproducible results. This technique is fluorescence spectroscopy. However, as the level of fluorescence will be very low an extremely sensitive machine is required.

3.2 Instrumentation for fluorescence spectroscopy.

To obtain fluorescence measurements the following components are required; a source of excitation radiation, a device for selecting the desired excitation, a sample cell, a device for selecting the required emission wavelength and a detector. The layout of these components is shown schematically in Figure 7. For analytical measurements in solution the detection system is usually placed at a 90 degree angle relative to the excitation radiation.

Fig. 7 Schematic of spectrofluorometer components.



3.2.1 Components of a spectrofluorometer.

Sources.[78] The source used in the spectrofluorometer is a xenon lamp. It produces intense radiation by the passage of current through an atmosphere of xenon. The spectrum is continuous over the range of about 250 to 600nm with the peak intensity occurring at 470nm. The xenon lamp requires a highly stable power supply to maintain constant intensity. Also, the UV emission from 180 to 210nm generates ozone, which must be either vented or decomposed. Most instruments have mirror or lens arrangements to maximise the amount of source radiation directed toward the sample.

Filters and monochromaters. Most spectrofluorometers are equipped with grating monochromators. The resolution of an excitation spectrum depends on the excitation monochromator, and the resolution of an emission spectrum depends on the emission monochromator.

Cells and cell compartments. Fused silica cells are used in the spectrofluorometer. In instruments where fluorescence is viewed at 90 degrees relative to the excitation radiation square cells can be positioned more reproducibly and are thus less subject to variations. Round cells can also be used, and are in fact generally preferable for contact lens analysis due to the problems involved with mounting a contact lens in a square cell. Care must also be taken in the design of the cell compartment to reduce the amount of scattered radiation reaching the detector. Baffles are often introduced into the compartment for this process.

Detectors. The typical fluorescent signal is of low intensity; large amplification factors are thus required for it's measurement. This amplification is achieved by using a photomultiplier tube as the detector.

The spectrofluorometer. One of the most popular spectrofluorometers (and the machine used in this investigation) is the Aminco-Bowman spectrophotofluorometer (SPF-125), an adaption of an instrument developed by Dr. Robert L. Bowman. The components of the spectrofluorometer are shown in Figure 8. The xenon source covers the range of excitation wavelengths from 200 to 1500nm. A blue or red sensitive photomultiplier tube is used to measure the spectral signal. Two gratings are used for monochromaticity, and the resolution of the instrument is less than 1nm.

Fig. 8 Components of a spectrofluorometer.

3.3 Experimental observables.

Fluorescence can be a particularly important analytical technique because of it's extreme sensitivity and good specificity. Fluorometric methods can detect concentrations of substances as low as one part in ten billion, a sensitivity 1000 times greater than that of most spectrophotometric methods. However, as stated previously a very sensitive machine is required to detect the low levels of fluorescence which are emitted from deposited contact lenses. In addition to this there are several other variables that must be taken into account when fluorescence spectroscopy is used as an analytical technique.

3.3.1 Excitation and emission spectra.

Any fluorescent molecule has two characteristic spectra; the excitation

spectrum (the relative efficiency of different wavelengths of exciting radiation to cause fluorescence) and the emission spectrum (the relative intensity of radiation emitted at various wavelengths).

The shape of the excitation spectrum should be identical with that of the absorption spectrum of the molecule, and independent of the wavelength at which fluorescence is measured. This is seldom the case however, the differences being due to instrumental artifacts. Examination of the excitation spectrum indicates the positions of the absorption spectrum that gives rise to fluorescence emission. The two spectra do not agree because:-

(a) photomultiplier sensitivity changes,

(b) the bandwidth of the monochromator changes,

(c) the slits remain constant in fluorescence.

To obtain the true or "corrected" spectra of the compound the apparent excitation curve would have to be corrected for these factors and then the absorption spectrum should be obtained.

A general rule is that the longest wavelength peak in the excitation spectrum is chosen for excitation of the sample.

The emission, or fluorescence spectrum of a compound results from the re-emission of radiation absorbed by that molecule. Here, fluorescence spectroscopy has advantages over absorption (Ultra-Violet) spectrophotometry. If the exciting radiation is at a wavelength that differs from the wavelength of the absorption peak, less radiant energy will be absorbed and hence less will be emitted. So, it

can be seen that to obtain the optimum emission spectrum the correct excitation wavelength is required.

Some compounds possess several excitation and/or emission peaks. This is of analytical usefulness. If two compounds have overlapping excitation bands both could be excited together and then differentiated by their emission spectra. Similarly, if two compounds emit radiation at the same wavelength, they can still be measured together in the same solution if they have different, non-overlapping excitation peaks. This is one of the major advantages that fluorescence spectroscopy has over absorption spectrophotometry. Other advantages are the specificity of fluorescence spectroscopy. This is the result of two main factors:

1. There are fewer fluorescent compounds than absorbing ones, all fluorescent compounds must necessarily absorb radiation, but not all compounds that absorb radiation will subsequently emit radiation.

2. Two wavelengths are used in fluorometry but only one in spectrophotometry. Two compounds that absorb radiation at the same wavelength will probably not emit at the same wavelength. The difference between the excitation and emission peaks ranges ranges from 10 to 280nm.

3.3.2 Choice of excitation wavelength.

As stated before, to obtain the optimum levels of fluorescent emission from a compound the correct excitation wavelength must be selected. From fluorescence

microscopy studies and informed guesswork it was found that 360nm was the optimal excitation wavelength to give maximum sensitivity for lipids. An excitation wavelength of 360nm results in a fluorescence emission peak at around 430nm for lipoidal species (see Figure 9). This was the first step towards the possibility that fluorescence spectroscopy could be used as a technique for contact lens analysis. The second step arose from protein fluorescence studies. Almost all proteins contain natural fluorophores, these being the amino acids tryptophan and tyrosine. These studies showed that the optimum excitation wavelength for these amino acids, and therefore for proteins was 280nm. This excitation wavelength gives a fluorescent emission peak at 360nm for proteins and 430nm for lipids (see Figure 10). This is advantageous as the photomultiplier tube is not sensitive below 360nm, so the primary excitation peak that occurs with an excitation wavelength of 360nm is not seen. This is demonstrated by comparing Figures 9 and 10.

This led to the use of two wavelengths for fluorescence analysis of worn contact lenses. At an excitation wavelength of 280nm the protein and lipid deposition peaks are both visible (Figure 10). An excitation wavelength of 360nm will only show the lipid deposition peak (Figure 9). However, excitation at this wavelength is of value because if a lens is very heavily deposited the protein peak can overlap the lipid deposition peak (Figure 11).

Fig. 9 Fluorescence spectrum of a worn contact lens excited at 360nm.

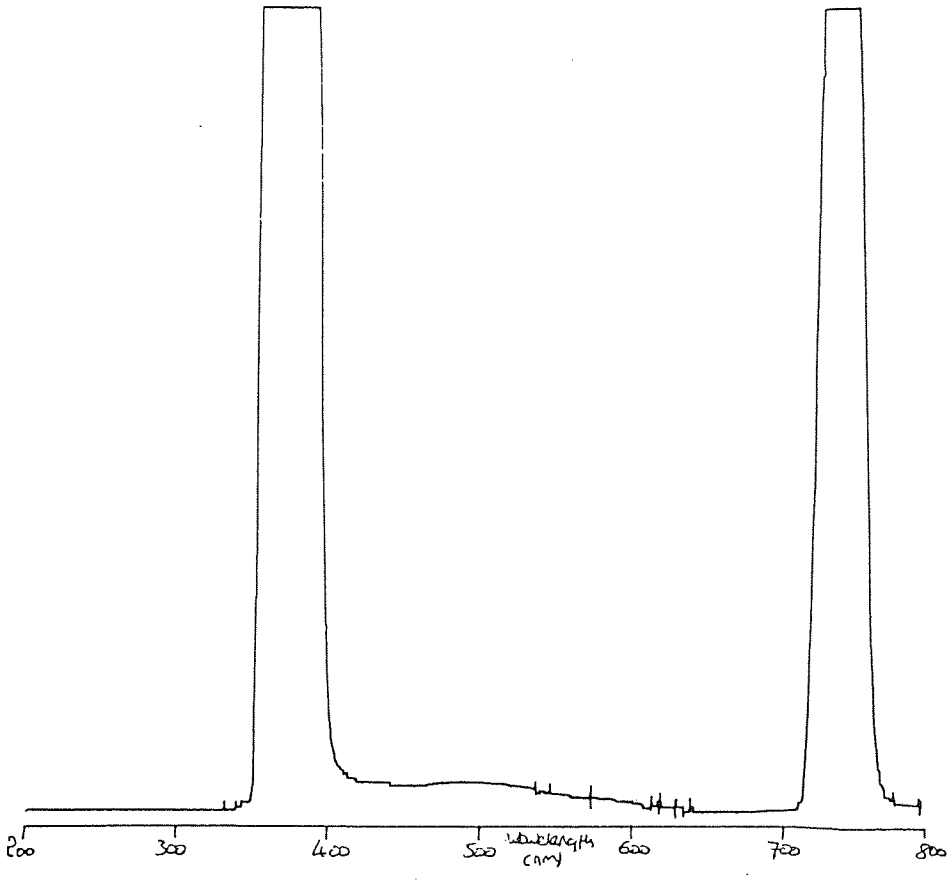


Fig. 10 Fluorescence spectrum of a worn contact lens excited at 280nm.

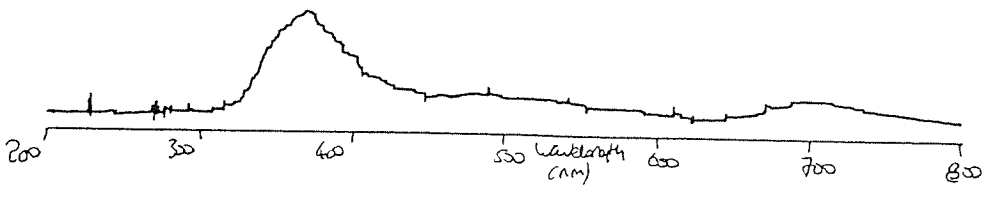
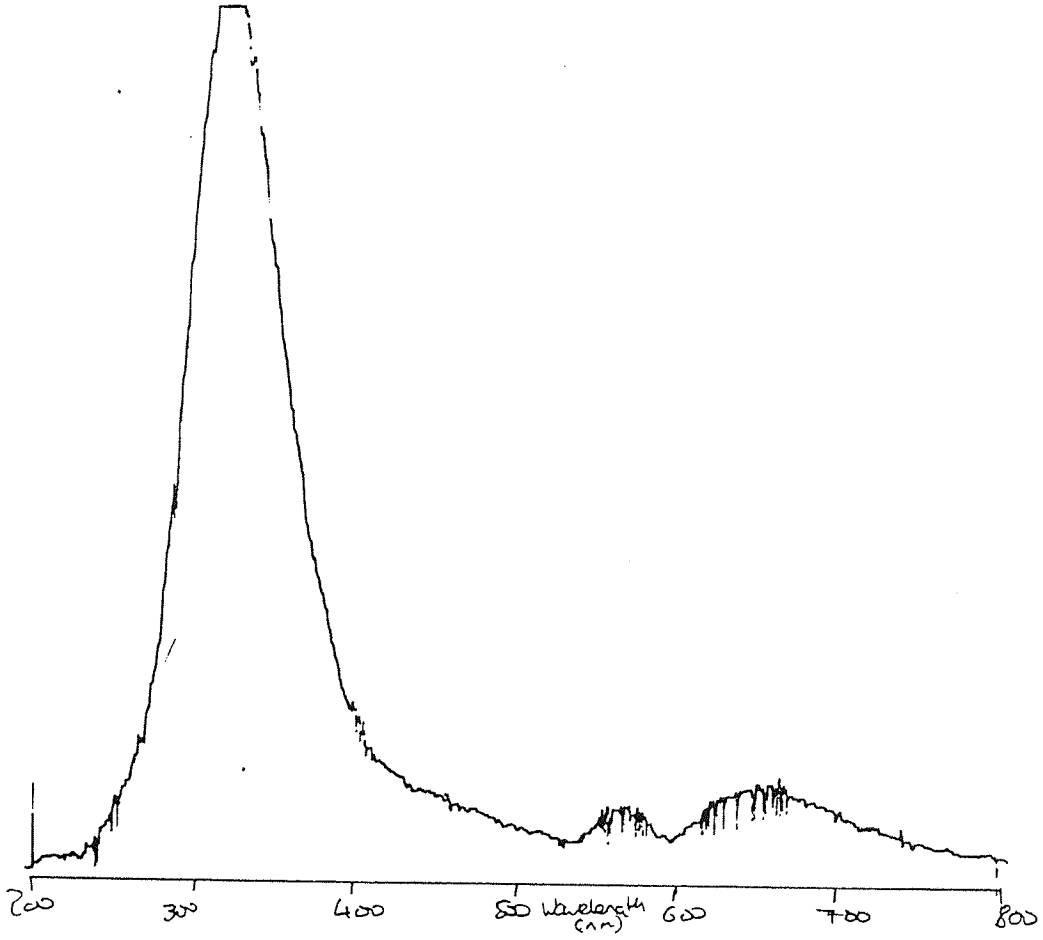


Fig. 11 Fluorescence spectrum of a heavily deposited contact lens excited at 280nm.



3.3.3 Cuvette and lens mounting

In this study a round fused silica glass cuvette was used. The clean, dry cuvette is first filled with distilled water. The lens is then positioned in the cuvette so that it curves round in a horse shoe shape, making sure that most of the lens comes into contact with the inside of the cell (Figure 12). Care must be taken with lens mounting in the cuvette, and positioning of the cuvette in the cuvette holder of the spectrofluorometer. Several conditions must be observed to avoid experimental inaccuracies. These are:-

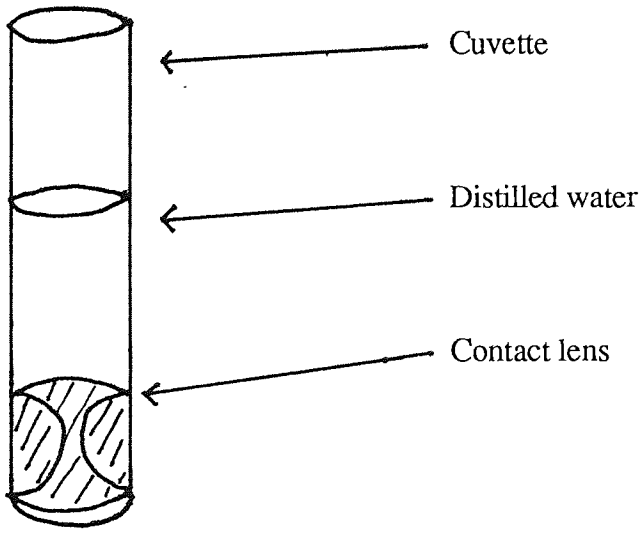
1. The cuvette must be totally clean and dry.

2. There must be no air bubbles in the distilled water in the cuvette.

3. The contact lens must be carefully mounted in the cuvette as described.

4. The cuvette containing the contact lens must be carefully placed in the cuvette holder of the spectrofluorometer. Variations in the glass of the cuvette can give rise to false readings. Therefore, before experiments can be performed the cuvette, containing only distilled water, must be evaluated in the spectrofluorometer to ensure that the cuvette itself will produce no fluorescent signal. Once a suitable orientation for the cuvette has been found it must always be placed in the cuvette holder in this specific position. This can be achieved by placing a mark on the rim of the cuvette and then ensuring that this mark is always in the same position in relation to the cuvette holder of the spectrofluorometer.

Fig. 12 Spectrofluorometer cuvette with lens in situ.

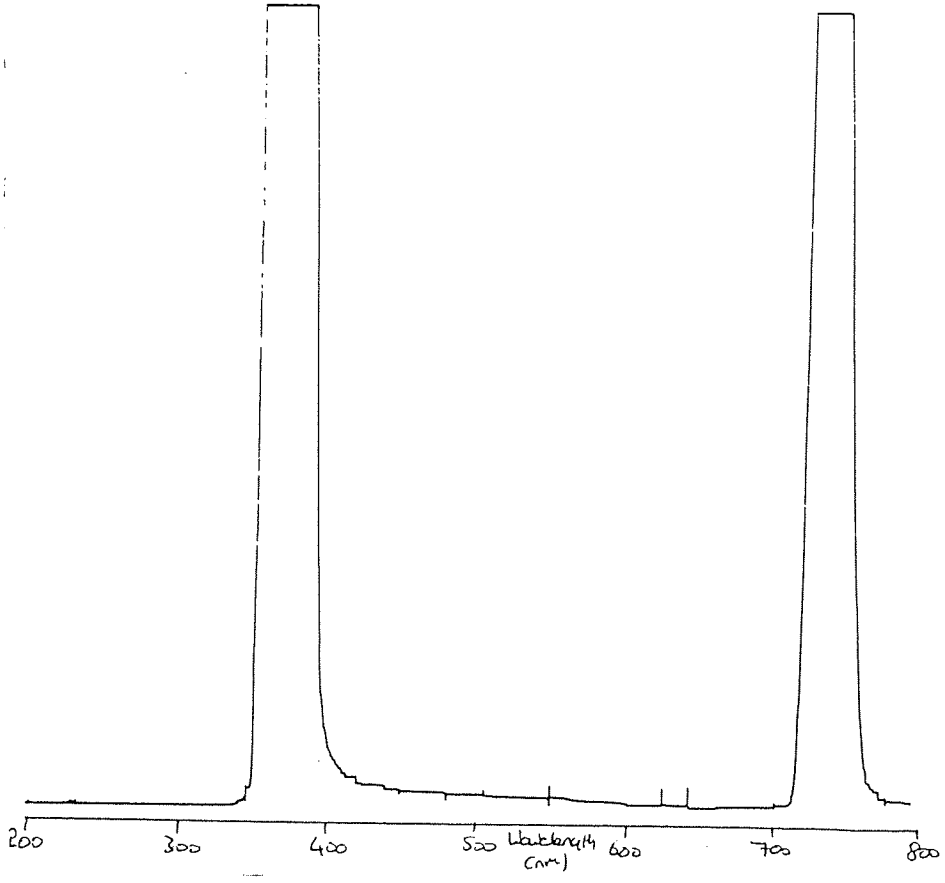


3.4 Initial experiments.

Several experiments were performed to assess the potential of fluorescence spectroscopy as an analytical technique. One of the first experiments involved recording fluorescence emission spectra at excitation wavelengths of 280nm and 360nm of unworn contact lenses. These lenses were made from several different lens materials. This was done to ensure that the unworn lenses did not themselves produce any significant fluorescence emission. Figures 13 to 15 show some of the results obtained. It can be seen that the unworn lenses produced very little fluorescent emission at either of the excitation wavelengths. Any peak observed in the spectrum of an unworn lens can subsequently be subtracted from the peak height recorded from worn lenses of the same material. Another very important early experiment involved assessing the sensitivity of the technique. This was performed in order to determine whether very low levels of deposition would be detectable. Spectra were again recorded at the two excitation wavelengths of 280nm and 360nm using lenses of differing wear times and levels of deposition. Figure 16 shows a spectrum obtained from a lens worn for only a week compared to a spectrum obtained from a heavily deposited lens discarded after several months wear. These and other results showed that low levels of deposition were detectable and also that it was possible to distinguish very sensitively between varying levels of deposition.

Fig. 13 Fluorescence spectra of unworn HEMA lens, excited at:- **a**, 360nm; **b**, 280nm.

a



b

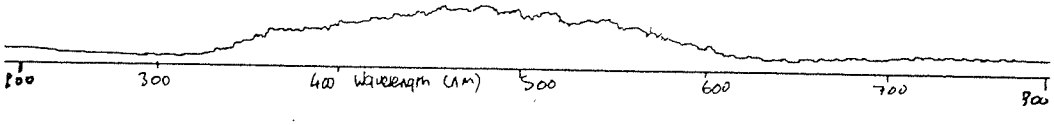
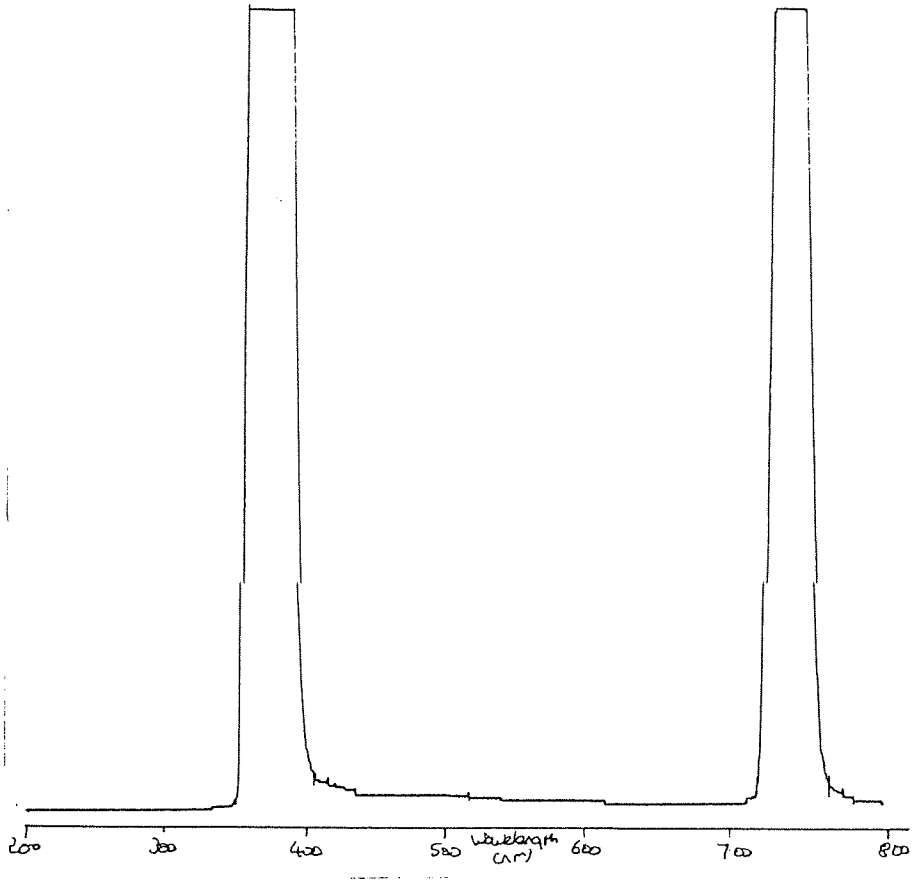


Fig. 14 Fluorescence spectra of unworn Etafilcon A lens excited at:- **a**, 360nm; **b**, 280nm.

a



b

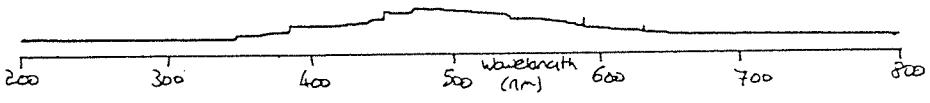
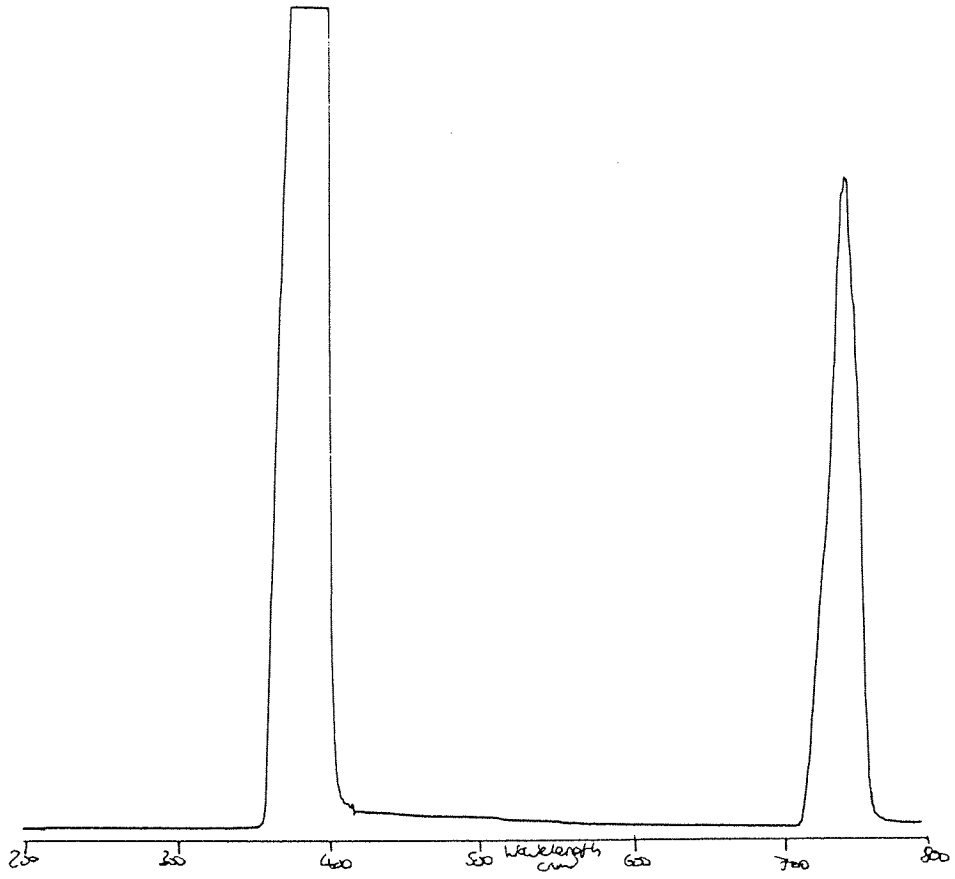


Fig. 15 Fluorescence spectra of unworn Tetrafilcon A lens, excited at:-**a**, 360nm; **b**, 280nm.

a



b

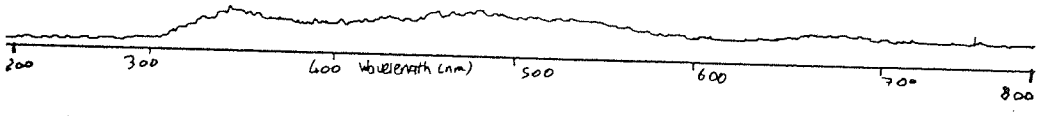
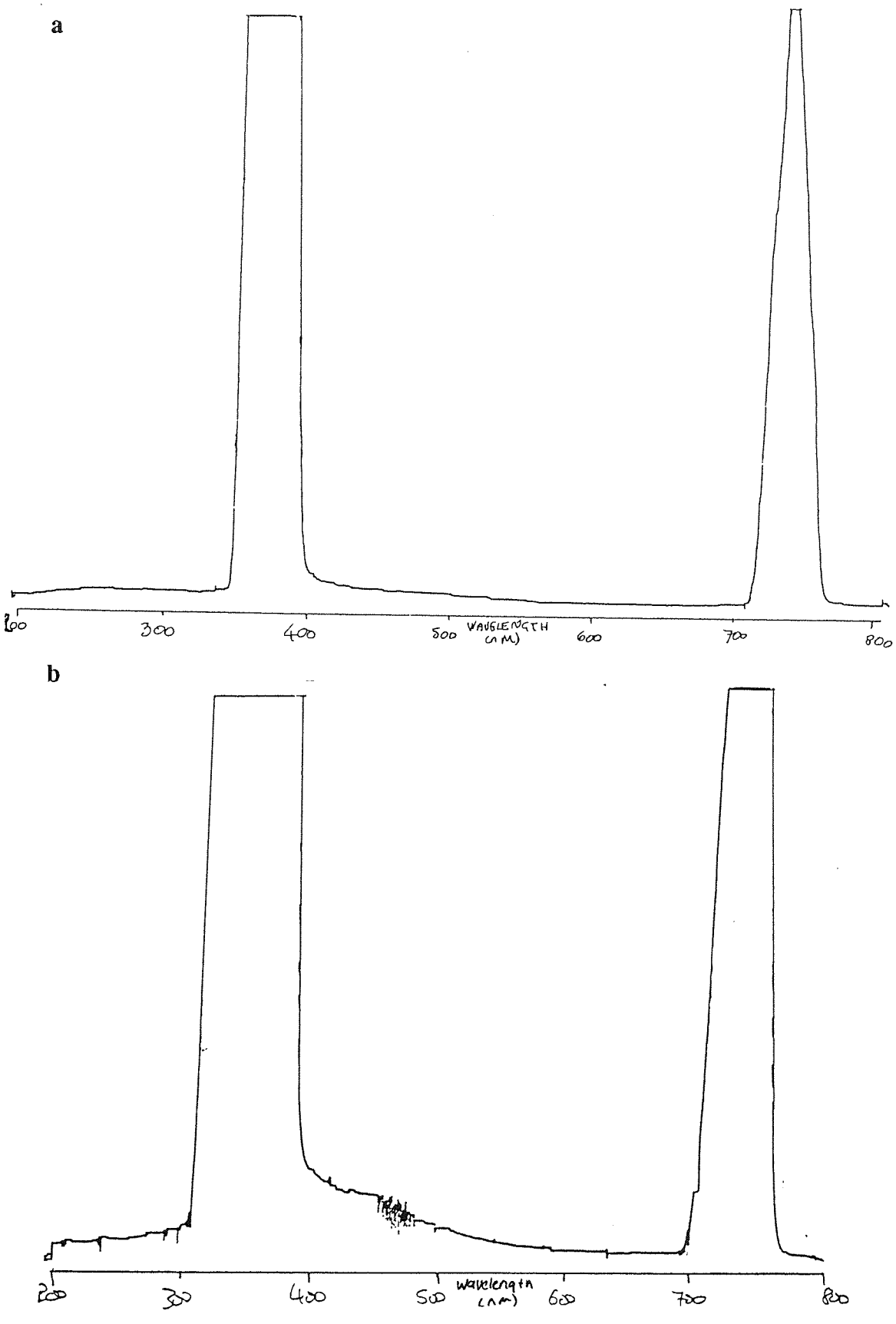


Fig. 16 Fluorescence spectra excited at 360nm:- **a**, One week wear period; **b**, several months wear period.



3.4.1 Studies involving fluorescence spectroscopy.

Following the success of these initial experiments it was realised that fluorescence spectroscopy could be used in many of ways to analyse contact lenses. As stated the peak heights recorded on fluorescence spectra can be used to give a direct indication of the amount of protein or lipid deposited on a lens surface. Monitoring these peak heights, either over a period of time, or across a patient population can yield information on a variety of areas important to contact lens research.

Once these early experiments had demonstrated that unworn contact lenses showed little or no fluorescence, and that lenses worn for even very short periods would show detectable increases in their lipid or protein peak heights it was realised that there was a great potential for research carried out using fluorescence spectroscopy. For example this fact could allow us to study the early stages of contact lens wear across a patient population. It is felt that research into the early stages of contact lens wear is extremely important, so it can be seen that fluorescence spectroscopy could be a very valuable research technique.

This study of the early stages of wear could be expanded, so that lenses of different materials could be compared as to their deposition profiles following identical wear schedules. This would give information about the effects of various factors e.g. water content and the surface charge of lenses on the early stages of deposition. This could then be related to the deposition characteristics of the same lenses in the later stages of wear. In addition, the fact that analysis using fluorescence spectroscopy had been seen to be able to distinguish between protein and lipid deposition would enable studies into the susceptibility of different lens

materials to deposit either protein or lipid preferentially to be undertaken.

These early results also indicated that a great many more studies could be undertaken using fluorescence spectroscopy. A few examples are:-

1. The effect of cleaning on cumulative deposition levels could be assessed, or, the efficiency of lens cleaning on a single testing could be determined i.e. what ability an individual cleaner or lens care product may have to decrease protein or lipid deposition levels.

2. Other possible experiments could involve looking at variations between deposition levels throughout a patient population, or, right eye/left eye differences. Lenses from wearers who are prone to deposit formation e.g. white spots could be compared to patients with no tendency for this sort of deposition. This could give useful information regarding any differences between these two sets of patients, particularly in the early stages of wear.

These are just a few examples of the proposed uses for fluorescence spectroscopy in contact lens analysis. Following on from these initial experiments several studies were proposed which would use fluorescence analysis in many different ways. These will be briefly summarised here, more detail will be given in the following chapters.

1. Early deposition trends of Etafilcon A. This study was set up to with the aim of investigating deposition trends in the early stages of wear of Etafilcon A. Two different lens types were used; a daily wear lathe cut lens ("B&L 58") and a

weekly extended wear/fortnightly daily wear disposable lens ("Acuvue"). This is fabricated via a stabilised wet moulded technique. Using fluorescence spectroscopy it was hoped to determine whether the different manufacturing techniques resulted in any difference in deposition characteristics.

2. Deposition trends of Group 1 (Polymacon, Tetrafilcon A) and Group 3 (Bufilcon) materials. This study was set up as a means of investigating the deposition trends of the ionic Group 3 material in comparison to the non-ionic Group 1 material during the early stages of wear. Using the same patient base it was also hoped to make an assessment of the effect of surfactant cleaning on cumulative deposition levels.

3. Deposition trends of Seequence versus Acuvue lenses. This study was set up with the aim of assessing the differences between the lipid deposition levels of these two lens materials.

4. In vitro analysis. The Artificial Tear Model formulated at Aston can be used to spoil lenses *in vitro*. This model can be used to give accelerated spoilation in comparison to conventional contact lens wear times. Therefore, using fluorescence spectroscopy in combination with *in vitro* spoilation of contact lenses it was hoped to measure changes in deposition levels (by monitoring the growth of the protein and lipid peaks) over simulated longer wear periods. Using this combination of techniques it was also expected that it would be possible to assess the efficiency of surfactant cleaners at removing deposits.

So, it can be seen that fluorescence spectroscopy is potentially an extremely versatile and useful technique for the analysis of contact lens deposits. Since the method was first set up many trials have been performed which have verified the fact that this technique is a reproducible and accurate way of determining the level of deposition on a contact lens. A few points have arisen from these studies which must be borne in mind when the technique is used. Firstly fluorescence spectroscopy will only detect material on and in the surface "layers" of the contact lens. This generally does not give rise to any problems. In the B&L 58 versus Acuvue trial it was noticed that lenses with very high levels of levels of lipid deposition had lower than expected levels of protein deposition. This was purely due to the deposited protein being masked by lipid on the lens surface. However, this only occurs rarely. The majority of Acuvue lenses analysed showed the high levels of protein deposition expected with respect to lipid deposition. This really just highlights the unusual patient to patient variations that can occasionally occur. Another problem arose due to microbiological (including fungal) contamination of stored lenses. This has been solved by storing the lense in "Renu" which contains a quarternary ammonium compound which has anti-bacterial activity.

3.5 Validation experiments.

Lipid families are much less fluorescent than protein, but both lipid and protein give linear calibration graphs up to about 20% fluorescence. Although "universal" calibration graphs give an excellent indication of surface protein, for absolute precision calibration graphs should be constructed for each material. This is because not all proteins are equally fluorescent, and the nature of protein

adsorption i.e. which proteins predominate at the lens surface differs somewhat from material to material.

In this project however, comparisons are made within similar families of materials and highlight patient to patient variations. In this case the calibration graph does not differ between patients. As a general guide, 20% fluorescence corresponds to 4000υg protein and 914υg of lipid. Specific comments will be made about quantification and patient to patient variations in the final discussion.

4. Deposition trends of Etafilcon A.

The recent introduction of disposable lenses into clinical practice has resulted in great interest into the deposition trends associated with such lenses.

This study was set up to investigate deposition in the early stages of wear of one of the materials (Etafilcon A) currently available as a disposable option.

Etafilcon A is a HEMA/MAA based 58% water content ionic material. It is classified as a Group 4 material according to U.S. categorisation. Two lenses of this material were used in the study; B&L 58 (a daily wear lathe cut lens) and Acuvue (a weekly extended wear/fortnightly daily wear disposable lens). This is fabricated via a stabilised wet moulded technique. Analytical examination was undertaken using fluorescence spectroscopy to assess whether the different manufacturing techniques (lathe cutting or wet moulding) resulted in any difference in deposition characteristics.

4.1 Clinical protocol.

The clinical work in this study was carried out by Lyndon Jones FBCO at his South London practice. After collection the lenses were sent to me at Aston University where the fluorescence analysis was undertaken.

Three male and three female myopic patients (mean age 28.8 years), who were existing high water content soft lens wearers were enrolled onto the trial. They were divided into equal groups of known white spot depositors and a control group

who had no previous problems.

The patients were fitted with an Acuvue lens in one eye and a B&L 58 lens in the other. They were advised to wear the lenses in their normal daily wear fashion for a period of two weeks whilst using Alcon Softab (an unpreserved free chlorine system; see Chapter 2) to disinfect the lenses. No surfactant cleaner or enzyme tablets were used.

The lenses were randomly allocated and a cross-over trial was performed in order that no bias could be introduced. After two weeks the subjects returned for evaluation and the lenses were collected for analysis. They were then issued with a further pair of lenses which were also worn for a fortnight prior to collection.

Following this initial study a further pair of Acuvue lenses were obtained from five of the enrolled subjects. These lenses had been worn in both eyes for a fortnight. Again the lenses were disinfected with Softab, no surfactant cleaner being used.

4.2 Analytical procedure.

Immediately after collection the worn lenses were placed in sterile vials containing preserved saline. Analysis of the lenses was performed within a week of collection.

Each of the lenses was examined by fluorescence spectroscopy, initially at an excitation wavelength of 360nm. This was because at this stage in the project fluorescence analysis was only being performed at this wavelength. Subsequently, when the value of an excitation wavelength of 280nm was realised several of the

lenses were re-evaluated at this second wavelength, as were the five pairs of Acuvue lenses that were obtained later.

This study involved the unique combination of a controlled wear regime over a short wear period, followed by reliable analysis using a novel technique which was performed within a short time after lens collection. This method of combined analysis should yield much more valuable and accurate information about the deposition profile of the contact lens material being used, and highlight patient to patient variations. This is in contrast to many of the early studies into deposit formation on contact lenses. Here, little information was available about the contact lens history i.e. how long the lens had been worn for and what (if any) method of lens cleaning had been employed. In addition these lenses were often stored for long periods before analysis was undertaken.

Using fluorescence spectroscopy as an analytical technique i.e. by comparing the fluorescence spectra obtained from each contact lens it was hoped to determine whether:-

1. The different manufacturing techniques resulted in different deposition profiles.

2. There was any difference in deposition levels between the known white spot depositors and the control subjects.

3. Left eye/right eye differences and patient to patient variations would be highlighted.

4. The use of Softab to disinfect the lenses would reduce the level of deposition.

4.3 Fluorescence spectroscopy results and discussion.

Analysis of the contact lenses was performed at excitation wavelengths of 360nm and 280nm. Before any fluorescence spectra can be recorded the spectrofluorometer has to be checked to ensure that the machine settings are correct, in addition, it must be allowed to warm up for half an hour. The Silica glass cuvette which will hold the contact lens must be completely clean and dry. Each contact lens is then carefully placed in the clean cuvette which is filled with distilled water. The lens must be positioned correctly inside the cuvette to ensure accurate and reproducible results. This involves the correct orientation of the contact lens with respect to the markers on the cuvette. After the lens is mounted in the cuvette it is important that the cuvette is precisely placed in the spectrofluorometer (these processes are described in more detail in Chapter 3). This procedure must be repeated every time a fresh contact lens is analysed. Each lens was analysed at 360nm before being returned to the correct storage vial. The lenses were then stored in the fridge in case any further analysis was required. Analysis of some of the contact lenses was repeated at an excitation wavelength of 280nm about a week after the initial analysis had been performed. The procedure for analysis at this wavelength is exactly the same as that described above for analysis at 360nm. The setting for excitation wavelength is simply altered to 280nm before fluorescence spectra are recorded.

The results of this study are shown in Tables 4, 5 and 6. Table 4 shows the initial results from the contact lenses obtained at an excitation wavelength of 360nm. Table 5 shows the results obtained from the subsequent analysis of some of the contact lenses at an excitation wavelength of 280nm. Table 6 show the results from

5 sets of Acuvue lenses obtained at a later date. These spectra were recorded at an excitation wavelength of 280nm. The results are given as "mm above baseline". Generally fluorescence is measured in arbitrary units i.e. a percentage of the fluorescence beam multiplied by a specific factor. In this project however, all fluorescence spectra were recorded under identical conditions i.e. the machine settings were unaltered throughout the length of the project. Therefore it is valid to simply measure the peak heights of the recorded spectra and these values (in mm above baseline) can be used as an indication of the amount of protein or lipid deposited on the surface of an individual contact lens.

4.3.1 Etafilcon A deposition levels.

Even after this short wear period (two weeks) protein and lipid begin to accumulate on the lens surface. Table 4 shows the lipid deposition levels of the two lens types excited at a wavelength of 360nm. It can be seen that the majority of the analysed lenses had increased peak heights on their recorded spectra in comparison to the peak heights obtained from the spectra of unworn lenses. This means that all these lenses had detectable levels of lipid deposition after the two weeks of wear.

4.3.2 Effect of different manufacturing techniques on deposition levels.

One of the aims of this study was to determine whether the different manufacturing techniques (lathe cutting for B&L 58 and wet moulding for Acuvue) would result in different deposition characteristics. Analysis of these results reveals

that the average increase in peak height (and thus the increase in deposition levels) for Acuvue lenses over the fortnight's wear period was 52%. The increase for the B&L 58 lenses was 65%. Although there is a noticeable difference between the two sets of results this should not be regarded as significant.

All the peak heights from fluorescence spectra are measured to within ±0.5mm, so if this is taken into account the difference of 13% seen between the two lens types in Table 4 is not really significant. Another factor which must be borne in mind is that the number of subjects involved in this study was small (only 6) therefore any unusual patient variations i.e. exceptionally high levels of lipid deposition, will greatly influence the overall view of the results.

It is therefore apparent that the different manufacturing techniques had no significant effect on deposition levels during the period of this study.

4.3.3 Effect of known white spot depositors on early deposition levels.

The second aim of this study was to determine whether there was any difference in deposition levels between the known white spot depositors and the control subjects. Three patients were known to be white spot depositors (patients 3, 5 and 6). When the lipid deposition results from these three subjects are compared to the results obtained from the non-depositors it is clear that no consistent difference is seen in gross deposition levels. This is consistent with the view that specific chemical steps, rather than simple bulk accumulation, are involved in white spot formation.

Table 4 Lipid deposition levels (mm above baseline) of B&L 58 and Acuvue lenses excited at 360nm.

	Peak height above baseline (mm)[a]			
	Fortnight 1		Fortnight 2	
Patient	Right eye	Left eye	Right eye	Left eye
1	5*	4	9	10*
2	10	10*	5*	6
3#	7	4*	10*	5
4	4	5*	4*	8
5#	5*	11	6	6*
6#	4*	5	4	5*

Peak height of unworn Acuvue: 4mm above baseline.

Peak height of unworn B+L 58: 4mm above baseline.

Key: [a], all results $\pm$0.5mm; #, white spot depositor; *, Acuvue lens.

Therefore it can be seen that it is not possible to predict whether or not a contact lens wearer will be a potential white spot depositor from fluorescence analysis of the levels of deposition in the very early stages of lens wear. Additional analysis using more detailed method (e.g. HPLC) is required to determine whether the deposition of specific lipids will initiate white spot formation.

4.3.4 Left eye/right eye differences and patient to patient variations.

Most of the contact lenses examined in this study showed similar levels of lipid deposition for left and right eye of the same patient. However, in one case differences were noticeable during both the fortnights wear periods. This demonstrates that analysis using fluorescence spectroscopy is able to detect left eye/right eye differences, even after only a very short period of wear. Subject number 3 had consistently increased levels of lipid in the right eye. This could have occurred for several reasons. The fact that lipid deposition levels were raised in the same eye over both wear periods may point to poor lens fit in this eye. However, this seems unlikely as a different lens had been in the right eye during both study periods. Other possible reasons for increased lipid deposition in one eye include rubbing the eye with the contact lens in situ or handling one lens more than the other. The lenses were not surfactant cleaned during this study therefore increased levels of lipid cannot be attributed to poor lens cleaning. Because the increases were seen over two wear periods it is probable that a combination of the factors mentioned above caused the increased levels of lipid deposition seen in the right eye of subject number 3.

In two other cases variations were seen in the levels of deposition between the eyes, although these were not consistent over the two periods of wear. In the first fortnight, subject number 5 had raised deposition levels in the left eye, and in the second fortnight subject number 4 showed an increase in deposition in the left eye. These increases were probably caused by the indirect patient related factors described above i.e. rubbing the eye with the lens in situ, or handling one contact

lens more than the other.

As well as highlighting left eye/right eye differences fluorescence spectroscopy was also able to demonstrate patient to patient variations in the analysed lenses. No patient had exaggerated deposition levels during both fortnights of lens wear. However, in two cases significant differences were seen between the first and second fortnight (subjects 1 and 2). These differences could have been caused by for example, diet variations in the two wear periods.

4.3.5 Effect of lens disinfection on deposition levels.

After the lenses obtained in this study had been analysed (by recording fluorescence spectra at an excitation wavelength of 360nm), it was important to determine whether disinfecting the lenses with Softab had any effect on their deposition profiles. This was done by recording a fluorescence spectra from a worn lens at an excitation wavelength of 360nm. The lens was then disinfected in Softab following the manufacturers instructions. After disinfection the fluorescence spectra of the lens was re-recorded. Results obtained clearly showed that Softab did not reduce the deposition levels of a worn contact lens. This indicates that the spoilation seen on the worn lenses in this study had accumulated over the fortnight of wearing, rather than simply being a result of wear on the day of collection.

4.3.6 Fluorescence analysis at 280nm.

Following initial fluorescence analysis at an excitation wavelength of 360nm, several of the lenses were re-examined at an excitation wavelength of 280nm. Analysis at this particular wavelength allows determination of protein and lipid deposition levels to be made. Spectra recorded at 280nm give two distinct peaks, a protein peak at around 360nm and a lipid peak at around 430nm. The results are shown in Table 5 and Figure 17.

The most noticeable observation from these results is the very high levels of protein deposition seen on all the analysed lenses (Acuvue and B&L 58), in comparison to the levels of lipid deposition recorded. This is due to the fact that Etafilcon A is an ionic Group 4 material, and like all the materials in this class, is very susceptible to large amounts of protein deposition. Analysis using fluorescence spectroscopy was able to demonstrate this very clearly; obvious differences were seen between the recorded peak heights for protein and lipid. Another feature of this analysis is that the lenses which demonstrated very high levels of protein deposition were the lenses that showed the lowest levels of lipid deposition and vice versa.

This is seen clearly in the graphical representation of the results (Figure 17). Here, the lens with the highest level of protein deposition (34mm above baseline) is also the lens with the lowest level of lipid deposition (6mm above baseline). In contrast the lens with the highest level of lipid deposition (36mm above baseline) has one of the lowest levels of protein deposition (14mm above baseline).

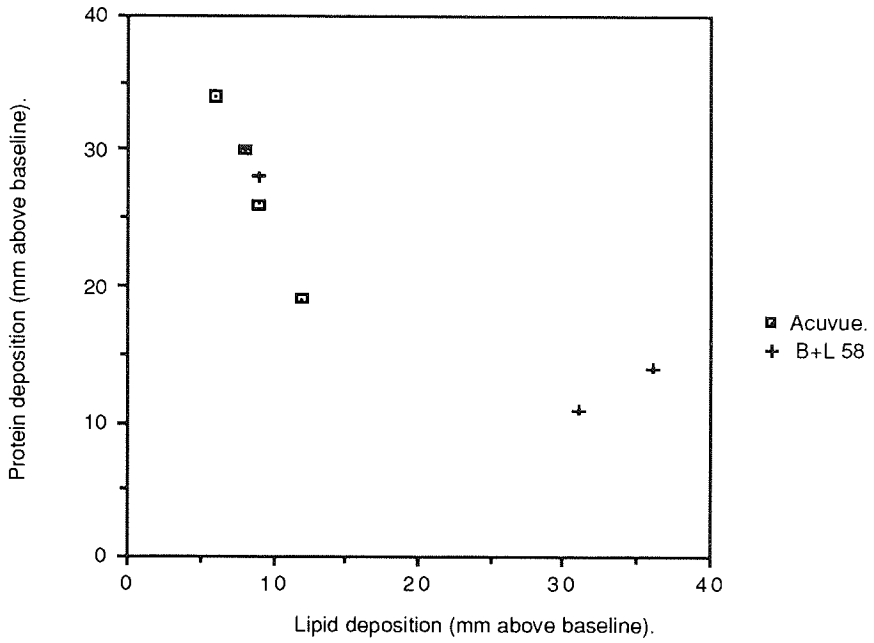
Table 5 Showing protein and lipid deposition levels (mm above baseline)* of Acuvue and B&L 58 lenses excited at 280nm.

Patient	Fortnight	Lens	Eye	Peak height above baseline (mm) Protein	Lipid
3	1	Acuvue	Left	30	8
3	1	B&L 58	Right	28	9
3	2	B&L 58	Left	11	31
3	2	Acuvue	Right	26	9
4	1	Acuvue	Right	19	12
4	1	B&L 58	Left	14	36
4	2	B&L 58	Right	30	8
4	2	Acuvue	Left	34	6

* All results ±0.5mm.

	"Protein"	"Lipid"
Unworn Acuvue: Peak height above baseline (mm).	2	5
Unworn B+L 58: Peak height above baseline (mm).	3	5

Fig. 17 Graph showing deposition levels on Acuvue and B&L 58 lenses (excitation wavelength 280nm).



It is believed that his phenomenon arises because of "masking" of one deposited species at the lens surface by another. As stated earlier, fluorescence spectroscopy will only detect deposited material on the lens surface, therefore, if a lens has a very high level of protein deposition on it's surface, this will to some extent "mask" the lipid at the lens surface. If this does occur, then very high levels of protein can be associated with low levels of lipid deposition and vice versa.

However, this is only observed to any great extent with unusually high levels of deposition of one species and will not in general have any great effect on experimental results. The "masking" effect is fairly weak so, if a lens did have for

example very high levels of protein deposition, but also had high levels of lipid deposition any masking would be overcome and both the deposited species would be detected at their correct levels.

4.3.7 Further analysis of Acuvue lenses at 280nm.

The final part of this study involved analysis of further sets of Acuvue lenses worn by five of the patients in this study; one subject (number 3) had dropped out at this stage. Here, the patients had worn Acuvue lenses in both eyes on a daily wear basis, again using Softab to disinfect the lenses and no surfactant cleaner. After a fortnights wear period the lenses were collected for fluorescence analysis. This was performed at an excitation wavelength of 280nm. The results are shown in Table 6 and Figure 18. Again the most noticeable observation is the much higher levels of protein deposition seen in comparison to the lipid deposition levels. Here, however the masking effect is not seen. This is probably due to the fact that in the majority of cases neither the lipid or protein deposition levels are particularly exaggerated, so masking of one component by another at the lens surface does not occur.

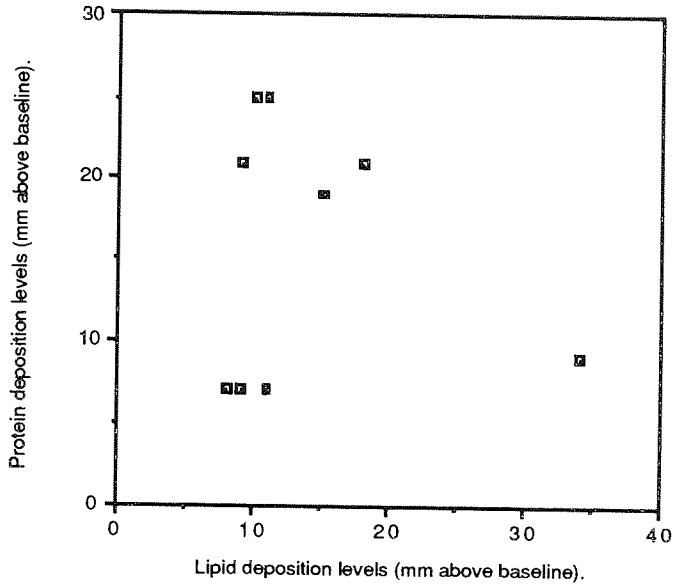
As these results are obtained from only one fortnights wear period comments about right eye/left eye differences and patient to patient variations are not really valid. These results are useful however as they again demonstrate that analysis of worn contact lenses is achieved very successfully using fluorescence spectroscopy. Analysis at 360nm will determine lipid deposition levels and analysis at 280nm will determine protein and lipid deposition levels.

Table 6 Showing protein and lipid deposition levels of Acuvue lenses (mm above baseline), excited at 280nm.

		Peak height above baseline (mm ±0.5)	
Patient*	Eye	Protein	Lipid
1	Right	25	10
1	Left	25	11
2	Right	7	9
2	Left	7	11
4	Right	19	15
4	Left	25	11
5	Right	9	34
5	Left	21	9
6	Right	21	18
6	Left	7	8

*: patient number 3 had dropped out of the study before these lenses were collected.

Fig. 18 Graph showing deposition levels on Acuvue lenses (excitation wavelength 280nm).



4.4 Conclusions.

In these analyses lenses of only one contact lens material were used. Further studies therefore needed to be performed in order to see whether fluorescence spectroscopy could be used to highlight differences in deposition characteristics of different contact lens materials. These are described in the following chapters. This investigation clearly demonstrated that both wavelengths (360nm and 280nm) can be used to successfully highlight variations between patients and right eye/left eye differences. The technique can also be used to assess whether different manufacturing techniques will effect the susceptibility of a lens material to

deposition. Fluorescence spectroscopy had therefore been shown to be a very powerful analytical technique in this study. The unique use of this accurate and sensitive method of contact lens analysis in combination with with a controlled wear programme will therefore prove very useful in all aspects of study into *in vivo* contact lens wear.

4.4.1 Effect of lens storage on analytical results.

The results of this study also highlighted some problems that were not investigated at this stage, but were felt to be important with regard to further studies. These uninvestigated aspects are mainly concerned with the length of time that the lenses were stored i.e. the period after collection but before analysis. At this stage it was not possible to combine evaluation of clinically derived lenses with studies of the ability of the adsorbed protein layer to remain at the lens surface, either in terms of it's confirmation or it's persistence at the lens surface. Therefore it was important to ensure a uniform storage and analysis programme. The results shown in Tables 4 and 6 are all obtained from lenses that had been stored and analysed under this type of regimen i.e. the lenses had all been collected and stored in identical conditions and analysis was carried out within a maximum period of a fortnight after collection. This was felt to be to be an acceptable time period which would not have any significant effect on the protein deposition levels. Subsequent studies have confirmed that this is an acceptable approach to the study of relative deposition levels.

As stated earlier, the data shown in Tables 4 and 6 was obtained from lenses that

had been analysed within a fortnight of their collection time. The data in Table 5 however, was obtained from some of the original lenses which had been stored for at least a month prior to being re-analysed. This second analysis was performed at 280nm, allowing the protein and lipid peaks to be distinguished. It is thought that the time period that had elapsed may have affected the results. In general the lipid peak is not reduced with storage time. However there was concern that the protein peak would be affected with time. This is because some of the smaller proteins, particularly lysosyme, will migrate into the lens matrix (in addition to being present on the lens surface). This lysosyme, on and within the lens will not remain static. It it thought that gradually over a period of time this unbound protein will diffuse out of (and possibly back into) the lens matrix. This process will also affect the protein levels on the lens surface as the diffusion from the matrix will cause quite large fluctuations in this surface protein. As stated before it is only the surface protein that is detectable by fluorescence spectroscopy. Therefore it can be seen that if analysis of contact lenses at 280nm is to be performed accurately, it must be conducted soon after worn contact lenses are received.

The results shown in Table 5 are from lenses that had been analysed after being stored for about a month in non-preserved saline. The results are not in fact greatly dissimilar to those expected i.e. they are comparable to the results seen in Table 6. However, in both cases there are several lenses that show lower than expected protein deposition levels. This could of course be due to patient variations or right eye/ left eye differences, which is probably the case for some if not all the lenses in Table 6 with low protein deposition levels. It is also equally possible though that these inconsistent results, and particularly those seen in Table 5 are due to the migration of protein from the lens matrix and surface. The lenses were stored in

non-preserved saline. With time this solution would have become acidic, which would cause the contact lens stored in this solution to shrink so dislodging the unbound protein at the lens surface. This would result in the low protein deposition levels seen; even though protein would still remain in the bulk of the lens this would not be detected by fluorescence spectroscopy. However, if the lens were re-analysed a day or so later it is possible that the protein deposition levels would have increased. This would be due to the fact that the protein held in the bulk of the lens would have diffused out to the lens surface. This replacement of the protein at the lens surface does not occur instantly, hence the low protein deposition levels are seen on stored contact lenses.

This phenomenon of protein interchange between the bulk of a contact lens, the surface of the lens and the lens' storage solution was studied in greater detail at a later stage in this project. From the results obtained in this study the importance of early analysis of lenses was realised.

4.4.2 Bacterial contamination of stored lenses.

Another potential problem was bacterial contamination of stored contact lenses. Provided the lenses were analysed fairly quickly after collection this should not cause any difficulties, but again in lenses stored for a long time problems may arise if the lenses were re-analysed. At a later stage in the project this problem was overcome by storing the lenses in Bausch and Lomb Renu multi-purpose solution. This solution contains a quarternary ammonium compound which gives it antibacterial activity. Another advantage of using this solution is that it does not

become acidic with time, therefore no lens shrinkage (and hence loss of protein at the lens surface) will occur.

So, at a later stage many of the problems encountered in this first study using fluorescence spectroscopy were overcome. However, for this and a few later studies potential difficulties were avoided by ensuring that lenses were not stored for long periods after collection and before analysis.

5. Early deposition trends on Group 1 and Group 3 materials.

A number of studies have concluded that lenses of an ionic nature deposit to a greater degree than lenses fabricated from non-ionic materials.[79,80] These studies have generally been concerned with high water content ionic materials (Group 4), however it is generally believed that all materials classified as ionic will exhibit enhanced deposition.

This study using fluorescence spectroscopy investigated the degree of spoilage associated with low water content ionic lenses during the early stages of wear, and the effect of surfactant cleaning of lenses at this stage. The lenses were worn under a strictly controlled regime with regard to lens wear times, disinfection and surfactant cleaning procedure. The results from this study can then be compared with the results from the previous investigation (Chapter 4). This involved assessment of the deposition levels in the early stages of wear of ionic high water contact lenses.

5.1 Clinical protocol.

The clinical work was performed by Lyndon Jones FBCO.

Ten healthy female myopic soft contact lens wearers were enrolled in the study after being screened for preexisting pathology, contact lens complications, or allergies. The mean age was 35.4 years. Refractive errors ranged from -4.0 to -0.75D with less than 0.75D of astigmatism. The patients were fitted to obtain

optimum fitting characteristics as defined by conventional criteria.

The patients were arbitrarily divided into two equal groups. Both groups were fitted with a control Group 1 HEMA lens (Eurothin) in one eye, and with either a Group 3 ionic lens (Softmate B) or, a Group 1 non-ionic lens (Classic) in the other eye.

The subjects were instructed to wear their lenses on a daily wear basis for one week. During this time they disinfected their lenses with Pilkington Barnes-Hind Perform (3% hydrogen peroxide with sodium thiosulphate neutralizer) but did not use surfactant cleaner or enzyme tablets. They were instructed to disinfect for 20 minutes and then neutralize overnight (minimum of 8 hours). After one week the lenses were collected for analysis.

At the time of collection a duplicate pair of lenses was issued. Subjects wore these lenses for one week and were instructed to clean them with Ciba-Vision Miraflow, a 20% isopropyl alcohol-based surfactant cleaner, before disinfecting with Perform. The Miraflow was rinsed off the lenses with buffered saline prior to disinfecting. At the end of one week, the lenses were again collected for analysis. The worn lenses were not cleaned on the day of collection.

The care regime employed throughout this study involved surfactant cleaning upon removal of the contact lenses at night. Therefore to maintain consistency with this regime it was decided that the worn contact lenses should not be cleaned on the day of collection.

5.2 Analytical procedure.

After collection the lenses were placed in sterile containers containing 3ml of preserved saline. Analysis was performed within a fortnight of receiving the lenses.

Each lens was examined by fluorescence spectroscopy at an excitation wavelength of 280nm. This process again involved careful lens handling and accurate positioning of the lens in the spectrofluorometer. Full details are given in Chapter 3. It was decided to use this wavelength because it was felt that after only a one week wear period the protein deposition levels would not be sufficiently high to "overrun" the lipid deposition peak. Therefore additional analysis at 360nm was not necessary. This was confirmed by observation.

This study was set up to determine:-

1. Whether the ionic Group 3 material would show enhanced deposition characteristics in comparison to Group 1 materials. As stated earlier many studies involving ionic high water content Group 4 lenses have concluded that these lenses will show higher levels of protein deposition than lipid deposition. This investigation, using fluorescence spectroscopy, hoped to demonstrate whether this phenomenon occurred in low water content ionic lenses (Group 3) as well. The sensitivity of the analytical method, and the fact that it is able to clearly distinguish between proteinaceous and lipoidal deposits should enable any differences in deposition characteristics between the ionic and non-ionic lens materials to be detected.

2. How the results from this study can be compared with those from the Etafilcon A study. This investigation (Chapter 4) involved an assessment of the early deposition trends of Acuvue lenses. These lenses, fabricated from Etafilcon A are categorised as Group 4 (ionic high water content) according to FDA classification. Comparing results from the study in Chapter 4 with the study being discussed in this Chapter will show whether the two ionic materials behave in a similar or dissimilar fashion with regard to their deposition characteristics. This will give an indication of how the proportion of ionic material and the level of water within the contact lens polymer will influence the deposition profile of an ionic material. Also, it will be interesting to compare the results obtained from the Group 1 HEMA lenses which have a tendency to deposit high levels of lipid and low levels of protein with the Group 4 lenses which have been shown to attract higher levels of protein than lipid.

3. The ability of surfactant cleaning to reduce deposition levels in these early stages of wear. It is hoped that the fluorescence analysis will demonstrate whether or not surfactant cleaning has any overall ability to decrease cumulative deposit formation during the first week of wear of a new contact lens.

5.3 Fluorescence spectroscopy results and discussion.

The fluorescence analysis results (measured in mm above baseline) from spectra recorded at 280nm are presented in Table 7.

5.3.1 Deposition levels on Group 1 and Group 3 materials.

It can be seen that the majority of the lenses had detectable levels of deposition after the week's wear period. This was seen consistently with all three lens types. In addition to all the lens types showing increased deposition levels, it was also apparent that over the period of the study, the three lens materials behaved in a fairly similar fashion. They all showed relatively little increase in protein deposition with time. The most marked change was seen in the lipid deposition levels. Both the Group 1 lens types (Classic and HEMA) showed a much greater increase in the amount of lipid deposited compared to protein.

Although none of the contact lens materials had markedly dissimilar deposition profiles the results show that both the Group 1 materials had greater levels of lipid deposition than protein. In contrast, when the peak height from an unworn Softmate lens is subtracted from the fluorescence peak heights of the worn Softmate lenses it is clear that the ionic Group 3 material (Bufilcon A) had marginally greater levels of protein deposition than lipid.

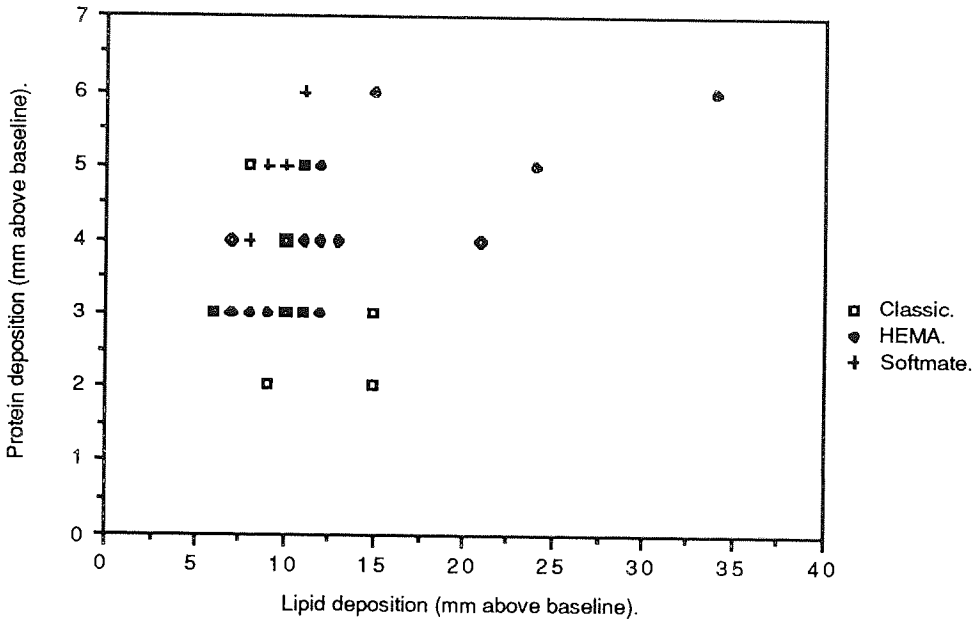
In this study using fluorescence spectroscopy as an analytical technique it was therefore possible to distinguish between the different contact lens materials. This was because this method of analysis is able to differentiate between the type of deposit on a lens surface, i.e. whether protein or lipid is deposited. In addition the amount of each deposited species can be determined. Therefore, information about the "characteristic" deposition profile of any contact lens material can be obtained.

Table 7 Showing protein and lipid deposition levels (mm above baseline) of Classic, HEMA and Softmate lenses at an excitation wavelength of 280nm (1= no surfactant cleaning, 2= with surfactant cleaning).

			1		2	
			Peak heights (mm above baseline ± 0.5).			
Patient	Lens type	Eye	Protein	Lipid	Protein	Lipid
	Classic	Unworn	3	7	-	-
	HEMA	Unworn	3	9	-	-
	Softmate	Unworn	2	8	-	-
1	Classic	Left	4	10	2	9
1	HEMA	Right	3	8	4	13
2	Classic	Right	5	11	5	8
2	HEMA	Left	5	24	3	10
3	Classic	Right	3	6	3	11
3	HEMA	Left	3	11	5	12
4	Classic	Left	3	15	3	10
4	HEMA	Right	3	7	3	9
5	Classic	Left	2	15	3	10
5	HEMA	Right	4	12	3	11
6	Softmate	Right	4	11	4	11
6	HEMA	Left	3	12	3	9
7	Softmate	Left	4	8	5	9
7	HEMA	Right	4	10	4	11
8	Softmate	Left	5	11	6	11
8	HEMA	Right	6	15	6	34
9	Softmate	Right	5	10	3	6
9	HEMA	Left	4	7	4	12
10	Softmate	Right	3	8	3	8
10	HEMA	Left	5	11	4	21

The results are shown graphically in Figure 19.

Fig. 19 Graph showing deposition levels of Classic, HEMA and Softmate lenses (excitation wavelength 280nm).



5.3.2 Patient to patient variations.

It is noticeable that in three cases HEMA lenses showed exaggerated levels of lipid deposition. This could be due to differences in the deposition profiles of the three lens materials. Alternatively these high levels of lipid deposition could be a result of patient to patient variations. For example, patient number 8 had high levels of lipid deposition on HEMA lenses during both of the week long wear periods. The other two patients with high levels of lipid deposition only showed this in one of the weeks of wear; patient 2 in the first week and patient 10 in the

second week. This could have arisen for example because of diet variations during the two weeks, or because of hormonal variations. However, in all these cases the increase in lipid deposition was only seen in the eye wearing the HEMA lenses. This might be as a result of sporadic effects. For example eye rubbing with the contact lens in situ can influence behaviour in one eye in relation to the other. In the case of patient number 8 this seems likely as the higher level of lipid deposition was seen in the right eye during both periods of lens wear. With patients 2 and 10 however, the difference in lipid deposition was not observed in both weeks of wear which makes the possibility of a right eye/left eye difference seem unlikely. However, in this particular study the fact that these higher levels of lipid deposition were only seen with HEMA lenses suggests that probably more than one factor is responsible. These higher lipid deposition levels are probably due to a combination of the fact that HEMA as a contact lens material is very susceptible to lipid deposition. Patient to patient variations will also influence lipid deposition levels, as will different behaviour in one eye in relation to the other (caused by rubbing the eye with the lens in situ). The variations discussed here (both patient to patient, and right eye/left eye) are representative of the variety of deposition levels you would expect to encounter across a patient population.

In this study it was only the HEMA lenses that, on a few occasions, showed unusually high levels of lipid deposition. Comparing these results with the lipid deposition results obtained from the previous study (Chapter 4-Early deposition trends on Etafilcon A lenses) it is clear that the Etafilcon A lenses demonstrated similar patterns of unusually high levels of lipid deposition in a few instances. This indicates that, like HEMA, Etafilcon A is susceptible to higher levels of lipid deposition due to patient to patient variations or sporadic influences such as rubbing

the eye with the lens in situ. These results imply that although Etafilcon A generally does not deposit lipid heavily during normal lens wear, in some unusual circumstances high lipid deposition levels can be detected. It is also interesting to note that the high levels of lipid deposition on Etafilcon A lenses were accompanied by unusually low levels of protein deposition. This is probably due to "masking' of one deposited at the lens surface by another. Fluorescence spectroscopy will only detect material deposited on the lens surface. Therefore if a lens has a very high level of lipid deposited on it's surface, this will to some extent "mask" the protein at the lens surface. Other possible causes of the high levels of lipid deposition and low levels of protein deposition detected on a few of the Etafilcon A lenses will be discussed later.

5.3.3 Comparison of Group 3 and Group 4 materials.

As stated previously, during the week long wear periods the three lens types did not behave in a greatly dissimilar fashion although, some differences were noticeable. One of the aims of this study had been to determine whether the ionic Group 3 lenses would show enhanced deposition characteristics in a similar manner to the greatly increased levels of protein deposition seen with ionic Group 4 lenses. The results clearly show that the levels of protein deposition for the Group 3 lenses are not high, in fact over the week's wear period the Group 1 HEMA lenses showed higher levels of protein deposition. In fact the Group 3 Bufilcon lenses in general showed slightly lower levels of deposition for both protein and lipid than the two sets of Group 1 lenses involved in this study. Despite these low overall

levels of deposition the Group 3 lenses were shown to deposit slightly more protein than lipid over the week long wear period.

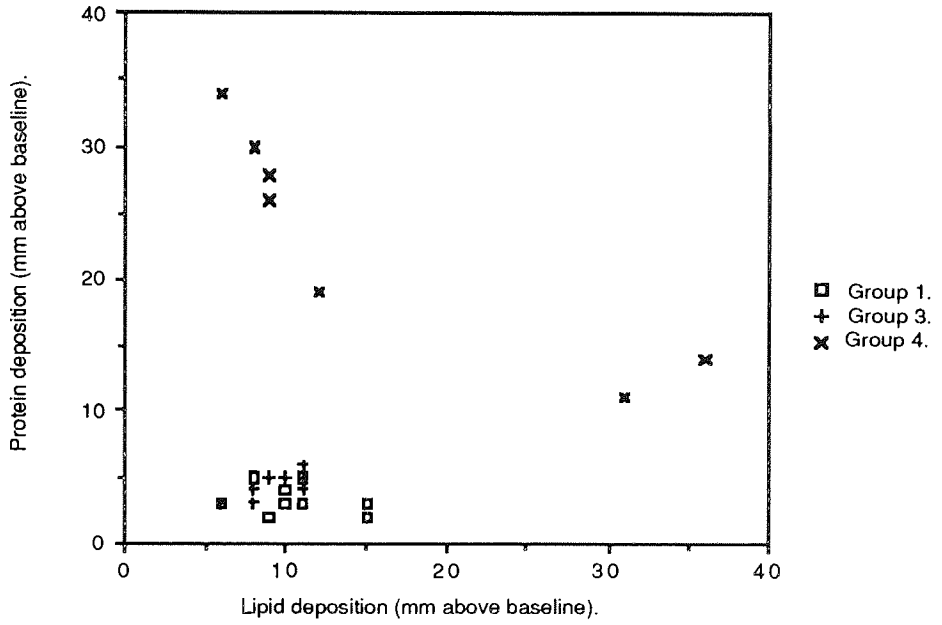
The low overall protein and lipid deposition characteristics observed with the Group 3 material probably occur as a result of several factors. For example if the surface area of the Group 3 Softmate lenses was slightly smaller than that of the other lens types, there would be "less" lens surface for protein and lipid to be deposited on. Consequently, lower levels of protein and lipid deposition would be detected on the Softmate lenses. In addition the surface finish of a contact lens can influence it's deposition profile. A very smooth surface on a contact lens will, to a certain extent, reduce deposit formation. In contrast, if the contact lens is slightly rough, this roughness will tend to encourage deposit formation. Therefore, it is possible that in this study, the Group 3 Softmate lenses had a slightly smoother surface finish than the other two lens types.

Another reason for the low levels of deposition detected on the Group 3 Softmate lenses is that a slight ionic charge (such as that on the Group 3 lenses being studied here) can be beneficial in reducing deposit formation.

Therefore it is apparent that although both Group 3 and Group 4 materials are termed ionic they do not both behave in a similar fashion; The Group 3 Softmate lenses did not show the greatly enhanced levels of protein deposition seen with ionic materials which are categorised as Group 4 (e.g. Etafilcon A). This is clearly shown in Figure 20. So, it is clear that a slight ionic charge, such as that on the Group 3 Bufilcon lenses can help reduce deposit formation. In contrast, a greater ionic charge (e.g Group 4 materials) will result in higher levels of lens deposition. This is particularly relevant with respect to protein deposition.

In Figure 20 some of the results from this study are compared to those from an earlier study involving Etafilcon A lenses (Acuvue). The Acuvue lenses analysed had been worn for a fortnight in comparison to the week long wear period used in this study. For the sake of comparison this discrepancy is not particularly important; even in the early stages of wear the increase in levels of deposition with time is not linear.

Fig. 20 Graph showing deposition levels of Groups 1, 3 and 4 lenses (Classic, Softmate and Acuvue/B+L 58), excitation wavelength 280nm.



This graph clearly shows the much higher levels of protein deposition seen with the Group 4 lenses and demonstrates the great difference in behaviour between the two ionic materials (Group's 3 and 4). Protein adsorption and absorption occurs much more dramatically on Group 4 Etafilcon materials (which contain appreciable

proportions of methacrylic acid) than on Group 3 Bufilcon materials (which have a very low proportion of ionic material). So, it appears that it is the proportion of ionic material contained within the contact lens polymer (as well as the water content of the lens material) that is important when determining which ionic contact lens materials will be particularly susceptible to heavy and preferential protein deposition.

Looking at Figure 20 it is clear that the Group 1 and 3 lens materials showed very little variation in protein deposition levels. However, there was a much wider variation in the levels of lipid deposition. These results highlight the fact that protein deposition is dependent on the contact lens material, and therefore will not vary greatly over a patient population. In contrast, lipid deposition is much more patient dependent, as demonstrated by the wider variation in recorded lipid deposition levels.

The deposition levels for the Group 4 lenses show a wide variation for both protein and lipid deposition levels. With a lens material that deposits protein as rapidly and heavily as Etafilcon A you would expect to see wider variations in protein deposition levels. However this fact cannot fully explain the extremely wide variations detected for both protein and lipid deposition levels. Figure 20 clearly shows that the highest levels of protein deposition are accompanied by the lowest levels of lipid deposition and vice versa. Because Etafilcon A is known to deposit protein heavily it is probable that rather than the high levels of protein deposition resulting in low levels of lipid deposition, it is the high levels of lipid deposition that produce the low levels of protein deposition detected. This implies that high levels of lipid deposition on Etafilcon A lenses can influence protein build

up. To a certain extent it is possible that these results arose as a result of masking of one deposited species at the lens surface by another. Because fluorescence spectroscopy is only able to detect material deposited on the lens surface, large amounts of one deposited species (e.g. lipid) can mask the other deposited species (e.g. protein).

However, it also appears possible that heavy lipid deposition on an Etafilcon A lens will "coat" the surface of the lens, thus preventing high levels of protein deposition. These results therefore indicate that there may be, to some extent, a preferential order of deposition on Etafilcon A lenses. If protein is deposited first on the lens surface (or very small amounts of lipid) then, as expected for Etafilcon A lenses, very high levels of protein deposition will be detected after a period of lens wear. However, if initially, large amounts of lipid are deposited on the lens surface, this will prevent protein being deposited, resulting in high levels of lipid deposition accompanied by low levels of protein deposition.

5.3.4 Factors affecting protein and lipid deposition levels.

Therefore, it is clear from this study using fluorescence spectroscopy that lipid deposition is largely patient dependent whereas protein deposition is much more material dependent. Variations in lipid deposition appear to be much more patient dependent than material dependent, but protein deposition levels will vary greatly depending on the choice of contact lens material. In contrast to this, the patient to patient variations in protein deposition are usually small, whereas the variations in lipid deposition between patients can be large. This highlights the fact that lipid

deposition levels on a contact lens material will vary considerably between individuals. Therefore it is impossible to determine specifically which lens materials will or will not deposit lipid heavily. Additionally, (as discussed earlier) on certain lens materials e.g. Etafilcon A high levels of lipid deposition will influence, and to some extent inhibit, protein build up on a lens surface. Thus, it is very difficult to accurately predict the deposition profile of a given contact lens material. Estimations can be made with respect to the expected deposition profile of a lens material, but it must always be remembered that these are generalisations, and not a definitive determination.

The differences among lens materials in adsorbing protein and lipid appears to increase with time. However, it is important to remember that long term deposit build up on hydrophilic lenses will also depend on patient related factors such as hygiene, compliance with instructions for cleaning, environmental factors, the care regime utilised and the patient's tear film quality (i.e. concentrations of tear components are not constant between individuals) and quantity.

5.3.5 Effect of surfactant cleaning on deposition levels.

A further aim of this study was to try and determine the effect of surfactant cleaning on cumulative deposition levels in the early stages of wear. Attempts were made to assess whether the use of a surfactant cleaner (Miraflow) during the second week's wear period would reduce the overall levels of deposition.

The results of the two week's deposition levels with the different wear schedules (no surfactant cleaning in the first week, surfactant cleaning in the second

week) are shown in Table 8. Here, the average increases in deposition levels (these values are obtained by subtracting the peak height of the unworn lens from the peak height of the worn lens) for the week's wear periods with and without surfactant cleaning are compared.

Table 8 Showing average increases in peak heights (excited at 280nm) with and without surfactant cleaning. Increases in peak heights are relative to the baseline value of unworn lenses.

	Average increase in peak heights (%).			
	Without surfactant		With surfactant	
Lens type	Protein	Lipid	Protein	Lipid
HEMA	33	30	27	58
Classic	13	63	7	37
Softmate	110	20	110	13

These results show that in general, surfactant cleaning in the second week's wear had relatively little effect at reducing deposition levels. As expected the lipid deposition levels were shown to be reduced by a much greater extent than protein deposition levels. Where the protein deposition levels were seen to be reduced this was probably partly caused as a result of the the rubbing and rinsing involved in the

digital surfactant cleaning of a contact lens. In addition, surfactants can be used to remove protein deposited on a lens surface e.g. sodium dodecyl sulphate (SDS) extraction of protein before electrophoresis. Generally the experimental conditions involved in this process are more severe than those encountered during surfactant cleaning of a contact lens, however, it can be seen that surfactants themselves do have some ability to remove protein from the contact lens surface.

The HEMA lenses appeared to show higher levels of lipid deposition when worn in conjunction with surfactant cleaning. This is probably due to the fact that in this period of wear two patients showed unusually high levels of lipid deposition. If these two patients are discounted then the average increase in lipid deposition for this second period of wear is only 21%. This highlights one of the advantages of fluorescence spectroscopy as an analytical technique; it permits individual variations in worn lens deposition levels to be detected and studied. These variations include; patient to patient variations, right eye/left eye differences, effect of lens wear period etc. However, it must be remembered that in this study the number of eyes (and number of lenses) analysed was not large enough to produce a statistically realistic average for all patients i.e. the normal patient population.

It can therefore be seen that although in most cases the deposition levels were reduced by surfactant cleaning, none of the lens types had their average value for protein or lipid peaks reduced by more than one half of the values obtained when the lenses were worn without surfactant cleaning, under the conditions of the study.

5.3.6 Assessment of the ability of surfactant cleaning to reduce deposition levels.

All the lenses had been cleaned with Miraflow on the evening prior to the day of collection. However, the period on the day of collection between when the lenses were inserted for wear and the time of collection would/could have varied between 7 ± 2 hours. Therefore it was important to establish the extent to which the surfactant cleaner had removed the lipid layer, in order to assess the rapidity and completeness of its re-establishment. At this stage in the project this was done by recording spectra from spoiled lenses at an excitation wavelength of 280nm, then cleaning the lenses with Miraflow under recommended conditions (15 seconds digital cleaning followed by copious saline rinse). The fluorescence spectra were then re-recorded. Later experiments involved *in vitro* spoilation of contact lenses combined with surfactant cleaning over longer time periods. These will be discussed in a following chapter.

The analyses performed here showed that surfactant cleaning with Miraflow did reduce the levels of deposition i.e. decreases in peak heights were seen. However, the peak heights were never reduced to the levels recorded for unworn lenses of the same type. This indicates that although surfactant cleaning does decrease deposition levels it will not completely remove all deposited material from the lens surface. Additional studies have also shown that there is a progressive increase in this residual baseline of deposited material.

It is known that deposited layers will quickly re-establish themselves when a cleaned lens is inserted into the eye. Therefore it is likely that the lenses analysed

from the second week of this study will have to some extent established a "fresh" deposited layer during wear on the day of collection. However, the study was set up to assess the effect of surfactant cleaning throughout a one week period of wear i.e. to determine the overall effect surfactant cleaning had on deposition levels rather than to evaluate the effect of cleaning on a single occasion. It can therefore be seen that even if there was some re-establishment of deposition on the day of collection the actual effect of surfactant cleaning over a week of wear could still be determined, and a valid comparison made between the deposition levels seen with and without surfactant cleaning.

From the results it was seen that surfactant cleaning did cause a reduction in deposition levels. This reduction, initially may seem quite large. However it must be remembered that the increase in deposition seen for all the lens types over both weeks of wear are, in comparison to other lens materials (e.g. Etafilcon A), or to lenses that have been worn for slightly longer periods of time, very small indeed.

From the results obtained in this study it appears that surfactant cleaning is only partially effective in removing lipid deposits, and that the level of lipid deposition is more dependent on individual patient tear chemistry than on time during the early stages of wear. However, surfactant cleaning is important in minimising the immobilasation of reactive lipids during the later stages of wear.

5.4 Conclusions.

Using fluorescence spectroscopy as an analytical technique in this study involving a strictly controlled lens wear programme it was possible to fulfil all the aims listed at the outset. Deposition levels had been detected on lens materials of different surface charges after only one week of wear. From an examination of the fluorescence spectra obtained it was possible to identify any differences or similarities between these lens materials. Patient to patient and left eye/right eye differences were clearly highlighted, and finally, the effect of surfactant cleaning on deposition levels was determined. Using conventional techniques (both destructive and/or non-destructive) it would have been very difficult, if not impossible to obtain these results so quickly and accurately. In addition, if further more detailed analysis of the contact lenses was required e.g. electrophoretic studies of the protein deposits or HPLC studies into the nature of the lipoidal deposits, then these processes could still be performed as fluorescence analysis will not disrupt the deposited layer on a contact lens. The results from this trial therefore, again demonstrate what an extremely valuable and versatile technique fluorescence spectroscopy is for contact lens analysis.

6. The deposition trends of Group 1 (Polymacon) and Group 4 (Etafilcon A) daily wear disposable lens systems.

This study was set up using strictly controlled wear programmes to investigate (using fluorescence spectroscopy) the lipoidal and protein spoilation associated with two commercially available daily wear disposable lens systems. Different lens care systems were used with each lens type. The lens care systems chosen were those recommended by the contact lens manufacturer for use with their disposable lenses. Using different lens care systems enabled estimations of patient preference to be made with regard to lens cleaning and disinfection.

In addition to the fluorescence analysis of the worn contact lenses, a clinically based study was also performed. The purpose of this was to investigate the total patient related performance of the lenses (e.g. vision, comfort etc.) and then relate this with deposition levels. Thus, deposition levels can be studied from both a material, and a patient viewpoint.

The lens materials chosen were:-

1. Vistakon (Johnson & Johnson) Acuvue. Fabricated from Etafilcon A this is a HEMA/Methacrylic acid based high water content ionic material (FDA category Group 4).

2. Bausch & Lomb Seequence, this HEMA based (Polymacon) lens is a low water content non-ionic lens in FDA category Group 1.

As in the previous studies the clinical work was undertaken by Lyndon Jones FBCO at his South London practice, with the fluorescence analysis being performed upon receiving the lenses at Aston University.

6.1 Clinical protocol.

Twelve patients (six male, six female), all of whom currently wore hydrogel lenses of a non-disposable type took part in this study. The subjects were all problem free wearers who had previously been using Ciba Miraflow as a surfactant cleaner, and Ciba 10:10 (a 3% hydrogen peroxide disinfection system), with fortnightly enzyme tablets, on their existing lenses. Each patient wore one of each type of disposable lens. These were worn for the wearing schedules recommended by the manufacturer, i.e. two weeks for Acuvue and four weeks for Seequence.

Each subject undertook four consultations:

1. Screening appointment.
2. Fitting of initial set of lenses.
3. Two/four weeks later return first pair. These lenses (and the lenses that were subsequently collected) were then sent to me at Aston University for analysis. Collect second pair of same design.
4. Two/four weeks later, return second pair and exit from trial.

During the wearing schedules the patients were recommended to use the following disinfection systems:-

Acuvue. The patients were instructed to disinfect their lenses using Alcon Softab (a chlorine based disinfection system). In addition they used Alcon Pliagel, a sorbic acid preserved surfactant cleaner to clean their lenses. The lenses were rubbed for 10 seconds and then rinsed with buffered saline before leaving in Softab overnight.

Seequence. The Seequence lenses were disinfected using Bausch and Lomb Optimeyes. This is a one step chlorhexidine based system currently recommended by Bausch and Lomb for use with their disposable system. It is based on tablet formulation, which is dissolved in rising mains tap water. Prior to using this system the patients were instructed to surfactant clean their lenses using one drop of B&L daily cleaner (a sorbic acid preserved surfactant). This was then rinsed off using rising mains tap water.

Subjects were instructed to refrain from using enzyme tablets throughout the course of the trial and were informed that they should attempt to wear their lenses for a minimum of ten hours, and a maximum of sixteen hours daily. A new lens container was given to the patient after each design of lens was changed, i.e. when the patients changed the care system.

At the end of the two/four week wear period the subjects returned to the test centre for collection of the lenses. To ensure adequate spoilation of the lenses by

each individual on the day of collection, the patients attended after having worn the lenses for a minimum of four hours, and a maximum of eight hours that day.

Upon return to the test centre the patients and their lenses were examined by the optomometrist. Visual acuity was measured and the degree of spoilation was ascertained, so that comparisons could be made with the fluorescence analysis results. These will be discussed later.

6.2 Analytical procedure.

The lenses were placed in sterile containers filled with 3ml of non-preserved saline and sent to Aston University for analysis. As in previous studies analysis was undertaken within a fortnight of receiving the lenses.

Analysis of the contact lenses was performed at excitation wavelengths of 360nm and 280nm. Each contact lens was carefully placed in the clean cuvette which was filled with distilled water. The lens was positioned accurately inside the cuvette before analysis was undertaken. After the lens was mounted in the cuvette it was important that the cuvette was precisely placed in the spectrofluorometer (these processes are described in more detail in Chapter 3). This procedure was repeated every time a fresh contact lens was analysed. Each lens was analysed at both wavelengths (360nm and 280nm) before being returned to the correct storage vial. The lenses were then stored in the fridge in case any further analysis was required.

The study had two main aims:-

1. To investigate the lipid and protein spoilation associated with the two lens types (ionic Etafilcon A and non-ionic HEMA). It was hoped that fluorescence spectroscopy would highlight the expected differences in the deposition profiles of the two lens materials. HEMA lenses are known to have a tendency to deposit more lipid than protein, particularly in the later stages of lens wear. In contrast Etafilcon A is known to be susceptible to high levels of protein deposition, and also to show comparatively low levels of lipid deposition. This occurs as a consequence of the ionic nature of the material. The charged surface of the lens will attract the oppositely charged groups in proteins resulting in protein deposition on the lens surface. Similarly, small charged proteins (e.g. lysosyme) will be able to migrate into the contact lens matrix.

2. To attempt to draw some inferences as to whether the spoilation profiles affect visual performance and patient preferences. This unique combination of clinical study and a novel analytical technique should yield accurate information about deposition levels on the disposable lenses which can then be compared to the practice based clinical findings.

6.3 Fluorescence spectroscopy results and discussion.

The fluorescence spectroscopy results are shown in Tables 9 and 10, and Figures 21 and 22. Table 9 and Figure 21 give the lipid deposition values

obtained from excitation at 360nm. Table 10 and Figure 22 are the results from excitation at 280nm and show both the protein and lipid deposition levels. This study enabled reliable laboratory based analysis of two disposable lens systems to be undertaken. This will give useful information about the relative performances of the two different lens systems under the Manufacturer's recommended wear schedules. In addition the laboratory analysis can be compared with the clinical analysis so that an estimation of the reliability of measurements of deposition levels in a practice based situation can be determined.

6.3.1 Deposition levels of Acuvue and Seequence lenses.

The most obvious point arising from this study is the difference in susceptibility of the two lens materials to deposit protein and lipid. Following the manufacturer's recommended wear schedules (two weeks for Acuvue and four weeks for Seequence) it can clearly be seen that the Acuvue lenses have a much greater ability to deposit protein (Figure 22).

In contrast, it was seen that the Seequence lenses deposited lipid preferentially (Figures 21 and 22). It is interesting to note that even though the Seequence lenses had been worn for twice as long as the Acuvue lenses the levels of protein deposition seen on the Seequence lenses were considerably lower than those recorded on the Acuvue lenses (Figure 22).

The results are in agreement with previous studies which have demonstrated that lenses fabricated from Etafilcon A have a much greater tendency to deposit larger amounts of protein than lipid, whereas lenses made from HEMA have a

tendency to deposit lipid to a greater degree than protein. The findings in this study are also in line with earlier studies which demonstrated that the patient to patient variations in protein deposition levels are small whereas the variations in lipid deposition levels between patients are large.

Although the wear periods of the two lens types were different, it is still valid to make comparisons between the sets of results. The aim of this study had been to investigate the deposition levels of the two lens types using the manufacturer's recommended wear time and care regime. This highlighted the fact that Acuvue lenses have a tendency to deposit larger amounts of protein than lipid, and that Seequence lenses tend to deposit more lipid than protein during the recommended wear schedules. Therefore these results indicate that if a patient was known to deposit lipid heavily, but only deposit small amounts of protein, then Acuvue could be the best choice of disposable lens, whereas, for someone who deposited large amounts of protein and relatively small amounts of lipid, Seequence could be the disposable lens of choice.

Figure 21 shows the wide patient to patient variations that can be seen in lipid deposition levels. The tendency of the HEMA (Seequence) lenses to deposit lipid to a greater extent than protein is also apparent. However, at this stage the lipid deposition levels of the HEMA lenses are not vastly different to those seen with the Etafilcon A Acuvue lenses, particularly when it is remembered that the HEMA lenses had been worn for twice as long as the Etafilcon A lenses. This indicates that it is only over longer wear periods that the ability of HEMA lenses to preferentially deposit lipid becomes really apparent.

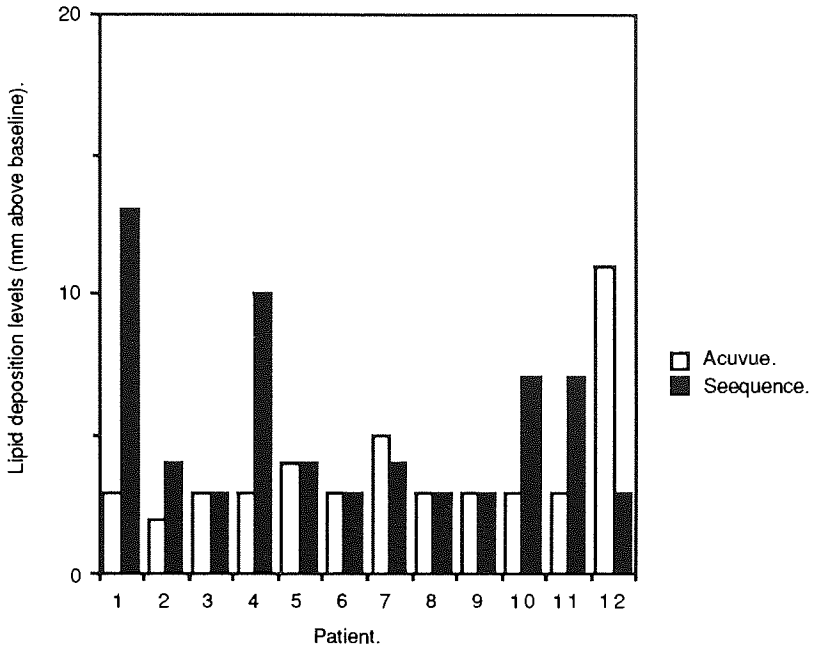
Table 9 Lipid deposition levels (mm above baseline) of Seequence and Acuvue lenses; excitation wavelength 360nm.

Patient	Eye	Lipid peak height (mm above baseline ±0.5) Acuvue	Seequence
Unworn	/	3	3
1	Left	3	13
	Right	3	7
2	Left	2	4
	Right	3	4
3	Left	3	3
	Right	3	4
4	Left	3	10
	Right	3	4
5	Left	4	4
	Right	4	3
6	Left	3	3
	Right	3	4
7	Left	5	4
	Right	3	3
8	Left	3	3
	Right	3	3
9	Left	3	3
	Right	3	3
10	Left	3	7
	Right	4	3
11	Left	3	7
	Right	4	13
12	Left	11	3
	Right	3	9

Table 10 Protein and lipid deposition levels (mm above baseline) of Seequence and Acuvue lenses; excitation wavelength 280nm.

Patient	Eye	Peak height above baseline (mm ±0.5)			
		Acuvue		Seequence	
		Protein	Lipid	Protein	Lipid
Unworn	/	2	6	2	6
1	Left	21	5	4	37
	Right	14	5	2	20
2	Left	9	4	3	10
	Right	3	4	3	6
3	Left	26	4	3	4
	Right	9	5	2	24
4	Left	10	4	3	21
	Right	23	4	3	6
5	Left	10	4	2	6
	Right	13	6	2	5
6	Left	18	5	2	8
	Right	13	6	2	5
7	Left	23	16	2	7
	Right	21	6	3	5
8	Left	9	5	3	5
	Right	9	6	2	5
9	Left	29	7	2	4
	Right	9	5	3	9
10	Left	25	5	3	25
	Right	30	5	3	7
11	Left	13	7	2	19
	Right	21	8	2	33
12	Left	20	31	2	6
	Right	20	7	3	20

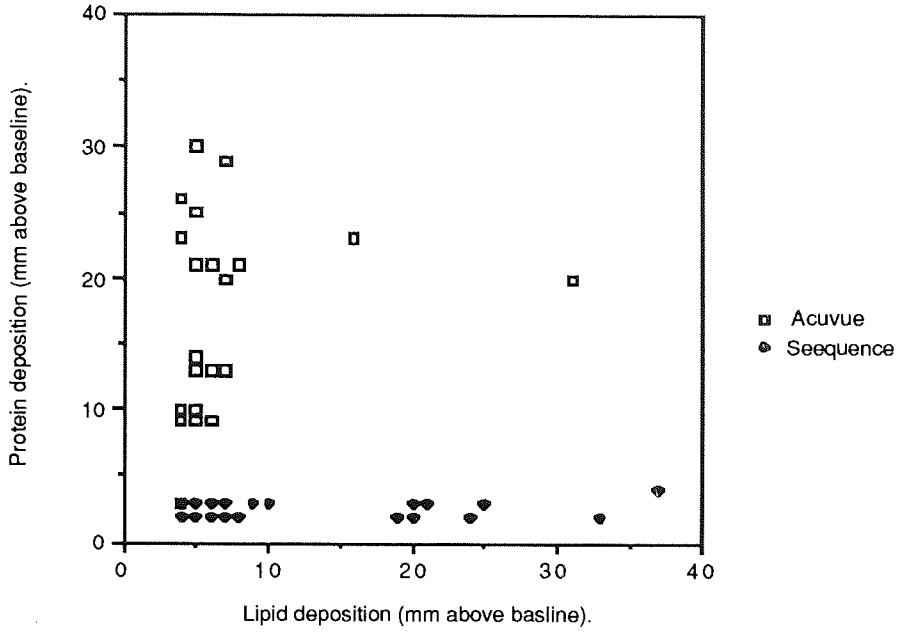
Fig. 21 Graph showing lipid deposition levels of Acuvue and Seequence lenses; excited at 360nm.



Even though the Acuvue lenses were worn for a fortnight less than the Seequence lenses, the results (Figure 22) show that the amounts of deposited protein i.e. the measured peak heights are on average greater than the amounts of deposited lipid seen on either of the lens types. This indicates that formation of protein deposits generally occurs more rapidly than the formation of lipid deposits.

Figure 22 also shows that protein and lipid deposition levels fall within a similar range of peak heights (0-40mm above baseline of the fluorescence spectrum).

Fig. 22 Graph showing protein and lipid deposition levels of Acuvue and Seequence lenses, excitation wavelength 280nm.



There are noticeable differences in the deposition profiles of the two deposited species. The majority of the analysed Acuvue and Seequence lenses showed lipid deposition levels of 10mm above baseline or less on their fluorescence spectra. In contrast the majority of the Acuvue lenses showed protein deposition levels between approximately 10 and 25mm above the baseline, with all the Seequence lenses having protein deposition levels of less than 5mm above baseline.

6.3.2 Etafilcon A deposition levels.

The results from this study demonstrate the fact that as a contact lens material, Etafilcon A is extremely susceptible to high levels of protein deposition, and also

that this deposition can occur very rapidly. It also shows that protein deposition is much more dependent on the contact lens material than on patient variations. Lipid deposition is, in general more patient dependent and Figures 21 and 22 both demonstrate that patient to patient variations in lipid deposition levels can be large. In contrast, variations in protein deposition levels between patients are generally small. However, Figure 21 demonstrates that the formation of lipoidal deposits at a lens surface is not entirely independent of the contact lens material.

6.3.3 HEMA deposition levels.

In general, the HEMA Seequence lenses showed greater levels of lipid deposition than the Etafilcon A Acuvue lenses (Figure 21), although this difference was not nearly as marked as the variations seen with the protein deposition levels of the two lens materials (Figure 22). This enhanced lipid deposition could have occurred due to the fact that the Seequence lenses had been worn for a longer period. However, other studies[73] have shown that HEMA lenses do show enhanced lipid deposition accompanied by low levels of protein deposition, particularly over longer wear periods. Therefore, it can be seen that although lipid deposition is generally patient dependent, there is also an element of material dependency involved.

6.3.4 Patient to patient variations.

The results shown in Tables 9 and 10 demonstrated no great overall differences between left and right eyes, although there were some inter-eye differences.

These will probably have been caused by factors such as poor surfactant cleaning of one lens, increased handling of one lens or, rubbing the eye with the lens in situ. In addition a few unusually high or low deposition levels were noticed in both lens types, for protein and lipid. These are examples of the unusual patient variations that would be expected over a patient population and could be related to factors such as diet and/or hormonal variations.

Looking at the right eye/left eye differences for lipid deposition levels (Table 9) in more detail, it is clear that, in most cases lipid deposition levels were similar for left and right eyes when the Etafilcon A Acuvue lenses were being worn. In two instances noticeable differences were detected between the left and right eye lipid deposition levels.

In contrast, five out of the twelve patients enrolled on the study showed left eye right eye differences when wearing the HEMA Seequence lenses.

There are several possible reasons for these observed variations in lipid deposition levels between eyes. For example, rubbing one eye with the lens in situ can cause increased lipid deposition on the surface of the contact lens. The lenses were also surfactant cleaned throughout this study, therefore, it is possible that poor cleaning of one lens during the wear period could lead to higher levels of lipid deposition on the poorly cleaned lens. In addition, excess handling of a contact lens can result in lipids being deposited onto the lens surface from the fingertips. This would result in higher levels of detectable lipid deposition on the contact lens surface. So, it can be seen that the indirect patient related factors discussed above can result in patients (who would normally be expected to show similar behaviour between right and left eyes) demonstrating discrepancies

between left and right eye deposition levels.

The fact that the majority of the left eye/left eye differences were detected on HEMA lenses suggests that, although lipid deposition is generally believed to be more patient dependent than material dependent, a certain amount of material dependency is also involved. It appears that lens materials, such as HEMA, which are known to deposit lipid preferentially, will tend to show a much wider range of patient to patient variations and/or left eye/right eye differences than lens materials such as Etafilcon A which generally deposit protein heavily accompanied by low levels of lipid deposition.

Looking at the variations in protein deposition (Table 10) it is clear that the protein deposition levels on the HEMA Seequence lenses showed very little variation between left and right eyes. This is in agreement with previous studies that have shown that protein deposition is generally more patient dependent than material dependent. However,the protein deposition levels on the Etafilcon A Acuvue lenses show wide variations between left and right eyes for the majority of subjects. Thus, it appears than when a lens material is known to deposit protein heavily, then levels of protein deposition on such a lens can vary widely, both between patients, and between eyes.

Because extremely large amounts of protein are deposited on and within Etafilcon A lenses you would expect to find a wider variation of deposition levels across a patient population. However, at least seven of the twelve subjects being studied showed extremely large differences between amounts of deposited protein in left and right eyes. This implies that there must be some other reason for the wide variations in left eye/right eye protein deposition levels that were detected in

this study.

Etafilcon A contact lenses are known to deposit protein very heavily, both on the lens surface, and within the contact lens matrix. It is generally believed that lysosyme is one of the major proteins deposited on and within Etafilcon A lenses. Lysosyme is a small, charged protein, thus it will readily be deposited on the surface of the ionic Etafilcon A lens. In addition, the small size of lysosyme means that it can be taken up into the contact lens matrix. So, it can be seen that over a period of wear, large amounts of lysosyme will be deposited on and within an Etafilcon A contact lens.

Fluorescence spectroscopy is only able to detect material deposited on the contact lens surface. Therefore, the wide differences in right and left eye protein deposition levels observed in this study will be caused by variations in the surface deposition levels of protein (lysosyme) on the Etafilcon A lens' surfaces. Lysosyme itself gives rise to a large fluorescence signal. Studies involving recording fluorescence spectra at 280nm from standard protein solutions of commonly deposited tear proteins (e.g. lysosyme, lactoferrin, albumin, globulins) show that lysosyme produces one of the largest fluorescence emission peaks. This indicates that relatively small variations in the amount of lysosyme deposited on a lens surface can lead to wide variations in the recorded fluorescence peak height.

It therefore seems probable that the wide differences in right eye/left eye protein deposition levels detected on Etafilcon A lenses in this study are due to variations in the amount of lysosyme deposited at the contact lens surface. Lysosyme is readily adsorbed and absorbed by Etafilcon A lenses. However the majority of

this lysosyme will not be permanently bound to the contact lens material i.e. under suitable conditions lysosyme that is not permanently bound can be easily removed from the contact lens. It is therefore felt that during lens storage (e.g. in saline) lysosyme will migrate from the contact lens matrix, to the lens surface, and then diffuse into the lens storage solution. So, it can be seen that even slight variations in lens storage times could lead to variations in the amount of lysosyme (protein) detected on an Etafilcon A lens surface.

This study highlighted the problem of lysosyme migration to and from ionic high water content contact lenses. In the following two chapters this phenomenon will be investigated and discussed in more detail.

6.4 Clinical results and discussion.

The second aim of this study involved a comparison of the fluorescence spectroscopy results with clinical analysis. As stated earlier, the clinical work was performed by Lyndon Jones FBCO at his South London practice. A summary of the clinical results will be given here, for purposes of comparison with the fluorescence analysis.

6.4.1 Clinical results.

Visual acuity with the contact lenses was tested at the initial visit and then again at the final visit. The results showed that there was no difference between right and left eyes at either initial or follow-up appointments. The data for right and left eyes was then summed to detect whether visual acuity was reduced with either

lens type after the designated wear period. The summed visual acuity test results indicated that overall the reduction in acuity was greatest with Seequence. However, the differences between the two lens types were only slight which makes any clinical significance unlikely. This reduction in acuity can be linked to the fluorescence spectroscopy results. The fluorescence analysis revealed that the Seequence lenses had the highest levels of lipid deposition. Therefore, it can be seen that even the levels of lipid recorded on the Seequence lenses after a months wear could result in a reduction in visual acuity. So, it can be seen that on the Seequence lenses it is primarily lipid deposition that causes the observed reduction in visual acuity.

For Acuvue lenses, comparison of measured visual acuity and measured deposit results indicate that reductions in visual acuity detected with Acuvue lenses were caused by protein deposition. Thus, it appears that it is not the deposition of a particular species (protein or lipid) at the lens surface that causes reductions in visual acuity during lens wear. From the results obtained in this study it seems that visual acuity is reduced by the species that is deposited most heavily on the contact lens surface. However, it must be remembered that the number of eyes/lenses examined in this study was very small. Further studies using many more patients/lenses would be required before any statistical significance could be ascribed to these findings.

The patients were asked to assess the visual quality, comfort, handling ability, care regimen and overall performance of the two lens types. The results indicated that in all aspects except the care regime Acuvue was better than Seequence. The care regime used with the Acuvue lenses was in fact found to be slightly

preferable to that used with Seequence, but not to any significant extent. No difference in handling ability was noticed during fluorescence analysis of the contact lenses.

As stated the care regime used with the Acuvue lenses was found to be preferable to that used with Seequence lenses. In addition a problem was encountered with the product used to disinfect the Seequence lenses (Optimeyes). Two patients exhibited a solution reaction to the Optimeyes system. These patients reported significantly reduced comfort with the system after 18-20 days. One patient exhibited a large amount of central coalescent corneal staining, whilst the other exhibited light punctate staining. No further problems were encountered when the patients were continued with Seequence lenses and Softab tablets.

Although this study had a small patient base, the level of solution reaction (17%) is worrying.

Finally slit-lamp spoilation of the worn lenses was undertaken by examining the deposits seen at the slit-lamp under 25x magnification. Results were classified according to type, coverage and degree. Analysis of these results revealed no apparent difference in deposition levels between the Acuvue and Seequence lenses. In addition, no differences in types of deposit i.e. proteinaceous or lipoidal, degree of deposition, or lens coverage were observed. These results differ widely from those obtained from fluorescence spectroscopy which indicated clear differences between the deposition profiles of the two lens types. This implies that practice-based analysis of deposition levels can be inadequate, particularly during the early stages of wear when deposit levels are low. This clearly demonstrates the need for novel analytical techniques like fluorescence

Spectroscopy which can give reliable information about the deposition trends of different contact lens materials.

6.5 Conclusions.

This study involved a unique combination of clinically and laboratory based analyses, using contact lenses worn under a strictly monitored regime. It enabled investigation of patient-related lens wear performance (e.g. vision, comfort, handling etc.) to be compared with practice based and laboratory based determinations of deposition levels.

The fluorescence analysis enabled accurate determinations of the deposition profiles for the two disposable lens types to be made. These detailed lens deposition profiles, combined with clinical findings regarding reductions in visual acuity following lens wear, provided information about how preferential deposition of protein or lipid may case reductions in visual acuity during contact lens wear.

One of the most striking observations from this study are the differences between the clinically based and laboratory based estimations of lens deposition levels. The clinically based results revealed no apparent difference in deposition levels between the Acuvue and Seequence lenses. In addition, no differences in types of deposit i.e. proteinaceous or lipoidal, degree of deposition, or lens coverage were observed. These results are in marked contrast to the laboratory based findings which showed clear differences in the deposition profiles of the two lens types. Low levels of lipid deposition and high levels of protein deposition were detected on Acuvue lenses. The Seequence lenses showed low

levels of protein and lipid deposition, although the lipid deposition levels were seen to be higher than the protein deposition levels. In addition, the Seequence lenses had slightly higher levels of lipid deposition than the Acuvue lenses.

This contrast between clinically based and laboratory based estimations of lens deposition levels implies that practice-based analysis of deposition levels can be inadequate, particularly during the early stages of wear when deposit levels are low. It also highlights the extreme sensitivity and selectivity of fluorescence spectroscopy compared to clinical analytical methods. The fluorescence analysis technique is able to detect very low levels of deposition, and can distinguish between deposited species on a lens surface, even after extremely short wear periods, when only extremely small amounts of material will be present on the lens surface. Therefore, it can be seen that fluorescence analysis is an extremely valuable method for analysis of contact lens deposition levels, particularly as it enables worn lenses to be returned to in eye lens wear following analysis.

Another interesting observation was that the lens care system recommended by the contact lens manufacturer is not necessarily the best with regard to patient satisfaction. This result again highlights the benefits obtained from a combined clinically and laboratory based study. In a purely laboratory based study information about ease of use of lens care systems would not be available.

A final point that arose from this study was the wide variations detected in protein deposition levels on the ionic high water content Acuvue lenses. It is felt that these variations could have arisen due to lysosyme migration to and from Acuvue lenses. This phenomenon will be investigated further, and discussed in more detail in the following two chapters.

7. *In Vitro* spoilation of Acuvue and Seequence lenses.

7.1 Introduction.

This study was set up to compare the deposition profiles of *in vitro* spoilt contact lenses with the deposition profiles of *in vivo* spoilt lenses. Results from the previous investigation (Chapter 6) provided the *in vivo* deposition profiles. This study involved the *in vitro* spoilation of the same lens types i.e. Seequence and Acuvue. It was important to try and reproduce the conditions from the *in vivo* study as accurately as possible in the *in vitro* study. Therefore, throughout the period of *in vitro* spoilation the lenses were cleaned using similar cleaning and disinfecting regimes to those that were used with the lenses analysed in Chapter 6. This will ensure that valid comparisons can be made between the two sets of results.

7.1.1 Method of *in vitro* spoilation.

The *in vitro* spoilation was performed using the "spiked" version of the Aston Tear Model (see Chapter 2). This model has been developed at Aston to closely mimic the composition of the tear fluid. It is based on an animal serum which is "spiked" with proteins and lipids in appropriate quantities. This produces a solution which contains ingredients similar to those found in tears and in comparable amounts (a list of ingredients is given in Chapter 2). Contact lenses

can then be artificially spoiled in this solution.

Spoilation by this method enables the deposition characteristics of any existing lens material (or of novel materials intended for contact lens use) to be evaluated in the laboratory as a preliminary screening before selecting candidates for a clinical study. In addition, the use of this artificial tear solution, and the method of spoilation developed at Aston University gives accelerated spoilation in comparison to conventional wear. Lenses are usually spoiled in the Model for 28 days without surfactant cleaning or disinfection. Preliminary experiments had indicated that 28 days in the *in vitro* model gives spoilation levels equivalent to longer than 6 months *in vivo* daily wear. However, cleaning and patient to patient variations will obviously affect this estimation. Therefore, the use of the *in vitro* spoilation method in combination with cleaning and disinfection regimes commonly used with *in vivo* lens wear should provide more accurate information about the *in vivo* wear period which is equivalent to 28 days *in vitro* lens spoilation.

7.1.2 Lens Materials.

As stated, the aim of this study was to monitor the *in vitro* spoilation of two different disposable lens types using fluorescence spectroscopy. The lenses used in this study were:-

1. Vistakon (Johnson & Johnson) Acuvue, fabricated from Etafilcon A this is a HEMA/Methacrylic acid based high water content ionic material (FDA Category Group 4).

2. Bausch & Lomb Seequence, this HEMA based (Polymacon) lens is a low water content non-ionic lens in FDA category Group 1.

Using these lens types enables a direct comparison to be made between the *in vivo* results obtained in Chapter 6, and the *in vitro* results obtained in this study. To ensure an accurate comparison cleaning regimes similar to those used in Chapter 6 were used to clean and disinfect the contact lenses spoilt in this *in vitro* study.

7.2 Experimental protocol.

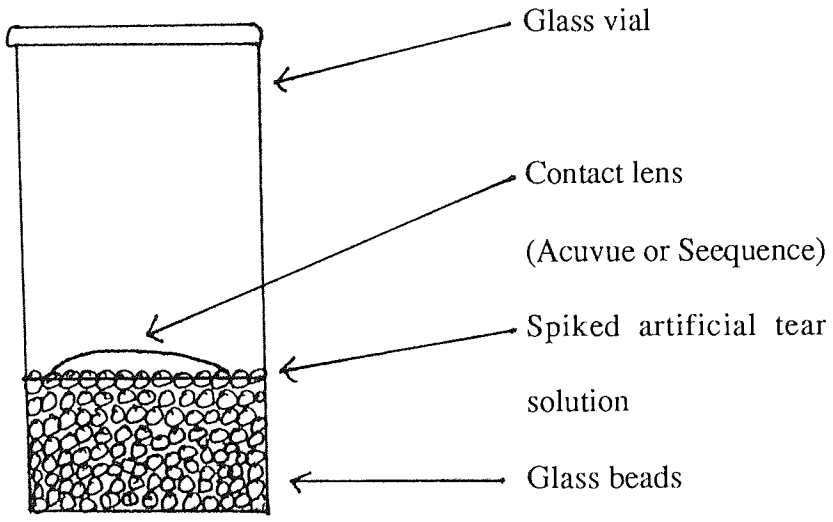
A total of six contact lenses were spoiled in the Spiked Artificial Tear Model (a description is given in Chapter 2); three Acuvue and three Seequence lenses. Before *in vitro* spoilation was started it was important to determine whether any of the clean lenses showed significant background fluorescence. Therefore at the start of the study each of the clean, unspoilt lenses had their fluorescence spectra recorded at 360nm and 280nm. After this the lenses were placed in numbered glass vials in preparation for *in vitro* spoilation

7.2.1 *In vitro* spoilation technique.

The clean unspoilt lenses were placed in numbered glass vials which were filled with clean glass beads to a depth of about 1.5cm. Both the beads and the glass vials were treated before use with a Siliconising solution. This helps to prevent deposition of components from the Artificial Tear solution onto the glass. The vials

were filled with the tear solution to a level just below the surface of the glass beads. The contact lens was then placed over the surface of the beads as shown in Figure 23. The vials containing the lenses were then placed on a shaker. Every 24 hours the vials were removed from the shaker, to enable the tear solution to be changed. The vials were then replaced on the shaker.

Fig. 23 *In vitro* spoilation using the Aston Tear Model.



Fluorescence spectra were recorded at 360nm and 280nm twice a week for four weeks during the *in vitro* lens spoilation. To mimic the *in vivo* study as closely as possible the *in vitro* spoiled lenses were cleaned twice a week using similar regimes to those employed in the *in vivo* study (Chapter 6), i.e. for both lens types, surfactant cleaning followed by disinfection with either a chlorine or hydrogen peroxide based system. A contact lens of each type (one Acuvue and one Seequence) was designated as a control. These lenses had no surfactant cleaning or disinfection throughout the spoilation period. It was intended to use these control lenses to determine whether or not surfactant cleaning and disinfection would

significantly reduce the lens deposition levels accumulated over the period of *in vitro* spoilation.

7.2.2 Experimental method.

To assess the effect of cleaning and disinfecting on deposition levels fluorescence spectra were recorded before surfactant cleaning of the lens took place, and then subsequently after lens disinfection had occurred.

As stated, of the three Acuvue and three Sequence lenses one lens of each type was designated as a control. The control lenses were not cleaned or disinfected during the four week spoilation period. Twice a week these lenses were placed overnight in a contact lens case containing only saline. This was done to mimic the period when the other contact lenses were placed overnight in disinfection or neutralising solutions. Of the remaining four lenses, one Acuvue and one Seequence were disinfected using a chlorine based system and cleaned with the recommended surfactant cleaner. The remaining Acuvue and Seequence lenses were disinfected with a hydrogen peroxide system. Again the lenses were cleaned with a surfactant cleaner recommended for use with the peroxide system.

The chlorine based disinfection system chosen was Alcon Softab. This self-neutralising solution has sodium dichloroisocyanurate as it's active ingredient. After surfactant cleaning the lenses were left in the disinfection solution overnight as directed by the Manufacturers. The surfactant cleaner used with this disinfection system was Alcon Pliagel.

The hydrogen peroxide based disinfectant was the Allergan Oxysept system.

This consists of two solutions, Oxysept 1 and Oxysept 2. Oxysept 1 is the hydrogen peroxide disinfecting solution, Oxysept 2 is the rinsing/neutralising solution. Throughout the study after surfactant cleaning the lenses were disinfected for one hour in Oxysept 1 before being stored overnight in Oxysept 2. The surfactant cleaner used with this system was Allergan LC-65. It was decided not to use Optimeyes (the hydrogen peroxide disinfection system used in the *in vivo* study) in this study because of the problems encountered with it's use in the previous study. These are discussed in Chapter 6.

Both types of disinfection system were used on the Acuvue and Seequence lenses in order to determine whether a particular disinfection system was more (or less) effective with a particular lens type.

7.3 Fluorescence analysis.

Before *in vitro* spoilation of the lenses was started fluorescence spectra of the clean unworn lenses were recorded at 360nm and 280nm. The lenses were then placed in numbered vials as described earlier. The allocation of lenses to vials was arranged as follows:-

Lens 1. Acuvue control. (No surfactant clean or disinfection).

Lens 2. Acuvue, chlorine based disinfection. (Surfactant clean with Alcon Pliagel, disinfect with Alcon Softab).

Lens 3. Acuvue, hydrogen peroxide based disinfection. (Surfactant clean with Allergan LC-65, disinfect with Allergan Oxysept).

Lens 4. Seequence control. (No surfactant clean or disinfection).

Lens 5. Seequence, chlorine based disinfection. (Surfactant clean with Alcon Pliagel, disinfect with Alcon Softab).

Lens 6. Seequence, hydrogen peroxide based disinfection. (Surfactant clean with Allergan LC-65, disinfect with Allergan Oxysept).

Once the background fluorescence of the contact lenses had been determined, it was important to establish whether or not the solutions that the lenses were to be stored in overnight during disinfection displayed any fluorescence themselves. Spectra recorded at 280nm and 360nm of these solutions e.g. saline, Softab and Oxysept 2 showed that none of them demonstrated any significant fluorescence.

Analysis of the lenses was performed as described in previous chapters. Before each lens was placed in the spectrofluorometer cuvette it was gently rinsed with distilled water to remove any excess artificial tears or disinfection/neutralising solution. Spectra were recorded before cleaning and disinfection, and then re-recorded the next day following overnight disinfection (or storage in saline for the controls). After disinfection the lenses were returned to their respective vials containing the glass beads and tear solution. The vials were then sealed and replaced on the shaker.

7.3.1 *In vitro* spoilation times for Acuvue and Seequence lenses.

As stated the expected period of *in vitro* spoilation was 28 days, in conjunction with twice weekly cleaning, disinfection and recording of fluorescence spectra. This would have given eight sets of results (before and after cleaning). However,

handling the Acuvue lenses proved very difficult, and most of them had torn badly by the end of 14 days. Therefore, there are only four sets of before and after cleaning results for the spoilation of Acuvue lenses. Similarly, the Seequence lenses started to tear slightly at the end of three weeks *in vitro* spoilation. The tears did not interfere with the *in vitro* spoilation and it was still possible to record accurate spectra. However, it was decided to only use the results from the Seequence lenses that were recorded before the lenses started to tear (i.e. up to the end of the third week of *in vitro* spoilation). In this way comparisons can be made between the deposition profiles of both lens types before any lens deterioration occurred. In addition this will ensure that the *in vitro* spoilation model mimics the *in vivo* study (Chapter 6) as closely as possible, as none of the lenses collected from the *in vivo* study had torn.

These reductions in the expected *in vitro* spoilation times did not prove problematical though; the *in vitro* spoilation method used in this study gives accelerated spoilation in comparison to conventional wear times, so the 14 and 21 days of spoilation achieved here will equate to a considerably longer wear period then the fortnight of *in vivo* wear in the previous study. Therefore, comparisons can still be made between the *in vitro* spoilation model and the *in vivo* results obtained in Chapter 6. In addition, an estimation of the spoilation profiles of both lens types over longer wear periods can be made.

7.3.2 Instrumental variables.

In this and the following study fluorescence spectra were recorded on a different

spectrofluorometer. This second machine is the same model as the original spectrofluorometer (Aminco-Bowman SPF-125), so spectra were recorded in exactly the same way. However, the two machines cannot be expected to give identical results i.e. the peak height recorded for a particular contact lens in one machine will not be the same as the peak height recorded for the same lens in the other machine. This is because of inherent instrumental factors. For example, the spectrofluorometer photomultiplier tube (see Chapter 3) will age and become non-linear with time. This will result in a drift in sensitivity over the range of excitation wavelengths. So, over time the sensitivity of an individual photomultiplier tube will start to vary. Therefore you cannot expect photomultiplier tubes in two different spectrofluorometers to have the same levels of sensitivity at a particular excitation wavelength.

All spectrofluorometers have to be precisely optically aligned before use. Again the alignment between two machines will not be identical, causing different results. The optical alignment of an individual machine will become less precise with time. To ensure accurate and consistent results the spectrofluorometer must be regularly calibrated. This is done by recording fluorescence spectra of standard protein solutions e.g. lysosyme, lactoferrin, albumin and globulins. At a later date spectra can be re-recorded using these standard protein solutions. Thus any inconsistencies in the spectrofluorometers' performance can be detected and corrected.

7.3.3 Rationale to enable comparison of results between two spectrofluorometers.

To enable comparisons of the results obtained from both machines to be made, various calibration experiments were performed. These involved recording spectra at 360nm and 280nm for standard solutions in both machines. In this way it was possible to determine the difference in sensitivity between the two machines. It was found that the second machine (the one used in this study) was more sensitive than the original spectrofluorometer at detecting deposited protein. This meant that a given standard protein solution (or a protein deposit on a contact lens) would give a larger peak on a fluorescence spectrum recorded on the second machine than would be seen on a spectrum recorded on the first spectrofluorometer. No significant difference was observed between the sensitivity with which the two machines detected lipid deposits.

Therefore the newer second spectrofluorometer will show a larger deposition peak than the original machine for the same amount of protein deposition. Theoretically it would be possible to work out a conversion factor to enable results from the two machines could be compared numerically. However, in this study only the amounts of protein or lipid being deposited on a lens surface (rather than actual quantities) were being analysed. Both machines enabled differentiations between high, medium or low levels of deposition to be made with extreme sensitivity. Therefore it was felt unnecessary to calculate an exact conversion factor. The numerical values of the peak heights measured from the fluorescence spectra of both machines cannot be directly compared, but as long as it is

remembered that the measured protein peak heights of spectra recorded on the second spectrofluorometer will be proportionately larger than those recorded on the first spectrofluorometer, then it is valid to compare results from the two machines.

7.3.4 Aims of the study.

In this study a comparison was made between fluorescence spectroscopy results obtained from a strictly controlled *in vivo* wear programme, and fluorescence spectroscopy results from an *in vitro* study using the same lens types, and the same cleaning and disinfection regimes (i.e surfactant clean followed by either chlorine based or hydrogen peroxide based disinfection). This will yield valuable information as to how efficiently the *in vitro* spoilation model is able to mimic the early stages of *in vivo* contact lens wear. In addition, the *in vitro* study will give further information about the effect of surfactant cleaning on deposit formation. It should be possible to determine the effect cleaning had on the cumulative deposition levels over a simulated longer wear period, and also to determine the ability of a single surfactant clean to reduce the level of deposition.

7.4 Fluorescence spectroscopy results and discussion.

The fluorescence spectroscopy results from the *in vitro* spoilation are shown in Tables 11 and 12 and Figures 24 and 25. During the fluorescence analysis it was noticed that on some occasions the measured fluorescence peak heights for both protein and lipid deposition were unexpectedly high. It was felt that this was due to instrumental errors on the day of recording. Obviously these erroneous results

would disrupt the overall pattern of the *in vitro* spoilation. To enable valid comparisons to be made between the *in vivo* and *in vitro* studies it was decided to aggregate the results for each of the lens types throughout the spoilation period being studied (14 days for Acuvue and 21 days for Seequence).

Therefore, there are just two sets of results for the protein and lipid deposition levels on the contact lenses; those recorded before cleaning and disinfection, and those recorded after cleaning and disinfection. The results from the fluorescence spectra that showed unusually high recorded peak heights were discarded. An estimation of the correct peak height was made from the peak heights of the fluorescence spectra that were recorded on the days before and after the inaccurate spectra.

The aggregated lipid deposition levels from lenses excited at 360nm are given in Table 11 and Figure 24. Table 12 shows the aggregated protein and lipid deposition levels from the lenses excited at 280nm, and Figure 25 shows the aggregated protein deposition levels. The length of time that lenses had been in the Tear Model is indicated in the "Level of spoilation" column of the results tables. Here, the period of spoilation is demonstrated numerically; a level of 1 represents the first set of fluorescence spectra, these are recorded two days after *in vitro* spoilation is started. Level 6 represents the last set of fluorescence spectra being studied here. These were recorded 21 days after spoilation was started.

The unspoilt lenses are given the same fluorescence peak height before and after cleaning (the after cleaning peak heights being given in brackets). This is because cleaning and disinfecting an unworn lens will not affect the baseline peak height of the lens.

Table 11a Aggregated lipid deposition levels (mm above baseline) of *in vitro* spoilt Seequence lenses throughout 21 day spoilation period. Excitation wavelength 360nm.

Level of Spoilation	Aggregated peak height (mm above baseline $\pm$0.5) A	B
Unspoilt	5	(5)
1	12	10
2	20	15
3	30	14
4	35	16
5	40	15
6	43	17

Key:- **A**, before cleaning; **B**, after cleaning
Level of spoilation column indicates the length of time the lenses had been in the spoilation model; 1= 3 days, 2= 7 days, 3= 10 days, 4= 14 days, 5= 17 days, 6= 21 days.

Table 11b Aggregated lipid deposition levels (mm above baseline) of *in vitro* spoilt Acuvue lenses throughout 14 day spoilation period. Excitation wavelength 360nm.

Level of Spoilation	Aggregated peak height (mm above baseline $\pm$0.5) A	B
Unspoilt	4	(4)
1	10	5
2	23	6
3	22	5
4	33	10

Key:- **A**, before cleaning; **B**, after cleaning
Level of spoilation column indicates the length of time the lenses had been in the spoilation model; 1= 3 days, 2= 7 days, 3= 10 days, 4= 14 days.

Table 12a Aggregated protein and lipid deposition levels (mm above baseline) of *in vitro* spoilt Seequence lenses throughout 21 day spoilation period. Excitation wavelength 280nm.

Level of Spoilation	Aggregated peak height (mm above baseline ±0.5)			
	A	**B**	**A**	**B**
	Protein		Lipid	
_Unspoilt	12	(12)	8	(8)
1	43	26	17	10
2	83	27	22	21
3	120	27	25	20
4	150	47	26	18
5	180	60	26	15
6	200	74	31	19

Key:- **A**, before cleaning; **B**, after cleaning
Level of spoilation column indicates the length of time the lenses had been in the spoilation model; 1= 3 days, 2= 7 days, 3= 10 days, 4= 14 days, 5= 17 days, 6= 21 days.

Table 12b Aggregated protein and lipid deposition levels (mm above baseline) of *in vitro* spoilt Acuvue lenses throughout 14 day spoilation period. Excitation wavelength 280nm.

Level of Spoilation	Aggregated peak height (mm above baseline ±0.5)			
	A	**B**	**A**	**B**
	Protein		Lipid	
_Unspoilt	9	(9)	3	(3)
1	81	40	18	7
2	245	200	18	15
3	291	220	23	13
4	429	237	28	14

Key:- **A**, before cleaning; **B**, after cleaning
Level of spoilation column indicates the length of time the lenses had been in the spoilation model; 1= 3 days, 2= 7 days, 3= 10 days, 4= 14 days.

Fig. 24a Aggregated lipid deposition levels of *in vitro* spoilt Seequence lenses; 21 days spoilation period. Excitation wavelength 360nm.

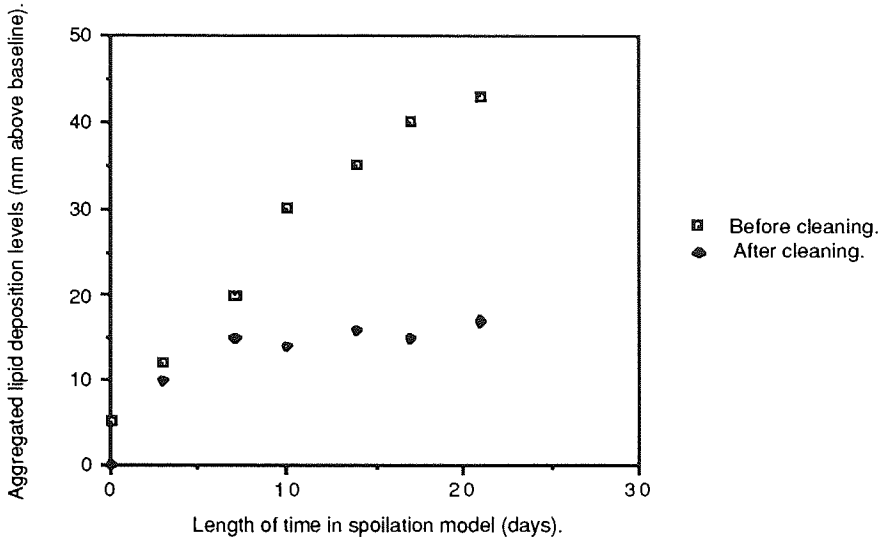


Fig. 24b Aggregated lipid deposition levels on *in vitro* spoilt Acuvue lenses; 14 days spoilation period. Excitation wavelength 360nm.

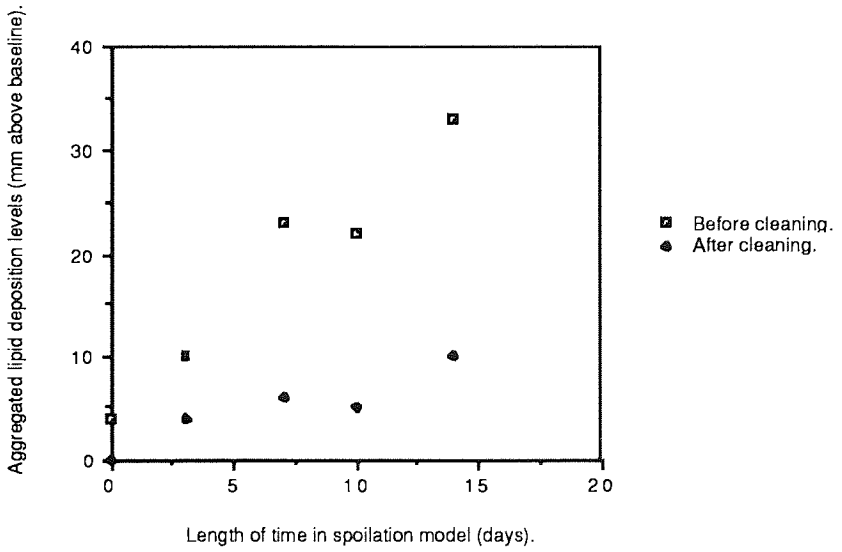


Fig. 25a Aggregated protein deposition levels of *in vitro* spoilt Seequence lenses; 21 days spoilation period. Excitation wavelength 280nm.

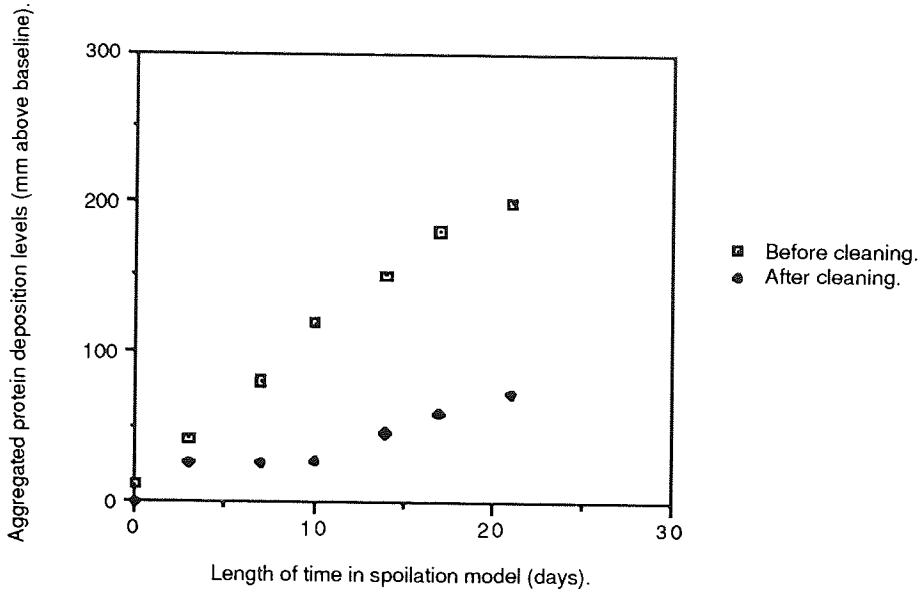
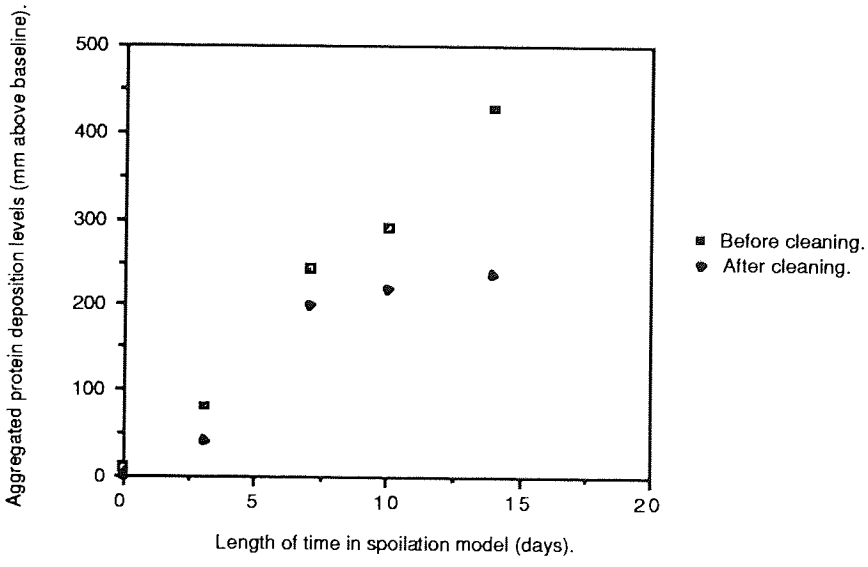


Fig. 25b Aggregated protein deposition levels of *in vitro* spoilt Acuvue lenses; 14 days spoilation period. Excitation wavelength 280nm.



7.4.1 Deposition levels on Acuvue and Seequence.

Looking at the gross deposition levels from the aggregated fluorescence results, it is clear that there is little difference in the amounts of deposited lipid between the *in vitro* spoilt Acuvue and Seequence lenses. As stated earlier, *in vitro* spoilation using the method developed at Aston University, gives accelerated spoilation in comparison to conventional wear times. An *in vitro* spoilation period of 28 days is equivalent to about 6 months *in vivo* daily wear. In this study the Seequence lenses were spoilt for 21 days. This period will equate roughly to just over four months *in vivo* daily wear. Similarly the 14 day period of spoilation for the Acuvue lenses in this study is equivalent to roughly 3 months *in vivo* daily wear.

Therefore, this *in vitro* study demonstrates that, during the first few months of "wear" there is little difference between the lipid deposition levels of Acuvue and Seequence lenses. These findings are in agreement with other gross deposition studies which indicate that it is only after longer wear periods that HEMA lenses (Seequence) start to deposit lipid heavily. However, the results are in contrast to the previous *in vivo* study (Chapter 6) which indicated that over the fist two to four weeks of wear Seequence lenses deposited lipid to a slightly greater extent than Acuvue lenses.

This discrepancy will have occurred because although the *in vitro* model mimics in eye lens wear as closely as possible, several factors such as pressure exerted on the lens during blinking and evaporation from the lens surface will not be reproduced in an *in vitro* spoilation situation. These factors will all contribute to lipid deposits forming on an *in vivo* worn contact lens surface. Thus, the absence

of these influences in the *in vitro* model will affect the results obtained, particularly when comparisons are made with the very early stages of in eye lens wear.

In contrast, the protein deposition levels from the *in vitro* spoilt Acuvue and Seequence lenses clearly show the greater tendency of the ionic Acuvue lenses to preferentially deposit protein, even during the first week of *in vitro* spoilation. These results compare favourably with the previous *in vivo* study (Chapter 6). Therefore it appears that the formation of protein deposition on an *in vitro* spoilt lens mimics more closely the in eye lens wear situation. This fact is in agreement with previous studies which showed that protein deposition is dependent on the contact lens material, rather than patient influenced factors, and therefore will not be so affected by the difference in conditions between the *in vitro* spoilation model and *in vivo* lens wear.

7.4.2 Effect of surfactant cleaning on gross deposition levels.

Looking at the effect of surfactant cleaning on lipid deposition levels it is clear that following surfactant cleaning, the amount of lipid deposited on the lens surface was substantially reduced on both the Seequence and Acuvue lenses, throughout the *in vitro* spoilation period. However, surfactant cleaning was shown to be only partially effective at removing lipoidal material from the lens surface. It is also interesting to note that surfactant cleaning was more effective at reducing lipid deposition levels on both lens types in the later stages of the *in vitro* spoilation. This result can be compared to earlier *in vivo* studies which showed that surfactant cleaning had little effect on lipid deposition levels in the early stages of wear, but that lens cleaning is important in the later stages of lens wear, particularly in

minimising the immobilisation of reactive lipids on the lens surface.

In addition to decreased levels of lipid deposition both lens types showed lower gross protein deposition levels following surfactant cleaning. This probably occurred as a result of the mechanical effects of digital cleaning of the contact lens. In addition, surfactants themselves can be used to remove protein deposited on a contact lens surface e.g. SDS extraction of contact lenses before electrophoresis. However, SDS extraction of protein from lenses requires much more severe experimental conditions than those encountered during surfactant cleaning of contact lenses. Therefore, in the lens cleaning situation, the ability of a surfactant to remove protein from a lens surface is only slight.

7.4.3 Effect of different disinfection regimes on deposition levels.

Throughout this study two different surfactant cleaners and disinfection systems were used. The Acuvue and Seequence lenses were both cleaned with products recommended by their respective lens Manufacturers i.e. a surfactant cleaner (Pliagel) and chlorine based disinfection system (Softab) for Acuvue, and a surfactant cleaner (LC-65) and hydrogen peroxide based disinfection system (Oxysept) for Seequence. Both these systems were used with both lens types in order to determine whether the recommended cleaning products were in fact better at removing deposits from one lens type than the other.

To determine the effect of the different disinfection regimes it is necessary to look at the original fluorescence data from lenses programmed through the *in vitro* spoilation and cleaning regimes. These results are shown in Figures 26 and 27.

The lenses are numbered as follows:-

Lens 1. Acuvue control. (No surfactant clean or disinfection).

Lens 2. Acuvue, chlorine based disinfection. (Surfactant clean with Alcon Pliagel, disinfect with Alcon Softab).

Lens 3. Acuvue, hydrogen peroxide based disinfection. (Surfactant clean with Allergan LC-65, disinfect with Allergan Oxysept).

Lens 4. Seequence control. (No surfactant clean or disinfection).

Lens 5. Seequence, chlorine based disinfection. (Surfactant clean with Alcon Pliagel, disinfect with Alcon Softab).

Lens 6. Seequence, hydrogen peroxide based disinfection. (Surfactant clean with Allergan LC-65, disinfect with Allergan Oxysept).

The spoilation period was designated by a combined Arabic/Roman numbering system. This indicates period of spoilation and, whether the lenses were uncleaned, or cleaned and disinfected i.e.:-

Level of spoilation

1i	Spoilation period 3 days, uncleaned,
1ii	Spoilation period 4 days, cleaned and disinfected,
2i	Spoilation period 7 days, uncleaned,
2ii	Spoilation period 8 days, cleaned and disinfected,
3i	Spoilation period 11 days, uncleaned,
3ii	Spoilation period 12 days, cleaned and disinfected, etc.

Fig. 26a Showing lipid deposition levels of *in vitro* spoilt Acuvue lenses; excitation wavelength 360nm; i= before cleaning and disinfection, ii= after cleaning and disinfection.

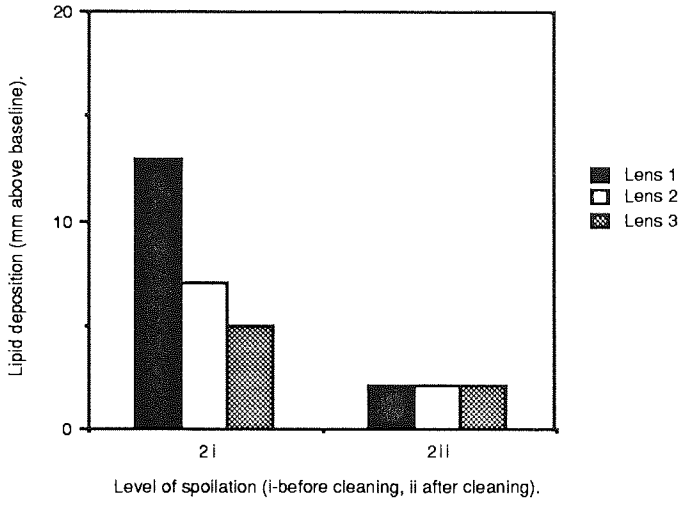


Fig. 26b Showing lipid deposition levels of *in vitro* spoilt Seequence lenses; excitation wavelength 360nm; i= before cleaning and disinfection, ii= after cleaning cleaning and disinfection.

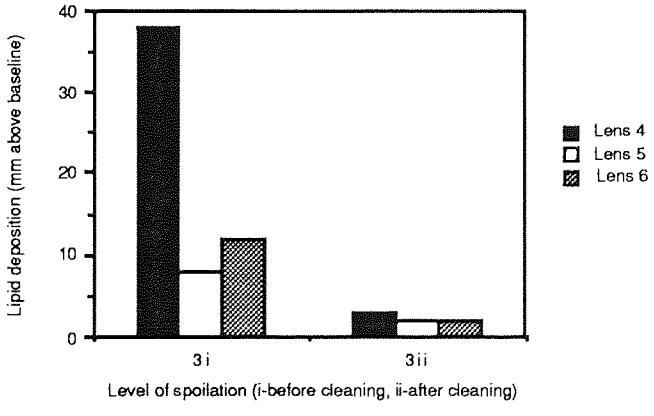


Fig. 27a Showing protein deposition levels of *in vitro* spoilt Acuvue lenses; excitation wavelength 280nm; i= before cleaning and disinfection, ii= after cleaning and disinfection.

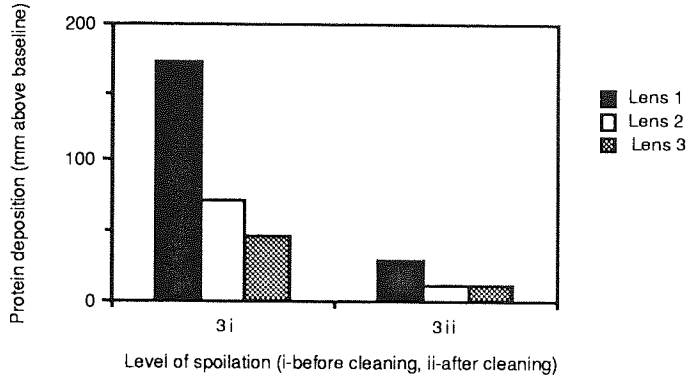
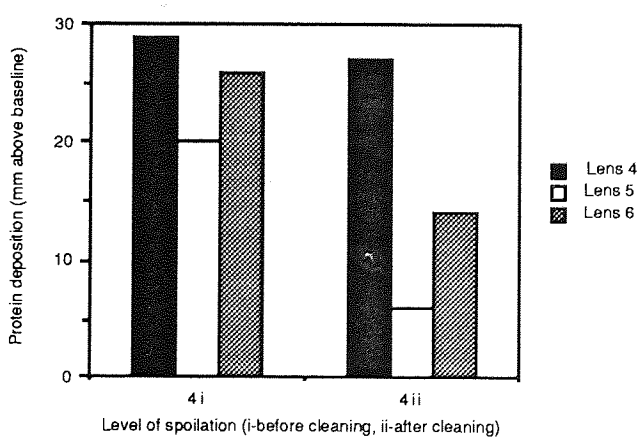


Fig. 27b Showing protein deposition levels of *in vitro* spoilt Seequence lenses; excitation wavelength 280nm; i= before cleaning and disinfection, ii= after cleaning and disinfection.



To minimise lack of correlation arising from the need to standardise peak heights when comparing sets of data, results were chosen from those data sets which were recorded on days when no innacuracies were detected in the working of the spectrofluorometer. This enables the effect of different care regimes to be compared. Therefore, to compare the effect of the different disinfection regimes on deposition levels it was decided to just use one set of results of protein and lipid deposition levels from both lens types. When selecting these results it was important to choose from those obtained as late as possible in the *in vitro* spoilation. This ensures that the *in vitro* spoilt lenses have fairly high levels of surface deposition. It was also important to select results that were recorded before the Acuvue lenses started to tear. The results chosen from Figures 26 and 27 are as follows:-

Lipid deposition levels on *in vitro* spoilt Acuvue lenses. From Figure 26a the results chosen were those labelled 2i and 2ii. These were recorded 7 and 8 days respectively after the start of *in vitro* spoilation.

Lipid deposition levels on *in vitro* spoilt Seequence lenses. From Figure 26b the results chosen were those labelled 3i and 3ii. These were recorded 10 and 11 days respectively after the start of *in vitro* spoilation.

Protein deposition levels on *in vitro* spoilt Acuvue lenses. From Figure 27a the results chosen were those labelled 3i and 3ii. These were recorded 10 and 11 days respectively after the start of *in vitro* spoilation.

Protein deposition levels on *in vitro* spoilt Seequence lenses. From Figure 27b the results chosen were those labelled 4i and 4ii. These were recorded 14 and 15 days

respectively after the start of *in vitro* spoilation.

The first point to make from these results is that, as expected in each case the control lenses (lens 1 and lens 4 in Figures 26 and 27) showed higher levels of protein and lipid deposition before cleaning and disinfection. After overnight storage in saline the control lenses did not have deposition levels lower than the cleaned and disinfected lenses, but, with the exception of protein deposition on Seequence lenses they did have substantially reduced levels of protein and lipid deposition.

This probably arose (particularly for deposited protein) as a result of the deposited layer diffusing off the lens surface during overnight storage. With respect to protein deposition levels this fact is in agreement with other studies which showed that protein deposition levels can change dramatically following lens storage (see Chapter 6-Deposition Trends of Group 1 and Group 4 lenses). This is particularly true for high water content ionic materials such as Etafilcon A. Previous studies have also indicated that, as seen in this *in vitro* investigation protein deposition levels of non-ionic low water content contact lenses (e.g. Seequence) are not substantially reduced following storage.

However, it has been observed that lipid deposition levels generally remain constant following a period of lens storage. These results were obtained from *in vivo* worn contact lenses. In this study the lenses were spoilt by an *in vitro* method. The method of *in vitro* spoilation is very intense i.e. lenses are spoilt very rapidly and heavily, so it is probable that the lipid deposited on the contact lens surface will not be as tightly bound as material that has been deposited on a lens

surface following several months of *in vivo* wear. So, it can be seen that lipid deposited on an *in vitro* spoilt lens will be able to either diffuse off, or fall off the lens surface during storage much more easily than lipid deposited on an *in vivo* worn contact lens.

It is interesting to look more closely at the protein deposition levels on the control lenses before and after storage (Figure 27a, 3i and 3ii for Acuvue; Figure 27b, 4i and 4ii for Seequence). As expected the Acuvue lenses, which are known to deposit protein very heavily (particularly lysosyme), had very high levels of protein deposition before overnight storage. After overnight storage, the protein deposition level had dropped substantially. This is due to lysosyme diffusing off the lens surface into the storage solution. In contrast, the *in vitro* spoilt Seequence lenses had low levels of protein deposition before overnight storage, and only a slight reduction in the level of protein deposition. This demonstrates clearly that Seequence lenses do not have a great tendency to deposit protein heavily. It also demonstrates that the protein that does deposit on the lens surface is fairly tightly bound, as the level of deposition is not substantially reduced following overnight storage.

Looking at the effect of the different disinfection methods (chlorine based compared to hydrogen peroxide based) on protein and lipid deposition levels it is clear that the lenses disinfected with a chlorine based product (Lens 2 and Lens 5) had similar levels of protein and lipid after disinfection to the lenses disinfected with a hydrogen peroxide based product (Lens 5 and Lens 6). Therefore it appears that particularly during the first few months of lens wear the method of disinfection will not affect lens deposition levels.

The method of disinfection can affect deposition levels in the later stages of wear. The active chemical constituents of the disinfection system itself will react with material deposited on and within the contact lens (particularly when a chlorine based disinfectant is used over long wear periods). This is especially true for deposited protein. This reaction of the disinfection system with the material deposited on the contact lens can lead to chemical changes of the deposited species which causes them to be irreversibly bound to the contact lens material. This can then lead to problems of increased deposition levels on the contact lens which cannot be removed or reduced by either surfactant cleaning of enzyme tablets. However, in this study the lenses were also surfactant cleaned, and this surfactant cleaning in combination with the low levels of deposition seen meant that the method of disinfection did not affect the protein and lipid deposition levels.

7.4.4 Comparison of *in vitro* and *in vivo* results.

One aim of this study had been to compare the results obtained from this *in vitro* study with the *in vivo* study described in Chapter 6. The *in vivo* analysis had highlighted differences between the deposition profiles of the two lens types. The ionic high water content Acuvue lenses showed high levels of protein deposition and low levels of lipid deposition. In contrast the non-ionic low water content Seequence lenses showed higher levels of lipid deposition and low levels of protein deposition. Looking at the gross deposition levels of the *in vitro* spoilt lenses, Figures 24 and 25 show that the *in vitro* spoilt lenses have the same deposition profiles as the *in vivo* lenses i.e high protein and low lipid deposition for Acuvue and high lipid and low protein deposition for Seequence.

This demonstrates that the method of *in vitro* lens spoilation used here is able to produce spoilation profiles similar to those seen with *in vivo* lens wear. It is also clear that this laboratory based spoilation method in combination with fluorescence analysis could be used to formulate a spoilation profile for any existing lens material (or of novel lens materials intended for contact lens use) as a preliminary screening before selecting candidates for a clinical study.

Another aim of this study was to compare the levels of deposition seen on the *in vivo* worn lenses with the deposition levels on the *in vitro* spoilt Acuvue lenses over a similar wear period. In the *in vivo* study, the Seequence lenses were worn for a period of four weeks . This equates to about 5 days in the *in vitro* spoilation model. Therefore, roughly the first two sets of fluorescence measurements taken in the *in vitro* study will correspond to the *in vivo* wear period in Chapter 6. The Acuvue lenses in the *in vivo* study were worn for two weeks. This equates to roughly 2 to 3 days in the *in vitro* spoilation model, so the first set of fluorescence measurements in the *in vitro* study will correspond to the *in vivo* wear period. Interestingly this corresponds with the earlier observation that 28 days in the *in vitro* spoilation model compares to approximately 6 months *in vivo* lens wear.

To compare the results from the *in vivo* and the *in vitro* study it is first necessary to subtract the fluorescence peak heights measured in each study of the unworn Seequence and Acuvue lenses. This will give results of only the protein and lipid deposition levels on the lens surface and thus eliminate any discrepancies caused by varying peak heights obtained from the unworn lenses. From the *in vitro* study the set of results obtained before lens cleaning and disinfection will be used. This is because on the day of collection the *in vivo* lenses were returned in

an uncleaned and disinfected condition.

Following subtraction of the unworn lens peak heights from the results, an average increase of 1.5mm for lipid deposition levels and 15mm for protein deposition levels is seen on the *in vivo* Acuvue lenses over the two week wear period. The corresponding results from the *in vitro* study are an increase of 6mm for gross lipid deposition levels, and 72mm for gross protein deposition levels. For the *in vivo* Seequence lenses an average increase of 3mm for lipid deposition levels, and 1mm for protein deposition levels for the four week wear period were seen. The corresponding results from the *in vitro* study are an increase of 11mm for gross lipid deposition levels, and 50mm for gross protein deposition levels.

Because the fluorescence results from the *in vivo* and *in vitro* studies were recorded on two separate spectrofluorometers a direct comparison cannot be made between the two sets of results. However, it is interesting to note that in general the fluorescence peak heights recorded in the *in vitro* study are about four times larger than the corresponding results obtained in the *in vivo* study. Therefore, although the results from these two studies are not directly comparable it can be seen, that provided the inconsistencies observed with the spectrofluorometer used in this chapter could be resolved, then the information from these *in vitro* and *in vitro* studies could be used, in combination with other experiments, to help determine a correlation factor. This would enable a direct comparison to be made between the two spectrofluorometers.

7.4.5 Effect of surfactant cleaning and disinfection on *in vitro* spoilation method.

Previous studies performed by other workers in the Group at Aston using this *in vitro* spoilation method have generally shown a steady increase in deposition levels throughout the spoilation period. These studies did not involve regular lens cleaning throughout the *in vitro* spoilation period. It appears that the cleaning and disinfection processes used in this study gave rise to inconsistent results. This probably arose as a result of the intensive conditions in the study (both cleaning, disinfection and spoilation) which caused both lens types to tear, and in the case of Acuvue, fall apart. Tears will change the refractive index of the contact lens, thus affecting the fluorescence spectroscopy results.

7.4.6 Lysosyme migration in Acuvue lenses.

A final interesting observation from this study is the fact that initial analysis if the fluorescence spectra indicate that some lenses appeared to show higher levels of deposition after cleaning and disinfection, (these increases are not apparent in the results tables due to the aggregation of fluorescence peak heights). The increases in protein deposition levels were only seen to a significant degree on the *in vitro* spoilt Acuvue lenses. The fact that this phenomenon was not seen consistently throughout the study, or with the protein deposition levels on Seequence lenses indicates that it does not occur as a consequence of the cleaning and disinfection process. Again, because this phenomenon was only really observed with the Acuvue lenses implies that it is unique to this particular lens material (EtafilconA).

Etafilcon A is an ionic high water content material. It is known that this material readily deposits high levels of protein. Involved in this deposition process is the migration of small protein species i.e. lysosyme, into the lens matrix. It is the ionic nature of the lens material and the small size of the protein lysosyme which enables this migration to occur. Considerable amounts of lysosyme can be taken up into the Etafilcon A matrix.

As stated in Chapter 3, fluorescence spectroscopy will only detect material deposited on the lens surface, so any lysosyme within the lens matrix itself will not be detected. It is also worth pointing out that fluorescence studies using dilute solutions of individual proteins show that of the tear proteins likely to be deposited on a lens surface (e.g albumin, globulins, lactoferrin and lysosyme), lysosyme gives a much greater emission peak when excited at 280nm than most other proteins.

The fact that such large increases are seen in the fluorescence peak height after cleaning and disinfection indicates that migration of lysosyme is responsible. If the increase was to be attributed to another protein species then a very large amount of e.g. albumin would be required to produce the large increases in peak height that were seen. This seems unlikely, particularly as most of the other protein species deposited during contact lens wear are too large to migrate into the lens matrix. It therefore appears that this phenomenon of increased protein deposition levels seen on Acuvue lenses after cleaning and disinfection is due to lysosyme leaching from the lens matrix to the surface of the contact lens. Here it would be detectable by fluorescence spectroscopy.

7.4.7 Effect of storage on lysosyme levels in Acuvue lenses.

The lens disinfection process involved overnight storage of the Acuvue lenses in either the disinfection or neutralising solution (the control lenses were stored overnight in saline). It is probable that it was during this period that lysosyme diffused out of the lens matrix into the lens storage solution. This process will take place in two stages; the lysosyme will first diffuse from a high concentration in the lens matrix onto the lens surface, then as the concentration of lysosyme on the lens surface increases, diffusion into the storage solution will start to occur. So, it can be seen that at any one stage during lens storage lysosyme will be diffusing to and from the lens surface; the process is not static. The time of recording of fluorescence spectra, and the amount of lysosyme deposited on and within the contact lens will therefore determine the level of increase seen, if any, after contact lens storage.

7.5 Conclusions.

This phenomenon, although having significant effect on the results obtained in this study cannot be said to have too great a bearing on the previous studies in this project. The increases in peak height after storage are only seen on high water content ionic lenses i.e. Acuvue, and only affect protein deposition levels. In these earlier studies spectra were recorded at the end of a specific wear period, and the period between cessation of wear and lens analysis was kept to a minimum. Thus, when analysis was performed the lenses had all been stored and worn for the same

length of time. This means that any diffusion of lysosyme from the lens matrix would have occurred consistently throughout the lenses to be analysed. Therefore, provided that contact lenses were collected and placed in storage within a short time period, the results obtained would still be representative of the deposition levels seen on Acuvue lenses after the particular wear regime being studied.

However, in Chapter 6 wide variations were seen between the levels of protein deposition detected on Acuvue lens' surfaces. In a previous study (Chapter 4), any variations in protein deposition levels on Acuvue lenses were accompanied by variations in lipid deposition levels. When unusually low levels of protein deposition were detected, these were accompanied by unusually high levels of lipid deposition (the reasons for these observations are discussed in Chapters 4 and 5). Where the expected high levels of protein deposition were seen, these were relatively constant throughout the contact lenses that were analysed.

In contrast to these findings, the study performed in Chapter 6 showed that although protein deposition levels varied widely, lipid deposition remained relatively low and constant. This implies that some lenses had been stored for longer periods than others. It is possible that even the fact that the worn lenses were collected from patients and placed in storage over a one day period, may have influenced the final detected levels of deposited protein on the Acuvue lens surfaces. Therefore it is clear that extreme care must be taken with regard to the storage and fluorescence analysis of ionic high water content contact lenses.

At the start of this project there was no understanding of lysosyme migration to and from ionic high water contact lenses, therefore the next section of this project

involved further investigation of this phenomenon. This will be discussed in the following chapter.

8. Lysosyme migration in Sureview lenses.

8.1 Introduction.

At the outset of this project little was known about the phenomenon of lysosyme migration to and from high water content ionic lenses. The previous chapters had highlighted the problem. Therefore, this study was set up to further investigate this phenomenon of lysosyme migration.

On several occasions throughout the *in vitro* study conducted in Chapter 7 increases were seen in protein deposition levels on Acuvue lenses following overnight storage. Also, quite large fluctuations were observed in protein deposition levels throughout the 14 day *in vitro* spoilation period. These large fluctuations were also observed with the *in vivo* worn Acuvue lenses analysed in Chapter 6. It was felt that these increases were due to lysosyme diffusing from a high concentration in the lens matrix onto the lens surface. Then, as the concentration of lysosyme on the lens surface increases, lysosyme will start to diffuse into the storage solution. These processes will result in the large fluctuations seen in the recorded levels of protein deposition on the Acuvue lens surfaces.

8.1.1 Aim of the study.

The aim of this study therefore, was to examine this pattern of variations in protein deposition levels. This was done by analysing variations in protein and lipid deposition levels on *in vivo* spoilt contact lenses. In the previous investigation (Chapter 7) migration of lysosyme was only observed to any great extent with lenses fabricated from Etafilcon A (Acuvue). Therefore it was important to use lenses manufactured from the same contact lens material in this study. In addition, as the *in vitro* spoilation method gives accelerated spoilation in comparison to conventional wear times it was felt that a longer wear period was required than the two weeks daily wear normally recommended for Acuvue lenses.

Because handling problems had been encountered with Acuvue lenses after two weeks spoilation in the *in vitro* model it was decided not to use Acuvue lenses in this study, but instead to use Vistakon Sureview lenses. These lenses are also made from Etafilcon A. They are intended to be worn as a disposable lens, but with a recommended wear period of four weeks daily wear. Consequently the lenses are slightly thicker and easier to handle than Acuvue lenses. Because both lenses are manufactured from the same material they can be expected to have similar deposition profiles. Therefore, you would expect that Sureview lenses, like Acuvue lenses, would show rapid and heavy levels of protein deposition accompanied by low levels of lipid deposition.

8.1.2 Lens type used in the study.

One pair of Vistakon Sureview lenses were used in this study. They had been worn on a daily wear basis for a total of 11 weeks. During this time the lenses were not surfactant cleaned but were disinfected with a hydrogen peroxide based disinfection system. This was Ciba 10.10. The lenses were collected at the end of the 11 week wear period and placed in a sterile container containing 3ml of non-preserved saline. They were then sent to me at Aston for analysis.

8.2 Fluorescence analysis.

To examine the pattern of variations in protein deposition levels thoroughly it was decided to record fluorescence spectra from the Sureview lenses over a period of time. The day on which the lenses arrived at Aston University was designated as day one of the study. Upon receiving the lenses, fluorescence spectra were recorded (as soon as was possible) at 360nm and 280nm for each contact lens. To monitor any diffusion of lysosyme from the Sureview lenses during transit, the saline in which the lenses had been stored was also analysed at 360nm and 280nm. The recording of these sets of fluorescence spectra represented the start of the study. The spectra were recorded three days after receiving the Sureview lenses. Therefore, the first set of results (i.e. the initial fluorescence spectra recorded 3 days after receiving the lenses) are described as having been recorded after a 3 day period of lens storage. Obviously this discounts the period of contact lens storage before the lenses arrived at Aston. However, for the sake of

experimental consistency (fluorescence spectra were to be recorded twice weekly over a five week period) it was felt that it was valid to start the study once the lenses had arrived at Aston.

After this initial analysis the lenses were placed in a clean contact lens container, the lens holders of which were labelled 1 and 2. From this stage on throughout the study the lenses were kept in the numbered lens holder to which they had originally been assigned.

8.2.1 Choice of lens storage solution.

The contact lens case was filled with Bausch and Lomb Renu. This multi-purpose solution can be used to surfactant clean, disinfect and store lenses. It contains a quarternary ammonium compound which gives antibacterial activity. It was hoped that storing the lenses in Renu throughout the study would help prevent any bacterial contamination of the contact lenses occurring. Another advantage of using this solution is that it does not become acidic with time, therefore no lens shrinkage (and hence loss of protein at the lens surface) will occur. One problem involved with the use of Renu as a lens storage solution is the fact that as stated earlier, Renu can also be used to surfactant clean contact lenses. Therefore, to minimise any loss of lipid (or protein) from the lens surface extreme care was taken to ensure, that as far as was possible, no agitation of the contact lenses occurred whilst they were in the Renu storage solution.

However, before using Renu in this study it was important to ensure that the Renu itself would not give rise to any significant fluorescence peaks. This was done by recording fluorescence spectra of the Renu solution at 360nm and

280nm. These recordings did not produce any significantly large peak heights. Spectra were then re-recorded a few days later to ensure that no chemical changes occurred in the Renu over the lens storage period which would themselves give rise to large fluorescence peaks. Again these results showed that nothing in the Renu demonstrated any significant fluorescence. Therefore it was decided that, provided sufficient care was taken, Renu would be suitable for lens storage throughout this study.

8.2.2 Monitoring lysosyme migration in Sureview lenses.

As stated it was decided that the best way to monitor the process of lysosyme diffusion from Etafilcon A lenses was to record fluorescence spectra over a period of time. It was also important to record spectra from the Renu storage solutions at the same time as spectra were recorded from the contact lenses. This would ensure that any diffusion of lysosyme from the lens surface to the storage solution could be detected.

After the initial spectra were recorded (3 days after receiving the contact lenses, i.e. following a three day period of lens storage) the lenses were placed in a contact lens case containing Renu as described. Subsequently spectra were recorded twice weekly from both contact lenses and the Renu storage solution, i.e. after 3 days of lens storage, 7 days of lens storage, 11 days of lens storage etc. Fluorescence spectra were recorded, twice weekly, for a total of 35 days lens storage period. After each set of spectra had been recorded, the contact lens case was filled with fresh Renu, and then stored until the next set of fluorescence

spectra were recorded.

8.3 Fluorescence spectroscopy results and discussion.

The fluorescence spectroscopy results from this study are shown in Tables 13, 14, 15 and 16 and in Figures 28, 29, 30 and 31. Table 13 and Figure 28 show the lipid deposition levels of the Sureview lenses excited at 360nm. Table 14 and Figure 29 show the protein and lipid deposition levels of the Sureview lenses excited at 280nm. Table 15 and Figure 30 show the lipid deposition levels and cumulative lipid deposition levels of the Renu storage solutions (excitation wavelength 360nm). Finally, Table 16 and Figure 31 show the protein deposition levels and cumulative protein deposition levels of the Renu storage solutions (excitation wavelength 280nm).

At this stage it must be remembered that the spectrofluorometer used in this study had shown some inconsistencies in the previous investigation (i.e. exaggerated peak heights). The reasons for these discrepancies are discussed in Chapter 7. Therefore, in this study any unusually high peak heights should be ignored (these will be indicated in the results tables). Where cumulative results are given, the innacurate peak height is ignored, and an average peak value (calculated from the preceding and following sets of results) is added on to the cumulative total.

Because lysosyme migration from the lens surface into the Renu storage solution can cause very high peak heights it is difficult to identify any inconsistencies in the protein deposition levels. Therefore anomalous protein

deposition levels are only highlighted when they clearly appear to be innacurate.

Table 13 Lipid deposition levels on stored Sureview lenses over 5 week period of analysis; excitation wavelength 360nm.

Lens	Period of storage (days)	Peak height above baseline (mm±0.5)
1	3	3
2	3	5
1	7	3
2	7	2
1	10	9*
2	10	2
1	14	4
2	14	4
1	17	7*
2	17	13*
1	21	3
2	21	3
1	24	5
2	24	5
1	28	2
2	28	1
1	31	10*
2	31	8*
1	35	3
2	35	3

Key: *; these peak heights appear unusually high and arise from spectrofluorometer inconsistencies, therefore they should be ignored.

Table 14 Protein and lipid deposition levels on stored Sureview lenses over 5 week period of analysis; excitation wavelength 280nm.

Lens	Period of storage (days)	Peak height above baseline (mm±0.5)	
		Protein	Lipid
1	3	85	4
2	3	164*	5
1	7	100	3
2	7	145*	4
1	10	53	2
2	10	78	2
1	14	158*	4
2	14	44	5
1	17	47	6
2	17	22	8
1	21	42	3
2	21	12	5
1	24	30	4
2	24	13	8
1	28	29	2
2	28	20	2
1	31	152*	10*
2	31	159*	10*
1	35	55	5
2	35	160*	5

Key: *; these peak heights appear unusually high and arise from spectrofluorometer inconsistencies, therefore they should be ignored.

Table 15 Lipid deposition levels and cumulative lipid deposition levels in Renu storage solutions, over 5 week period of analysis; excitation wavelength 360nm.

Number of Renu storage solution	Period of storage (days)	Peak height above baseline (mm±0.5)	
		Individual	Cumulative
1	3	3	3
2	7	4	7
3	10	1	8
4	14	3	11
5	17	12*	13
6	21	16*	15
7	24	1	16
8	28	10*	19
9	31	6	25
10	35	3	28

Key: *; these peak heights appear unusually high and arise from spectrofluorometer inconsistencies, therefore they should be ignored.

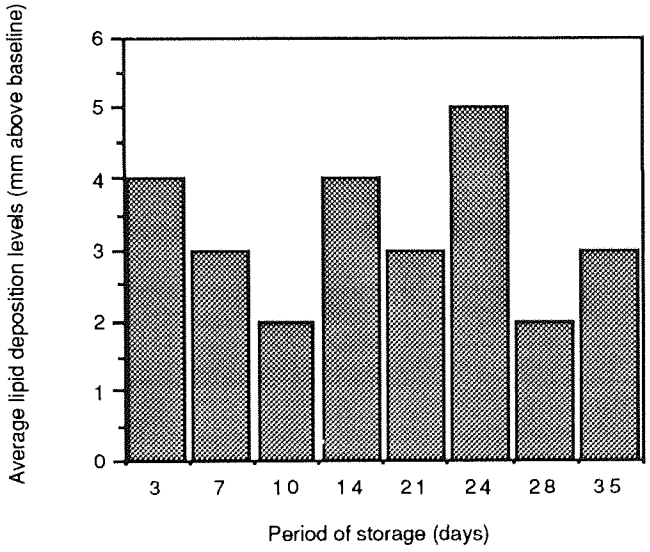
Table 16 Protein deposition levels and cumulative lipid deposition levels in Renu storage storage solutions, over 5 week period of analysis; excitation wavelength 280nm.

Number of Renu storage solution	Period of storage (days)	Peak height above baseline (mm±0.5)	
		Individual	Cumulative
1	3	164*	42
2	7	84	126
3	10	52	178
4	14	67	245
5	17	104	349
6	21	125	474
7	24	101	575
8	28	30	605
9	31	85	690
10	35	16	706

The results are shown graphically in Figures 28, 29, 30 and 31. Figure 28 shows the variations in lipid deposition over the five week study period. Figure 29 shows the variations in protein deposition levels throughout the study period. Figure 30 shows the cumulative lipid deposition levels of the Renu storage solutions, and Figure 31 shows the cumulative protein deposition levels of the Renu storage solutions over the five week study period. For the graphical

representation of the results any unusually high peak heights will be ignored. Also, an average peak height value from the combined deposition levels (protein or lipid) of Lens 1 and Lens 2 will be used in Figures 28 and 29. This should help to give a better overall impression of the results, and minimise any errors due to spectrofluorometer inconsistencies.

Fig. 28 Lipid deposition levels of stored Sureview lenses over five week study period; excitation wavelength 360nm.



8.3.1 Lipid deposition levels on stored Sureview lenses.

Fluorescence spectra were recorded at 360nm as well as 280nm to verify that, as in the *in vitro* study (Chapter 7), no significant variations in lipid deposition levels were seen during lens storage. Figure 28 shows that the average lipid deposition levels did vary throughout the five week study period, but not to any great extent. The averaged fluorescence peak heights only varied slightly; from 2mm above baseline to 5mm above baseline, with an average value of 3.25mm above baseline.

It is possible that these variations occurred as a result of slight diffusion of the lipid layer deposited on the contact lens surface into the Renu storage solution. The lipid layer that is deposited on the contact lens surface is composed of many different classes of lipid. Of these, some will be water soluble. Therefore it is probable that the majority of the lipids diffusing of the surface of the stored Sureview lenses will be water soluble lipids.

However, it must be remembered that the lens storage solution used, Renu, is a multi-purpose solution and therefore can also be used to surfactant clean lenses. So, it is also possible that there was a slight reduction of the lipid layer due to the surfactant properties of Renu. This effect would only have been very small though, because there was very little agitation of the lenses whilst they were being stored in Renu.

The variations in lipid deposition levels could, on some instances have arisen due to either accidental touching of the lens during analysis, or because the cuvette used for the fluorescence was not completely clean. These facts highlight the need

for extreme care whilst performing fluorescence spectroscopy of contact lenses.

Fig. 29 Protein deposition levels of stored Sureview lenses over five week study period; excitation wavelength 280nm.

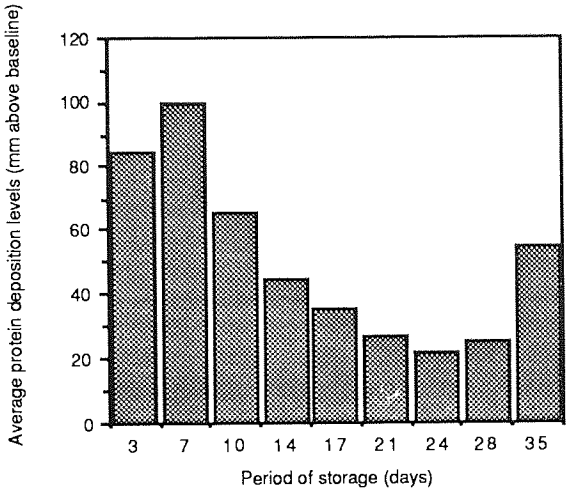


Fig. 30 Cumulative lipid deposition levels in Renu storage solution over five week study period; excitation wavelength 360nm.

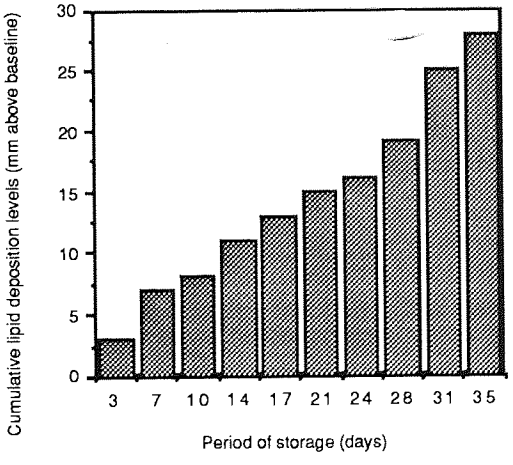
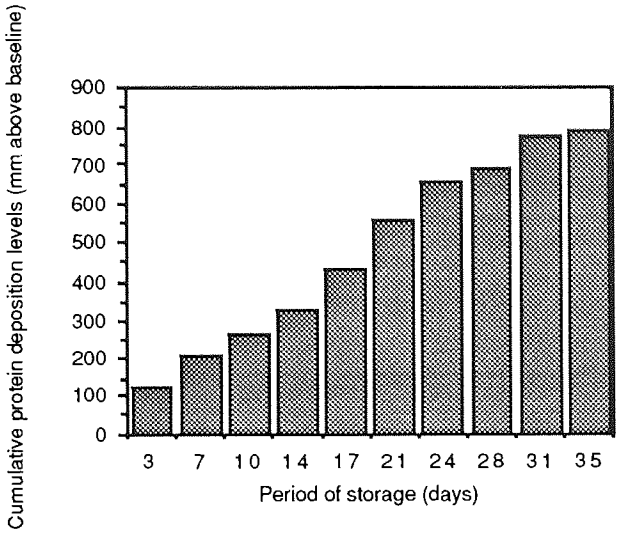


Fig. 31 Cumulative protein deposition levels in Renu storage solution over five week study period; excitation wavelength 280nm.



8.3.2 Protein deposition levels on stored Sureview lenses.

In contrast to the lipid deposition levels, a wide variation was seen between the levels of protein detected on the Sureview lenses throughout the study (Figure 29). Over the period of five weeks the minimum averaged level of protein detected was 22mm above baseline. The maximum level was 100mm, with an average peak height of 55mm above baseline throughout the study.

The undulating pattern of deposition levels observed in the *in vitro* spoilation study in Chapter 7, and with the Acuvue deposition levels in Chapter 6, is clearly shown in Figure 29. Here, the amounts of protein detected on the lenses can be seen to rise and fall with time. In the first week of the study the amounts of

protein detected were high. Early in the second week they started falling, and continued to fall throughout the second, third and fourth week of analysis. A minimum level of surface protein deposition was achieved at the end of the fourth week of study. The levels of surface deposition detected on the Sureview lenses then started to build up again in the fifth and final week of study. These results indicate that lysosyme migration was occurring constantly throughout the investigation.

8.3.3 Cumulative lipid deposition levels in Renu storage solutions.

Figure 30 shows that the lipid deposition levels in the Renu storage solutions rose slowly and steadily throughout the five week study period. This indicates that during each period of storage there was a small amount of diffusion occurring between the lipid deposited on the contact lens surface, and the lens storage solution.

The majority of the lipid species diffusing off the surface of the stored Sureview lenses will probably be water soluble lipids. However, it must also be remembered that as stated earlier, the lens storage solution used (Renu) is a multi-purpose solution and therefore can also be used to surfactant clean lenses. So, it is possible that some of the lipid detected in the lens storage solution could have arisen from a slight reduction of the lipid layer on the lens surface due to the surfactant action of Renu. This effect would only have been small though, because there was very little agitation of the lenses whilst they were being stored in Renu.

It is interesting to compare the extremely low level of cumulative lipid detected in the Renu storage solutions with the much higher level of cumulative protein. After the five week study period, the cumulative level of lipid in the Renu storage solutions was only 28mm above baseline. This compares to a cumulative total of 706mm above baseline for protein detected in the Renu storage solutions. This again highlights the fact that there is very little reduction in the deposited lipid layer during lens storage, but that there can be a substantial amount of protein diffusing from a stored ionic high water content contact lens into the lens storage solution.

8.3.4 Cumulative protein deposition levels in Renu storage solutions.

Figure 31 shows that there was a steady rise in cumulative protein deposition levels in the Renu storage solutions. This indicates that lysosyme was constantly diffusing from the stored Sureview lenses into the lens storage solution, throughout the period of study. A comparison of Figures 30 and 31 shows that the amount of protein (lysosyme) diffusion from the stored lenses is many times greater than than the amount of lipid diffusion. Therefore it is clear that over a period of lens storage, the lipid deposition levels will not reduce to any significant degree. In contrast, the protein deposition levels can be affected to a very great extent by lens storage.

It is interesting to note that the stored Sureview lenses did appear to be becoming depleted of lysosyme over the study period. This is demonstrated by looking at Figure 31. Here, the cumulative protein deposition level in the Renu

storage solutions continued to rise throughout the five week study period. However, the rate of increase in the cumulative protein deposition levels was slowing, and had almost stopped during the fifth week of analysis. This indicates that less and less lysosyme was diffusing from the contact lens into the storage solutions as the period of lens storage increased.

Therefore, it appears that lenses can be almost totally depleted of lysosyme following a period of storage, provided that the lens storage solution is changed regularly. It is unlikely though that a contact lens would be able to be completely denuded of protein in this manner. Some protein will be irreversibly bound to the contact lens and therefore will not be able to diffuse from the contact lens into the storage solution.

8.3.5 Lysosyme migration in Sureview lenses.

It is interesting to note that high levels of lysosyme at the surface of the contact lenses (i.e. large recorded peak height) are often accompanied by low levels of detected protein in the Renu storage solutions. This indicates that as suggested in Chapter 7, the diffusion of lysosyme out of the contact lens matrix onto the lens surface, and then from the lens surface into the lens storage solution is in fact a two stage process.

The lysosyme will gradually diffuse from the matrix of the Etafilcon A contact lens and start to build up on the surface of the lens. During this process little diffusion of lysosyme from the surface of the lens to the storage solution will take place. The level of lysosyme at the lens surface will continue to build up until it

reaches a maximum. At this point the layer of deposition at the lens surface will start to "overload". When deposits first start to form on a contact lens surface the first layer of deposition will be strongly bound to the polymer matrix of the lens, subsequent layers of deposition will be bound less strongly, and the outermost deposited layers will be very loosely bound, both to the contact lens itself and to other deposited layers on the contact lens. Therefore, it appears that when the lens becomes heavily deposited, the forces which usually hold the deposited layer on the lens surface are not strong enough to maintain the layer, so it will diffuse off the surface of the lens. This "removal" of the deposited layer from the lens surface is the second stage of the pattern of lysosyme diffusion.

The diffusion off the lens surface of the outermost lysosyme "layers" will be rapid. This accounts for the large drops in fluorescence peak height recorded on Lens 1 and Lens 2 (see Figure 29). It also explains the fact that often a sudden drop in protein deposition levels on the contact lens surface is accompanied by a rise in detected protein in the Renu storage solutions. In this situation, the built up lysosyme layer will rapidly drop off the lens surface into the lens storage solution, causing the increase in peak heights observed in the Renu solutions. Once the accumulated lysosyme layer has dropped off the surface of the contact lens, the remaining lysosyme in the lens matrix will start to diffuse from a high concentration in the bulk of the lens to a low concentration at the lens surface, so re-forming a layer on the surface of the contact lens.

8.3.6 Importance of lens material in lysosyme migration.

It must be remembered that migration of lysosyme will only arise with lens materials such as Etafilcon A which are both ionic and have a high water content. It is this combination of factors which are required to attract, and then hold substantial amounts of lysosyme within the contact lens matrix.

Therefore, it can be seen that this two stage undulating process will continue indefinitely, with layers of lysosyme continuously building up and dropping off the lens surface. The rate of build up of layers on the lens surface will start to slow down with time as the lens matrix becomes depleted of lysosyme. However, it is felt that when the concentration of lysosyme within the lens matrix becomes significantly depleted there is likely to be some diffusion back from the lens surface into the bulk of the lens. This will only occur if there is a greater concentration of lysosyme at the lens surface, and diffusion will stop when equilibrium is reached between the lens surface and the bulk of the contact lens.

8.3.7 Lysosyme diffusion to and from contact lenses.

This study has shown how during the storage of Sureview lenses lysosyme will diffuse from the lens matrix to the surface of the lens, and then from the surface of the lens into the storage solution. It has also suggested that when the level of lysosyme in the lens matrix becomes less than that at the lens surface, diffusion will occur from the lens surface back into the bulk of the contact lens. Following on from this, it is also probable that as well as lysosyme diffusing back

from the lens surface into the lens matrix, lysosyme will also diffuse from the lens storage solution onto the surface of the contact lens. This will only occur if the lens storage solution has a concentration of lysosyme greater than that on the lens surface.

In this analysis of stored Sureview lenses it is unlikely that any significant amount of diffusion of lysosyme from the storage solution to the lens surface occurred. This is because the Renu storage solutions were changed twice a week, so it is improbable that the concentration of lysosyme in solution would become high enough to overcome the diffusion gradient from the surface and bulk of the contact lens. Apart from the first fluorescence measurements, large fluorescence protein peak heights for the contact lenses and the Renu solutions are not usually seen during the same time period. In the case of the first set of recorded peak heights, high levels of lysosyme were detected both on the contact lens surfaces, and in the saline storage solution. This indicates that an equilibrium has been reached between the lysosyme in solution, and the lysosyme on and within the contact lens surface. So, it appears that during transit enough time passed to allow the levels of lysosyme in the contact lenses and in the storage solution to equilibrate.

8.3.8 Effect of lysosyme migration on fluorescence analysis.

It therefore seems probable that in previous studies in this project, the length of time that the worn Acuvue lenses had spent in storage before analysis (one to two weeks), would be sufficient to enable the lenses to be in a state of equilibrium

with their storage solutions (the lens storage solutions were not changed during this period). In view of the undulating build up and then drop off the lysosyme layer at Acuvue and Sureview lens surfaces, it appears that probably the optimum time to record fluorescence spectra from lenses fabricated from Etafilcon A is at this "equilibrium" state. When the contact lenses are at equilibrium with their storage solutions, the fluorescence results will not be affected by the dropping off and building up of the lysosyme layer at the lens surface, therefore it will be possible to obtain consistent results throughout a group of Etafilcon A lenses.

However, problems can still arise with regard to this approach to fluorescence analysis of ionic high water contact lenses. In the study undertaken in Chapter 6, Acuvue lenses were analysed after a fortnights wear period. The fluorescence spectroscopy results showed wide variations in the levels of protein detected on the Acuvue lens' surfaces. It is felt that these wide variations in protein deposition levels occurred because of variations in the storage times of the Acuvue lenses. As in other studies undertaken in this project, care was taken to ensure that contact lens analysis was performed as soon as possible after the contact lenses arrived at Aston. However, this study involved analysis of twenty-four contact lenses at two excitation wavelengths. The analysis of all of these lenses took two to three days to complete. In addition, the worn lenses were collected at the test centre over a one day period. Therefore, it can be seen that some of the Acuvue lenses could have been stored for up to four days longer than others, before any fluorescence analysis was performed. It is probable that this difference in lens storage times led to the wide variations in protein deposition levels detected on the Acuvue lenses.

Therefore, it can be seen that extreme care must be taken with regard to the

storage periods of ionic high water content contact lenses. If at all possible identical (or virtually identical) lens storage periods should be maintained for all high water content ionic contact lenses intended to be analysed by fluorescence spectroscopy. To ensure that analysis of a sample of lenses can be performed within a short period of time, it is important that not too many lenses are involved in a particular study. With respect to accuracy in recording fluorescence spectra from ionic high water content contact lenses, it is better to repeat a study several times using small numbers of contact lenses, rather than performing a single study which involves a large number of contact lenses.

8.4 Conclusions.

This study therefore demonstrates that there is considerable migration of lysosyme from Etafilcon A contact lenses. This phenomenon was not noticed in any of the other lens materials analysed in this investigation. The results indicate that care must be taken when recording fluorescence spectra from lenses fabricated from Etafilcon A. Ideally any such lenses should be left for at least a week after collection, and stored for equal time periods, before any analysis is undertaken.

It is also important that the lens storage solution is not changed before analysis is performed. By not changing the lens storage solution, a worn Etafilcon A lens will be able to reach equilibrium with it's storage solution, with minimum loss of protein from the lens surface and matrix. If the storage solution was replaced with fresh solution, more lysosyme would have to diffuse from the contact lens in order to re-establish equilibrium between the contact lens and the storage solution.

This required storage period of at least a week could result in bacterial contamination of the contact lens, therefore it is essential that worn contact lenses should be placed in sterile containers, which preferably contain a solution that has antibacterial activity.

8.4.1 Fluorescence analysis of Etafilcon A lenses.

These facts should always be remembered when performing fluorescence analysis on lenses fabricated from Etafilcon A. This will ensure that consistent and repeatable results are obtained. Obviously the recorded fluorescence peak height will not be representative of the total amount of protein that was on the surface and within the bulk of the contact lens and when it was removed from the eye; some of the lysosyme that was present initially will have diffused into the storage solution. However, fluorescence spectroscopy is only able to detect material deposited on the surface of the contact lens, so any lysosyme within the lens matrix would not be detected anyway.

Therefore, the most accurate way to record fluorescence spectra from Acuvue and Sureview lenses is to wait until they have reached equilibrium with their storage solution, as this is the only way to ensure that no effects will be seen from the migration of lysosyme to and from Etafilcon A lenses.

9. Assessment of the efficacy of different surfactant cleaners.

Surfactant cleaners are used, generally on a daily basis to reduce deposition levels on the lens surface and, to help prevent the formation of large deposits. Their main purpose is to reduce lipoidal deposition levels, but they also have some ability to reduce proteinaceous deposition levels as well. This is probably a result of the mechanical effect of digital rubbing of the surfactant cleaner on the lens surface, as well as the effect of the surfactant itself. There are many different surfactant cleaners available, and some will probably be more effective than others. In general they all have a fairly similar composition; most have a viscous polymeric base containing a surfactant. It is this surfactant, in combination with the mechanical effects from the digital rubbing of the lens surface, which are responsible for reducing lipoidal (and proteinaceous) deposition levels on the contact lens. Surfactant cleaning will not completely remove all the deposition from a lens surface, but is useful in the long term as a method of preventing large deposit formation and thus prolonging the life of a contact lens.

9.1 Introduction.

This study was set up to assess the efficacy of several surfactant cleaners. Because there are such a wide range of surfactant cleaners available, it was felt that it would be interesting to compare the abilities of a sample of cleaners to reduce deposition levels on spoiled contact lenses. Fluorescence spectroscopy was the

chosen method for lens analysis in this study. The non-destructive nature of fluorescence analysis means that is possible to record spectra from spoiled lenses, the re-record them following surfactant cleaning. In addition the extreme sensitivity of fluorescence spectroscopy will enable even small reductions in the lens deposition levels to be detected.

9.2 Experimental protocol.

This investigation was run in conjunction with the study described in Chapter 5 (Early deposition trends of Group 1 and Group 3 materials). Worn contact lenses from the Group 1 versus Group 3 study were used to assess the ability of a selection of surfactant cleaners to reduce deposition levels. The study in Chapter 5 had involved determination of the protein and lipid deposition levels on Group 1 (HEMA and Classic) and Group 3 (Softmate) contact lenses. The lenses were worn for a one week period, either with or without surfactant cleaning. Even after this short wear period protein and lipid deposits built up quite considerably on the surface of the contact lenses.

To assess the ability of different surfactant cleaners to reduce deposition levels a selection of the lenses collected in the above study were used. It was important to select lenses that had shown high levels of lipid deposition over the week long wear periods. This will ensure that there is sufficient level of "removable" lipid at the lens surface. Surfactant cleaning does not completely remove all lipid at the lens surface, it simply reduces the lipid deposition level to a "baseline" level. This represents the expected more "tightly bound" lipid at the lens surface that cannot be

removed by surfactant cleaning. Therefore, it was important to choose a contact lens material that showed fairly heavy levels of lipid deposition throughout the study period i.e. considerably greater than the "baseline" level of lipid deposition.

9.2.1 Choice of contact lens material.

The lens material that had shown the highest levels of lipid deposition throughout the study in Chapter 5 was HEMA. Therefore, a selection of worn HEMA lenses were chosen for the investigation into the effectiveness of different surfactant cleaners. The lenses were selected from both week long wear periods. The only criteria for lens selection was that the worn HEMA lenses should have sufficiently high levels of lipid deposition i.e. greater than the estimated "baseline" level of lipid deposition. For HEMA lenses the "baseline" level of lipid deposition was taken to be represented by a fluorescence peak height of 3-4mm above baseline e.g. the lowest recorded lipid peak heights for the worn HEMA lenses.

9.2.2 Surfactant cleaning protocol.

Five commercially available surfactant cleaners had their ability to reduce deposition levels assessed. For the purposes of this study the cleaners were simply labelled A-E. A specific cleaning protocol was developed to assess the ability of the test products to surfactant clean under different experimental conditions e.g. mechanical agitation versus digital clean. The final step in the cleaning protocol involved digital cleaning of the lens with a cleaner (Cleaner B)

that had been found to be the most effective at reducing lipid deposition levels in previous laboratory based studies. The purpose of this final step was to return the lenses to their "baseline" level of lipid deposition i.e. the level at which no further lipid can be removed from the lens surface by surfactant cleaning. The cleaning protocol was as follows:-

1. 20 seconds of mechanical agitation of lens and 5ml of cleaner in a 15ml vial, followed by copious saline rinse.
2. Repeat step 1.
3. 20 seconds of digital cleaning with 2ml of cleaner, followed by copious saline rinse.
4. As in step 3, but digital clean with Cleaner B.

After each cleaning process fluorescence spectra were recorded to allow an estimation of the decrease in lipid deposition levels to be made.

9.3 Fluorescence analysis.

Fluorescence analysis was performed at an excitation wavelength of 360nm. This excitation wavelength was chosen because the aim of this study was to monitor the decreases in lipid deposition following various surfactant cleaning regimes. These decreases will be shown most clearly using fluorescence analysis at an excitation wavelength of 360nm. Although surfactant cleaning has been shown to reduce protein deposition levels this is felt to occur more as a result of the mechanical effect produced at the lens surface by the rubbing and rinsing

process, rather than as a direct effect of the surfactant cleaner on deposited protein. Therefore it was felt that the best way to monitor the effectiveness of different surfactant cleaners was to determine the decreases in lipid deposition levels throughout the cleaning protocol described above, using fluorescence analysis at an excitation wavelength of 360nm.

Fluorescence spectra were recorded from the worn HEMA lenses before any cleaning occurred. Although spectra had already been recorded from these worn lenses (as part of the study in Chapter 5), it was felt that spectra should be re-recorded at the start of this study to allow for any changes in the surface deposition layer during the period of lens storage between studies. The lenses were in fact only stored for about a week between the two studies. This period of storage is unlikely to have resulted in any reductions in the level of surface lipid deposition, but to ensure as high a level of accuracy as possible spectra were re-recorded at the start of this study. Subsequently fluorescence spectra were recorded following every step in the cleaning protocol.

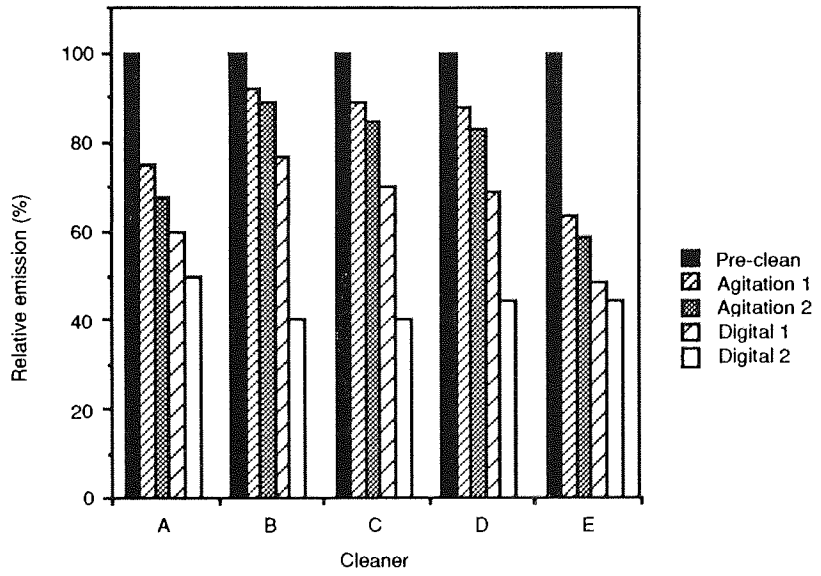
9.4 Fluorescence spectroscopy results and discussion.

As stated fluorescence analysis was performed at an excitation wavelength of 360nm. A total of five worn HEMA lenses were studied, i.e. one lens for each of the five surfactant cleaners being tested. The surfactant cleaners were labelled A-E, with surfactant cleaner B being used to clean all the lenses down to their baseline level of lipid deposition.

The fluorescence intensities from the initial spectra of the worn lenses were

given a relative emission value of 100%. The reductions in peak height following each cleaning step were determined as percentage decreases relative to the original assigned value of 100%, obtained from the worn but uncleaned contact lens. The results are shown in Figure 32.

Fig. 32 Comparative efficacy of five commercially available surfactant cleaners (A-E) to reduce lipid deposition levels on worn HEMA contact lenses. Excitation wavelength 360nm.



The results show clearly that a reduction was seen in the lipid deposition levels after each step of the cleaning protocol. This demonstrates that surfactant cleaning, whether by mechanical agitation or digital rubbing will result in a reduction in the lipid layer deposited at a lens surface.

In general the biggest reduction in lipid deposition levels on the worn HEMA lenses was seen after the first mechanical agitation. This is because at the start of

the cleaning protocol the lenses had the highest amount of deposited lipid. When a lens has a relatively high level of lipid deposition, it is fairly easy to remove lipid from the lens surface (i.e. reduce the level of deposited lipid). The second mechanical agitation did further reduce the level of lipid deposition, but not to as great an extent as the reduction seen following the first mechanical agitation. This indicates that the lipid was becoming "harder" to remove from the lens surface. So it is clear that mechanical agitation alone is not a reliable method of surfactant cleaning contact lenses. Although both mechanical agitation steps did consecutively reduce the level of lipid deposition, they did not reduce it to anywhere near the baseline level of lipid deposition (i.e. the percentage emission recorded after the final digital clean).

A further reduction was seen in the lipid deposition levels on all the worn HEMA lenses following the first digital clean. This reduction was generally greater than that seen following the second mechanical agitation. This indicates that digital rubbing of the lens with a surfactant cleaner is a much more effective method of reducing lipid deposition levels than simple mechanical agitation. From looking at the final set of results (digital clean with Cleaner B) it is clear that after the first digital clean, all of the five lenses still had appreciable amounts of removable lipid at their surfaces. So, although 20 seconds of digital cleaning with the five surfactant cleaners was able to produce further reductions in the lipid deposition levels, it wasn't able to completely take off all the removable lipid from the lens surface. It was only following a further digital clean with a cleaner shown to be effective in previous studies (Cleaner B) that the worn lenses had their lipid deposition levels reduced to a "baseline" value.

9.4.1 Differences in effectiveness of surfactant cleaners.

Each of the five commercially available surfactant cleaners were able to reduce the lipid deposition levels following each step in the cleaning protocol. However, some variations were seen in the magnitude of these reductions. This indicates that some of the surfactant cleaners were more effective at reducing lipid deposition levels than others. From the results shown in Figure 32 it appears that the most effective surfactant cleaner (i.e. the one that caused greatest reductions in the lipid deposition levels) was Cleaner E. Cleaner A was the second most effective, followed by cleaners D and C. Interestingly, the cleaner found to be most effective in previous studies (Cleaner B) was seen to be the least effective in this study throughout all of the steps of the cleaning protocol excepting the last.

However, in the last step of the cleaning protocol (for all the contact lenses) Cleaner B showed itself to be the most efficient at reducing deposition levels. A much bigger reduction in deposition levels was seen following the final digital clean with Cleaner B for all the worn HEMA contact lenses. This indicates that although Cleaner B was not the most effective surfactant cleaner of the five tested using the cleaning protocol described earlier, it is by far the most effective at reducing lipid deposition levels back to their baseline value, i.e. the level of lipid deposition at which no further lipid will be able to be removed from the lens surface by surfactant cleaning.

This study has therefore shown that not all surfactant cleaners are equally effective at reducing lipid deposition levels. It is also clear that care must be taken with the length of time that contact lenses are surfactant cleaned for. The results from this study showed that irrespective of surfactant cleaner effectiveness, all the

worn lenses still had considerable amounts of removable lipid on their surfaces following 20 seconds digital cleaning. It was only after a further 20 seconds digital cleaning that the lens lipid deposition were reduced to a baseline value.

None of the surfactant cleaned lenses had their lipid peak height reduced by more than one half of it's original value. This result is consistent with previous studies. The "remaining" peak height recorded after the finish of the surfactant cleaning protocol will be composed from the true baseline fluorescence peak height of the contact lens (i.e. the peak height that would be recorded from the clean unworn contact lens) and the fluorescence peak height of the remaining tightly bound lipid at the lens surface.

9.5 Conclusions.

This study has clearly shown that all surfactant cleaners are not equally effective. In addition, it is also clear that, regardless of the effectiveness of surfactant cleaners, they are never able to completely remove all deposited lipid from the contact lens surface. This means that whenever a contact lens is surfactant cleaned, a baseline level of deposited lipid will always remain at the lens surface. Using this information, a "model" of deposit build up, and removal can be formed. In this model, deposition levels build up rapidly throughout the period of contact lens wear. The level of deposition will continue to increase until the lens is removed from the eye. If the contact lens is then surfactant cleaned, the level of lipid deposition will decrease considerably, to a baseline level. This model of deposition build up and removal can be visualised as resembling the blade of a

saw. The tip of the saw tooth represents the high levels of deposition found on a lens following a period of wear. The troughs between the saw teeth represent the baseline levels of deposition following surfactant cleaning of a contact lens.

Therefore, it can be seen that, during contact lens wear, lipid deposition levels will continue to rise and fall, in conjunction with lens wear and surfactant cleaning. The saw-tooth model of deposit formation and removal highlights the need for efficient surfactant cleaners. A surfactant cleaner that can maintain a low baseline level of lipid deposition (i.e. remove all, or nearly all, "removable" lipid at each surfactant clean) will ensure low deposition levels over a long period of lens wear. This will help prevent the immobilisation of reactive lipids at the lens surface, thus reducing the possibility of large deposit formation. In contrast, if a surfactant cleaner is not able to remove all the removable lipid, the higher level of baseline deposition will, in the long term, result in reactive lipids remaining at the lens surface. This can then facilitate the formation of more complex deposits on the contact lens surface.

Although surfactant cleaning will not substantially affect deposition levels during short periods of contact lens wear (i.e. the use of disposable lenses) it is extremely important during longer wear periods. To prolong the period of lens wear it is therefore vital that lenses are cleaned regularly with an efficient surfactant cleaner (i.e. one that can produce and maintain the lowest possible level of baseline lipid deposition possible). It can therefore be seen how important it is to have a reliable method , such as the one described in this study, for determining the effectiveness of existing, or novel surfactant cleaners.

The method of evaluating the effectiveness of surfactant cleaners using a rigid cleaning protocol and fluorescence analysis, can also be used to test the ability of a wide range of lens care products, or novel lens care products, to reduce both protein and lipid deposition levels. Any lens care products (surfactant cleaners, disinfection systems, enzyme tablets) could be tested in a suitably adapted cleaning or care protocol using fluorescence analysis before, during, and after the appropriate cleaning or care regime. An excitation wavelength of 360nm would show the effect of a particular lens care product on lipid deposition levels, while an excitation wavelength of 280nm would highlight any decreases in protein deposition levels. In this way the ability of a whole range of lens care products to affect protein and lipid deposition levels could be evaluated.

This method of analysis could also be used to assess the efficiency of any novel lens care product, particularly surfactant cleaners and enzyme tablets to reduce protein and lipid deposition levels.

So, it can be seen that as well as being an extremely important method of analysis of contact lens deposition levels, fluorescence spectroscopy is also extremely useful in evaluating the effectiveness of contact lens care products.

In this study worn contact lenses were analysed throughout the cleaning protocol. However, it is also possible (and probably easier in a laboratory based situation) to use *in vitro* spoilt contact lenses. Lenses can be artificially spoiled in the *in vitro* model described in Chapter 2, and then used in a cleaning protocol, in exactly the same way as an *in vivo* spoiled contact lens. In this way an evaluation of the effectiveness of lens care products to reduce deposition levels on a wide range of contact lens materials can be made in a purely laboratory based

experiment. So, it would be possible to determine not only which lens care products are most effective, but also whether some products are more effective at reducing deposition levels on certain types of contact lens materials.

10. Conclusions.

10.1 Introduction.

This project set out to assess the potential value and effectiveness of fluorescence spectroscopy as a method for analysis of contact lens deposition.

10.1.1 Techniques available for contact lens analysis.

At the outset of this study it was necessary to review the value and shortcomings of techniques currently available for contact lens analysis. The main problem involved in the analysis of early lens spoilation is the fact that minute quantities of deposited species are involved. In addition, it was felt that there was no suitable non-destructive analytical technique that could be used in conjunction with clinical studies. The advantages of a non-destructive method of lens analysis are that it is not necessary to remove deposited species from the contact lens surface prior to analysis. This means that the lens could, potentially, be returned to in eye wear following analysis. Alternatively, non-destructive analysis will enable further, more detailed analysis of deposits to be performed at a later date.

Techniques available for analysing spoilation can be divided into optical methods used for looking at the surfaces of the lens, and chemical methods used for analysing the extracts from these surfaces.

Techniques for analysing the surfaces include optical microscopy, histological

and histochemical analysis, fluorescence spectroscopy, Fourier-transform infra-red spectroscopy (FTIR), scanning confocal laser microscopy and electron microscopy. FTIR is a very useful technique for looking at small areas of deposits but this method is not at present capable of quantifying low levels of deposition in the early stages of wear.

Techniques for analysing the extracts include high performance liquid chromatography (HPLC), fluorescence spectroscopy, electrophoresis and thin-layer chromatography.

In addition, clinical methods for determining lens deposition levels are available e.g. RUDKO classification.

Another technique that could be used for contact lens analysis is Ultra-Violet (UV) spectroscopy. However, there are several disadvantages associated with this technique. Firstly UV spectroscopy is a transmission technique. This means that it can only be used to detect relatively large amounts and is therefore generally too insensitive to detect the very low levels of deposition found in the early stages of wear. Secondly, UV spectroscopy relies on the fact that compounds absorb light in the ultra-violet wavelength range. Since a vast number of compounds will absorb light in this region, UV spectroscopy has fairly low specificity as an analytical technique.

10.1.2 Problems involved in contact lens analysis.

Problems can arise with the techniques used for contact lens analysis. These usually are destructive; they rely on taking material off the lens to examine it. This gives rise to the question is all the material off the lens or not? Therefore, at the outset of this study it was felt that some attempt should be made to develop a non-destructive technique for use in parallel with a controlled clinically supervised wearing programme.

This project involved fluorescence spectroscopy used as an analytical technique in a way that has not previously been possible. Fluorescence spectroscopy can be used to non-destructively assess the levels of deposition on a contact lens both quantitatively and qualitatively, even after only very short wear periods. For the purposes of this project only qualitative analysis was performed, i.e. from fluorescence spectra of contact lenses, it was possible to determine the levels of protein or lipid deposited at the lens surface. This was achieved by comparing the fluorescence emission peak height to the baseline of the fluorescence spectrum. The level of protein or lipid deposition is indicated by the height of the fluorescence emission peak height (results are given as mm above baseline). However, it was not possible to determine the actual amounts of deposited protein or lipid from the recorded fluorescence spectrum. Since the finish of this project, further experiments have been performed which enable the actual amounts of protein or lipid deposited at the lens surface to be determined following fluorescence analysis. These will be discussed later.

10.1.3 Early studies of contact lens spoilation.

Many studies[48,54,69,73] have been performed in an attempt to gain more information about the processes involved in contact lens spoilation. However, in general these studies were carried out with lenses that were heavily spoilt i.e. at the end of their useful lives, had been worn for differing time periods, or, had poorly documented wear histories.. There is a parallel here with studies performed with biomaterials that are inserted into the body. As with contact lenses these are generally only examined when problems occur, or when the patient dies. Another disadvantage with contact lenses that have been worn for long periods is that the clinical histories of the lenses is often poor, and they may have been stored for long periods after wear was stopped, but before any research was performed. During this storage period the deposition profile of the lens may alter quite substantially, i.e. both protein and lipid may diffuse from the lens surface, into the lens storage solution. This diffusion of deposited species will vary according to the period of lens storage. Generally, short periods of storage (up to two weeks) will not greatly affect deposition levels. However, longer periods of storage can lead to considerable changes in lens deposition levels. In addition, long contact lens storage periods can result in bacterial contamination of the lens.

This project set out to perform analysis on contact lenses that had been worn for short periods under clinically supervised conditions. Therefore, in contrast to many early studies, the lenses analysed in this study all had well documented wear histories. In addition lens analysis was performed within a short period of cessation of lens wear (i.e. one to two weeks). Therefore, this project hoped to provide reliable information about the early stages in contact lens deposition using

contact lenses that had well documented wear histories, in combination with a novel, non-destructive method of analysis.

10.1.4 Initial experiments involving fluorescence spectroscopy.

Before any clinically based studies involving fluorescence analysis were set up it was important to perform some preliminary experiments to assess the capabilities of the technique.

Initial experiments (Chapter 3) had demonstrated that analysis using fluorescence spectroscopy was able to detect protein and lipid deposited on a contact lens surface, and that the level of deposition could be determined by measuring the peak height of the recorded fluorescence spectrum. It was also established that fluorescence spectra could be recorded at two distinct wavelengths; an excitation wavelength of 360nm gives rise to a lipid deposition peak at an emission wavelength of 430nm, whereas an excitation wavelength of 280nm gives rise to two separate protein and lipid deposition peaks at emission wavelengths of 360nm and 430nm respectively.

These early experiments demonstrated the potential of fluorescence spectroscopy as a method for analysing surface deposition on contact lenses. Following these initial investigations various studies were carried out using lenses worn in clinically controlled programmes, and *in vitro* spoilt lenses,

10.2 Observations from analytical studies involving fluorescence spectroscopy.

The analytical studies involving fluorescence spectroscopy undertaken in this project have involved assessing the early deposition levels of various contact lens materials under different wear conditions. In addition an *in vitro* study was performed using an artificial lens spoilation method. Final studies involved assessing the effect of lens storage on deposition levels and estimations of the efficacy of various surfactant cleaners.

These studies have all involved fluorescence spectroscopy as the method of lens analysis. The extreme sensitivity of this technique allows protein and lipid deposition levels to be detected on the lens surface even after very short wear periods. In addition, the use of fluorescence spectroscopy enables any variations in lens deposition levels resulting from in eye lens wear i.e. patient to patient variations, effect of water content and ionicity, effect of surfactant cleaning and disinfection etc. to be detected Thus, information has been obtained throughout this project about the potential uses (and any limitations) of fluorescence spectroscopy.

One of the first studies in this project involved determining whether different manufacturing techniques would affect the deposition characteristics of an ionic high water content contact lens material. Contact lenses can be manufactured by different techniques i.e. lathe cut or cast polymerised. These differing manufacturing methods could potentially give rise to very slight variations in the

contact lens surface. Such variations may in turn result in different deposition profiles between contact lenses formulated from the same material, but manufactured by different methods.

A study was set up to investigate this potential problem using lathe cut and wet moulded Etafilcon A lenses worn in a clinically controlled wear programme. The fluorescence spectroscopy results for both protein and lipid deposition levels clearly showed that there was no difference in the deposition profiles of the differently manufactured Etafilcon A lenses. Therefore it is clear that certainly during the early stages of lens wear, the different lens manufacturing techniques did not affect the deposition levels of the Etafilcon A lenses. However, this is not always the case. For example, the edges of a contact lens (which can vary according to method of manufacture) can affect lipid deposition levels due to meibomiam gland stimulation.

This study also enabled an estimation of the effect of known white spot depositors on early deposition levels to be made. White spot deposits are the most chemically complex manifestations of ocular incompatibility. They form on the surface of the lens with no specific site. White spot formation involves particularly lipid deposition, but all tear components are involved in the later stages. Therefore to obtain information about the formation of white spots it is important to study the early stages of lens wear before deposits become isolated and insoluble.

A study was set up to investigate the early deposition levels of known white spot depositors compared to contact lens wearers with no previous history of white spot formation. Over a fortnights wear period no consistent difference was seen in gross deposition levels between the known white spot depositors and the non-depositors. This is consistent with the view that specific chemical steps, rather

than simple bulk accumulation, are involved in white spot formation. Therefore it can be seen from this study involving fluorescence analysis, that it is not possible to predict whether or not a contact lens wearer will be a potential white spot depositor, simply from analysis of lens gross deposition levels in the very early stages of wear.

Fluorescence spectroscopy will allow determination of the level (or amount) of lipid deposited on the lens surface, but cannot be used to detect specific classes of lipid. Therefore, although there may have been differences in the content of the lipoidal deposits between the known white spot depositors and the control subjects (i.e. deposition of specific lipids may initiate white spot formation), these would not be detectable by fluorescence analysis.

10.2.1 Patient to patient variations and right eye/left eye differences.

All of the *in vivo* studies performed in this project involved monitoring the early stages of contact lens spoilation. All lenses were worn under strict clinically controlled programmes.

Studies were performed with lenses of different water contents and ionicity, and with lenses that had, or had not been surfactant cleaned. In most cases, regardless of contact lens material, or whether or not the lenses had been surfactant cleaned, patient to patient variations were clearly observed in lipid deposition levels. These variations were detected as unusually high lipid deposition levels in a particular subject, compared to the level of lipid deposition demonstrated by the majority of patients in that particular study. In each of the *in vivo* studies performed in this

project (Chapters 4, 5 and 6) 20-30% of the patient population had lipid deposition levels that were greater by 50% or more than the remaining 70-80% of subjects. In contrast, protein deposition levels remained relatively constant throughout the patient populations (protein deposition levels were seen to vary with different contact lens materials, but generally remained constant across a patient population wearing a given contact lens material). In studies that involved two separate wear periods, patient to patient variations in lipid deposition levels were seen most commonly for only one of the wear periods. However in two cases patient to patient variations were detected in both scheduled wear periods.

Therefore, the results obtained from the *in vivo* studies using fluorescence spectroscopy undertaken in this project (Chapters 4, 5 and 6) imply that lipid deposition levels can vary widely across a patient population ,and therefore are largely patient dependent, whereas protein deposition levels remain relatively constant across a patient population, but will vary widely between different contact lens materials. Thus, protein deposition appears to be much more material dependent.

Lipid deposition levels were seen to remain relatively constant in the early stages of contact lens wear, irrespective of which contact lens material is used, but protein deposition levels were seen to vary greatly depending on the choice of contact lens material. Very high levels of protein deposition were detected on the Group 4 ionic high water content lenses studied in Chapters 4, 6, 7 and 8. In contrast, following similar wear periods, low water content ionic and non-ionic lenses showed protein deposition levels 70-80% lower than those detected on the Group 4 lenses.

Patient to patient variations in protein deposition levels are usually small, whereas the variations in lipid deposition between patients can be large. In one study involving ionic high water content lenses (Chapter 6) wide variations in protein deposition levels were seen throughout the patient population. However, it was felt that these variations arose due to problems with lens storage times, these will be discussed later.

The differences among lens materials in absorbing protein and lipid appears to increase with time. However, it is important to remember that long term deposit build up on hydrophilic lenses will also depend on patient related factors such as hygiene, compliance with instructions for cleaning, environmental factors, the care regime utilised and the patient's tear film quality and quantity.

Left eye/right eye differences were detected in the studies performed throughout this project. These differences were mostly demonstrated as increased lipid deposition levels in one eye during one of the scheduled wear periods (if the lenses had been worn for two separate wear periods). The fact that when two separate wear periods were scheduled in a study (Chapters 5 and 6), these differences were only detected in one of the scheduled wear periods, indicates that the majority of the left eye/right eye differences detected were probably caused by indirect patient related factors i.e. one eyelid being tighter than the other, rubbing one eye with the contact lens in situ, handling one lens more than the other or, if surfactant cleaning was involved, cleaning one lens less effectively than the other. In a few cases left eye/right eye differences were detected in two consecutive wear periods. Again these differences could have arisen due to the factors listed previously. It is also

possible that they occurred due to difference in lens between the eyes, which could lead to enhanced lipid deposition levels.

Left eye/right eye differences in protein deposition levels were only really detected in ionic high water content contact lenses (Etafilcon A). Etafilcon A is known to deposit protein (particularly lysosyme) very rapidly and heavily. The reasons for this are discussed in the following section of this conclusion. Fluorescence spectroscopy is a very sensitive technique so it is able to detect even very slight changes in protein deposition levels. In addition lysosyme, which is avidly taken up by Etafilcon A lenses, is in itself extremely fluorescent.

Therefore, it is possible that the left eye/right eye differences detected in Etafilcon A deposition levels could have arisen due to slight differences in lens wear times i.e. if one contact lens was put in first and removed last during a period of wear. Alternatively, lysosyme migration can occur rapidly from stored Etafilcon A lenses, so it is also possible that the left eye/right eye differences were due to one lens being stored for a slightly longer period than the other.

However, two of the Etafilcon A lenses analysed in Chapter 4 that showed left eye/right eye differences in protein deposition levels, had very low levels of protein deposition, accompanied by unusually high levels of lipid deposition. This implies that the high levels of lipid deposition detected on these lenses will somehow affect the detected level of protein deposition. Fluorescence spectroscopy is only able to detect material deposited on the surface of a contact lens. Therefore it is probable, that if one species is deposited heavily (in this case lipid), it will to a certain extent "mask" the other deposited species (protein), thus rendering it undetectable by fluorescence analysis.

10.2.2 Effect of water content and ionicity on contact lens deposition profiles.

In an attempt to increase the hydrophilicity and wettability of soft content lenses various "wetting agents" can be incorporated into the contact lens polymer. However, this can result in the surface of the lens becoming charged. In the case of methacrylic acid (one of the principal monomers of Etafilcon A lenses) the overall net charge of the lens is highly negative. A number of studies[79,80] have shown that such a charge causes the lens to attract a far greater number of deposits, particularly protein, than lenses that are relatively inert.[81] This is believed to be due to the fact that the positive charge on the surface of protein thermodynamically favours such attraction. The charges on proteins arise because of amino acid residues on protein side chains. The amino acid residues consist of NH_4^+ or OH^- groups. The proportion of these groups on the protein side chains will determine the overall net charge of the protein.

So, it can be seen that the surface charge of a lens, and the overall net charge of proteins will affect the levels of protein deposition. In addition, the size of a protein, and the mean interchain space and/or number of pores of a contact lens polymer, will determine whether a protein is simply deposited on the lens surface, or will migrate into the polymer matrix itself. This is particularly relevant for high water content lenses; these lenses have larger pore size and/or number of pores, and are therefore more susceptible to tear film constituents entering the lens matrix.[79] Although high water content lenses do have a larger pore size than lenses with lower water content, most deposited tear proteins are still too large to enter the polymer matrix e.g. tear albumin and tear lactoferrin have molecular

weights of 90 and and 67Kd respectively. These molecular weights can be contrasted with that of lysosyme (14.3Kd).[47] It is also interesting to compare the molecular dimensions of these protiens. For example, albumin has a molecular dimension of 11.6x2.2x2.7nm^3. This contrasts with the much smaller molecular dimension of lysosyme (4.5x3.0x3.0nm^3).[82] The much lower molecular weight (and therefore smaller size) of lysosyme enable it to pass through the pores of ionic high water content lens materials, e.g. Etafilcon A, thus being absorbed in large amounts within the contact lens matrix. As part of this project various analytical studies were undertaken in an attempt to obtain information about the affect of water content and ionicity on deposition levels. These studies were particularly concerned with the early stages of lens wear. Fluorescence spectroscopy studies using Group 4 Etafilcon A contact lenses were able to show clearly the heavy protein and low lipid deposition profiles discussed above, even after only short wear periods (i.e. two weeks). These studies also highlighted problems relating to length of storage time of lenses before analysis, choice of storage solution and lysosyme migration. These points will be discussed later. Further studies using Group 3 ionic low water content lenses (Bufilcon A) showed that these ionic lenses also deposited protein preferentially i.e. they deposited more protein than lipid. However, the level of protein deposition on the Bufilcon A lenses was found to be similar to the levels of protein deposited on non-ionic, low water content ionic lenses (HEMA). Results from studies involving fluorescence analysis of Group 3 and Group 4 lenses indicate that lenses termed as ionic will not necessarily show similar deposition profiles. This is especially true for protein deposition. Fluorescence analysis of lenses worn under strictly controlled

conditions, demonstrated that ionic Group 3 lenses do not show enhanced deposition characteristics in a similar manner to the greatly increased levels of protein deposition seen with ionic Group 4 lenses. The results clearly demonstrated that the levels of protein deposition on the Group 3 lenses were not high; over an identical wear period (one week), Group 1 HEMA lenses showed higher levels of protein deposition. In fact the Group 3 Bufilcon lenses in general showed slightly lower levels of deposition for both protein and lipid, than the Group 1 or Group 4 lenses involved in these studies. Despite these low overall levels of deposition the Group 3 lenses were shown to deposit slightly more protein than lipid over a week long wear period. Therefore it is apparent that although both Group 3 and Group 4 materials are termed ionic they do not both behave in a similar fashion; The Group 3 lenses did not show the greatly enhanced levels of protein deposition seen with ionic materials which are categorised as Group 4 (e.g. Etafilcon A).

So, it appears that both the water content, and the ionic charge, of a contact lens will affect it's deposition profile. A high water content lens is much more susceptible to deposition of tear constituents within the lens matrix because of the larger pore size and/or number of pores associated with such lens types. An ionic charge causes the lens to attract a large number of deposits, particularly protein. A slight ionic charge, such as that on the Group 3 Bufilcon lenses, in combination with a low water content, can help reduce deposit formation. In contrast, a greater ionic charge, and high water content (e.g Group 4 materials), will result in higher levels of lens deposition. This is particularly relevant with respect to protein deposition.

Studies performed by other workers at Aston University have indicated that

non-ionic low water content lenses (HEMA) show greater levels of lipid deposition than protein deposition, following lens wear periods of six months or more. Fluorescence spectroscopy studies performed in this project involved analysis of the protein and lipid deposition levels on HEMA lenses during the very early stages of wear (one to two weeks). These studies showed that the HEMA lenses showed low levels of protein deposition. However, it was clear that in the early stages of wear the levels of lipid deposited were also low. This fact indicates that it is only in the later stages of wear that HEMA lenses start to deposit protein heavily.

These results were backed up by *in vitro* spoilation studies. These studies involved spoilation of HEMA lenses using the method of *in vitro* lens spoilation developed at Aston (see Chapter 2). The results demonstrated that following short periods of *in vitro* spoilation (i.e. equivalent to about one months *in vivo* daily wear), HEMA lenses had similar levels of deposition to Group 4 Etafilcon A lenses that had been in the *in vitro* spoilt model for the same time period. These results imply that during the early stages of wear, HEMA lenses do not show the heavy levels of lipid deposition associated with longer wear periods. Therefore, it appears that as lenses age (i.e. when HEMA lenses are worn for periods of six months or more), they attract more deposits. This is because it is impossible to totally remove all deposited material from a lens surface. Surfactant cleaning will reduce the levels of lipid deposition lipid deposition on a lens surface, but will never completely remove all deposited lipid. Over time, the surface layer of spoilation will act as a site for further deposition.

In addition to the water content and ionicity of a contact lens affecting deposition levels, several others factors relating to the contact lens itself can affect levels of deposition. For example, differences in lens surface areas. A contact lens with a smaller surface area will have "less" lens surface for protein and lipid to be deposited on. Consequently, lower levels of protein and lipid deposition would be detected. In addition the surface finish of a contact lens can influence it's deposition profile. A very smooth surface on a contact lens will, to a certain extent, reduce deposit formation. In contrast, if the contact lens is slightly rough, this roughness will tend to encourage deposit formation.

10.2.3 Effect of surfactant cleaning and disinfection on deposition levels.

To assess the effect of surfactant cleaning on early cumulative deposition levels a clinically controlled programme was set up in which lenses were worn for two separate week long wear periods. In the study, deposition levels were assessed on two Group 1 lens materials (HEMA and Tetrafilcon A), and a Group 3 material (Bufilcon). In the first wear period no surfactant cleaner was used, the lenses were only disinfected daily with a chlorine based disinfection system. In the second wear period the lenses were surfactant cleaned before disinfection.

The results showed that in general, during a one week period of wear, surfactant cleaning did reduce deposition levels, but these reductions were not substantial. As expected, when a reduction in deposition levels was seen on the surfactant cleaned lenses, it was the lipid deposition levels that were shown to be reduced to a greater extent than the protein deposition levels. Where the protein

deposition levels were seen to be reduced, this was probably caused as a result of the the rubbing and rinsing involved in the digital surfactant cleaning of a contact lens. In addition, surfactants can be used to remove protein deposited on a lens surface e.g. sodium dodecyl sulphate (SDS) extraction of protein before electrophoresis. Generally the experimental conditions involved in this process are more severe than those encountered during surfactant cleaning of a contact lens, however, it can be seen that surfactants themselves do have some ability to remove protein from the contact lens surface.

Following surfactant cleaning, the HEMA Group 1 lenses showed an average decrease of 30% in lipid deposition levels and 18% in protein deposition levels. The Group 1 Tetrafilcon A lenses showed 41% and 46% decreases in lipid and protein deposition respectively. The Group 3 lenses showed a 35% decrease in lipid deposition levels following surfactant cleaning. No reduction in protein deposition levels was detected.

It can therefore be seen that although in most cases the deposition levels were reduced by surfactant cleaning, none of the lens types had their average value for protein or lipid peaks reduced by more than one half of the values obtained when the lenses were worn without surfactant cleaning, under the conditions of the study.

All the lenses had been surfactant cleaned on the evening prior to the day of collection. However, the period on the day of collection between when the lenses were inserted for wear and the time of collection would have been 7 ± 2 hours. Therefore as a part of this study it was important to establish the extent to which the surfactant cleaner had removed the lipid layer, in order to assess the rapidity

and completeness of its re-establishment. This was done by recording spectra from spoiled lenses at an excitation wavelength of 280nm, then surfactant cleaning the lenses under recommended conditions (15 seconds digital cleaning followed by copious saline rinse). The fluorescence spectra were then re-recorded. These analyses showed that surfactant cleaning did reduce the levels of deposition i.e. decreases in peak heights were seen. However, the peak heights were never reduced to the levels recorded for unworn lenses of the same type. This indicates that although surfactant cleaning does decrease deposition levels it will not completely remove all deposited material from the lens surface.

It is known that deposited layers will quickly re-establish themselves when a cleaned lens is inserted into the eye. Therefore it is probable that the lenses analysed from the second wear period of the study (when the lenses were surfactant cleaned) would have to some extent established a "fresh" deposited layer during wear on the day of collection. However, the cumulative lipid deposition levels (i.e. the total amount of lipid deposited throughout the week wear period) indicate that surfactant cleaning does not substantially reduce lipid deposition levels in the early stages of lens wear; the lipid deposition levels were only 30-41% lower on the surfactant cleaned lenses as compared to the non-surfactant cleaned lenses. Surfactant cleaning does however become much more important in minimising the immobilisation of reactive lipids during the later stages of wear.

The study also showed that surfactant cleaning is only partially effective in removing lipid deposits; the surfactant cleaned lenses had 13-37% greater lipid peak heights than unworn lenses of the same materials. When spoiled contact lenses had fluorescence spectra recorded before and after cleaning the fluorescence peak height was never reduced to a level comparable to the fluorescence peak

heights recorded for unworn contact lenses of the same material. This showed that even after surfactant cleaning, 60-70% of deposited lipid will remain deposited at the lens surface.

To assess the effect of disinfection systems on deposition levels, an *in vitro* spoilation study was performed. Two different lens types were disinfected with two disinfection systems throughout the period of *in vitro* spoilation (see Chapters 2 and 7 for details).

Disinfection systems aim to prevent any bacterial contamination of the contact lens occurring. To achieve this the lens must remain in the disinfection (and/or neutralising) solution for a specific length of time. The most commonly used disinfection systems are oxidising disinfectants. In this project the effect of two different disinfection systems on deposition levels were studied; a chlorine based system and a hydrogen peroxide based system. Lenses were also surfactant cleaned throughout the period of *in vitro* spoilation.

Early experiments showed that the disinfection process did not itself cause any reduction of deposition levels on a spoiled contact lens. Fluorescence spectra were recorded from a spoilt contact lens before and after disinfection. No reduction was seen in the fluorescence peak height following the disinfection process.

To assess the effect of disinfection systems on deposition levels contact lenses were spoiled in the spiked version of the Artificial spoilation model formulated at Aston University (see Chapters 2 and 7). The results from this study clearly showed, that following an *in vitro* spoilation period equivalent to the first few months of in eye lens wear, the lenses disinfected with a chlorine based product

had similar levels of protein and lipid deposition to the lenses disinfected with a hydrogen peroxide based product. Therefore, particularly during the first few months of lens wear it appears that the method of disinfection will not greatly affect lens deposition levels. It must be remembered though that in the study the lenses were also surfactant cleaned, and this surfactant cleaning in combination with the low levels of deposition seen meant that the method of disinfection does not affect the protein and lipid deposition levels.

The method of disinfection can affect deposition levels in the later stages of wear. The active chemical constituents of the disinfection system itself will react with material deposited on and within the contact lens (particularly when a chlorine based disinfectant is used over long wear periods). This is especially true for deposited protein. This reaction of the disinfection system with the material deposited on the contact lens can lead to chemical changes of the deposited species which causes them to be irreversibly bound to the contact lens material. This can then lead to problems of increased deposition levels on the contact lens which cannot be removed or reduced by either surfactant cleaning of enzyme tablets.

Therefore, the choice of lens disinfection system is important, particularly when lenses are intended to be worn over long periods i.e. more than a few months.

10.2.4 Assessment of the effectiveness of different surfactant cleaners.

A study was set up using fluorescence analysis in combination with a strict cleaning protocol to assess the effectiveness of five commercially available surfactant cleaners. The cleaning protocol is described in Chapter 9. Five worn

HEMA lenses were assessed. The initial lipid peak height of these lenses (from fluorescence spectra recorded at an excitation wavelength of 360nm) was given a relative emission value of 100%. Following surfactant cleaning, by mechanical agitation and digital cleaning, decreases in lipid deposition levels were shown as reductions in the percentage of relative fluorescence emission. The recorded decreases in relative fluorescence emission varied between 22% and 50% depending on which surfactant cleaner had been used.

Therefore, the study showed that not all surfactant cleaners are equally effective at reducing lipid deposition levels. It was clear that 20 seconds mechanical agitation of the lens in the surfactant and/or 20 seconds digital cleaning of the lens did reduce the levels of lipid deposited on the lens surface. However, these processes themselves were not able to take all the "removable" lipid off the lens surface. A further 20 seconds digital clean with a surfactant cleaner (which had been shown to be effective in previous investigations) was required to remove all the removable lipid from the contact lens surface. This final surfactant clean further reduced the relative fluorescence emissions of the contact lenses by 5-40%. Even after this final surfactant clean it was apparent that all the test lenses still had some lipid deposited on their surfaces; the fluorescence peak heights were still greater than those recorded for unworn HEMA lenses. This represents the tightly bound lipid that will always remain at the lens surface even following surfactant cleaning.

The method of evaluating the effectiveness of surfactant cleaners using a rigid cleaning protocol and fluorescence analysis could also be used to test the ability of a wide range of lens care products or novel lens care products to reduce both protein and lipid deposition levels. Any lens care products (surfactant cleaners,

disinfection systems, enzyme tablets) could be tested in a suitably adapted cleaning or care protocol using fluorescence analysis before, during and after the appropriate cleaning or care regime. An excitation wavelength of 360nm would show the effect of a particular lens care product on lipid deposition levels, while an excitation wavelength of 280nm would highlight any decreases in protein deposition levels. In this way the ability of a whole range of lens care products to affect protein and lipid deposition levels could be evaluated.

So, it can be seen that as well as being an extremely important method of analysis of contact lens deposition levels, fluorescence spectroscopy is also extremely useful in evaluating the effectiveness of contact lens care products.

10.2.5 Lysosyme migration in ionic high water content lenses (EtafilconA).

At the outset of this study little was known about lysosyme migration in ionic high water content contact lenses. The phenomenon was first noticed in Chapters 6 and 7. In Chapter 6 wide variations in protein deposition levels (from 3-30mm above baseline) were detected on fluorescence spectra recorded from high water content ionic lenses (Acuvue) following a fortnights wear period. It was felt that these variations could be linked to slight (four day) variations in lens storage times. In Chapter 7, initial analysis of the fluorescence data showed, that on a number of occasions, Acuvue lenses had increased levels of surface protein deposition following overnight storage (the results given in Chapter 7 are from aggregated fluorescence data, therefore these variations are not apparent). In addition, control Acuvue lenses, which were not surfactant cleaned or disinfected, showed a

decrease of around 80% in protein fluorescence peak height following overnight storage in saline.

It was felt that these variations in surface protein deposition levels were due to migration of protein from the stored lenses. Minarik and Rapp[79] showed that high water content ionic lenses (e.g. Acuvue) can absorb large amounts of protein, particularly lysosyme. Thus, a worn high water content ionic contact lens will contain large amounts of lysosyme, both deposited on the lens surface, and absorbed within the lens matrix. In addition, fluorescence studies using dilute solutions of individual proteins show, that of the tear proteins likely to be deposited on a lens surface (e.g albumin, globulins, lactoferrin and lysosyme), lysosyme gives a much greater emission peak when excited at 280nm than most other proteins.

As described earlier, extremely large variations were seen in the protein fluorescence peak heights following periods of lens storage. This fact, combined with the fluorescence data obtained from recording spectra at 280nm of standard protein solutions, indicates that migration of lysosyme is responsible for the variations in protein deposition levels. If these variations were to be attributed to another protein species, then a very large amount of e.g. albumin would be required to produce the large variations in peak height that were seen. This seems unlikely, particularly as many of the other protein species deposited during contact lens wear are too large to migrate into the contact lens matrix. It therefore appears that the increased protein deposition levels seen on Etafilcon A lenses after cleaning and disinfection, and the variations in protein deposition levels following differences in Acuvue storage times, are due to lysosyme leaching from the lens

matrix to the surface of the contact lens, and then from the surface of the contact lens into the lens storage solution.

A study was therefore set up to further investigate the processes involved in lysosyme migration (Chapter 8). The study involved monitoring the changes in protein deposition levels of stored Etafilcon A lenses (Vistakon Sureview), and the lens storage solutions (Bausch & Lomb Renu), over a five week period of analysis. Fluorescence spectra were recorded from the stored lenses, and the lens storage solutions, twice weekly at an excitation wavelength of 280nm. After each set of spectra had been recorded, the lenses were stored in fresh Renu. Therefore, over a period of five weeks, information was obtained about variations in the surface protein deposition levels of the stored Etafilcon A lenses. In addition, placing the lenses in fresh storage solution twice weekly indicated whether or not lysosyme was migrating from the stored lenses, into the storage solution throughout the study period. The information obtained from this study is presented below.

Lysosyme migration from the Etafilcon A contact lenses was seen to occur consistently throughout the study. Protein was detected in all of the analysed lens storage solutions, and the protein deposition levels on the stored lenses were seen to vary throughout the period of study.

Therefore, it appears that during lens storage, lysosyme will gradually diffuse from the matrix of the Etafilcon A contact lens and start to build up on the surface of the lens. The level of lysosyme at the lens surface will continue to build up until it reaches a maximum. At this point the layer of deposition at the lens surface will start to "overload". When deposits first start to form on a contact lens surface, the

first layer of deposition will be strongly bound to the polymer matrix of the lens. Subsequent layers of deposition will be bound less strongly, and the outermost deposited layers will be very loosely bound, both to the contact lens itself and to other deposited layers on the contact lens. Thus, when the lens becomes heavily deposited, the forces which usually hold the deposited layers on the lens surface are not strong enough to maintain them, so, all but the most strongly bound will become detached from the surface of the lens.

The fall off of the lysosyme layers is rapid. The built up lysosyme layers will rapidly drop off the lens surface into the lens storage solution. Once the accumulated lysosyme layer has become detached from the surface of the contact lens, the remaining lysosyme in the lens matrix will start to diffuse from a high concentration in the bulk of the lens to a low concentration at the lens surface, so re-forming a layer on the surface of the contact lens.

Fluorescence analysis of the lens storage solutions showed that there was a steady rise in cumulative protein deposition levels in the Renu storage solutions throughout the study period. This indicates that lysosyme was constantly diffusing from the stored Sureview lenses into the lens storage solution throughout the period of study. It was clear that the amount of protein (lysosyme) diffusion from the stored lenses is many times greater than than the amount of lipid diffusion. Therefore, over a period of lens storage, the lipid deposition levels will not reduce to any great extent. In contrast, the protein deposition levels can be greatly affected by lens storage.

It is interesting to note that the stored Sureview lenses did appear to be becoming depleted of lysosyme over the study period. The cumulative protein deposition level in the Renu storage solutions continued to rise throughout the five

week study period. However, the rate of increase was slowing and had almost stopped during the fifth week of analysis. This indicates that less and less lysosyme was diffusing from the contact lens into the storage solutions as the period of lens storage increased.

Therefore, it appears that lenses can be almost totally depleted of lysosyme following a long period of storage, provided that the lens storage solution is changed regularly. It is unlikely though that a contact lens would be able to be completely denuded of protein in this manner. Some protein will be irreversibly bound to the contact lens and therefore will not be able to diffuse from the contact lens into the storage solution.

So, it can be seen that the undulating process of lysosyme migration will continue indefinitely, with layers of lysosyme continuously building up on, and becoming detached from, the lens surface. The rate of build up of layers on the lens surface will start to slow down with time as the lens matrix becomes depleted of lysosyme. However, it is felt that when the concentration of lysosyme within the lens matrix becomes significantly depleted there is likely to be some diffusion back from the lens surface into the bulk of the lens. This will only occur if there is a greater concentration of lysosyme at the lens surface, and diffusion will stop when equilibrium is reached between the lens surface and the bulk of the contact lens.

It must be remembered that migration of lysosyme will only arise with lens materials such as Etafilcon A which are both ionic and have a high water content. It is this combination of factors which are required to attract, and then hold substantial amounts of lysosyme within the contact lens matrix. As discussed

earlier, the principle monomers of Etafilcon A are HEMA and methacrylic acid. The presence of methacrylic acid results in the overall net charge of an Etafilcon A lens being highly negative. A number of studies[79,80] have concluded that such a charge will cause the lens to attract a far greater number of deposits, particularly protein. This is believed to be because the positive charge on the surface of protein will thermodynamically favour such attraction. The charges on proteins arise because of amino acid residues on protein side chains.

Therefore both the surface charge of a lens, and the overall net charge of proteins will affect the levels of protein deposition. In addition, the size of a protein, and the pore size and/or number of pores of a contact lens polymer, will determine whether a protein is simply deposited on the lens surface, or will migrate into the polymer matrix itself. This is particularly relevant for high water content lenses because these lenses have a larger pore size and/or number of pores so are therefore more susceptible to tear film constituents entering the lens matrix.[79] However, although high water content lenses do have a larger pore size than lenses with a lower water content, most deposited tear proteins are still too large to enter the polymer matrix. For example tear albumin and tear lactoferrin have molecular weights of 90 and and 67Kd respectively. These molecular weights can be contrasted with that of lysosyme (14.3Kd).[47] The much lower molecular weight (and therefore smaller size) of lysosyme enable it to pass through the pores of ionic high water content lens materials, e.g. Etafilcon A, thus being absorbed in large amounts within the contact lens matrix.

During the storage of Etafilcon A lenses lysosyme will diffuse from the lens matrix to the surface of the lens, and then from the surface of the lens into the storage solution. It is also probable that when the level of lysosyme in the lens matrix becomes less than that at the lens surface, diffusion will occur from the lens surface back into the bulk of the contact lens. Following on from this it is likely that as well as lysosyme diffusing back from the lens surface into the lens matrix, lysosyme will also diffuse from the lens storage solution onto the surface (and then into the lens matrix) of the contact lens. This will only occur if the lens storage solution has a concentration of lysosyme greater than that on the lens surface i.e. if the lens is stored in an unchanged solution over a long period of time.

For a protein to take part in an interfacial reaction (i.e. be adsorbed on a contact lens surface), it must first arrive at the surface of the lens by a process of diffusion. If the lens is stored in a solution with a high protein concentration, all the protein molecules in the vicinity of the lens surface will be rapidly adsorbed, provided that the level of lysosyme at the lens surface is lower than the concentration of lysosyme in solution. As a result of this absorption, the protein concentration in a juxtapositioned interfacial sublayer, of several molecular diameters in thickness will be reduced to zero. A diffusion process will then occur from the bulk solution phase into the sublayer, and then from this sublayer onto the lens surface.

Multiple layers of protein can be adsorbed onto the lens surface. The high concentration of adsorbed protein at the lens surface will allow diffusion from the lens surface into the contact lens matrix. These processes are not static, diffusion will continue to, or from, the lens matrix, surface and storage solution, depending on the concentrations of protein (lysosyme) within these areas. Diffusion will only

cease once an equilibrium has been reached between the lens storage solution, the surface of the lens, and the contact lens matrix.

It is felt that lenses for long periods in the same solution will probably reach an equilibrium between the lysosyme in solution, and the lysosyme on and within the contact lens surface.

Throughout this project, fluorescence spectroscopy has been shown to be a very powerful non-destructive method of analysing the interaction of contact lens materials with the eye. The unique ability of this technique to distinguish clearly between protein and lipid deposited on the lens surface, even after only very short periods of wear, means that fluorescence spectroscopy will be extremely valuable in enabling the processes involved in the early stages of contact lens spoilation to be understood. It is only once these processes of early deposit formation are fully understood that steps can be taken to minimise deposit formation on contact lenses i.e. through the use of more deposit resistant lens materials, or improved cleaning systems.

10.3 Problems encountered with the use of fluorescence spectroscopy.

The overall aim of this project was to assess the potential of fluorescence spectroscopy as a method of contact lens analysis. It was also important to determine any problems involved with the use of this technique. Generally, very few difficulties were encountered. Those that were will be discussed below.

10.3.1 Effect of lens storage times on analytical results.

The first problem encountered in this project was the effect of the storage period of contact lenses, i.e. the length of time between removal of the contact lens from the eye and fluorescence analysis being performed. Obviously the ideal situation would involve removing the lens from the eye followed by immediate fluorescence analysis. However, this was not often possible due to the need for lenses to be sent to Aston University from the Optometrist's practice in London. Therefore it was important to ensure that the lenses were analysed as soon as possible after arrival at Aston. This will minimise any bacterial contamination of the contact lenses, and limit any disruption to the deposited layer on the contact lens.

If the study being carried out involved fluorescence spectra being recorded from contact lenses over a period of time, the lenses being analysed were stored in Bausch and Lomb Renu. This is a multi-purpose solution which has anti-bacterial activity, which will limit any bacterial contamination of stored contact lenses. Renu also can be used as a surfactant cleaner. This could lead to reductions in the deposited lipid layer during lens storage. Therefore extreme care was taken when storing spoiled contact lenses in Renu to ensure that no agitation of the contact lens occurred during storage. In this way it is unlikely that any loss of deposited lipid layer occurred.

As stated the lenses were usually stored for one to two weeks before analysis. Wherever possible, the storage times for a group of contact lenses involved in a particular study were kept equal i.e. lenses from one study may have been analysed following seven days storage and lenses from another study may have

been stored for nine days before analysis. However, if possible, all the lenses from one study were analysed on the same day.

Studies involving storage and re-analysis of contact lenses (Chapters 7 and 8), showed that this one to two week period of storage had little effect on the lipid deposition levels of all lens types studied. The protein deposition levels also remained relatively constant for all lens types studied, with the exception of the ionic high water content lenses (Etafilcon A). The problems encountered with storage of Etafilcon A lenses will be discussed later. However, it was clear from these studies that a lens storage period of one to two weeks before analysis would not significantly affect the deposited layer on the contact lens.

10.3.2 Reproducibility of results.

To ensure reproducibility of results several steps must be followed in relation to the cuvette used for fluorescence analysis, positioning of the contact lens within the cuvette and orientation of the cuvette within the spectrofluorometer.

Throughout this study a round fused silica glass cuvette was used. The clean, dry cuvette is first filled with distilled water. The lens is then positioned in the cuvette so that it curves round in a horse shoe shape, making sure that most of the lens comes into contact with the inside of the cell (Figure 11, Chapter 3). Care must be taken with lens mounting in the cuvette, and positioning of the cuvette in the cuvette holder of the spectrofluorometer. Several conditions must be observed to avoid experimental inaccuracies. These are:-

1. The cuvette must be totally clean and dry.

2. There must be no air bubbles in the distilled water in the cuvette.

3. The contact lens must be carefully mounted in the cuvette as described.

4. The cuvette containing the contact lens must be carefully placed in the cuvette holder of the spectrofluorometer. Variations in the glass of the cuvette can give rise to false readings. Therefore, before experiments can be performed, the cuvette containing only distilled water, must be evaluated in the spectrofluorometer to ensure that the cuvette itself will produce no fluorescent signal. Once a suitable orientation for the cuvette has been found it must always be placed in the cuvette holder in this specific position. This can be achieved by placing a mark on the rim of the cuvette, and then ensuring that this mark is always in the same position in relation to the cuvette holder of the spectrofluorometer.

10.3.3 Fluorescence spectroscopy of ionic high water content lenses.

The process of lysosyme migration from Etafilcon A contact lenses has been described earlier. This phenomenon was not noticed in any of the other lens materials analysed in this investigation. Lysosyme migration will occur rapidly from Etafilcon A contact lenses leading to large variations in the amount of lysosyme present at the lens surface at any one time. This would obviously affect fluorescence spectroscopy measurements taken from Etafilcon A lenses (fluorescence spectroscopy will only detect material deposited on the lens surface). So it is clear that care must be taken when recording fluorescence spectra from lenses fabricated from Etafilcon A.

The study performed to investigate lysosyme migration (Chapter 8) indicated

that after a storage period of one to two weeks in an unchanged storage solution the concentration of lysosyme on and within an Etafilcon A lens will equilibrate with the concentration of lysosyme in the storage solution. This occurs due to the steady diffusion of lysosyme from the contact lens to the storage solution. At this equilibrium point no further diffusion will occur from the contact lens, so the concentration of lysosyme at the lens surface will remain constant.

Therefore it was felt that the most accurate way to record fluorescence spectra from Etafilcon A lenses (e.g. Acuvue and Sureview) is to wait until they have reached equilibrium with their storage solution, as this is the only way to ensure that no effects will be seen from the migration of lysosyme to and from Etafilcon A lenses. Ideally any such lenses should be left for at least a week after collection before any analysis is undertaken. In addition, when recording fluorescence spectra from high water content ionic contact lenses, it is also important that lens storage times are not allowed to vary greatly. If possible fluorescence spectra from a group of such lenses should be recorded on the same day .

It is also important that the lens storage solution is not changed before analysis is performed. By not changing the lens storage solution, a worn Etafilcon A lens will be able to reach equilibrium with it's storage solution with minimum loss of protein from the lens surface and matrix. If the storage solution was replaced with fresh solution, more lysosyme would have to diffuse from the contact lens in order to re-establish equilibrium between the contact lens and the storage solution. This required storage period of at least a week could result in bacterial contamination of the contact lens therefore it is essential that worn contact lenses should be placed in sterile containers which preferably contain a solution that has antibacterial activity.

These facts must always be remembered when performing fluorescence analysis on lenses fabricated from Etafilcon A. This will ensure that consistent and repeatable results are obtained. Obviously the recorded fluorescence peak height will not be representative of the total amount of protein that was on the surface and within the bulk of the contact lens and when it was removed from the eye; some of the lysosyme that was present initially will have diffused into the storage solution. However, fluorescence spectroscopy is only able to detect material deposited on the surface of the contact lens, so any lysosyme within the lens matrix would not be detected anyway.

Therefore it can be seen that any potential problems with the use of fluorescence spectroscopy as a method of analysing contact lens deposition levels can be overcome provided that sufficient care is taken when the technique is used.

10.4 Concluding discussion.

This project out to assess the potential of fluorescence spectroscopy; a non-destructive technique intended to be used for analysing contact lens deposition levels. This technique is unique, it enables detailed analysis of the surface deposition levels of a contact lens to be made at any stage during contact lens wear. Fluorescence spectroscopy has many advantages over other methods available for analysing contact lens deposition.

Firstly, the analytical procedure involved in performing fluorescence spectroscopy is relatively quick and easy to perform. Several experimental

conditions must be adhered to to ensure accuracy and reproducibility of results, but these are all fairly straightforward and do not present any great problem. The ease of use of fluorescence spectroscopy is one of it's major advantages over other analytical methods e.g. electrophoresis and HPLC. These methods can be both time consuming and costly to perform.

Comparing fluorescence spectroscopy with techniques like electrophoresis and HPLC, highlights another advantage involved with the use of fluorescence analysis. This is the non-destructive nature of fluorescence spectroscopy. During fluorescence analysis contact lenses are simply placed in the cuvette (which contains distilled water) for one to two minutes i.e. the time taken to record a fluorescence spectrum. The lens is then returned, undamaged to the storage solution. The non-destructiveness of this technique means that following the initial determination of lens protein and lipid deposition levels further more detailed analysis of lens deposits can be made using destructive techniques such as electrophoresis and HPLC. Alternatively, following fluorescence analysis a contact lens could be returned to in eye wear. In this way a detailed picture of deposit formation could be built using a long wear period in combination with regular fluorescence analysis.

One problem with the use of destructive analytical techniques is the need to extract deposited material from the contact lens before analysis can be performed; there is always doubt as to how much deposited material will actually have been removed by the extraction method. Fluorescence analysis of a lens following extraction will determine how much deposited material remains at the lens surface.

Other advantages of analysis using fluorescence spectroscopy are the extreme sensitivity and selectivity of the technique. These compare very favourably with

other optical methods of lens analysis, e.g. optical microscopy, UV spectroscopy. Fluorescence spectroscopy is able to clearly distinguish between protein and lipid deposited on a lens surface. This enables information about deposition profiles of contact lens materials to be accumulated i.e. which lens materials have a tendency to deposit protein and/or lipid heavily. The ability to distinguish between protein and lipid on the lens surface will also highlight unusual patient to patient variations and left eye/right eye differences.

The extreme sensitivity of fluorescence spectroscopy enables even very low levels of deposition to be detected at the lens surface. Therefore using fluorescence spectroscopy it is possible to make detailed studies of the early stages of contact lens deposition. It is vital to be able to study these early stages, as it is only once they are fully understood that steps can be taken to minimise deposit formation on contact lenses during longer wear periods.

Because fluorescence spectroscopy can detect even small changes in deposition levels at a lens surface it is possible to determine the effectiveness of lens cleaning and disinfection products at reducing the deposited layer. Fluorescence spectra can be recorded before and after e.g. surfactant cleaning. In this way the ability of the lens care product to reduce the level of deposition can be ascertained.

This project has shown what an extremely valuable technique fluorescence spectroscopy can be and how it can be used with great effectiveness to further our understanding of the processes involved in contact lens spoilation. Any problems arising from the use of the technique were highlighted (and overcome) in the various studies performed during the course of this project.

Perhaps the only drawbacks to the technique as used in this project are the fact

that it can only be used to detect material deposited on the surface of a contact lens, and that the technique was qualitative rather than quantitative i.e. it was not possible to determine the actual amount of protein or lipid detected at the lens surface. These points will be discussed in the final sections of this conclusion.

10.5 Quantification of fluorescence spectroscopy.

Since the end of this project experiments performed by Dr. Valerie Franklin have enabled quantification of the peak heights recorded on fluorescence spectra. These experiments involved recording spectra from clean, unworn contact lenses and then dehydrating the lenses in a microwave. The lenses were then weighed, and left to dehydrate fully. After rehydration the lenses were spoiled in standard solutions of protein or lipid. The spoilation method used was similar to that described in Chapter 2. The artificial tear solution was replaced by the standard solutions, and the period of spoilation varied according to the set experimental conditions. At regular intervals (e.g. one hour, two hours etc.), fluorescence spectra were recorded from the spoilt lenses. Following this the lenses were again dehydrated and weighed. After full rehydration the lenses were replaced in the appropriate standard solution. Repeating these processes over a period of time enabled the change in weight of protein or lipid deposited on the contact lens surface to be related to increase in fluorescence peak height. In this way calibration graphs can be plotted for amount of deposited protein or lipid versus fluorescence peak height. This should then enable any protein or lipid peak height recorded from a spoiled contact lens to be related to the actual amount of deposited

protein or lipid.

Lipid families are much less fluorescent than protein, but both lipid and protein give linear calibration graphs up to about 20% fluorescence. Although "universal" calibration graphs give an excellent indication of surface protein, for absolute precision calibration graphs should be constructed for each material. This is because not all proteins are equally fluorescent, and the nature of protein adsorption i.e. which proteins predominate at the lens surface differs somewhat from material to material.

In this project however, comparisons are made within similar families of materials and highlight patient to patient variations. In this case the calibration graph does not differ between patients. As a general guide, 20% fluorescence corresponds to 4000υg protein and 914υg of lipid.

10.6 Suggestions for further work.

This project has raised several interesting issues (many of which are now being studied by the Research Group at Aston). Suggestions for further work are as follows:-

1. One of the earliest studies in this project involved investigating the early deposition stages of patients who were known white spot depositors. The results from these patients were compared with patients not known to be white spot depositors. Both sets of patients cared for their lenses in an identical manner over a two week wear period. The results from this study showed that no consistent

difference was seen in gross deposition levels between the known white spot depositors and the non-depositors. This is consistent with the view that specific chemical steps, rather than simple bulk accumulation, are involved in white spot formation. Therefore, it can be seen that it is not possible to predict whether or not a contact lens wearer will be a potential white spot depositor, simply from fluorescence analysis of gross lens deposition levels in the very early stages of wear.

However, for further work it would be interesting to monitor deposition profiles of white spot depositors and non-depositors over longer wear periods i.e. performing regular fluorescence analysis, returning the lens to in eye wear between analyses. The fluorescence analysis could be combined with regular use of more detailed analytical methods (e.g. HPLC), on some of the worn lenses. This would give information relating to the stage in contact lens wear at which processes that result in white spot formation start to occur, and help to determine whether deposition of certain lipids will initiate white spot formation..

2. As stated earlier one of the main drawbacks with fluorescence analysis as it was used in this project is that it can only be used to detect material deposited on the surface of a contact lens. This can be a particular problem with contact lens materials such as Etafilcon A which take up large amounts of protein into the lens matrix. It is hoped that using fluorescence spectroscopy in combination with UV spectroscopy should be able to overcome this problem.

There are several disadvantages to using only UV spectroscopy as a method of analysing contact lens deposition levels. Firstly UV spectroscopy is a transmission technique. This means that it can only be used to detect relatively

large amounts and therefore can be too insensitive to detect the very low levels of deposition found in the early stages of lens wear. Secondly, UV spectroscopy relies on the fact that compounds absorb light in the ultra-violet wavelength range. Since a vast number of compounds will absorb light in this region, UV spectroscopy has relatively low specificity as an individual analytical technique.

However, although the selectivity and sensitivity of UV spectroscopy are too low to make it valuable as as an individual method of analysing contact lens deposition levels, it can be used to detect protein and/or lipid contained within the contact lens matrix. Therefore it can be seen that the sensitivity and selectivity of fluorescence spectroscopy to detect material deposited at the lens surface could be used in combination with the ability of UV spectroscopy to detect material within the contact lens matrix. In this way an overall picture of the levels of protein and lipid deposited on the lens surface and within the lens matrix can be built up.

3. The effect of different disinfection methods on deposition levels during the first few months of wear has been discussed earlier. This study showed that there was no difference in deposition levels between lenses disinfected with a chlorine based product and lenses disinfected with a hydrogen peroxide based product. However, it is felt that the choice of disinfection system is important in minimising deposit formation during long wear periods.

Therefore it would be interesting to monitor the deposition levels of contact lenses that had been disinfected with different systems over a longer wear period e.g. one year of more. Recording fluorescence spectra regularly and then returning the lens to in eye wear would produce a detailed picture of when and

how the method of disinfection starts to influence lens deposition levels.

4. The fact that fluorescence spectroscopy is a non-destructive method of analysis allows further, more detailed analysis of the material deposited on a contact lens to be performed following initial estimations of the protein and lipid deposition levels. This would be particularly useful to increase understanding of the very early stages of contact lens spoilation. For example electrophoresis or HPLC could be performed following fluorescence analysis to determine which proteinaceous and/or lipoidal species are deposited on a particular contact lens material following very short periods of wear, and in what quantities.

Clinically based studies could then be set up in which lenses worn under strictly monitored conditions were collected after increasing periods of wear. These lenses could then have initial estimations of protein and lipid deposition levels determined by fluorescence spectroscopy. More detailed analysis could then be performed using techniques such as electrophoresis and HPLC which would give information relating to the changes in amount and type of deposited material occurring as the lens wear time is increased.

5. Further experiments could be performed to increase our understanding of lysosyme migration to and from high water content ionic lenses. Using the knowledge from quantification experiments (possibly in conjunction with UV spectroscopy) and a similar study to that described in Chapter 8 it may be possible to determine the amounts of lysosyme taken into the lens matrix over a given wear period. Similarly the amount of lysosyme leaching from the lens matrix over a

specific period of storage could also be determined. This would enable more accurate determination of the total protein deposition levels of lens materials such as Etafilcon A.

6. A study in this project has already described how fluorescence analysis can be used to evaluate the efficiency of surfactant cleaners. The fact that this method of evaluation using a rigid cleaning protocol and fluorescence analysis could be used to test the ability of a wide range of lens care products or novel lens care products to reduce both protein and lipid deposition levels has also been discussed. For further work, any lens care products (surfactant cleaners, disinfection systems, enzyme tablets) could be tested in a suitably adapted cleaning or care protocol using fluorescence analysis before, during and after the appropriate cleaning or care regime.

In the study undertaken in this project (Chapter 9) worn contact lenses were analysed throughout the cleaning protocol. However, it is also possible (and probably easier in a laboratory based situation) to use *in vitro* spoilt contact lenses. Lenses could be artificially spoiled in the in vitro model described in Chapter 2, and then used in a cleaning protocol in exactly the same way as an *in vivo* spoiled contact lens. In this way an evaluation of the effectiveness of lens care products to reduce deposition levels on a wide range of contact lens materials can be undertaken in a purely laboratory based experiment. So it would be possible to determine not only which lens care products are most effective, but also whether some products are more effective at reducing deposition levels on certain types of contact lens materials.

10. References.

1. Hoffman, A S, Ratner, B D: (1975) Synthetic hydrogels for biomedical applications. Polym. Prepr. Am. Soc. Div. Polym. Chem. 2, pp272-275.

2. Graham N B, Wood D A: (1984) Polymeric inserts and implants for controlled release of drugs, in Macromolecular Biomaterials. (Eds. Hastings, G,W,; Ducheyne, P.) CRC Press, Boca, Raton, Florida.

3. Tighe B J: (1976) The design of polymers for contact lens applications. Br. Polym. J. 8, pp71-76.

4. Tighe, B J: (1986) The role of permeability and related properties in the design of synthetic hydrogels for biomedical applications. Br. Polym. J. 18 (1), pp8-13.

5. Wichterle O, Lim D: (1960) Hydrogels for biological use. Nature 185, pp117-118.

6. Baier R E: (1977) The organisation of blood components near interfaces. Ann. N.Y. Acad. Sci. 283, pp17-36.

7. Baker R E, Tighe B J: (1980) Polymers in contact lens applications VIII. The problem of biocompatibility. Cont. lens J. 18, pp3-14.

8. Tripathi R C, Tripathi B J, Ruben M: (1980) The pathology of soft contact lens spoilage. Ophthalmol. 87, pp365-380.

9. Tripathi R C, Tripathi B J, Millard C B: (1988) Physico-chemical changes in contact lenses and their interactions with the cornea and tears: A review and personal observations. CLAO J. 14 (1), pp23-32.

10. Tighe B J: (1987) Hydrogels in medicine and pharmacy. (Vol.3) (Peppas N A, ed.) pp53-82, CRC Press, Boca, Raton, Florida.

11. Tighe B J: (1988) in Contact Lens Practice (3rd edition), Stone J and Phillips A J eds. pp72-124, Butterworths, London.

12. Refojo M F: (1978) The chemistry of soft contact lens materials. In: Soft Contact Lenses: Clinical and Applied Technology, Ruben M ed. Wiley, New York, pp19-39. Enclyopedia of Polymer Science and Technology (Vol. 1) p195, Wiley Interscience.

13. Franklin V J, Bright A M, Tighe B J:(1983) Hydrogel polymers and ocular spoilation processes. Trends in Polymer Sciences Vol.1, No.1, pp9-16.

14. Burstein N L: (1985) The effects of Topical Drugs and Preservatives on the Tears and Corneal Epithelium in Dry Eye. Trans. Opthalmol. Soc. U.K. 104, pp402-409.

15. Holly F J, Lemp M A: (1977) Tear physiology and dry eyes. Surv. Ophthalmol. 22, pp69-87.

16. Lamberts D W: (1987) Physiology of the tear film. In: Smolin G, Thoft R A, eds. The Cornea. Little, Brown & Company. pp38-52.

17. Wolff E: (1954) Anatomy of eye and orbit. Fourth edition, New York, Blakiston Co., pp207-209.

18. Holly F J, Lemp M A: (1971) Surface chemistry of the tear film: implications for dry eye syndromes, contact lenses and opthalmic polymers. J. Amer. Contact Lens Soc., 5, pp27-34.

19. Botello S Y: (1964) Tears and the lacrimal gland. Sci. Am. 211, pp78-86.

20. Holly F J: (1978) Surface chemical evaluation of artificial tears and their ingredients II. Interaction with a superficial layer. Cont. lens J. 4(3), pp52-65.

21. Andrews J S: (1970) Human tear film lipids I. Composition of the principal non-polar component. Exp. Eye Res. 10, pp223-227.

22. Nicolaides N, Kaitaranta J K, Rawdah T N, Maey J I, Boswell III F M, Smith R E: (1981) Meibomian gland studies. Comparison of steer and human lipids. Invest. Ophthalmol. Vis. Sci. 20(4), pp522-537.

23. Nicolaides N, Santos E C, Papadakaski K: (1984) Double bond patterns of fatty acids and alcohols in steer and human meibomian gland lipids. Lipid 19(4), pp264-277.

24. Tiffany J M: (1978) Individual variations in human meibomian lipid composition. Exp. Eye Res. 27, pp289-300.

25. Kikkawa T: (1969) Studies on the mechanism of tear secretion II on the secretory potential and the mechanism of it's establishment in the lacrimal gland of the rabbit. Acta. Soc. Ophthalmol. Jap. 73, pp1016-1073.

26. Bron A J: (1988) Eyelid secretions and the prevention and production of disease. Eye 2, pp164-171.

27. Mircheff A K: (1989) Lacrimal fluid and electrolyte secretion: a review. Current Eye Res. 8(6), pp607-617.

28. Rismondo V, Edelhauser H F, Ubels J L: (1987) Ionic composition of tears and lacrimal gland fluid. ARVO abstracts p157.

29. Huth S W, Hirano P, Leppold I H: (1980) Calcium in tears and contact lens wear. Arch. Ophthalmol. 98, pp122-125.

30. Van Haeringen N J: (1981) Clinical biochemistry of tears. Surv.

Ophthalmol. 26(2), pp84-95.

31. Iwata S: (1973) Chemical composition of the Aqueous phase. Int. Opthalmol. Clin. 13(1), p29-46.

32. Holly F J: (1981) Tear film physiology and contact lens wear I. Am. J. Optom. & Phys. Optics 58(4), pp324-330.

33. Janssen P T, Van Bijsterveld O P: (1983) Origin and biosynthesis of human tear fluid proteins. Invest. Ophthalmol. Vis. Sci. 24, pp623-630.

34. Creeth J M: (1978) Constituents of mucus and their separation. Br. Med. Bull. 34(1), pp17-21.

35. Botello S Y: (1964) Tears and the lacrimal gland. Sci. Am. 211, pp78-86.

36. Tripathi R C, Tripathi B J: (1981) The role of lids in soft contact lens spoilage. Contact Intraocular Lens Med. J. 7, pp234-240.

37. Bowers R W J, Tighe B J: (1987) Studies in ocular compatibility of hydrogels. A review of the clinical manifestations of spoilation. Biomaterials 8, pp8-13.

38. Lowther G E, Hilbert J A, King J E: (1975) Appearance and location of hydrophilic lens deposits. ICLC 2(4), pp30-34.

39. Bowers R W J, Tighe B J: (1987) Studies in ocular compatibility of hydrogels. White spot deposits-chemical composition and geological arrangement of components. Biomaterials 8, pp172-177.

40. Hart D E: (1984) Lipid deposits which form on extended wear contact lenses. ICLC 2, pp318-362.

41. Klein P: (1983) Review of deposits on extended wear contact lenses. ICLC 10(3), pp161-165.

42. Caroline P J, Robin J B, Gindi J J, Pickford M S, Olsen A P, Schanzlin D J: (1985) Microscopic and elemental analysis of deposits on extended wear soft contact lenses. CLAO J. 11(40), pp311-316.

43. Karageozian HL: (1976) Use of an amino acid analyser to illustrate the efficacy of an enzyme preparation for cleaning hydrophilic lenses. Contacto 20, pp5-10.

44. Karageozian H L: (1974) Chemical identity of opaque deposits on human worn hydrophilic contact lenses. Allergan Report Series 92, Allergan Pharmaceuticals, Irvine, California.

45. Allen J, Botting R, Sharp A, Tuffery A A: (1978) Composition of deposits on extended wear soft contact lenses. Optician 8, pp8-9.

46. Baily N J: (1975) Contact lens coatings: the effects on service life. J Am. Optom. Assoc. 46, pp214-218.

47. Wedler F C: (1977) Analysis of biomaterials deposited on soft contact lenses. J. Biomed. Mater. Res. 11, pp525-535.

48. Bloomfield S E, David S D, Rubin A L: (1978) Acute Corneal Calcification. Ann. Opthalmol. 10, pp355-360.

49. Hart D E, Shih K L: (1987) Surface interaction of hydrogel extended wear contact lenses: microflora and microfauna. Am. J. Optom. Physiol. Optics 64(10), pp739-748.

50. Roth H W: (1978) The etiology of ocular irritation in soft contact lens wearers: distribution in a large clinical sample. Contact Intraocular Lens Med. J. 4, pp38-47.

51. Ganju S N, Cordrey P: (1976) Removal of adsorbed preservative in soft

contact lenses. Eye 1, pp17-21, CLM publication.

52. Kleist F D: (1979) Appearance and nature of hydrophilic contact lens deposits. Part 1: protein and other organic deposits. ICLC 6, pp120-130.

53. Fowler S A, Mathea M S, Allansmith M R: (1980) Evolution of Soft Contact Lens coatings. Arch. Opthalmol. 98, pp95-99.

54. Trager S Y: (1972) Solutions of Soft Lenses. Manufacturing Optics International May, pp403-405.

55. Krezanoski J Z: (1972) Pharmaceutical aspects of cleaning and sterilizing flexible contact lenses. The Opthalmic Optician 12, pp1035-1037.

56. Cumming J S: (1973) The future of Soft Contact Lenses. Manufacturing Optics International 26, pp309-312.

57. Krezanoski J Z: (1972) The significance of cleaning Hydrophilic Contact Lenses. Journal of the AOA 43(3), pp305-307.

58. Koetting R A: (1973) Coping with Coating-the most serious Soft Lens problem. Opt. J. Review of Optometry 4(4), pp20-27.

59. Loran D F C: (1973) Surface corrosions of hydrogel contact lenses. The Contact Lens 4(4), pp3-8.

60. Feldman G L: (1972) From the Editors Desk. Contact Lens Soc. of Am. J. 6(1), pp8-11.

61. Author Unknown: (1972) Through a Soft Lens brightly. Medical World News 13, pp7, 17-19.

62. Stein H A, Slatt B J: (1973) Clinical impressions of hydrophilic lenses. Canadian Journal of Opthalmol. 8, pp83-91.

63. Wiley T M: (1975) Discrete lens opacities on hydrophilic contact lenses.

Optometric Weekly July 10, pp34-34.

64. Doughman, Mobilia, Drago, Havener, Gavin: (1975) The nature of "spots" on Soft lenses. Ann. Opthalmol. 7, pp3-10.

65. Johnson D G: (1973) Keratoconjunctivitis associated with wearing hydrophilic lenses. Can. J. Opthalmol. April, pp92-96.

66. Lowther G E, Hilbert J: (1975) Deposits on hydrophilic lenses: differential appearance and clinical causes. Am. J. Optom. and Physiolog. Optics 52(10), pp687-692.

67. Hathaway R A, Lowther G E: Soft Lens Cleaners: Their effectiveness in removing deposits. J. Am. Optom. Assoc. Vol.49 no.3 3/78 pp259-265.

68. Korb D R, Henriquez A S: (1980) Meibomian gland disfunction and contact lens intolerance. J. Am. Optom. Assoc. 51, pp243-251.

69. Tripathi R C, Tripathi B J, Ruben M: (1978) Soft lens spoilation. In: Ruben M, ed. Soft contact lenses: Clinical and applied technology. Wiley, New York, pp299-334.

70. Dohlman C H, Boruchoff S A, Mobilia E F: (1973) Complications in use of Soft contact lenses in corneal disease. Arch. Opthalmol. 90, pp367-371.

71. Bilbaut T, Gachon A M, Dastugue B: (1986) Deposits on Soft Contact Lenses: Electrophoresis and Scanning Electron Microscopic examinations. Exp. Eye Res. 43, pp153-165.

72. Hosaka S, Ozawa H, et al: (1983) Analysis of deposits on high water content contact lenses. J. Biomed. Mat. Res. 17, pp261-277.

73. Franklin V J: (1990) Lipoidal species in ocular spoilation processes. PhD Thesis.

74. Lakowicz J R: (1983) Principles of fluorescence spectroscopy. Plenum

Press, New York.

75. Guilbault G G: (1973) Practical Fluorescence, Theory, Methods and Techniques. Marcel Dekker Inc., New York.

76. Walton A J, Maenpa F C: (1979) Applications of fluorescence to the study of proteins at interfaces. J. Colloid and Interface Science 72(2), pp265-278.

77. White C E, Arquaer R J: (1970) Fluorescence analysis: A practical approach. Marcel Dekker Inc., New York.

78. Mendes E: (1991) The analysis of contact lenses using fluorescence spectroscopy. Final Year Project.

79. Minarik L, Rapp J: (1989) Protein deposits on individual hydrophilic contact lenses: Effects of water and ionicity. CLAO J. 15, pp185-188.

80. Sack R A, Jones B, Antignani A, et al: (1987) Specificity and biological activity of the protein deposited on the hydrogel surface. Invest. Opthalmol. Vis. Sci. 28, pp842-849.

81. Jones L W J: (1992) Contact lens deposits: their causes and control. Contact Lens Journal 20, (1) pp6-13.

82. Norde W, Favier J P: (1992) Structure of adsorbed and desorbed proteins. Colloids and Surfaces (64) pp87-93.

Appendix: List Of Abbreviations.

Bufilcon A:- 45% ionic low water content lens polymer. Principal components; HEMA, DAA, MA. Lens types of this polymer used in this project; Softmate.

DAA:- diacetone acrylamide.

Etafilcon A:- 58% ionic high water content lens polymer. Principal components; HEMA, MA. Lens types of this polyner used in this project; Acuvue, Sureview.

FTIR:- Fourrier Transform Infra Red Spectroscopy.

HEMA:- 2-hydroxyethyl methacrylate.

HPLC:- high performance liquid chromatography.

IR:- infra-red.

MA:- methacrylic acid.

MMA:- methyl methacrylate.

Polymacon:- 38% non-ionic low water content lens polymer. Principal component; HEMA. Lens types of this polymer used in this project; Eurothin, Seequence.

Tetrafilcon A:- 42.5% non-ionic low water content lens polymer. Principal components; HEMA, VP, MMA. Lens types of this polymer used in this project; Classic.

UV:- ultra violet.

VP:- *N*-vinyl pyrrolidone.