OCULAR COMPATIBILITY OF HYDROGELS

by

RODERICK WILLIAM JOHNATHAN BOWERS

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SUMMARY

The primary objective of this research was to investigate the effects of long term wear on the ocular compatibility of hydrogel contact lenses with reference to the incidence of deposits. A number of spoilt lenses, representative of a wide range of lens chemistries were examined. A variety of analytical and histological techniques were developed in order to characterise the morphology, chemical composition and geology of lens deposits. Four major manifestations of hydrogel spoilation, discrete elevated deposits, lens discolouration, and lens surface coatings and plaques were examined.

Discrete elevated deposits and surface plaques were composed substantially of, but by no means solely, tear derived lipids. The morphology, geology and chemical composition of these deposits was unaffected by variations in the lens chemistry, wear protocol or patient identity. The rate of deposit formation was, however, a function of the interfacial tension of hydrogels with tears. These findings suggest that a common interfacial process, resulting in the general modification of lens properties, is responsible for deposit formation. Although not the most rapid process, lipid adsorption is considered to be the most important in the modification of the lens surface. Furthermore, it is considered that the presence of matrix-imbibed lens fabrication artefacts accelerate lipid adsorption and therefore deposit formation.

Lens discolouration results from the presence of matrix-bound melanin-like particulate deposits. Deposit formation is independent of the lens chemistry or wear protocol. The intensity of discolouration is a function of deposit size and population density. Lens coatings examined are composed of inorganic calcium phosphate crystals overlain by a proteinaceous coating. Deposit formation is independent of the lens chemistry and in this case resulted from the employment of inadequately de-ionised water in lens buffer formulations.

KEY WORDS

Biocompatibility
Ocular Environment
Hydrogel Contact Lens Spoilation
Light and Scanning Electron Microscopy
Histological and EDXA Techniques

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CHAPTER ONE

CHAPTER I

AN OVERVIEW OF THE PROBLEM OF HYDROGEL BIOCOMPATIBILITY

1.1 GENERAL INTRODUCTION

The formation of deposits on and within the expanded gel network of hydrogel soft contact lenses is a major and significant problem encountered during lens wear. This project is principally concerned with the development and application of analytical techniques suitable for the characterisation of such deposits. As a result of these investigations it is hoped to comment critically on the interfacial reactions that occur between hydrogel contact lenses and the ocular environment.

This deposition phenomenon is however, not unique to soft contact lenses, indeed the underlying processes are common to other examples in which materials come into contact with biological solutions. The deposition of tear components on contact lenses, the clotting of blood at foreign surfaces, the formation of dental plaque and marine fouling are all examples of the same phenomenon, the adsorption of bio-organic species at solid interfaces.

To date most studies of these manifestations have been primarily concerned with blood contact materials, and in particular, the study of thromboresistance or biocompatib-

ility of these materials. As a result of the apparent similarity of the various types of interactions of materials with biological environments, it is obvious that an appreciation of one area will facilitate progress in another. Chapter I reviews the problem of biocompatibility, and attempts to relate known blood contact behaviour to the associated problem of soft contact lens biocompatibility. A brief summary of synthetic hydrogels and their biomedical applications, as well as a review of the historical development and applications of soft contact lens materials will also be presented. Chapter 2 provides a characterisation of the ocular environment, whilst the various manifestations of hydrogel contact lens intolerance are outlined in Chapter 3.

1.2 BIOMATERIALS AND BIOCOMPATIBILITY

1.2.1 Biomaterials: A biomaterial may be defined as a material that is used in the treatment of patients and which at some stage interfaces with tissue for a significant length of time so that the interaction between the tissue and the material is an unimportant factor in the treatment[1]. The emphasis on this definition is on the time of contact between the material and the tissue of the patient, for it is this that distinguishes a biomaterial from any other material used only transiently where there is little time for nonfunctional interactions to have any significance. Thus, the definition excludes all materials that contact tissues for a short period of time, but encompasses all

those that interface with tissues for a length of time greater than a few hours.

1.2.2 Biocompatibility:

Biocompatibility is the term used to describe that state of affairs when a material exists within a physiological environment without either the material adversely and significantly affecting the body, or the environment of the body adversely and significantly affecting the material [2]. This definition implies there are two broad aspects of biocompatibility, the effects on the material, and the effects on the tissue. These are, however, very much interrelated, for it is often the adverse effect on a material by the physiological fluids that precipitates an adverse response from the tissue. Biocompatibility is primarily concerned with the chemical interactions that occur between the materials and the body fluids, and the physiological responses to these materials. As will become evident, the chemical interactions that occur between the biomaterial and its working environment are clearly dominated by the initial events at the molecular level and at the interface; thus it is the surface properties of the biomaterial that governs its acceptability within a defined environment.

1.3 CRITERIA FOR BIOCOMPATIBILITY

The criteria that govern the the biocompatibility of a material remain unclear. Estimations have been made of the

contributions of surface charge and density [3-8], critical surface tension and surface free energy [9-17], to the biocompatibility of hydrogels. Although no general correlation has been agreed on, a relationship between the critical surface tension of a material and its biocompatibility has been established [18].

The critical surface tension of a solid is defined as the surface tension of a liquid that will just wet the surface and spread. When any surface comes into contact with a liquid an interface is formed. The difference between the critical surface tension of the solid and the surface tension of the liquid is referred to as the interfacial tension. It is this concept that is often invoked by workers to describe the relationship between a surface and a biological fluid. Indeed several workers have formulated a Minimal Interfacial Surface Energy Hypothesis [19-22]. This suggests that in order for a material to achieve maximum biocompatibility it is necessary that it exhibits a low interfacial tension (of the order of 5 dynes.cm^{-1} or less) with the biological system in which it is placed.

An alternative approach to this 'Minimal Interfacial Surface Energy' hypothesis, which rests largely on theoretical considerations, is that of Baier et al [9-13]. Thromboresistant or blood compatible materials, were found to be coated with a thin glycoprotein film, with a critical surface tension of 20-30 dynes.cm^{-1}. Further work on the surface characterisation on the endothelial surfaces of blood vessels showed

that the critical surface tension also fell within this above range. From these findings they deduced that for a material to exhibit thromboresistance, the critical surface tension of the biomaterial must lie within the range of 20-30 dynes.cm^{-1}. This is known as the 'Moderate Surface Energy' hypothesis.

1.4 ASPECTS OF PROTEIN ADSORPTION ON POLYMERS

There are many examples of protein adsorption in daily events in which materials come into contact with various biological solutions and in so doing demonstrate the phenomenon of protein adsorption. The initial adsorption process exerts a profound and significant influence on further events in the relevant biological environment giving rise to familiar processes such as thrombus formation (in blood), the formation of dental plaque (in saliva) and marine fouling (in sea water). Recent evidence suggests that this phenomenon plays a role in fertility control by intra-uterine devices.

In addition to the aforementioned phenomena, must be added the progressive formation of deposits on and within the expanded gel network of contact lenses. This latter problem has only been highlighted recently, following the increased use of hydrophilic 'soft' contact lenses. A more complete discussion of this problem, together with specific considerations relating solely to hydrophilic soft contact lens wear is given in Chapter 3.

1.4.1 The Blood Clotting Process:

A comprehensive discussion of blood clotting, thrombogenesis, is outside the scope of this thesis. Several analogies may be drawn however, between the process of blood clotting at polymer interfaces and the deposition processes occurring at the surface of soft contact lenses.

Within a few seconds of first exposure to blood, all non physiological materials acquire a rapidly thickening coat of essentially pure protein, which significantly alters the surface properties of these materials [23]. The complexity of this adsorbed protein layer has been demonstrated by the electrophoretic separation of proteins in detergents [24-26]. These studies emphasise the fact that the adsorbed layer is not dominated by any particular protein. It has also been shown that the composition of this layer does not remain static once a peak thickness of some 200 Å has been established [27-32]. Protein adsorption is under thermodynamic constraints with the adsorption process following Langmuir type isotherms, that is to say monolayer formations [33]. Recent evidence challenges the concept of monolayer formation [34]. Indeed the exact situation with regards to the morphology and distribution of adsorbed proteins at interfaces remains unclear.

The conformational changes in structure that proteins undergo in response to their environment has been the subject of many studies [35-43]. The precise conformation changes that occur on adsorption remain unknown. It has

been shown that configuration states and anchoring sites are variously affected by temperature, pH and concentration in solution. Morrisey et al [36-38], who used infrared measurements on bound fractions to determine the carbonyl exchange sites, found that the internal bonding of globular proteins, such as albumin, were sufficient to hold the protein in its natural or bulk configuration, but for fibrinogen this was not the case [10,27,44-49]. This correlates with the view that primary albumin adsorption leads to a greater thrombo-resistant material.

1.5 SYNTHETIC HYDROGELS AND BIOMEDICAL APPLICATIONS

1.5.1 Hydrogels - Some General Considerations

Hydrogels are defined as polymeric materials which have the ability to swell in water and retain a significant fraction within its three dimensional network. They can be natural, or modified natural biopolymers as well as synthetic polymers. This study is specifically concerned with synthetic hydrogels that were originally developed as potential biomaterials as a result of their similarity to living soft tissue.

Hydrogels posses an inherent permeability to small molecules as a result of their expanded gel network. Such a property facilitates the elimination of polymerisation artefacts before the hydrogel is placed in the respective biosystem [50]. Leaching of fabrication additives, has been cited by

Lyman [51] as a cause of inflammation and ultimate rejection. An example of this phenomenon is highlighted by the phthalate plasticiser, used in the fabrication of poly(vinyl chloride) [52]. The phthalate was shown to produce a decrease in albumin adsorption and an increase in fibrinogen adsorption, which is indicative of a thrombogenic agent.

The incorporation of drugs into hydrogels and their controlled release has been widely studied [53]. This technique is particularly useful in the controlled release of prostaglandin PGE_2, in the form of vaginal pessaries used in the induction of labour in female patients suffering from an unripe cervix at the full term of pregnancy. The pre-delivery use of PGE_2 has been shown to ease and shorten these difficult deliveries while at the same time reducing by half the number of Caesarian sections required for this cause.[54]

A comprehensive review of hydrogels as biomaterials is given by Ratner and Hoffman [22], and from this review a list of hydrogel biomedical applications is given in Table 1.1. The monomeric compositions of the various hydrogel contact lenses examined in this study are considered in Chapter 4.

1.6 HISTORICAL DEVELOPMENT OF CONTACT LENS MATERIALS

Although the original concept of a contact lens may be traced back to Leonardo da Vinci, some five hundred years

Table 1.1 BIOMEDICAL APPLICATIONS OF SYNTHETIC HYDROGELS [22]

Coatings	Homogenous Materials	Devices
Sutures	Electrophoresis Gels	Enzyme Therapeutic Systems
Catheters	Contact lenses	Drug delivery systems
I.U.D's	Artificial corneas	Artificial organs
Blood Detoxicants	Vitreous Humour replacements	(Haemodialysis/ Haemoperfusion)
Sensors (electrodes)	Breast or other soft tissue substitutes	
Vascular Grafts	Burn dressings	
Electrophoretic Cells	Bone ingrowth sponges	
Cell Culture Substrates	Dentures	
	Ear-drum plugs	
	Synthetic cartilage	

ago [55], it was not until 1887 that the first example of lenses being fitted to the eye can be found. These original lenses were ground from glass and it was not until the 1940's that poly(methyl methacrylate), (PMMA), emerged as a widespread alternative, initially as a scleral form and finally as a corneal lens in 1948 [56].

The choice of PMMA as the thermoplastic to supercede glass, was somewhat empirically based upon its properties of 'apparent' physiological inactivity, ease of processability and material toughness [57]. The use of PMMA lenses is restricted to daily wear only, due to the rigidity of the lens and oxygen impermeability. In view of these disadvantages, PMMA is referred to as a 'hard' lens material and must be fitted in such a way as to allow a flow of oxygen to the cornea, by rocking on a layer of the pre-corneal tear fluid which transports dissolved oxygen by way of a pumping action [58,59].

Such problems as those outlined above, led to the development of rigid thermoplastic such as poly(4 methyl pent-1-ene and cellulose acetate butyrate having a far greater oxygen permeability than that of PMMA. However, these materials have not superceeded PMMA as the most widely used hard contact lens material due to other complications arising from their structures. In the case of poly(4-methyl pent-1-ene), the material is not wettable by tears unless prior surface treatment is undertaken to increase surface wettability. This problem does not arise with certain varieties of cellulose acetate butyrate, however, due to dimensional

instability of this material, difficulties arise in obtaining correctly fitted lenses [60].

Rigid hard lenses induce corneal epithelial erosion over a period of time. Although the corneal epithelial cells possess self-regenerative powers, prolonged contact, leading to irritation, may cause permanent damage of the non regenerative Bowman's layer. Such a drawback has led to the development of polymers for potential soft contact lens materials, having a far greater degree of comfort. Two types of polymer that have been widely investigated as potential 'soft' contact lens materials are synthetic elastomers, of which the most studied example is silicone rubber poly(dimethyl siloxane) and hydrogels, namely poly(2-hydroxy ethyl methacrylate), poly(HEMA). However, a fundamental drawback in the use of silicone rubber and other synthetic elastomers is that, like poly(4 methyl pent-1-ene) a surface treatment is required to ensure the material is wettable by tears. This however, is not the case with hydrogel polymers since they possess hydrophilic groups that give rise to the absorption of water, ultimately producing a wettable surface.

Since the early work of Wichterle on the application of poly(HEMA), as a contact lens material, numerous claims have been filed, citing many hydrogel compositions and methods of preparation. An extensive review of the patent literature relating to the use of hydrogels as contact lens materials has been compiled by Pedley, Skelly and Tighe [61].

Many soft contact lens materials based on poly(HEMA), having a water content of approximately 40 percent are marketed by various optical manufacturers in Western Europe, North America and Australia. Complementing these materials are those with a water content less than 40 percent. Such materials are manufactured by co-polymerising HEMA with monomers of reduced hydrophilicity. Of great interest are those soft contact lenses manufactured from high water content hydrogel polymers which offer suitable compatibility for extended wear. A more complete appraisal of the functional properties of monomers employed in the manufacture of daily and extended wear soft contact lenses examined in this study is given in Chapter 5.

1.7 SOFT CONTACT LENS FUNCTIONS AND APPLICATIONS

The primary application of a contact lens device is the correction of refractive defects in vision, such as myopia, hypermetropia and astigmatism. In the myopic condition, light from an object focuses in front of the retina, due to the focal length of the eye being too short since the eyeball is too long, or conversely, hypermetropia for the opposite reasons given for myopia. Astigmatism is characterised by an image of an object formed on the retina in which the image is focused in widely differing planes due to differing curvatures of the cornea.

With the advent of high water content hydrogel lenses, and attendent oxygen permeability levels, the possibility of

extended wear soft contact lenses has been realised. Apart from the obvious cosmetic and handling benefits that extended wear lenses provide, such devices have been used in the treatment of aphakia and in numerous therapeutic applications [62-68]. It is necessary to define a therapeutic lens, since it could be argued that even a power contact lens to correct aphakia is therapeutic, in the sense that it re-establishes normal vision. However, in the context of this work a therapeutic lens is defined as one that is used to treat tissue abnormality, to protect diseased and injured tissue, so that the tissue is able to return to its normal anatomical and physiologically functional state [63]. Power correction, when also obtained, is a secondary benefit and is not the primary treatment.

It is unnecessary to search the literature for examples of the applications of therapeutic lenses. Without doubt, almost every disease and operation on the anterior and posterior segment offers an opportunity for the employment of such devices [63]. Extended wear therapeutic lenses have been successfully used in the treatment of conjunctivitus and infiltration in infants [64], kerratitis sicca [65], and in the treatment of dry eyes [66]. Although conflicting reports on the success of therapeutic lenses in the treatment of kerratoconnus have been cited [67,68], the use of such devices in the treatment of this condition is still commonplace.

CHAPTER TWO

CHAPTER II

THE STRUCTURE AND FUNCTION OF THE CORNEA

2.1 INTRODUCTION

In order to appreciate the complex interfacial reactions that occur when a hydrogel soft contact lens is placed in the eye, it is necessary to characterise the components of the ocular environment. This chapter is concerned with an overview of the structure and function of the cornea, whilst the composition of the pre-corneal tear film that bathes the lens, is considered in Chapter 3.

2.2 THE CORNEA

The cornea is the main refractive element of the human visual system. It consists mainly of fibrous protein collagen and is distinguished from skin by its optical clarity and lack of keratinisation. This transparent, supportative, avascular membrane consists of five layers: the outer epithelium, Bowman's layer, stroma, Descemet's membrane and the endothelium as displayed in Figure 2.1

2.2.1 Corneal Epithelium

The epithelial layer of the cornea is composed of flattened, non-keratinised, squamous epithelium of 5 to 7 cell layers. It is the most regularly arranged of all squamous epithelia

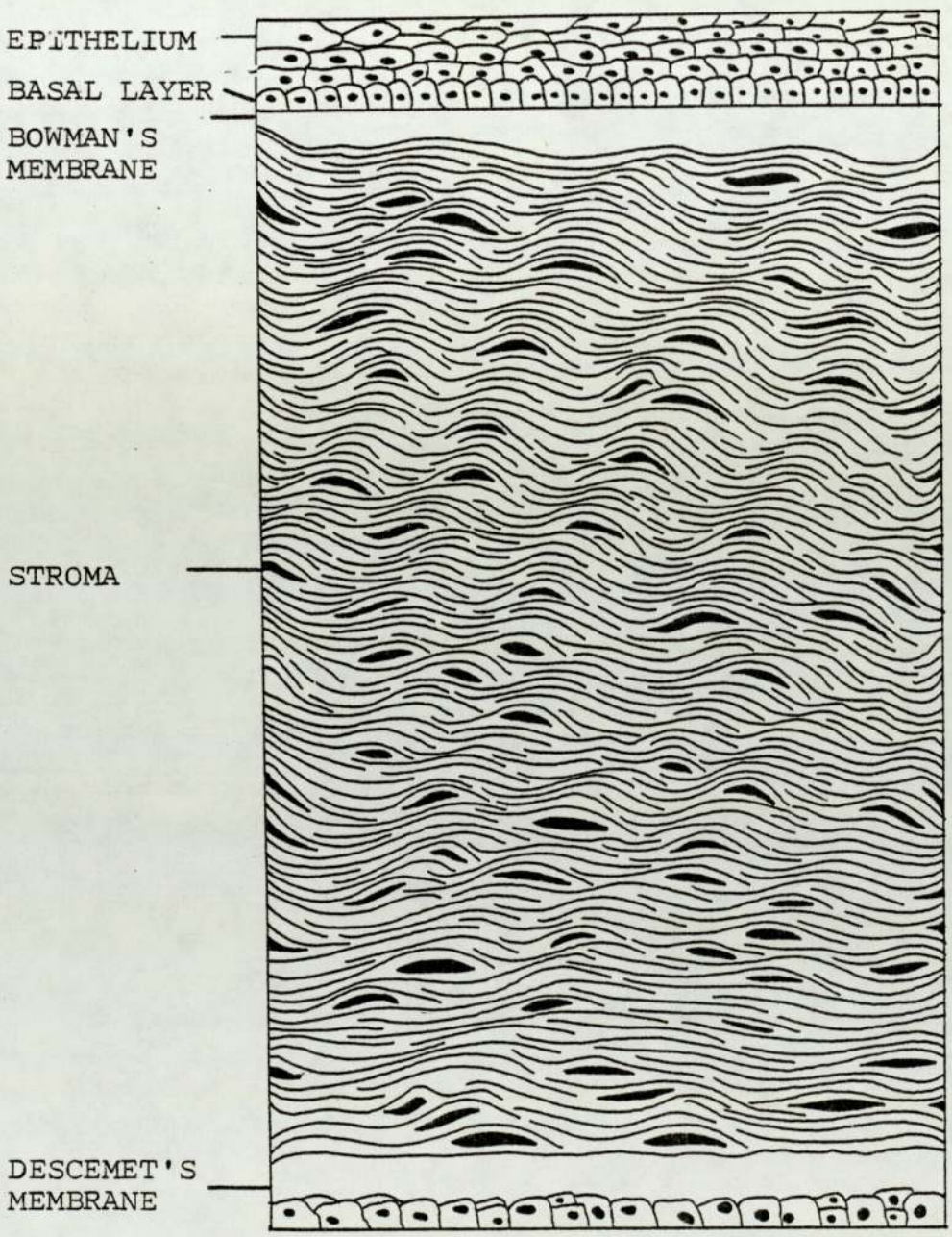


Figure 2.1 STRUCTURE OF THE CORNEA

in the human body[69]. The basal layer of the epithelium is composed of a single layer of columnar cells that are prominent in mitosis. Secreted by these cells and closely applied to their undersurfaces is a thin uniform basement membrane, that together with attendant hemidesmosomes, comprises the attachment complexes responsible for the tight adhesion of the epithelium to the underlying Bowman's layer. The epithelium is highly innervated, with multiple branches of the trigeminal nerves terminating as free, unmyelinated nerve endings between cells of the basal layer.

Like epithelial cells at other sites they are covered by a microvilli. These projections are relatively short, some 100-200 nm in length, with diameters ranging from 200-300 nm and are irregularly spaced over the cornea[70-72].

Interspaced between the microvilli are microplicae. The function of these structures is as yet unknown, although it has been proposed that they play a mechanical role in preventing gravitational flow of the tear film across the cornea[73]. Yet others believe that the tear film is sufficiently stable that it does not require such support[74,75]. It is evident that the microvilli and microplicae increase the surface of the epithelium and thereby increase transportation effectiveness across the epithelium[73].

It has been noted that corneal epithelial microvilli have cores of microfilaments that extend into the superficial cytoplasm and join a mass of horizontally dispersed filaments called the 'terminal web'. Analysis of isolated intestinal

brush borders have revealed the presence of myosin and calmodulin and brush borders contract in the presence of calcium and ATP [76,77]. Using heavy neuromysin as a label, Gipson and Anderson [78] demonstrated actin filaments in the microvilli of normal corneal epithelium. They postulate that actin filaments play a role in the migration of corneal cells during wound healing.

2.2.2 Corneal Epithelial Glycocalyx

Scanning electron microscopy has revealed the microvilli and microplicae to display a filamentous glycocalyx. Corneal epithelial cells do not appear to possess the necessary cellular organelles to secrete and maintain the glycocalyx and it has been found that this function is undertaken by the conjunctival epithelium.

Although the glycocalyx is believed to be a component of all cell membranes [79], there are few cell types in which the filamentous structure of the glycocalyx is seen as clearly as that in the eye. Analysis of the intestinal glycocalyx reveals the filaments of the cell coat to be carbohydrate side-chains of glycoprotein enzymes in the cell membrane [71]. The protein moieties of the enzymes are believed to be integral membrane proteins. The enzymes include sucrase, maltase, alkaline phosphotases, amino-peptidases and lactase which hydrolyse nutrients prior to transport into the intestinal epithelial cell [71].

By the employment of ruthenium red, a histological stain, the glycocalyx has been shown to be dense in highly acidic glycoproteins. It is possible that the high acidity is due to the extreme density of sialic acid residues[80]. The glycocalyx is considered to play a fundamental role in the initial interactions between invading bacteria and the epithelium. It is thought that the glycocalyx may determine the specific sites on the epithelia where bacteria first adhere, the requisite first step in the initiation of an infection[81,82].

2.2.3 Corneal Epithelial Biochemistry

The primary metabolic fuels of the corneal epithelium are glucose and glycogen that are derived mainly from the aqueous humour. In addition to anaerobic glycolysis, via the Embeden Myerhof pathway in the absence of oxygen the corneal epithelium utilises two aerobic pathways as sources of energy. The Tricarboxylic Acid Cycle and the hexose monophosphate shunt.

Oxygen, necessary for glucose metabolism, is acquired directly from the atmosphere and carried to the cornea by four recognised routes[83]. It is now accepted that of primarial importance in the transport of oxygen is the pre-corneal tear film, which contains dissolved air and oxygenates the cornea by a bathing process during the blink cycle. The other pathways that enable oxygen transportation are via the limbal blood vessels, the aqueous humour and the blood of the conjunctival vessels. During sleep, oxygen for the mainten-

ance of normal corneal metabolism is provided by the capillaries of the palpebral conjunctiva.

2.3 BOWMAN'S LAYER

Posterior to the epithelial basal cell layer, lies Bowman's layer varying from 8-14 um in thickness. Electron microscopy revealed the presence of fine collagen fibres, about 250 um in diameter[84]. The fibres are buried in a matrix and are arranged in a randomly orientated network[85]. Bowman's layer would appear to have a common origin with the underlying stroma, due to the similarity of its structure, and in fact the two layers cannot be separated.

2.4 THE STROMA

The stroma is approximately 480-500 um thick and constitutes about 90% of the total corneal thickness. It is composed of two structural components, namely cells and lamellae. Stromal lamellae consist of bundles of fibrils, themselves composed of fibres of tropocollagen. The stroma contains collagen of types I and III. X-ray diffraction has shown that the collagenous fibres show molecules of tropocollagen forming a semi-crystalline structure[86]. The lamellae, approximately 300 Å in diameter, lie parallel to the surface and to each other, being embedded in a water matrix.

The structure of the stroma is proposed to be responsible

for corneal transparency. The lattice theory, which is now the most widely accepted of several pertaining to corneal transparency, was put forward by Maurice[86]. He suggested that the perfectly regular arrangement of collagen fibrils causes them to behave as a series of diffraction gratings. Hence any light scattering arising from differences in refractive indices between the collagen fibrils and the mucoid matrix is eliminated.

2.5 DESCEMET'S MEMBRANE

Descemet's membrane is reported to be the normal basement membrane of the corneal endothelium, interposed between stroma and endothelium. It is composed of collagen, namely types IV, VI and VII and glycoproteins, including fibronectin[87]. After birth, the endothelial cells secrete a second form of Descemet's membrane on the posterior surface of the foetal banded portion. This posterior non-banded layer appears ultrastructurally homogeneous, with a finely granular quality and thickens with age, the thickness increasing from approximately 2 μm at age 10 to 10 μm at age 80[88,89].

2.6 CORNEAL ENDOTHELIUM

The corneal structure is completed by the endothelium. This layer lacks the distinctive thin basement membrane, usually associated with endothelia. It consists of a single layer of hexagonal cells, 5 μm in height and 18-20 μm in length. Scanning electron microscopy of human corneas has shown the selective binding of ruthenium to the posterior endothelial cell surface[90], suggesting the presence of a glyco-

calyx similar to that found with corneal epithelial cells. With advancing age, endothelial cells gradually increase in size, and are less frequently replaced by mitosis. These spaces resultant from cell loss are filled by the thinning and spreading of adjacent cells, thus increasing the irregularity of the cell layer [91].

2.6.1 Maintenance of Corneal Clarity

The endothelium undertakes two main functions; firstly in acting as a barrier membrane and secondly the maintenance of stromal clarity [87]. Although the endothelium has been equated with the detergescent role, the mechanism by which this occurs is still unclear [92]. Harris and Nordquist [93] suggested that water itself is pumped out of the cornea. However active water transport as a primary event is unknown in biological systems [94]. It seems more likely that active transport of electrolytes occurs and water follows passively with electrolytes. Several workers have suggested that, as an analogy with other biological systems, transport of cations occurs [95,96]. Such a cation would generate a potential across the endothelium with the lens side positive. Although a spontaneous potential has been demonstrated across de-epithelialised corneas it was found to have the wrong polarity [97]. Further work by Hodson led to the proposal of the existence of a bicarbonate pump, which is in good agreement with the polarity [98]. The authors have found that one third of bicarbonate ions are provided by intracellular conversion of exogenous carbon dioxide to bicarbonate and two thirds by bicarbonate conversion in the stroma.

CHAPTER THREE

CHAPTER III

THE PRE-CORNEAL TEAR FILM

3.1 INTRODUCTION

The pre-corneal tear film is composed of three layers; an outer superficial lipid layer, an intermediate aqueous phase, and a tertiary mucoid layer. (Figure 3.1) Derived from a number of sources, it fulfils a number of roles. The presence of a continuous film significantly increases the optical resolution of the eye by 'smoothing' the many fine irregularities of the corneal surface. As well as providing the major oxygen source for the avascular cornea, the tear film acts as a lubricative agent, allowing the eyelids to remove cellular debris from the cornea, by sweeping across its surface. The tear film also ensures the necessary degree of hydration required for corneal functioning and defines the transport characteristics across the cornea [99]. By the provision of a wide spectrum of anti-microbical proteins, the tear film provides powerful and effective protection of the cornea.

3.2 TEAR FILM THICKNESS, VOLUME AND DISTRIBUTION

Tear film thickness has been variously estimated as 6 μm, 10 μm and just under 10 μm [100-102]. The film attains its thickness 'from the moment after the blink' [100] and from that point onwards shows no evidence of drainage, although

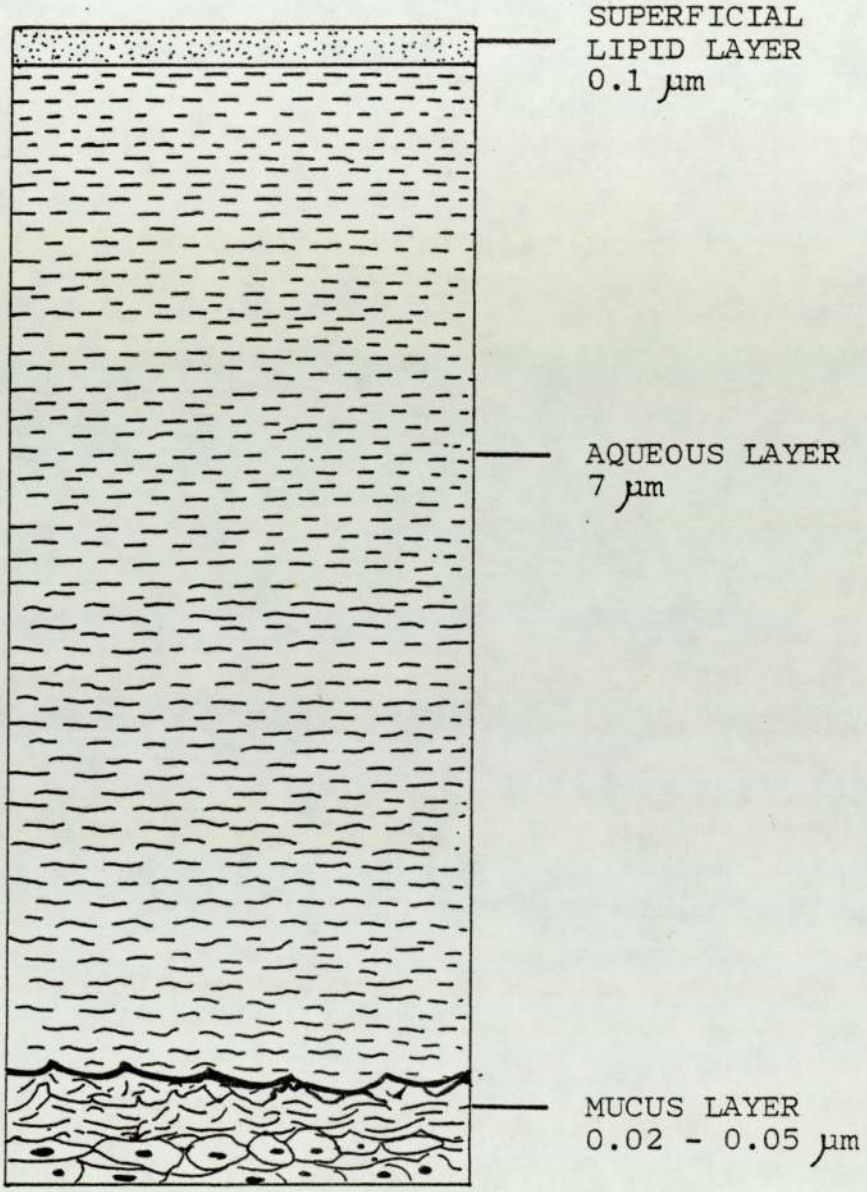


FIGURE 3.1 STRUCTURE OF THE TEAR FILM

in the absence of further blinking, thinning of the film occurs as a result of evaporation from the aqueous layer. Estimation of tear volume and flow rates are fraught with technical difficulties. It is proposed that the decay characteristics of sodium fluorescein instilled into the lower marginal tear strip provides the most reliable estimate of reflex tear production. This method provides a value for tear volume of 7.0 μl. By comparison of the data for tear volume with that achieved from an objective fluorophotometer, an estimate of 1.2 $\mu l.min^{-1}$ has been made for tear production rates. The production of tears decreases with age, falling from an average of approximately 2 $\mu l.min^{-1}$ in the early years to less than 1 $\mu l.min^{-1}$ in the middle sixties [103]. The tear film is unevenly distributed over the ocular surfaces, although it forms a distinct meniscus at the lid margins. It is estimated that the volume of tears in this area is approximately 3 μl [100]. The volume of tears covering the cornea has been estimated to be 1 μl, whilst a further 3 to 4 μl are distributed in an even manner over the conjunctiva.

3.3 TEAR FILM STRUCTURE AND COMPOSITION

A comprehensive treatise of the structure and composition of tear fluid has recently been given by Larke [104]. Based upon this review a characterisation of the various bio-organic and inorganic components of each layer of the tear film will now be given.

3.3.1 The Superficial Lipid Layer

The superficial lipid layer is primarily derived from the meibomian glands. The upper lid commonly has from thirty to forty of these glands that are somewhat smaller. Each gland has an orifice at the lid margin on the inner side of the intermarginal sulcus. Since the meibomian glands are located between the tarsal plates in each lid, the simple act of blinking is sufficient for the expression of the secretion onto the tear film. The lipid layer provides a hydrophobic barrier, preventing tear overflow on the lids, a watertight seal for the closed lids during sleep and contributes to the optical properties of the tear film[105]. It is also suggested that the lipid layer retards evaporation of water from the aqueous phase and contributes to tear film stability by preventing contamination of the tear film with highly polar skin lipids.

A number of investigators have attempted to analyse the composition of human meibomian lipids.[106] These were largely qualitative investigations and with one exception, no attempt was made to define the constituent fatty acids and alcohols. As will be seen the most striking feature of these reports is the lack of agreement of the nature and relative amounts of the different lipid classes in the secretion.

Pez[107] identified cholesterol, fatty acids and 'fats'. Linton et al[108] reported that the major part of the

material was 'neutral fats' and other unidentified material, plus not more than 10% of phospholipid, but could not confirm the presence of cholesterol. Later work by Ehlers[102], using more reliable techniques, detected both cholesterol and cholesteryl esters, fatty acids, phospholipids and a trace of triglycerides. Nicholaides[105] identified wax esters and cholesteryl esters among the neutral lipids, as well as squalene and other hydrocarbons, fatty acids and mono-, di- and triglycerides. Keith[109] compared normal meibomian lipids, sebum, and meibomian lipids from patients with seborrhoeic blepharo-kerato-conjunctivitis, by thin layer chromatography. The major components were identified as wax and cholesteryl esters, with small amounts of triglycerides, fatty acids and sterols. Prior to 1970, Andrews[110] performed the only reported quantitative analysis of fatty acids and alcohols making up the principal neutral lipid fraction (wax ester and cholesteryl esters) of human meibomian secretions, but made no attempt to separate the classes of esters prior to hydrolysis. In a definitive study, Tiffany[106], identified wax esters, cholesterol esters and triglycerides in all samples studied, although great variations in the relative proportions of the above classes were observed. Appreciable amounts of hydrocarbons and free fatty acids were detected in several cases. However, the fatty acids did not seem to be derived by hydrolysis from triglycerides as in skin lipids. Fatty acids were of both odd and even numbers, and both straight and branched chains were evident, the unsaturated content was however low. Tiffany concluded that no 'typical'

composition can be discerned and suggested that 'mean' values would be meaningless, since in terms of physical function, of the meibomian secretions, a synthetic mixture of the average composition might be less desirable than any one of several widely differing compositions.

Most recently work undertaken by Nicholaides et al [105], using glass capillary and column gas liquid chromatography, of pooled human samples has shown the lipid composition to be more complex than has been hitherto reported. A comparative table of the major lipid classes detected is given in Table 3.1 . Glass capillary chromatography revealed a complex pattern of odd and even chain hydrocarbons, with unsaturated fatty acids in total lipids and various lipid classes, ranging from C_{12} to C_{31}. Fatty acids in total lipids and wax esters ranging from C_{18} to C_{31} were detected. Nicholaides suggests that free fatty acids are derived from the triacyl glycerols in human sebum. Such findings contradict those of Tiffany.

3.3.2 The Aqueous Phase

The aqueous phase, derived from the main and accessory lacrimal glands, constitutes 99 percent of the total thickness of the tear film. It is this layer that contains the wide variety of both organic and inorganic components to be found in tears that contribute to the normal functioning and protection of the cornea.

TABLE 3.1

COMPARATIVE LIPID COMPOSITION FOR BOVINE AND HUMAN MEIBOMIAN SAMPLES[105]

	Sterol Esters	W A Esters	Material In The Diester Region	Triacyl Glycerols	Material In The Post Triacyl Glycerol Region	Free Sterols	Free Fatty Acids	Polar Lipids
Bovine Samples	31.7	31.2	11.4	1.6	2.8	3.0	5.1	13.3
Samples From Man	29.5	35.0	8.4	4.0	3.2	1.8	2.1	16.0

All Values % by Weight

3.3.2.i Tear Proteins

Electrophoretic techniques have revealed some 60 protein components in the aqueous phase [111], some of which are immunologically indistinguishable from serum homologues, whilst others are clearly distinguishable and of specific origin. It is estimated that of the 60 components, 20 proteins are secreted by the lacrimal gland [112]. There is little agreement on the way serum proteins enter the tear fluid. Chao et al could not exclude the synthesis of some serum proteins within the lacrimal gland [113]. With the aid of calcium dobesilate, a drug that increases vascular permeability, Janesson [112] concluded that serum proteins are mixed with the tear fluid by leakage of the conjunctival capillaries. Although tear proteins have been considered to consist of a mixture of albumin, globulin and lysozyme, it is now clear that this classification is oversimplified. The major tear proteins recently identified in tears are discussed below.

3.3.2.ii Lysozyme

Mucopeptide-N-acetyl muramyl hydrolase, or more commonly lysozyme, represents a major tear protein. Lysozyme, a non specific tear protein, with a molecular weight of 14,400, is found in saliva, serum, nasal secretions, polymorphonuclear leucocytes and urine as well as tears. Lysozyme was the first tear protein whose anti-microbial activities were identified and described [114]. The ability of the protein to cause the dissolution of the bacterial cell wall, results

from the hydrolysis of the B1,4, glycosidic linkage of N-acetylglucosamine-N-acetyl neuraminic acid polymers. Although the enzyme possesses some lengths of helix, most of the molecule lacks secondary structure. The tertiary structure is stabilised by three disulphide bonds and is arranged in such a way that a deep cleft runs through the enzyme. The polysaccharide chain of the substrate site sits into this cleft, being held into the correct orientation by a number of hydrogen bonds, and the specific linkage is then cleaved [115].

Lysozyme differs from other tear proteins in its electrophoretic mobility. As a result of its very low iso-electric point, the protein migrates to the cathode under most electrophoretic conditions, whilst the majority of tear proteins migrate to the anode. Two iso-enzymes of lysozyme have been detected that may be electrophoretically resolved under appropriate conditions [116]. Lysozyme is secreted by the lacrimal acinar cells [117] and there is little or no correlation between serum and tear lysozyme concentrations. Several workers have estimated the concentration of lysozyme in tears at between 0.83 and 2.06 mg/ml [116], although this value, as with others stated for principal tear proteins, is somewhat affected by the method of collection [118]. Although lysozymal levels appear largely unaffected by circadian patterns, marked reductions in concentrations have been observed in polluted environments and diseased states [119].

3.3.2.iii Non-Lysozymal Anti-Bacterial Factor

Lysozyme has long been considered to be the major anti-bacterial component in tears, however, on heating tears, the protein loses its anti-staphylococcal activity, although the lysozymal content is unaffected [120]. Reports of a low molecular weight protein, known as non lysozymal anti-bacterial factor (NLAF) with potent bacteriocidal properties, have been made [121]. It has been suggested that NLAF is in fact βlysin, an anti-bacterial component, due to its immunological similarity to serum βlysin [122], although it is present in higher concentrations in tears than in serum [123]. NLAF acts on the cytoplasmic membrane, and is active against a wider range of Gram positive and Gram negative rods and cocci than lysozyme, including common ocular pathogens against which lysozyme is ineffective even in higher concentration [124].

3.3.2.iv Lactoferrin

Lactoferrin was first identified in tears by Broekhuyse [125] by sodium dodecyl sulphate electrophoretic and immuno-electrophoretic techniques. As is the case with lysozyme, the acinar epithelium of the main and accessory lacrimal glands has been shown to be the source of this protein [126]. Lactoferrin has a reported concentration of 20 mg/ml and therefore represents a major protein [127]. Work by McGill [128] has shown that both lactoferrin and lysozyme levels in tears decrease with age and disease conditions [128]. Lacto-

ferrin has both bacteriostatic and complexing properties and can reversibly bind two atoms of iron. In addition to its known bacteriostatic effects, Kijlistra et al have shown lactoferrin to exert a strong inhibitory effect on the classical complement system[129,130], and therefore, may have anti-inflammatory capacities. The protein is reported to play a major role in the non-specific defence against a variety of bacteria[131,132], although it was initially considered that the anti-bacterial effects were due to its iron binding capacity, recent studies indicate that lactoferrin also has a direct effect on certain bacterial strains[133]. Besides its own bacteriocidal role it has been shown to play a role in the regulation and production of granulocytes and macrophage-derived colony stimulating factors[134,135]. Kijlistra has further shown that tear lactoferrin can inhibit the formation of the classical C_3 convertase of the complement system and thus can prevent the formation of the biologically active complement fractions C_{3A} and C_{5A}. This latter function of lactoferrin may prevent complement activation on the external surface of the eye in those inflammatory conditions when some leaks into the tear film[130].

3.3.2.v Ceruloplasmin

Although Gachon et al[111] failed to detect ceruloplasmin, a copper carrying glycoprotein in tears, recent work by McGill detected the presence of ceruloplasmin with a mean age adjusted value of 16 mg/ml[128]. McGill et al found a

significant positive correlation between IgG and ceruloplasmin, suggesting a common source of origin and differing from that of lactoferrin and lysozyme. They hypothesize that the protein is secreted from serum, either by the lacrimal gland, or via the conjunctiva, which suggests that the blood-tear barrier is incomplete, allowing a small leakage of serum proteins to occur. Although the exact role of ceruloplasmin in tears is not yet clear, as a result of its copper binding capacity, it may well play a role in the non-specific defence of the eye.

3.3.2.vi Transferrin

As with lactoferrin, transferrin is an iron binding protein present in tears[136,137]. It is suggested that transferrin constitutes one of the major components in tears, however, an accompanying identification of lactoferrin was not made[138]. The possibility that transferrin can become confused with lactoferrin, due to their electrophoretic mobility has been suggested[139]. However, the presence of both lactoferrin and transferrin in tears has been confirmed, although transferrin is present in a much lower concentration[111].

3.3.2.vii The Albumins

Specific tear pre-albumin, synthesized by the lacrimal gland, is a protein unique to tears and is not found in serum, spinal fluid, saliva or urine[137]. The molecular weight of tear albumin is estimated between 15-20,000, which

is considerably less than the reported molecular weight of serum albumin. Although tear albumin has been demonstrated to produce a single line on conventional gel column electrophoresis, improved techniques have demonstrated the presence of more than one component and genetic polymorphism with five discernable types of tear albumin has been observed [111,114]. Although the exact role of tear pre-albumin in tears remains unclear, it has been linked along with other tear proteins, in the defence mechanisms of the eye.

3.3.3 The Immunoglobulins in Tears

The immunoglobulins, secreted by the lacrimal gland, comprise one of the principal groups of tear proteins and play a major role in ocular protection [122]. The IgG, IgM, IgD and IgE tear components are identical to those of serum immunoglobulins. Secretory IgA of tears is the principal immunoglobulin and differs from that in serum. Secretory IgA is a dimer, formed from two IgA units, each identical with serum IgA plus a J chain and a 'secretory piece'. On incorporation of the secretory piece with the dimer, the immunoglobulin migrates to the lumen of the lacrimal gland whereupon it is incorporated in the tears [126]. The concentration of secretory IgA has been variously estimated as 9 to 50 mg $100\mu l^{-1}$ [141], 17 mg $100\mu l^{-1}$ [142] and 10.7 mg gm^{-1} of total protein [143]. The presence of secretory antibody means that local resistance to invading micro-organisms can be achieved more rapidly than the general

systemic response, but with the added possibility that serum IgA directed against antigens at other sites in the body may also be incorporated into ocular secretory IgA.

3.3.4 Proteinase Inhibitors, Prostaglandins and Histamines

Proteinase inhibitors Alpha I, antichymotrypsin, Alpha I antitrypsin and Apha II Macroglobulin have been detected in tears[111]. It is thought that they are involved in the inhibition of proteolytic enzymes derived from both ocular tissue and bacterial sources. Radioimmunoassay techniques with specific-antisera have detected the presence of prostaglandin F in tear fluid[146]. The role of the prostaglandins is that of one of the mediators of the inflammatory process. Histamine concentration in tears from 'normal' subjects and those suffering from vernal conjunctivitis have been reported. 'Normal' subjects were found to have levels of $10.3\,ng\,ml^{-1}$ whilst patients suffering from vernal conjunctivitis were found to have levels in excess of $38.2\,ng\,ml^{-1}$[147].

3.3.5 Low Molecular Weight Organic Substances

Glucose is present in tears at very low concentrations. Raised glucose levels may be seen in diabetes, however these levels have been attributed to raised tissue fluid levels, rather than raised tear levels. The existence of free amino acids in tears remains unclear, although it has been suggested that they may be present in a concentration

greater than levels reported in serum[104]. The concentration of urea is reported to be similar to that of plasma, suggesting unrestricted passage across the blood-tear barrier of the lacrimal gland[148].

3.3.6 Tear Electrolytes, Tear pH, and Buffering Capacity

Sodium is the main cation in tears, with a concentration of 145 microequivalents per millilitre, and is similar to that reported for serum. Potassium, the other principal cation, is found at levels of 3 to 6 times higher in tears than its concentration in serum. Magnesium and calcium are also found in tears at low levels. Calcium is of interest to contact lens practitioners as it is often found to be present in lens deposits. The concentration of calcium is low; approximately 2.11 $mg.dl^{-1}$ [149]. Chloride and bicarbonate anions are found in tears at concentrations similar to those in serum[150]. Sodium, potassium and chloride ions, contribute towards the osmotic pressure exerted by the tear fluid. Differences between individual levels of tear ions and between the open and closed eye environments have been reported[151]. It is thought that bicarbonate and carbonate ions contribute towards the maintenance of pH. Differences have been observed in the pH of the waking and closed eye[152]. The acidic 'shift' observed in lid closure is thought to be due to the obstruction of carbon dioxide effluxing through the cornea, with the formation of an increasing concentration of weakly dissociated carbonic

acid when the lids are closed[153]. The observed changes in tear film pH would probably be greater were it not for the presence of bicarbonate ions in the tear film.

3.4 THE MUCOUS PHASE

The mucous phase of the tear film constitutes the interface between tears and the corneal epithelium and is estimated to be 0.02-0.04 μm in thickness. Although primarily derived from the conjunctival goblet cells[113], it is suggested that a proportion of tear mucin may be contributed by the lacrimal gland. Analysis of human ocular mucus has revealed the presence of lipoglycoproteins of both high and low molecular weight, and plasma proteins. The glycoprotein moieties of the high molecular weight lipoglycoproteins contain 40 percent protein and 60 percent carbohydrates. N-acetyl hexosamine and N-acetyl neuraminic acid comprise approximately 500 residues per 1000 amino acid residues. At physiological pH, N-acetyl neuraminic acid is completely ionized and contributes both a high negative charge density and hydrophilic nature to the molecule. Plasma proteins contribute 60 percent of the macromolecular components in ocular mucus. These proteins also appear to be complexed with lipids[154]. Although mucus plays a significant role in the removal of lipids and debris from the surface of the anterior eye[155], its primary function is to render the corneal epithelium wettable by tears as outlined below.

3.4.1 Wetting of the Corneal Epithelium

The corneal epithelium is relatively hydrophobic in nature, with a critical surface tension of approximately 30 dynes. cm^{-1}. In order that a stable tear film should be maintained, the liquid film must completely wet the corneal surface. The major constituent of tears is water, with a surface tension of 72.8 dynes.cm^{-1}. Synthetic wetting agents which are capable of reducing the surface tension of water to 30 dynes.cm^{-1}, also have the inherent disadvantage of lowering the interfacial tension of oil and water to zero, which would lead to spontaneous emulsification and solubilisation of cell membrane lipids. Mucins, the natural wetting agents in tears, cannot lower the surface tension so dramatically, but neither do they cause the undesirable lipid solubilisation at their normal physiological levels. In simple terms, mucins may be regarded as acting in two combined ways to bring about epithelial wetting.

(i) by reduction of the surface tension of the water component of tears to approximately 40-46 dynes.cm^{-1}

(ii) by increasing the critical surface tension of the cornea to approximately 40 dynes.cm^{-1}, by adsorption onto its surface.

Once the tear film is established, the anterior superficial lipid layer is believed to play a large part in stabilising the film, and this itself is thought to be stabilised by the mucins in the aqueous phase.

CHAPTER FOUR

CHAPTER IV

NATURE AND OCCURRENCE OF LENS SPOILATION

4.1 INTRODUCTION

This chapter is concerned with a review of the nature and occurrence of lens spoilation. Lens spoilation is the term invoked to encompass physical and chemical changes in the nature of hydrophilic soft contact lenses and various extraneous deposits which may impair the optical properties of the lens or produce symptoms of discomfort and often intolerance to the wearer [156]. The extent of the situation may be appreciated by considering the reported incidence of lens spoilation. According to several reports, lens spoilation has been observed in 7-82 percent of extended wear lenses [156-159]. Lens spoilage may occur in some cases as early as 48 hours of wear, whilst the majority of cases, 3-6 months of daily or extended wear [160]. The degree of spoilation appears to be independent of the level of lens hydration, with both moderate and high water content lenses being equally affected. It is convenient in a review of this type to divide the observed types of spoilation into different classes. It must be appreciated, however, that such a classification is largely artificial. There is considerable overlap between the classes and systemic analyses of the results of spoilation are rarely undertaken. The classification is best regarded, therefore, as a grouping of various related types of clinically observed

phenomena and is shown in Table 4.1. The various classes of lens spoilation are outlined below.

4.2 DISCRETE ELEVATED DEPOSITS

Probably the most chemically complex manifestations of ocular incompatibility are discrete elevated deposits, the so called 'white spots' found on the anterior lens surface. They are also referred to in the contact lens literature as mucoprotein-lipid and mucopolysaccharide deposits. Ruben and others cite the deposition of mucoprotein-lipid deposits on lenses as a major cause of spoilation, with a reported incidence of up to 82 percent of patients fitted.[156,161-166] In a study by Tripathi et al[156], 80 percent of lenses examined by light and electron microscopy, displayed such deposits with or without calcareous material. It would appear that the main contributing factors include inherent or acquired defects in the lens material, altered ocular secretions and tear chemistry. There is considerable variation in the composition of this group of deposits from patient to patient. This has given rise to the speculation of the origin of the components.[167-170] The composition of the tear film has been reviewed in Chapter 3. Tear solutes include proteins, amino acids, glycoproteins, glucose and lipids and tear electrolytes at concentrations comparable to those of plasma.

It is therefore obvious that the tears are a constantly available source for many of the components for deposit

Complex Deposits	Lens Coatings	Microbial	Extrinsic Factors
Discrete elevated deposits - 'White Spots'	Proteinaceous Films. Granular Deposits. Specific Calcium Deposits	Fungal and Bacterial Contamination	Mascara Contamination. Deposition of Iron Particles

Table 4.1 CLINICALLY OBSERVED MANIFESTATIONS OF HYDROGEL LENS SPOILATION

complexes. To this, however, must be added the products of normally desquamating epithelial cells, and in pathological conditions, necrotic tissue as well as altered tear components. Hydrophilic lens materials have an affinity for tear proteins, especially lysozyme, amino acids and glycoproteins, and they readily adhere to the lens surface, especially if the character of the latter is altered. The composition of mucoprotein-lipid deposits probably results from the interplay of various factors of this type[161]. Low pH, for example, encourages deposition of protein. Improper cleaning and dry spots, contribute to the formation of gelatinous deposits. Patients with an early stage of dry eye syndrome tend to have higher levels of mucus than the norm. This is considered to be the result of a reduced amount of lysozyme. Several factors, such as hormonal contraceptives, induce adhesion and precipitation of mucus, due to the abnormal mucin formation. Discrete deposits, composed predominantly of lipid, result from a combination of the following conditions: dryness and stress on the lens surface, during blinking, is thought to alter the structural integrity of the lens surface, an accumulation of normal or altered lipid secretions from the Meibomian glands and those of Zeiss and Moll. Although enzyme cleaners are useful in removing protein-lipid residues, they cannot be recommended on a full time basis for two reasons: firstly because of the potentially harmful effects on the eye and secondly, once encrusted debris is removed from the lens, pits, crevices and cracks are left that may enhance secondary spoilage.[156,171,172]

Apart from the problem of lens decentering leading to poor fit, mucoprotein-lipid deposits lead to a decrease in visual acuity, giving rise to injury of the cornea and palpebral conjunctiva. Patients with such deposits on their lenses often show clinical conditions such as giant, papillary, follicular and other allergic conjunctivitis. It is thought that these conditions result either from mechanical irritation of the deposits on the conjunctiva or the adsorbed proteins providing an antigenic stimulus initiating an auto-immune response[173-175].

4.3 LENS COATINGS

A major class of spoilations includes the surface films, coatings and plaques. Related to discrete deposits, although somewhat more geographically dispersed are the so-called protein films. Such films are characterised by a thin, semi-opaque, white superficial-layered appearance. These layers appear to consist of denatured protein. The films vary in their extent of lens coverage, ranging from small patches to complete covering of the lens surface. The general accumulation of protein films on the soft lenses leads to an increase in surface haziness and surface rugosity. A decrease in visual acuity results, due both to lens opacity and to poor lens movement on the eye. Red eye, increased irritation and conjunctivitis are typical patient responses to protein covered lenses. These deposits have been variously attributed to mucoproteins, albumin, globulins, glycoproteins and mucin[176-178].

Karageozian[169], with the aid of an amino acid analyser, has reported that protein films consist mainly of denatured lysozyme. Since lysozyme comprises only 18 percent of the total tear protein, in comparison with 60 percent albumin and 22 percent globulin, it is, according to the author, evidently selectively adsorbed and denatured on the lens surface. However, Hathaway and Lowther have reported that albumin, as well as lysozyme, forms marked deposits in a short period of time[179]. Furthermore it was found that ionic solutions such as calcium and sodium chloride enhanced the rate of, and degree of deposit formation of organic tear components.

Kleist[180,181], reports that protein films are found on both thermally and chemically disinfected lenses. Not surprisingly, the level of proteinaceous films was higher on thermally disinfected lenses. Morgan et al[182] suggest that lens surface coatings are more likely to occur in those patients who have a higher level of phosphatase, lactic acid dehydrogenase and cholesterol. Conversely, patients with low levels of creatine in plasma are more likely to show coatings on the lens. Hathaway and Lowther found that there was no correlation between protein concentration and the rate of deposit formation. However, a correlation was found between the rate of deposition and tear break up time (B.U.T.). A short B.U.T. was found best correlated with rapid deposit formation[183]. Several workers report that drying of the lens surface due to poor blinking or low tear volume may also enhance deposit formation.

As a point of reference one should note that B.U.T. and lens drying are not synonymous. Tear film B.U.T. occurs in a matter of seconds and therefore cannot be a function of drying alone, since it has been estimated that it would take approximately 10 minutes to eliminate the entire film by evaporation [184]. Briefly B.U.T. is caused by the migration of polar lipids from the superficial tear film layer, since when the lids open, the new tear-air interface has a high surface tension. When the mucous layer has become sufficiently contaminated it ruptures, producing a 'dry spot' or a discrete area of hydrophobicity on the lens surface.

Koetting [184] reports that the time before the detection of visible protein is made, decreases drastically when the lenses are rubbed and rinsed in saline before disinfection. There are conflicting reports in the literature on this subject, several workers indicate that the regular use of surfactant cleaners reduces the incidence of spoilage, whilst others suggest that regular use makes no difference at all. Protein films may be prevented. Many workers advocate the use of strong oxidising agents such as per-sulphates, perborates and even hypochlorite bleaching agents. Oxidative cleaners may indeed remove protein films, however Eriksen points out that in many cases irreversible damage to the lens may result from their use [169,185]. The use of enzymes, such as papain, in soaking solutions provides a safe

and effective method of film removal [186]. Heavy white visible films may be removed by the enzyme but at this stage the lens may have other types of deposits that are unaffected by the cleaner. The removal of the overlying protein film may cause eye irritation [173].

4.4 INORGANIC FILMS

Inorganic films are similar in gross appearance to protein films, however, they are composed of insoluble non crystalline materials [181]. Heavy inorganic films often cause damage to the lens surface, since the material may penetrate into the lens matrix [187-189]. These films are generally covered with protein that smoothes the underlying rough inorganic material. Inorganic films are composed mainly of calcium phosphate and co-precipitated protein [187]. The deposit may well be hydroxy apatite, the thermodynamically stable phase of calcium phosphate in biological conditions.

The incidence of these films is less than its organic counterpart. Kleist reports that of 370 lenses 10 percent highlighted inorganic films compared to 19 percent with protein films. These films are more common in thermally disinfected lenses. These deposits are common on high plus lenses worn by aphakics [188]. The main cause of these deposits appears to be the precipitation and growth of calcium phosphate from tears while the lens is in the eye. Tear chemistry and tear production are important factors. Poor blinking and insufficiency of tears, or low B.U.T. seems

to be associated with these deposits[184]. The presence of protein films is thought to predispose the lens to the build up of inorganic material, since the protein surface will become more hydrophobic and more likely to dry between blinks.

Once nucleated hydroxy apatite will grow directly from slightly supersaturated solutions like tears. The mechanism discussed by Bloomfield[190], that leads to corneal calcification may contribute to hydrophilic lens calcification. Briefly the release of calcium and phosphorus from injured cells could increase the calcium phosphorus product locally. The activation, together with release of such cellular enzymes as alkaline phosphatase and adenosine triphosphatase which are present in corneal cells, also may lead to local calcification. These enzymes have pyrophosphatase activity; they cause the hydrolysis of pyrophosphate to inorganic phosphate. Pyrophosphates are inorganic compounds that are widely distributed in the body and act as physiological inhibitors of calcification in soft tissues.

4.5 GRANULAR DEPOSITS

Another major class of inorganic lens deposits are the so-called granular deposits which are a special form of crystalline deposit. These elevated white or translucent formations vary in size[191]. Several hypotheses concerning the potential etiology of such deposits have been discussed in the literature. Lowther suggests that tears may not be

directly involved since crystalline formations penetrating the lens surface will develop when an unused lens is boiled in undistilled water. Koeting et al suggest that the crystalline formation disrupts or 'breaks down' the polymer and once this has occurred, components of the tear fluid, normally too large to penetrate the matrix, become incorporated into the deposit. Hilbert et al [192] further intimate that these deposits form as a result of the crystallisation of some ionic component of the tear fluid, possibly during periods of surface drying. The influence of drying is substantiated by the fact that such deposits always occur on the exposed anterior surface of the lens [167].

The 'drying concept' is further reinforced by Doughman et al [161]. Excrescent deposits were only found on the anterior surface; the highest density of deposits being at the centre of the lens. They concluded that a combination of dryness and stress on the lens surface probably alters the structural integrity of the lens polymer. Thus these deposits may be considered as localised versions of an inorganic film and are often covered with a layer of protein.

A paper by Gasset et al [193] emphasises the fact that the incidence of these deposits is significantly higher in aphakic patients compared with those with other refractive errors, although the author was unable to offer an explanation for the observation. A tendency for these deposits to re-occur has been noted even on new lenses from the

same or another manufacturer, which leads to the conclusion that their formation originates mainly from the wearer and not from the lens. Thus it appears that lens calculi are very dependent on the lens wearer's tear chemistry. Microscopic observations have shown that crystalline growths appear to nucleate on the lens surface as a thin amorphous structure that grows by crystallisation of calcium phosphate from the tears.

It is interesting to note that calcareous deposits that manifest themselves as discrete or large areas of cloudiness are known to occur in the cornea, especially in Bowman's membrane [194]. This is analagous to calcium deposition in the soft lens, where the deposition appears to occur just below the anterior surface. In certain circumstances, relatively specific microcrystalline deposits are formed with selective patterns of distribution. These observations would tend to suggest that definable and specific factors may be involved in calcium deposition.

4.6 SPECIFIC INORGANIC CALCIUM DEPOSITS

Calcium carbonate deposits consist of crystalline growths that display a definite needle-like form. When large areas of the lens surface are covered with clusters of crystals the lens has a film-like appearance. The crystals, consisting mainly of calcium carbonate grow into the lens surface, producing small pits which may be covered with a protein film [195]. With continued wear the deposits metamorphose

into the more insoluble calcium phosphate and take on the appearance of multiple lens calculi. Kleist[181] found that 9.5 percent of 370 lenses with deposits exhibited calcium carbonate deposits. Only 4 percent of 201 thermally disinfected lenses were found with this type of deposit in contrast to 15 percent of 109 chemically disinfected lenses which were found with this deposit. It was suggested by Kleist that calcium carbonate deposits form when the lens is in the eye.

Freiberg[195] found that thermal disinfection of the lenses dissolved the calcium deposits if they were of recent origin. This may go some way in explaining why there is a low percentage occurrence of this type of deposit on thermally disinfected lenses.

4.7 MICROBIAL SPOILATION

One of the most serious hazards of soft lens wear is spoilage due to invasion and contamination by microbial organisms[196]. Two separate studies have produced similar figures for the occurrence of this type of spoilation. These have indicated that in one set of patients some 24 percent of patient lenses showed problems related to the presence of pathogenic organisms[197], and in another that about 30 percent of patients fitted with soft lenses carry such organisms[198].

Normally the lens surface can be expected to have traces

of micro-organisms due to the presence of harmless flora, including fungi of the conjunctival sac. Many species of fungi and yeasts have been identified on soft lenses[199-203]. Amongst these are the yeasts of the Rodotonita species, Candida tropicales, fusarium and albicans together with fungi such as Aspergillus fumigatus and niger and Penicillium sp.

Yamamoto et al[204] have reported the presence of the fungus Cephalosporium acremonium on and within a therapeutic soft lens. They suggest factors which may influence soft lens infiltration by fungi include enzymatic activity produced by the fungus, which leads to polymer degradation providing a matrix and nutrient source for fungal growth. Microbial growth is especially prevalent when unpreserved saline is used. Kleist[180] found that of 54 lenses using unpreserved saline that had deposits, 28 percent exhibited microbial growth. The incidence is much lower when preserved saline is used. Protein deposits and other debris are thought to predispose lenses to fungal invasion[197]. Prevention through adequate cleaning and disinfection procedures must be practised since microbial colonies, once established are extremely difficult to eradicate.

Several workers have reported the presence of bacteria on both the anterior and posterior surfaces[167,205-206]. Fowler et al[206] found bacterial cells on the anterior surface of several contact lenses, each lens surface was found to be contaminated with dried mucus. Some bacteria were scattered

randomly over the surface, with no apparent attachment to the surface of the lens, whereas others were attached to the coated surface by thin, flagella-like pseudopodia 0.1-1.3 um in diameter. The distal ends of these structures were free and unattached to the surface. Several bacteria were actually embedded in the surface coating, whilst others were aggregated around clumps of mucus. It would therefore appear that bacteria may use the lens coating as a nutrient source, and as a result of their attachment and proliferation may provide antigenic mass.

4.8 LENS DISCOLOURATION

Discolouration of hydrophilic soft contact lenses appears to be a major drawback in the use of such materials although the frequency of its occurrence is not well documented. Discolouration or loss of lens transparency has been linked with several factors. Among possible causes are nicotine from cigarettes [207], topical adrenalin and topical vasoconstrictors as well as components from the tear fluid. The possible nature of discolouration is described in various papers of which the most seminal appears to be that of Kleist [181]. Yellow or brown discoloured lenses have been found to contain granular particles deposited just below the lens surface, which fluoresced under U.V. light. Strong absorption peaks at 280 nm were taken to suggest an aromatic structure for the component. These observations, and those of others, have led to the belief that the discolouration is caused by melanin, the human skin pigment [208]. However,

tests with common melanin stains proved negative, although positive results were obtained with ferricyanide. This stain indicates the presence of sulphahydral containing melanins. Thermal disinfection leads to a higher percentage of discoloured lenses. This may be attributed to the rate of formation of the coloured pigment being speeded up by higher temperatures. Adrenalin and other similar compounds used in eye medications may play a potential role in lens discolouration since they may be taken up by the lens. This effect can be demonstrated in vivo by the incubation of a lens in an adrenaline solution [209]. Additionally, adrenochrome is believed by some to be a possible precursor of melanin [210]. Therefore the presence of adrenochrome in eye medications may enhance the natural production of melanin. Smoking gives rise to smokers melanosis [211]. Melanin production is stimulated by nicotine and the other polycyclic aromatic compounds in tobacco smoke. Thus it is not a direct discolouration but nicotine-stimulated biochemical mechanism. Chlorhexidine, usually as the digluconate salt that is found in several solutions for hydrophilic lenses, has been reported to discolour lenses [181]. It is believed that it is chlorhexidine decomposition compounds that stain the lens yellow to yellow-green. It has been determined that chlorhexidine may reach a concentration in lenses, some 200 times greater than that in the lens solution, and hydrophilic lenses become cloudy and non-wettable following storage in solution containing chlorhexidine [212]. It appears that the tears act as a sink for chlorhexidine taken up by the lens during storage. The

rate of desorption during wear is probably a function of blink rate and tear flow. Once chlorhexidine is desorbed from the lens, the probability of its secondary adsorption to tear proteins is great because of its strong affinity for soluble proteins. Chlorhexidine molecules complexed with tear proteins would be expelled from the eye via the canaliculus. The replacement tears contain unbound proteins which are available for binding with desorbed chlorhexidine [213]. Plaut et al [214] found that the uptake of gluconate and acetate salts was almost entirely irreversible over the concentration range studied. In the case of the digluconate salt with uptakes of up to or less than 7 mMols.Kg^{-1} the interaction of the above salt was completely irreversible with no preservative being eluted from the polymer during the desorption step. It is considered that the digluconate salt within the above concentration range in the matrix, is responsible for the discolouration. However, at concentrations greater than 7 mMols.Kg^{-1} desorption and interaction occurs with proteins.

The greying discolouration of hydrophilic lenses has been equated with the deposition of mercury from Thimerosal [215]. It is found in many hydrogel lens care systems, including surfactants, preserved saline. Thimerosal is classified as an organic mercurial antiseptic and is primarily bacterostatic. Ganju and Cordrey [216] using ultra violet light absorption, reported mild to considerable uptake of thimerosal. Uptake was found to be dependent on the following factors: the duration of the contact lens within the

solution; the concentration of the solution; the surface area of the lens and the water content of the lens. These deposits have been described as grey to black, are of mottled texture and are probably insoluble mercuric sulphide. It is thought that the deposits are formed from the precipitation of sulphur from the rubber gaskets during thermal disinfectation and then combination with mercury. Kleist [181] found that the black deposits were common with thermally disinfected lenses. He refers to well documented cases where multiple re-use of preserved saline led to mercury deposition.

4.9 EXTRINSIC FACTORS

Several extrinsic factors have been cited in the literature that contribute to lens spoilage [151]. Cosmetics, for example, may act as simple foreign bodies which adhere either directly to the lens or the lids which then sweep cosmetic debris over the lens surface on blinking. Such cosmetic debris is often mistaken for a heavy mucoid secretion. This type of deposit may result during the application of make-up, during normal wear, or when make-up is being removed. Various creams and oils which are applied to remove make-up may also enter the eye and precipitate deposits which are as severe as those caused by make-up itself. Globules of mascara are very tenacious and remain on the lens surface for an extended period of time. Such a coating of mascara precipitates a breakdown in the integrity of the tear film due to a severe decrease in the hydrophilicity

of the lens surface. Airborne foreign bodies and dust can readily spoil a lens. They pose special problems in patients who are exposed to environments with high levels of metallic particles, toxic fumes and dust. An accumulation of foreign material over a period of time, as well as polymer breakdown, affects gas permeability and may carry a variety of infective agents. Of relatively common occurrence are so called 'Rust Spots'. These deposits are small, raised, superficial spots coloured orange to rust red by iron salts [217]. Loran has suggested that they may be related to siderosis caused by metallic foreign bodies embedded in the eye. When in contact with the lens, metallic iron-containing particles may oxidise to form iron salts that penetrate and disrupt the surface of the lens as the slightly soluble iron salts oxidise to form the insoluble ferric. Kleist reports that the incidence of rust coloured spots is fairly high on thermally disinfected lenses and lower in chemically disinfected lenses. Since the spots are usually small and only found on the anterior surface, they are not considered to have a deleterious effect on the eye. Often the spots are covered with a protein film that smooths the disrupted areas of the lens. On treating the lens with an enzyme cleaner, the reducing agents may turn the spots from orange to black. The iron compounds in the spot can be removed by strong sequestering agents, although the raised, disrupted spot on the lens remains [181].

4.10 SCOPE AND OBJECTIVES OF THE PRESENT WORK

It is apparent that the literature related to hydrogel contact lens intolerance is diffuse and is primarily concerned with the clinical manifestations of lens spoilation. Indeed few analytical studies of this deposition phenomenon are cited in the literature. Thus, the aims of this project are to identify the chemical composition, geological structure and possible etiologies of deposits found on and within commercially available hydrogel contact lenses worn during daily and extended wear programmes. As a result of these investigations it is hoped to comment critically on the interfacial reactions that occur between hydrogel contact lenses and the ocular environment. An overview of the bulk and surface properties of hydrogel contact lenses examined during this study is presented in the following chapter, whilst a discussion of the analytical techniques and methodologies employed in this project is presented in Chapter 6.

CHAPTER FIVE

CHAPTER V

COMMERCIAL SOURCE, BULK AND SURFACE PROPERTIES OF HYDROGEL LENSES

5.1 INTRODUCTION

From previous discussions in Chapter 1, it is plausible to suggest that the biocompatibility of a hydrogel contact lens is governed by the surface reactions that occur between the lens polymer and components of the ocular environment. In order to establish the possible reactions that may occur between a prosthetic device and its operative biological environment, it is necessary not only to characterise the functional components and composition of that environment, but also those properties that govern the functional characteristics of the prosthetic device. A characterisation of the ocular environment has been given in Chapters 2 and 3. This chapter, will therefore, attempt to provide an overview of the bulk and surface properties of the various hydrogel contact lenses examined during the course of this study.

5.2 COMMERCIAL SOURCE AND IDENTITY OF LENSES

A total of 350 worn hydrogel contact lenses of several commercial designs were examined during the course of this project. Analysed lenses were classified as those worn on a daily wear basis and, those employed in extended wear

programmes. Daily wear lenses are those that are worn during waking hours only, whilst extended wear lenses may be worn continuously for the 24 hour period and beyond, without the normal requirement for removal prior to sleep. Since the extended wear contact lens is in contact with the ocular environment for a significantly greater period of time than daily wear devices, it is plausible to suggest that the criteria for ocular biocompatibility are more exacting than those for daily wear lenses. As a result of these considerations this project was primarily concerned with the study of the biocompatibility of extended wear lenses.

Spoiled hydrogel lenses examined in this project were derived from a number of commercial and medical sources as outlined in Tables 5.1-2. A significant proportion of worn lenses were supplied by Kelvin Lenses Ltd., a large Manchester based lens manufacturing company. Indeed the Kelvin X-TEN lens was originally formulated in these laboratories as part of a collaborative continual wear research and development programme between the afore-mentioned and Kelvin Lenses Ltd. Spoiled X-TEN lenses employed in the treatment of aphakia were also supplied by practitioners at the Moorfield and Croydon Eye Hospitals. A number of spoiled Permalenses were donated by a large ophthalmic practice in London.

Commercial Outlet	Lenses Prescribed	Wear Regimes	Application
Kelvin Lenses Ophthalmic Optician (Clinical Trial)	X-TEN, Permalens	Extended	Cosmetic
P.Lewis	Permalens	Extended	Cosmetic
Moorfields and Croydon Eye Hospitals	X-TEN, Scanlens	Extended	Aphakia
Cooper Vision	O_2T	Daily	Cosmetic
Kelvin Lenses Retail Outlets	X-TEN	Extended	Cosmetic
Hydron U.K.	Hydron	Daily	Cosmetic

Table 5.1 COMMERCIAL AND MEDICAL SOURCES OF SPOILED LENSES

Table 5.2 COMMERCIAL SOURCE, IDENTITY AND MONOMER COMPOSITION OF SPOILED LENSES

Lens Manufacturer	Name of Product	Lens Wear Programme	Composition of Lens Material	% Water Content
Cooper Vision Optics	O_2T Permalens	D X	HEMA HEMA,NVP,MAA	39
Hydron U.K.	Hydron	D	HEMA	39
Contact Lens Manufacturing	Sauflon 85	X	NVP,MMA	50
Scanlens	Scanlens	X	AMINO/AMIDO CO POLYMER	73.5
Kelvin Lenses	Eurolens	X	COMPLEX MONOMERS	72
Kelvin Lenses	X-TEN	X	COMPLEX MONOMERS	72

Abbreviations

HEMA - Hydroxy ethyl methacrylate

NVP - N vinyl pyrrolidone

MMA - Methyl methacrylate

MAA - Methacrylic acid

D - Daily wear

X - Extended wear

5.3 CLINICAL TRIAL PROGRAMMES

In collaboration with Kelvin Lenses Ltd. a number of adult patients of both sexes, who were experienced in hydrogel lens wear, were selected to form a controlled clinical trial group of lens wearers. Such a group was established prior to the initiation of this project by Kelvin Lenses Ltd. in order to assess the wear tolerance (biotolerance) of the X-TEN lens, and to compare the biotolerance of the lens against established and successful extended wear devices. The existence of such a facility does however confer several unique features on this project. The ocular environment provides a rare opportunity to assess the in vivo reactions that occur between a hydrogel polymer and a complex biological fluid. As a result of the ease of insertion and removal of lenses, the traumatic effects that are otherwise induced by the surgical implantation of polymeric devices are minimalised. Control over the length of time in which the device comes into contact with the biological environment is more precise since lenses may be worn for periods as short as several minutes or as long as several months. It would be quite impracticable to surgically implant a device only to remove it 10 minutes later.

As a result of the similarity in the tear composition and structure of the left eye and right eye, and the ability to subject the respective lenses to identical wear times and cleaning regimes, the ocular environment presents a unique facility to assess the relative ocular biocompat-

ibilities of various commercially available hydrogel lenses that differ in their bulk and surface properties. A more concise account of the exact experimental conditions employed in these studies is given in Chapters 8 and 9.

5.4 POLYMERIC COMPOSITION OF DAILY AND EXTENDED WEAR LENSES

The monomeric composition of the various commercial hydrogel contact lenses examined in this project is given in Table Although a comprehensive evaluation of the monomeric compositions of the various lenses lies outside the scope of this thesis, four classes of monomers that are particularly relevant to the design of lenses studied in this project are considered below.

5.4.1 Poly(Hydroxy alkyl Methacrylates and Acrylates)

Poly(HEMA), the most widely used and studied hydrogel, was originally synthesized by the Du Pont Scientists in 1936 [218], it was not until the early 1960's however, that Wichterle and Lim [219] polymerised the monomer in an aqueous media, with a cross-linking agent, and from this beginning arose the advent of hydrogels for contact lens and general biomaterial applications. Poly(HEMA), although mechanically weak, is chemically stable at extremes of pH and temperature, two major requisites for a potential biomaterial [220-222]. The optical properties of poly(HEMA) are dependent on the polymerisation solvent. A bulk polymerisation reaction is therefore undertaken where, along with a cross-linking

agent, the monomer acts as its own solvent and produces a dehydrated hydrophilic polymer, which on hydration becomes an optically transparent, homogeneous hydrogel. An isomer of HEMA, 2 hydroxy-propyl acrylate, (HPA), is utilised in extended wear formulations such as the X-TEN lens. Although polymers containing HPA exhibit slightly weaker mechanical properties than poly(HEMA), they possess a higher water content (50 percent) than those based on its isomer (40 per cent) [223-225]. As a result of this function, recent lens formulations have been preferentially based on HPA instead of poly(HEMA) [226].

5.4.2 N-Vinyl Pyrrolidone

N-Vinyl pyrrolidone,NVP, present in the formulation of several lenses examined was originally utilized as a plasma expander [227]. However, in contact lens formulations NVP is used as an adjuvant or modifying group to both increase water content and to some extent the strength of the resultant gel, due to its hydrogen bonding capacity. For these reasons NVP is commonly employed in the preparation of gels of sufficiently high water content for extended wear [226].

5.4.3 Anionic and Cationic Monomers

These monomers are added to the composition to alter the charge characteristics of a gel. In the case of methacrylic acid (MAA), it is capable of hydrogen bonding, thus

increasing the equilibrium water content and mechanical strength of the resulting gel, which are the main roles of MAA in contact lens applications [226].

5.4.4 Acrylamide and Derivatives

Acrylamide and its derivatives (methacrylamide and diacetone acrylamide) are incorporated in hydrogel compositions in order to boost the water content, as a result of intermolecular hydrogen bonding, of the resulting gel. It is as a result of this function that monomers of this class are used in the formulation of extended wear materials.

5.5 EQUILIBRIUM WATER CONTENT OF HYDROGELS

Probably the most important single property of hydrogels with respect to contact lens applications is the 'dissolved' oxygen permeability of such devices, since the cornea is avascular and therefore derives oxygen principally from the atmosphere. The dissolved oxygen permeability of hydrogel contact lenses is governed by the equilibrium water content, (EWC), of the gel. Indeed the dissolved oxygen permeability of hydrogels has been found to increase exponentially with increasing water content [228].

The EWC of a hydrogel may be defined as the ratio of the weight of the water contained within the hydrogel to the weight of the hydrogel at equilibrium hydration expressed as a percentage [229]. The weight of water in the hydrogel is

obtained by subtracting the weight of a polymer sample dehydrated to constant weight from the weight of the gel at equilibrium hydration. Hence the EWC may be expressed in terms of the following equation:

$$\% \text{ EWC} = \frac{\text{Weight of water (Ww)}}{\text{Weight of gel (Wg)}} \times 100$$

$$= \frac{\text{Wt of hydrated sample - Wt of dehydrated sample}}{\text{Wt of dehydrated sample}} \times 100$$

$$= \frac{Wg - Wp}{Wg} \times 100$$

where Wp = weight of dehydrated polymer (5.2)

From experimentation it has been concluded that a hydrogel suitable for extended wear should have an EWC of 70 percent or more in order to maintain corneal clarity.

5.6 MODIFICATION OF SURFACE TENSION OF HYDROGELS

Hydrogels of sufficiently high water content for use as extended wear contact lenses depend upon the presence of polar groups containing oxygen or nitrogen to confer hydrophilicity, and therefore tissue compatibility, upon the polymer. Their presence however, gives rise to a high surface energy in hydrogels of high water content since the critical surface tension of these lenses is dominated by the presence of water within the matrix. Such a rise in surface energy results in increased adhesive characteristics leading to a greater degree of bio-organic adsorption[226].

The surface tension of tears is also related to the significant water component of tears, although it has already been discussed how the presence of surfactant mucins lowers the surface tension to a level of 40-46 dynes.cm^{-1}. In this study several of the extended wear lenses considered, exhibit high surface energies, in the order of 60 dynes.cm^{-1}. for the reasons outlined above. In consequence the interfacial tension of these devices is relatively high. Radio-labelled protein adsorption experiments conducted in these laboratories have shown these lenses to adsorb large amounts of protein [230].

In Chapter 1 the interfacial energy hypothesis, a theory pertaining specifically to the biocompatibility of blood contact devices, was considered. In summary it was proposed that in order for a biomaterial to exhibit blood biocompatibility, the biomaterial must exhibit an interfacial tension in the order of 5 dynes.cm^{-1}. Indeed it is this concept that was employed in the conception and development of the Kelvin X-TEN lens whereby efforts were made to maintain the equilibrium water content at a level suitable for extended wear (i.e 70 percent), yet at the same time lowering the interfacial tension of the hydrogel lens with tear fluid. These goals were attained by the incorporation of hydrophobic fluorinated monomers in the lens composition. The monomeric composition of the lens and methods of synthesis are described in the relevant patent literature [228]. Further protein adsorption studies revealed that the X-TEN lens adsorbed substantially less protein than the previously

examined commercial extended lenses[230]. The clinical trial group provides further opportunities, not only to establish the relative biotolerance (biocompatibility) of the purpose designed X-TEN lens (against non-modified commercial designs),but also to assess as to whether the Minimal Interfacial Energy hypothesis is applicable in the prediction of the ocular biocompatibility of hydrogels.

CHAPTER SIX

CHAPTER VI

DEVELOPMENT AND APPLICATION OF ANALYTICAL TECHNIQUES

6.1 INTRODUCTION

This chapter is concerned with the development and application of techniques that were employed in the analysis of various manifestations of hydrogel lens spoilation. Techniques were designed in order to assess the morphology and geographical location of lens deposits, alterations in the surface rugosity of lenses during wear, and to the identity, chemical composition and structure of the various types of deposits observed on and within hydrogel lenses examined during this project. A more detailed discussion of the respective techniques and their methodologies is given below.

6.2 LIGHT MICROSCOPICAL TECHNIQUES

Various light microscopical techniques were used in this study to assess the surface integrity of worn and unworn lenses, and the morphology, fluorescence and geographical location of lens deposits formed both on and within hydrogel contact lenses.

6.2.1 Lens Cleaning and Handling

Prior to microscopical examination, lenses were removed

from storage vials and subjected to a cold clean and disinfection regime. After examination, lenses were then stored in sealed glass vials containing 10 mls of preserved isotonic saline at room temperature. (The constituents of the preserved saline are given in Appendix 1.). This was undertaken in order to prevent microbial contamination of lenses during long periods of storage. During examination, disposable surgical gloves were worn, and surgical tweezers employed, to handle lenses in order to avoid damage to the lenses and to minimise digital contamination.

6.2.2 Lens Mounting

From experimentation, a reliable technique was developed for mounting spoiled lenses on glass microscope slides (Jencon's Ltd). Prior to use, slides were cleaned in a 10 percent solution of sodium dodecyl sulphate (Sigma Ltd), thoroughly rinsed with freshly distilled water and oven dried. A drop of preserved saline was then placed in the centre of a clean slide and the lens was then placed concave surface down and flattened with a glass coverslip. There are two main advantages in mounting lenses in such a way. Firstly, the lens surface may be inspected with only minor adjustments in focus and secondly, surface distortion that results from lens dehydration is minimised. Most hydrophilic lenses may be repeatedly examined in this way without damage to the lens. Aphakic lenses are however, unsuitable for this method of mounting. Instead they were

mounted on a hanging drop slide (Jencon's Ltd) with a concave depression ground into it. The lenses were then placed in the depression, convex surface down, immersed in preserved saline, and covered with a coverslip.

6.2.3 Stereomicroscopy

A Leitz stereo microscope, with a magnification range of X10 - X140, was initially used to gain an overall impression of the lens surface. Although simple, this technique provides a useful, non-destructive overview of the nature, occurrence and geographical location of lens deposits. Various types of illumination were employed, allied with custom designed reflective stages. Optimum results were obtained with indirect illumination of the lens. Although useful, this technique is limited since only gross or pigmented deposits are observed. Therefore more informative techniques were developed.

6.2.4 Phase Contrast and Interference Contrast Microscopy

Phase contrast and interference contrast microscopy render otherwise transparent objects visible, by converting phase changes in the light passing through them into changes of wave amplitude. Regions of differing refractive index are therefore made visible as regions of differing brightness. Interference microscopy differs from phase contrast microscopy only in that, whereas in phase contrast light refracted by the object is made to interfere with light which

has passed through the same field, in interference microscopy, the light, which has passed through the object, is made to interfere with a beam which has travelled along a different path.

Phase contrast studies were carried out with a Leitz Dialux 20 microscope, whilst a Zeiss Interference microscope was employed for interference studies. The major advantages of these techniques are that they enable the detection and assessment of the morphology, density and extent of surface coverage by lens coatings. The detection of these semi-transparent coatings is otherwise impossible without employing such techniques. As a result of the increased depth of focus that these techniques provide, studies of the nature and occurrence of matrix-bound deposits were also made possible. Studies of spoilt lenses were also undertaken employing darkfield techniques.

6.2.5 Fluorescence and Polarisation Microscopy

Fluorescence studies were carried out on spoiled lenses and unstained histological sections with a Leitz Dialux 20 microscope fitted with a Ploempak 2.4 Fluorescence Vertical illuminator. A 50 watt ultra high pressure mercury lamp was employed as the light source. Polarisation studies were conducted on spoiled lenses with a Zeiss polarising microscope. The relevance of fluorescence and polarisation microscopy in the analysis of spoilt hydrogel lenses is considered more fully in Chapter 9.

6.3 HISTOLOGY

6.3.1 Introduction

Histological techniques were developed and employed in this study in order to identify the organic components of lens deposits, the geology and the chemical composition of the hydrogel matrix-deposit interfacial region. From these studies it was hoped to characterise those biological species that initially react with the polymer lens. The various techniques are outlined below.

6.3.2 Reagents

Unless otherwise stated all reagents employed in the histological analysis of lens deposits were of analytical grade and were obtained from B.D.H. Ltd. The compositions of fixation solutions and respective buffers are given in Appendix I.

6.3.3 Paraffin Histology

Initially routine paraffin methodologies were attempted. However, in common with other workers[231], it was found to be extremely difficult to prepare sections of hydrogel contact lenses using this technique[232]. The major problems encountered were; the extensive dissolution of deposits in the lipid soluble wax, combined with lens distortion and shrinkage. These problems are thought to arise from

the incomplete dehydration of the hydrogel and thus insufficient paraffin wax impregnation of the expanded matrix. Attempts to remedy the situation involved the use of matrix-swelling agents, such as dimethyl formamide. The extent of matrix swelling was however, extremely difficult to control and the paraffin technique was therefore abandoned.

6.3.4 Cryohistology

From experimentation, it was found that cryomicrotomy provides a reliable technique that produces hydrogel lens sections without either significant leaching of deposit components or significant alteration in the geological structure. A major advantage of this technique is that the need for an impregnating, embedding agent to support the specimen is made obsolete, since matrix imbided water is frozen during the method. This technique was also found to minimalise lens shrinkage and distortion. A Leitz 1320 bench top, freezing microtome was used exclusively throughout this study. The methods of lens preparation employed in this study are outlined below.

Spoiled areas of the lens to be examined, were removed with the aid of a custom built drill rig that produced longitudinal sections of dimensions 5 mm x 3 mm. Excised lens samples were washed several times in freshly distilled water prior to fixation.

6.3.5 Lens Fixation

Formaldehyde, in the form of commercially available Formalin was used as the primary fixative throughout this study. Formalin consists of free formaldehyde, methylene glycol and polyoxymethylene glycols[233]. As a result of the formation of low levels of formic acid, formaldehyde were made up with phosphate-buffered saline (pH 7.2) using analytical grade reagents according to standard techniques. The primary fixative action of formaldehyde is on proteins. Formaldehyde is thought to act by the cross-linking of peptide chains, amino, amido and thiol functional groups. Although it was originally thought that formaldehyde was unable to fix lipids[234], recent evidence suggests that formaldehyde reacts with unsaturated fatty acids during fixation. The site of reaction is thought to be double bond of the acid[235]. Although formaldehyde is unable to preserve soluble polysaccharides, it is very effective in fixing mucoproteins and acid mucopolysaccharides bound to proteins[236]. 10 percent formaldehyde fixatives were used in histological studies. From experimentation it was found that fixation of lens samples for a period of 12 hours in 10 mls of the fixative at room temperature produced optimum results. After fixation, lens samples were thoroughly washed in distilled water prior to freezing.

6.3.6 Section Cutting

Prior to sectioning, lens samples were embedded in agar.

1 g of agar was dissolved in 100 mls of PBS (pH 7.2), using conventional techniques. Semi-solid agar was then poured into a transparent perspex mould (1 cm x 1 cm x 1 cm). The lens sample was then positioned with the longitudinal side of the sample positioned horizontally. The use of agar as a mounting medium provides a repeatable, precise and reliable method of sample orientation. When set, the agar block was released from the mould, mounted on the microtome specimen stage and frozen with carbon dioxide. Sections, 10 μm thick, were then cut from the frozen agar block with tungsten carbide knives (Leitz Ltd), floated out on distilled water, examined by various microscopical techniques and finally stained.

6.3.7 Staining Techniques

Various staining techniques were employed for the chemical identification and localisation of components in contact lens deposits. The stain recipes and methodologies employed in this study are described in various texts[237,238]. In all cases normal dehydration procedures were removed from the methods, since it was found from experimentation that such steps minimalise the shrinkage and distortion of lens sections caused by this process. To assess the viability of stains employed, control sections of unworn lenses of identical monomeric composition to the spoiled lens were treated in a similar manner to sections of spoiled lenses. The chemical specificity of the stains employed was wide ranging; from general stains such as

Oil Red O for lipids, to the highly specific Von Kossa calcium detection method. More detailed discussions of the nature and histochemistry of the stains are given in Chapters 7 - 10.

6.3.8 Section Mounting and Examination

Prior to mounting, sections were washed in distilled water and then centrally positioned on clean glass slides. Sections were then immersed in a drop of glycerin-albumin solution, covered with a glass cover-slip and oven dried at 60°C. Mounted sections were then examined by the microscopical techniques already discussed.

6.4 RESIN EMBEDDING

6.4.1 Introduction

As previously discussed two major aims of this project were the study of the geology and chemical characterisation of the interfacial components of hydrogel contact lens deposits. A preparatory technique was developed that facilitated a dual histological and electron microscopical study of lens deposits.

Acrylic resin embedding of specimens, in combination with light microscopy, offers significant advantages over conventional embedding media in that the technique provides greatly improved morphological definition. However the

majority of acrylic resins are unstable in an electron beam and are therefore unsuitable for use in electron microscopy studies. Conversely epoxy resins, commonly used for electron microscopy studies are incompatible with conventional histological techniques without complicated pre-treatment. An embedding medium, L.R.White resin (London Resin Company) is compatible with conventional stains due to the hydrophilic nature of the resin and is also beam stable. As a result of these properties this resin was used extensively in this study.

6.4.2 Lens Fixation and Embedding

Formaldehyde based fixatives were used when conventional histological stains were to be applied to lens sections. When dual light and electron microscopical studies were to be conducted, lenses were fixed in a 5 mls solution of 4 percent freshly depolymerised gluteraldehyde in a phosphate buffer (pH 7.2), with 2.5 percent sucrose (w/v). Lens samples were dehydrated according to the method outlined in the section concerned with scanning electron microscopy.

6.4.3 Infiltration of Lens Specimens

From experimentation, it was found that the infiltration schedule significantly affected the morphology of lens deposits. Optimum results were obtained when lens samples were placed in a light-proof glass vial, containing 5 mls

of the resin monomer solution for 1 hour at 20°C. Lens samples were then removed and the schedule repeated, although fresh monomer was used.

6.4.4 Polymerisation

Lens samples were removed from the infiltration medium and placed in gelatin capsules, (Polyscience Ltd.), that were then filled up with resin accelerator mixture, made up according to the manufacturer's data sheet.

From experimentation it was found that polymerisation time and temperature are fundamental to the physical character of the final block. Homogeneous and transparent blocks were obtained under the following conditions. Sealed gelatin capsules were placed in a custom designed support rig, in a water bath maintained at a temperature of 12°C (Figure 6.1). At this temperature, polymerisation is complete after 20 minutes. Post curing of the specimen block was carried out at 60°C for 24 hours, after which the specimen block was removed from the gelatin capsule and stored in a dessicator until sectioning was performed.

6.4.5 Specimen Cutting and Mounting

Specimen blocks were cut on a Leitz 1320 cryomicrotome with a tungsten carbide knife at 25°C. Under these conditions it was possible to cut sections 2-3 μm thick. Cut sections were floated out on 30 percent acetone on a hot-

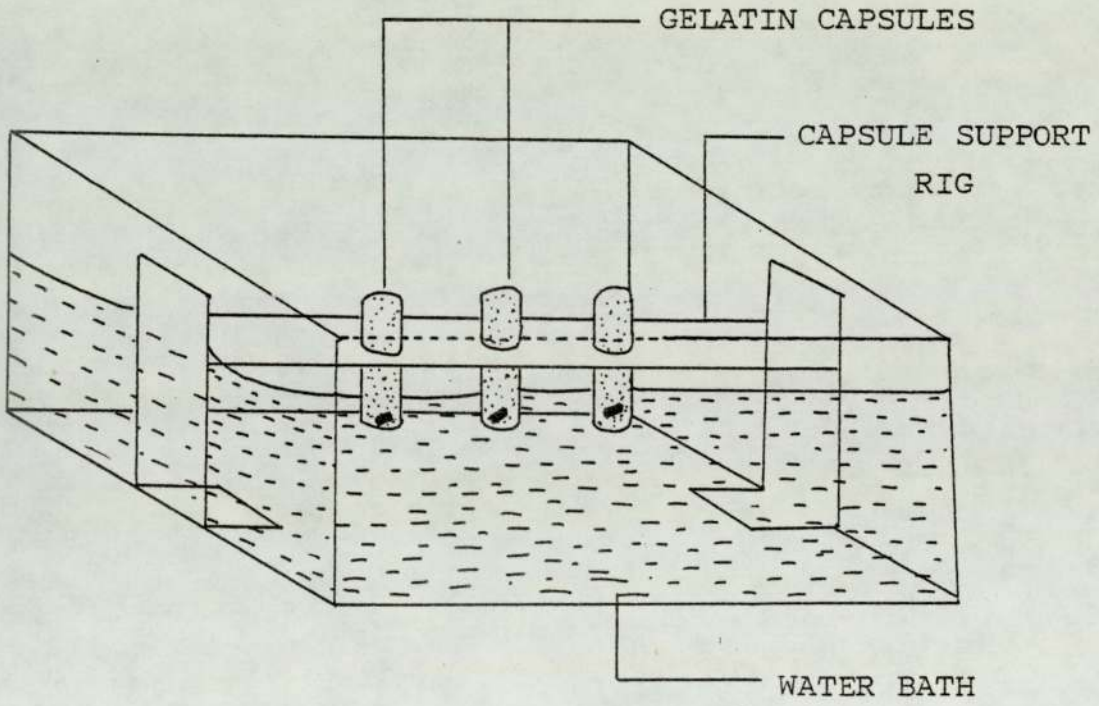


FIGURE 6.1 RESIN POLYMERISATION WATER BATH

plate maintained at 60°C and were then allowed to dry at this temperature. Staining of the sections was then undertaken, although dehydration through graded alcohols after staining was avoided. Stained sections were then blotted, air dried and mounted in glycerin-albumin. Sections that were to be studied by electron microscopy were placed in 1 percent osmium tetroxide (Sigma) for 10 minutes, oven dried at 60°C and coated with gold-palladium by the techniques outlined below.

6.5 SCANNING ELECTRON MICROSCOPY

6.5.1 Introduction

Scanning electron microscopy (S.E.M) provided a powerful tool in the determination of the surface topography of hydrogel lenses. This technique was extensively used in this project in order to characterise the various morphologies of lens surface manifestations of hydrogel lens intolerance. S.E.M studies of sections through the hydrogel matrix-deposit material interfacial region were also carried out. The lens preparatory techniques employed in this study are discussed below.

6.5.2 Lens Fixation

From experimentation with worn and unworn lenses it was found that a buffered gluteraldehyde-picric acid fixative (BGPA) was most effective in preserving the morphology and

compositional integrity of lens deposits without causing any deliterous effects on the lens polymer.

6.5.3 Preparation of a Gluteraldehyde Fixative

A 25 percent (v/v) solution of highly purified monomeric gluteraldehyde was obtained from Sigma. From experimentation it was found that the use of a purified form of depolymerised, gluteraldehyde greatly increased the reliability of the technique and avoided contamination of the lens with impurities such as cyanide and arsenic. There is also considerable variation in the purity, content and shelf-life of commercial grades of gluteraldehyde [236]. 2 mls of 25 percent gluteraldehyde was added to 83 mls of 0.1 M phosphate buffered saline (pH 7.4) that was made up with analytical grade reagents (Sigma) according to standard techniques. To this was added 15 mls of saturated picric acid in order to increase the preservation of lipid associated protein that is otherwise lost when gluteraldehyde alone is used as a fixative [239]. The resulting buffered gluteraldehyde-picric acid was stored at -4°C, in a glass stoppered vial until use.

6.5.4 Fixation Protocol

Areas of the lens to be examined were removed by the technique previously discussed and were then placed in a stoppered glass vial containing 5 mls of fresh buffered gluteraldehyde-picric acid fixative for 1½ hours at 4°C.

It is necessary to maintain the pH of the fixative in the region of 7.2-7.4 in order to maximise the binding of the aldehyde groups with proteins [236]. Lens sections were then removed and post fixed in osmium tetroxide as outlined below.

6.5.5 Osmium Tetroxide Fixation

Although gluteraldehyde-picric acid fixative is extremely efficient in cross-linking proteins and mucopolysaccharides, its ability to preserve lipoidal components is slight [239]. The excessive loss is however, considerably reduced by treatment with a 1 percent solution of osmium tetroxide. Osmium tetroxide also imparts electron density to osmophilic structures in its reduced form.

6.5.6 Preparation of Osmium Tetroxide Solution

Yellow crystals of osmium tetroxide were obtained from Sigma. The glass ampule containing the crystals, a glass stoppered bottle, and a heavy glass rod were all cleaned with concentrated nitric acid, thoroughly washed in distilled water and oven dried. It is essential that all organic matter is removed from preparative and storage glassware involved in the preparation of the solution since such contaminants may reduce osmium tetroxide to its hydrated dioxide, which is ineffective as a fixative.

A volume of 100 mls of freshly distilled water was added

to a pre-cleaned glass bottle, into which a glass ampule containing 1 g of osmium tetroxide was gently lowered. The ampule was then broken with the glass rod and the bottle was quickly stoppered and shaken vigorously. Dissolution of the crystals was facilitated by gentle heating on a steam bath over a magnetic stirrer. The bottle was then tightly stoppered, wrapped in aluminium foil and refrigerated until use.

6.5.7 Fixation Protocol

Lens samples previously treated with buffered gluteraldehyde-picric acid, were placed into pre-cleaned, light proof, stoppered glass vials containing 5 mls of 1 percent osmium tetroxide. Since prolonged osmium fixation causes extraction of proteins a short fixation is preferred. From experimentation, it was found that a period of 90 minutes was optimum. Post-fixation was carried out at $4^{o}C$. Post-fixed lenses were then removed from the fixative and thoroughly washed in freshly distilled water prior to dehydration.

6.5.8 Lens Dehydration and Drying

Boyde and Wood, in an excellent review of the preparatory techniques for specimen observation by S.E.M., stated that air drying of hard tissues of specimens is adequate [240]. However, it is the opinion of the author and others, that soft biological specimens should not be studied in such

a manner, due to the loss of fine detail and distortion that occurs in the specimens. Although soft contact lenses are obviously not biological specimens, they have many features in common. The dehydrated lens is brittle like some hard tissues, whereas the hydrated lens has certain similarities to soft tissues. Indeed, from experimentation, it was found that air drying of soft lenses, led to lens shrinkage and distortion, and indeed severe artefacts similar to those seen when soft biological specimens are treated in such a manner. Thus a more efficient dehydration protocol was designed.

Initially it was considered that freeze and critical point drying induce relatively little distortion and that these techniques are comparable in producing specimens for S.E.M. However, a major drawback in the freeze-drying technique, is the movement of phase boundaries through the specimen that leads to the production of artefacts. In freeze-drying such boundaries move through the specimen twice; first, a liquid-solid boundary moves rapidly in the freezing process, and a second, solid vapour boundary, moves during sublimination of the frozen matrix. As a result of these considerations critical-point drying was employed as the main preparatory technique for lens observation.

Prior to critical-point drying, hydrogel lenses were dehydrated in a purpose built, perspex dehydration chamber (Figure 6.2). After washing, in distilled water, the osmicated lenses were placed in the chamber, previously

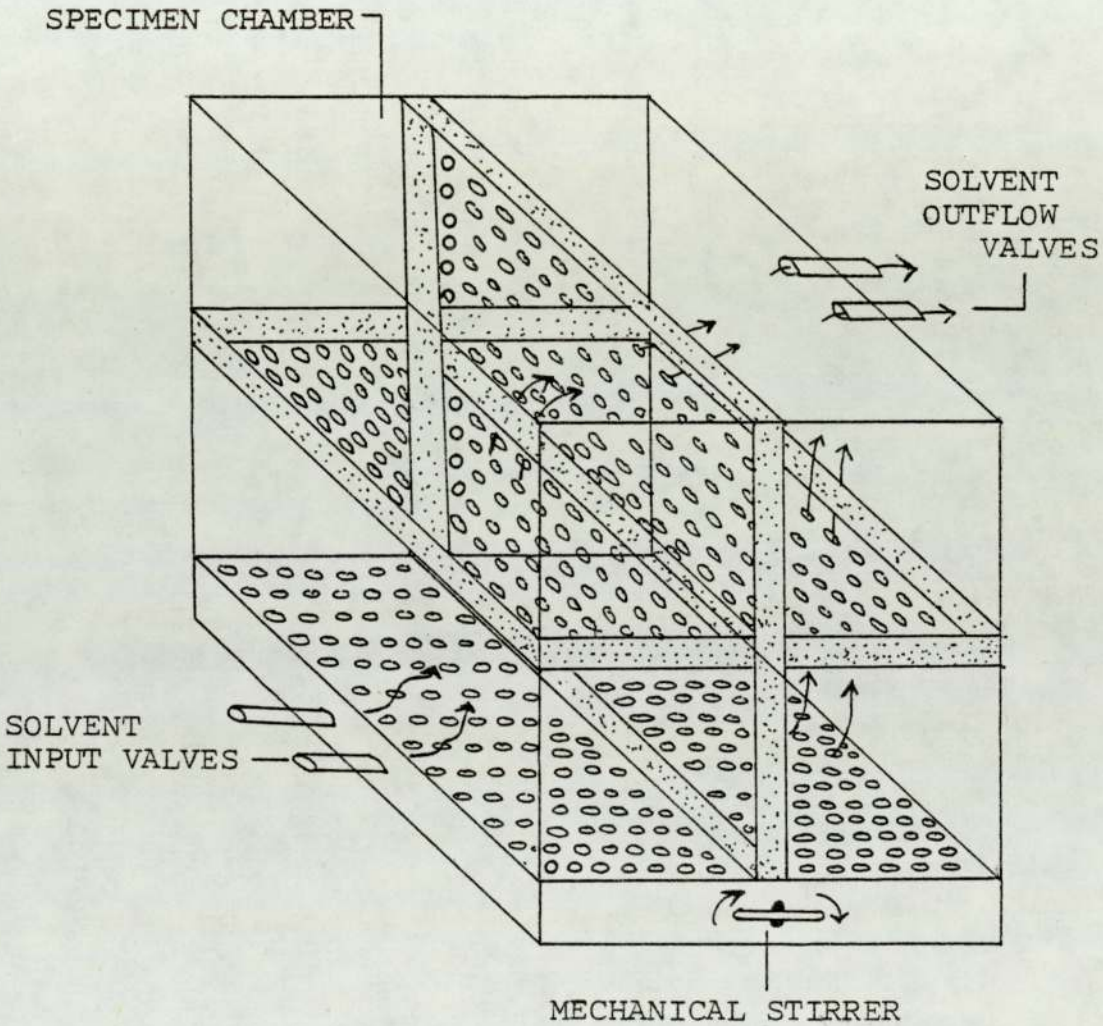


FIGURE 6.2 GRADIENT FREE DEHYDRATION CHAMBER

filled with freshly distilled water. A volume of 100 percent ethanol, twice that of the dehydration chamber, was gradually introduced over a period of 24 hours, during which the dehydration solvent was continually stirred. It has been found that this method eliminates the extreme shrinkage and distortion of lenses observed during sequential dehydration regimes. After ethanol dehydration, lenses were subjected to a series of amyl acetate solutions, ranging from 25 percent acetate to 100 percent pure solvent, for a period of 30 minutes respectively. Final dehydration was achieved with the aid of a critical-point dryer, (Polaron Equipment Ltd.) that was operated according to the method of Anderson [241].

Dried lenses were then mounted on aluminium stubs and coated with a thin layer of conducting gold-paladium, in a sputtering device (Nanotech Ltd.) according to standard metallurgical procedures. If not examined immediately, coated lenses were placed in a dessicator under high vacuum. Lenses were examined with a Stereoscan Electron Microscope (Cambridge Instruments Ltd.) at operating voltages of 5, 10, 15 and 20 KeV.

6.6 PHOTODOCUMENTATION OF SPOILED LENSES AND LENS SECTIONS

Lenses examined by stereomicroscopy were photographed with the aid of an eye-piece mounted camera (Periflex). Calibration of light intensities and exposure times was undertaken with a purpose-designed photoelectric-cell

detection system, mounted on the second eye piece. From experimentation it was found that slow black and white films, such as ILFORD HP5 and PAN F, provided optimum results.

A Leitz Vario-Orthomat photomicroscopy system mounted on a Leitz Dialux microscope, combined with a Wild MPS 45 Photoautomat (Leitz Ltd.), was used for the photodocumentation of lenses studied by phase and interference contrast microscopy and fluorescence microscopy. This system was also employed in the photodocumentation of stained histological sections. This system is totally automatic, adapting the exposure length to the types of light format used and film employed. Kodacolour V400 Film (Kodak Ltd.) was used for colour studies of lenses and sections. Electron micrographs were obtained with a Nikon camera mounted on the electron microscope. From experimentation it was found that ILFORD HP5 films and exposure lengths of 60 seconds provided optimal results.

6.7 ENERGY DISPERSIVE X-RAY ANALYSIS

The histological techniques employed in this study were implemented in order to detect the major organic components of lens deposits. These techniques are however, limited in their ability to detect inorganic constituents within either deposit structure or the lens matrix. Energy dispersive x-ray analysis (EXDA) was the principal analytical technique used in the elemental analysis of the surfaces

of worn lenses and deposit structures. By this technique elements with an atomic number greater than that of fluorine may be detected.

Since EDXA is a surface analytical tool, sections of spoiled lenses bearing deposits were submitted to analysis in order to provide an elemental geological profile of the deposit material and the underlying lens matrix. From experimentation, it was found that the preparatory techniques already discussed for S.E.M. studies provided optimum results. Lenses were however, thoroughly washed in distilled water after fixation procedures in order to remove contaminating buffer salts from the lens matrix. Studies were conducted with the aid of a Link System Autoanalyser, mounted on a Cambridge steroescan electron microscope. Analytical investigations were carried out according to standard metallurgical procedures. Optimum results were obtained with an electron beam accelerating voltage of 20 KeV and a sample collection time of 100 seconds. Both qualitative and semi-quantitative elemental analyses were performed along with the construction of elemental x-ray maps. By this technique the surface and geological density of an element, found to occur on and/or within the deposit structures or lens polymer may be assessed. Various illustrations of this technique are found in plates 9.18-20.

CHAPTER SEVEN

CHAPTER VII

THE DISCOLOURATION OF HYDROGEL CONTACT LENSES

7.1 INTRODUCTION

The discolouration and loss of lens transparency is a major and significant problem encountered during both daily and extended wear programmes. The nature and occurrence of this problem has been reviewed in Chapter 4. Although several attempts have been made to establish the chemical identity of the species responsible for lens discolouration, as yet no definitive report has been published as to the exact nature of the causative agent or agents [181]. Allied to this is the fact that although the incidence of discolouration has been well documented, questions as to whether alterations in the bulk and surface properties of hydrogel lenses effect the nature and occurrence of lens discolouration remain unanswered. This chapter, is therefore, concerned with the chemical analysis of discoloured lenses of various commercial derivations and attempts to establish as to whether a relationship exists between the bulk and surface properties of a lens and the nature of the discolouration.

7.1.1 Sources of Discoloured Lenses

A total of 69 yellow-brown discoloured lenses, involving a range of wear protocols, were examined in this study.

The commercial identity of the lenses, their numbers and wear programmes are outlined below.

Table 7.1

LENS MANUFACTURER	COMMERCIAL IDENTITY	NUMBER OF LENSES	WEAR PROTOCOL
Kelvin Lenses Ltd	X-TEN	41	Extended
	JW2	5	Extended
Cooper Vision Ltd	Permalens	6	Extended
Hydron U.K.Ltd	Hydron	17	Daily

7.2 EXPERIMENTAL TECHNIQUES

A Beckman SP800 spectrometer was employed in the determination of the U.V. and visible light spectra of discoloured lenses. From experimentation optimum results were obtained by mounting discoloured lenses on a custom designed Melinix, poly(ethylene terephalate) (I.C.I.Ltd.) 'scaffold' placed in a quartz cell with a 1 cm light path that was filled with freshly distilled water. The 'scaffold' was positioned in such a way as to eliminate any spectral interference from Melinix. Prior to spectral analysis of a discoloured lens, the U.V. and visible spectra of an unworn control lens of identical monomeric composition was obtained in order to identify which peaks were characteristic of a particular monomeric composition.

The spectra of various discoloured lenses are found in Appendix 2. From these it can be seen that there is a large amount of adsorption occurring at approximately 266 nm in

the U.V., tailing off gradually into the visible but still absorbing at high wavelengths. The main peak though, is found to be at 327 nm, with a shoulder at about 608 nm, at which point the absorption tails off completely. Such an absorption profile was common among all discoloured lenses examined. The U.V.and visible spectra of unworn control lenses displayed very little absorption in the spectrum. Therefore the absorption seen in the case of discoloured lenses, must be due to absorbed species and not to any component of the lens polymer.

7.2.1 Microscopy

Microscopical analysis of discoloured lenses was undertaken with a Leitz Dialux microscope fitted with phase contrast objectives. The methods employed to mount and photodocument the lenses is discussed in the previous chapter.

Plates 7.1 - 6 depict photomicrographs of discoloured extended and daily wear lenses previously examined by spectrometry. A most significant feature is the apparent presence of diffuse, particulate deposits. Such a finding is in accordance with other workers who have conducted similar studies[181]. Of particular interest are plates 7.5 - 6, that are representative of a patient's right and left eye lenses respectively. Both lenses were worn continuously for a period of 3 months. The lenses were removed at this time as a result of the severity of the discolouration. On

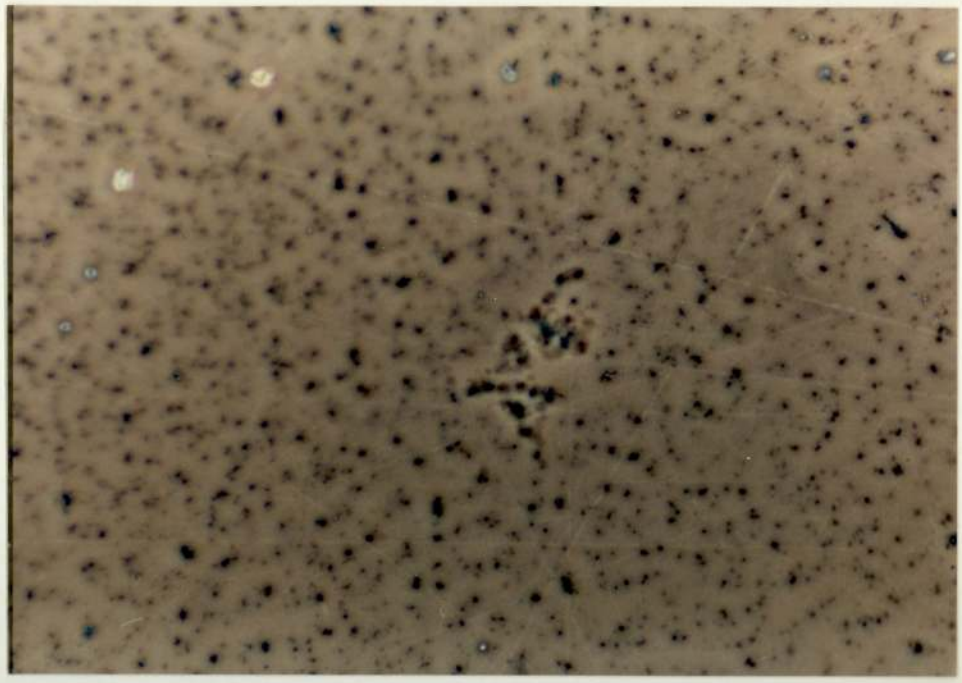


Plate 7.1 Particulate Deposit Density, Permalens, Phase Contrast, X240

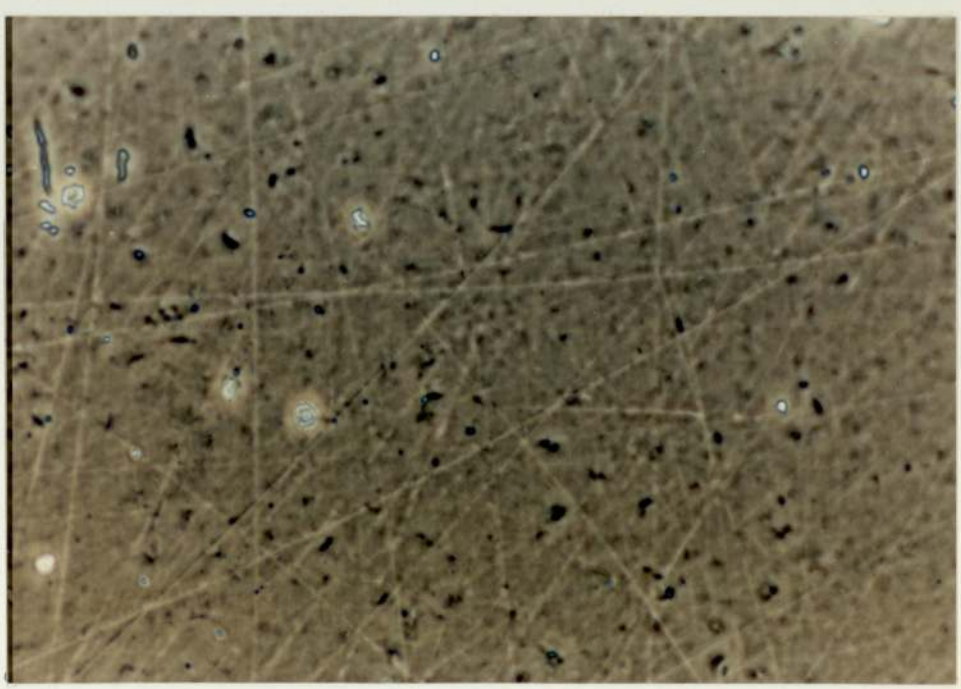


Plate 7.2 Particulate Deposit Density, X-TEN Lens, Phase Contrast, X 240

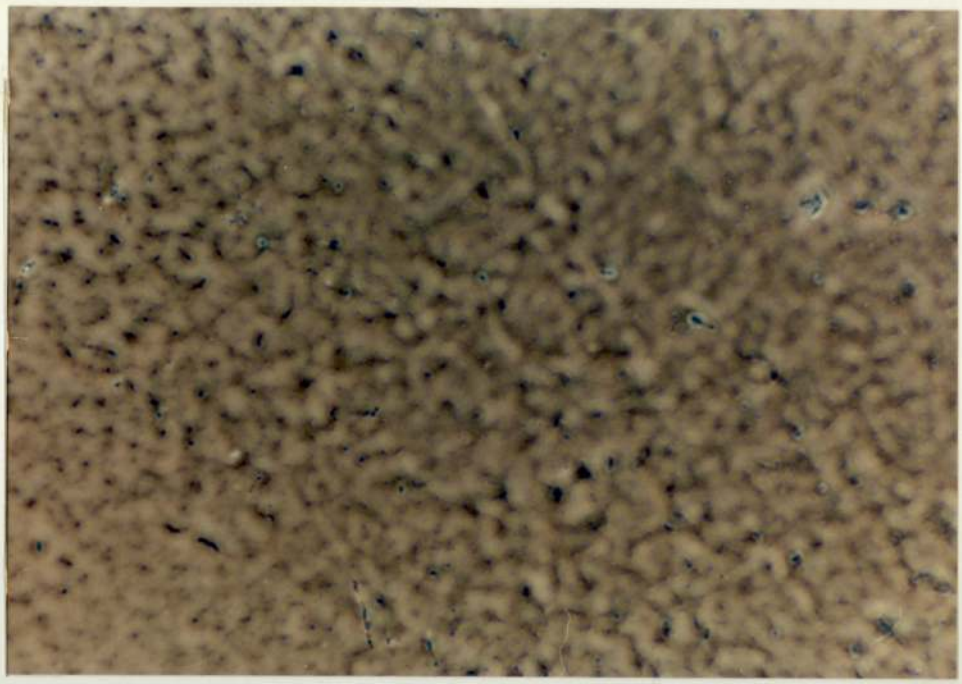


Plate 7.3 Particulate Deposit Density,
X-TEN Lens, Phase Contrast, X 240

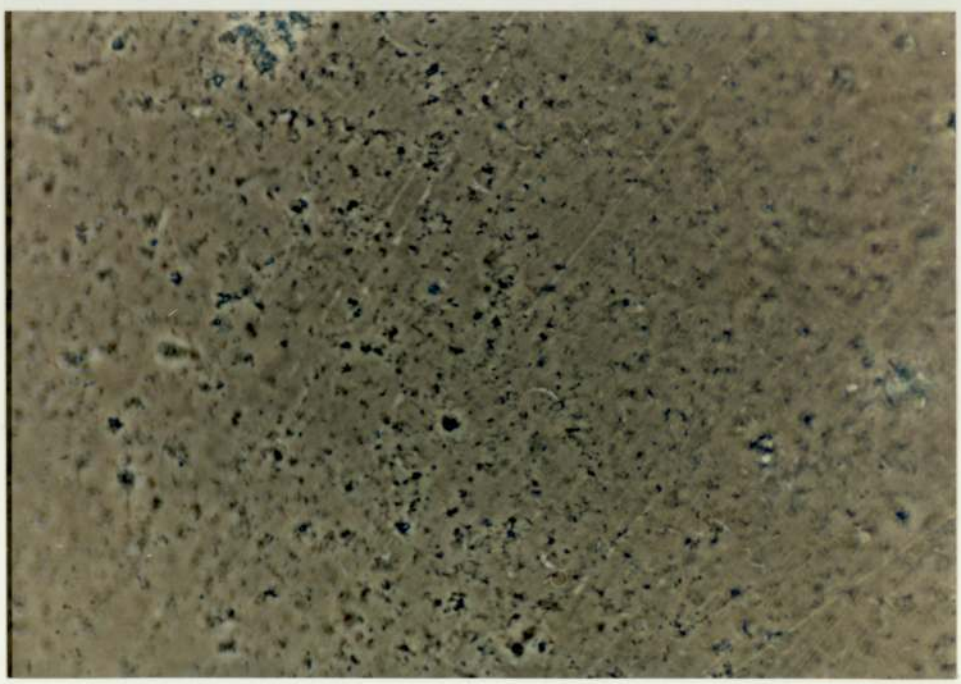


Plate 7.4 Particulate Deposit Density,
Hydron Lens, Phase Contrast, X 240

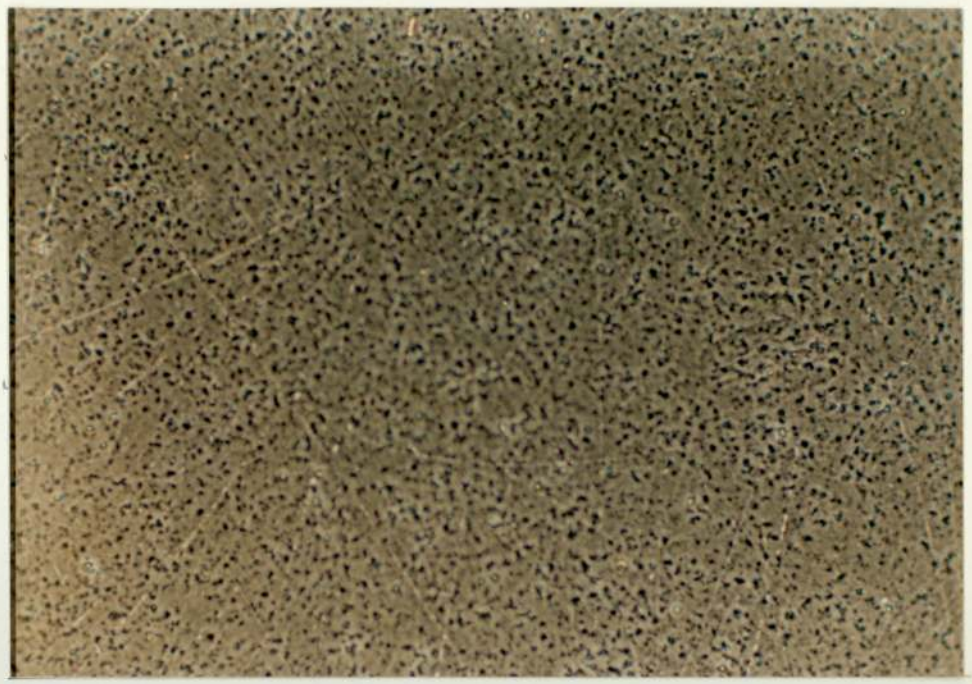


Plate 7.5 Particulate Deposit Density,
X-TEN Lens Right Eye Phase Contrast X 240

Plate 7.6 Particulate Deposit Density
X-TEN Lens Left Eye Phase Contrast X 240

inspection with the naked eye, in the case of the right eye lens, the intensity of the discolouration appeared to be considerably greater than that of the left lens. Indeed U.V.spectra of the right lens displayed an absorption profile similar to that of the left, although the right lens spectra displayed peaks of considerably greater magnitude. From spectral evidence and microscopical analysis, it is evident that there is a direct relationship between the intensity of discolouration and the population density of matrix-bound, particulate deposits.

7.2.2 Histological Studies

Histological studies were conducted on discoloured lenses in order to confirm the presence of matrix-bound deposits and to identify the chemical composition of these structures.

Discoloured lenses were fixed in formaldehyde, frozen, sectioned and mounted on glass slides by the techniques outlined in Chapter 6. Initially longitudinal sections, 10 μm thick, were mounted and examined by phase contrast microscopy and photodocumented. The results of this study are displayed in plates 7.7 - 12. From these it is obvious that pigmented particulate deposits are found within the lens matrix of all discoloured daily and extended wear lenses examined by this technique. Plates 7.11 and 7.12 depict the unstained sections of the right eye, left eye lenses portrayed in plates 7.5 and 7.6. In accordance with spectral

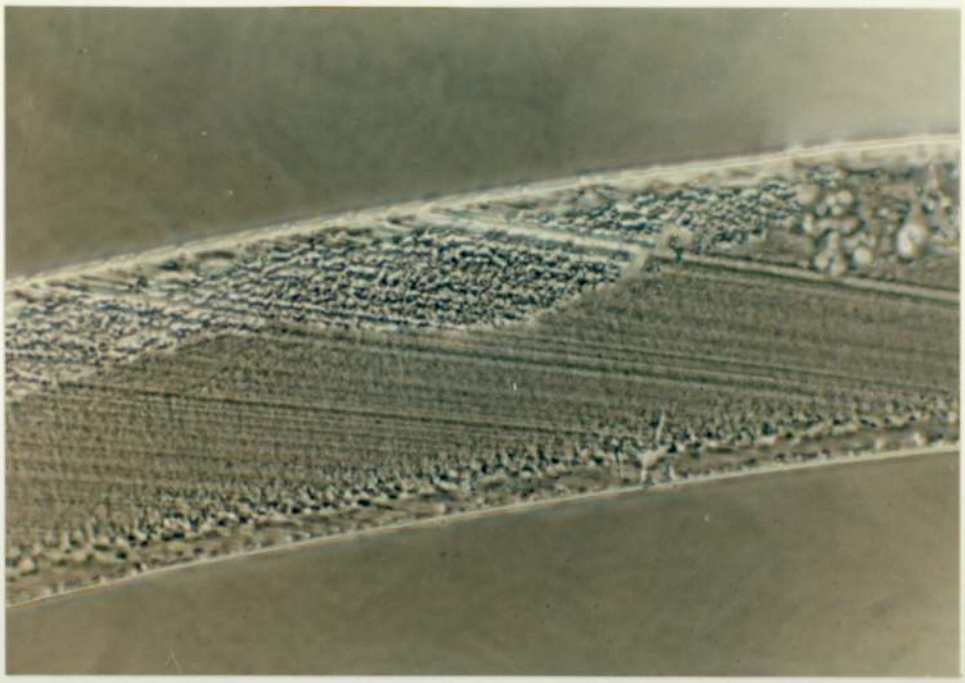


Plate 7.7 Particulate Deposit Distribution
Hydron Lens Phase Contrast X 65

Plate 7.8 Particulate Deposit Distribution
Permalens Phase Contrast X 240

Plate 7.9 Particulate Deposit Distribution
Hydron Lens, Phase Contrast, X 65

Plate 7.10 Particulate Deposit Distribution
X-TEN Lens, Phase Contrast, X 240

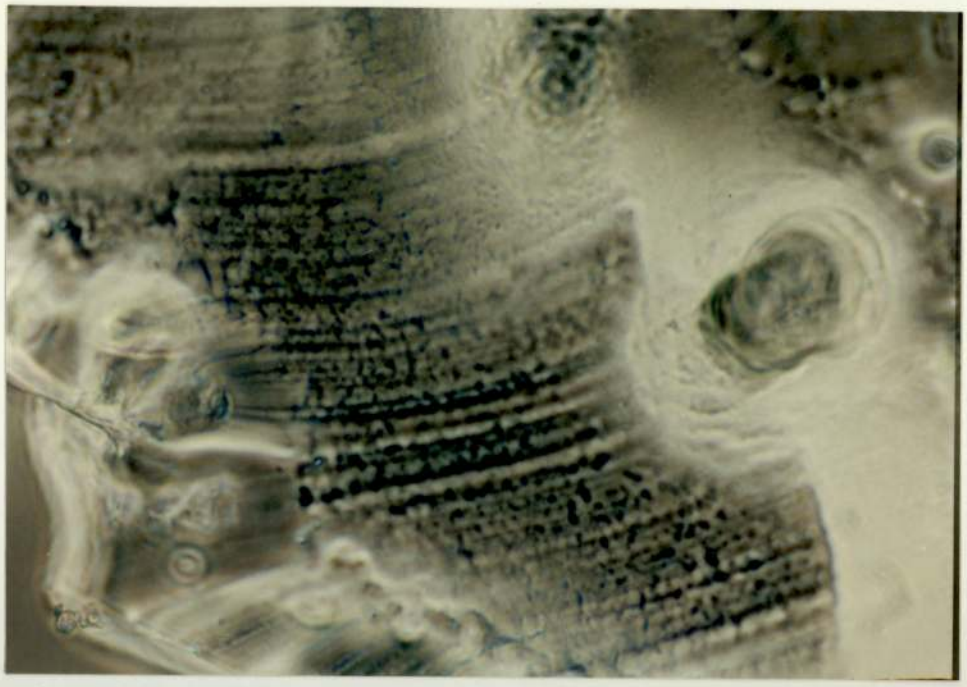


Plate 7.11 Particulate Deposit Distribution, Right Eye, X-TEN Lens, Phase Contrast, X 240

Plate 7.12 Particulate Deposit Distribution Left Eye, X-TEN Lens, Phase Contrast, X 240

findings and lens surface studies, the population density and size of the particulate deposits that occur within the right eye lens matrix, is greater than that of the left lens. It would appear therefore, that the relationship between the intensity of lens discolouration and the population density and size of the particulate deposits holds true.

The presence of matrix-bound particulate deposits in discoloured lenses has been confirmed by microscopical studies. U.V.spectra of discoloured lenses further suggest that the deposit structure contains conjugated systems. Indeed U.V. and visible spectral profiles of discoloured lenses are consistent with a melanin type polymer, since melanin black has been reported as having a maximum of 327 nm with the adsorption tailing off to approximately 640 nm [242]. As a result of these findings it was decided to further examine sections of discoloured lenses with stains suitable for the detection of melanin or melanin-like compounds. Two staining procedures were employed in this study, the Masson-Fontana method that is designed specifically for the detection of melanin, and the Schmorl reaction that is specific for species such as aromatic precursors of melanin. Throughout this study control sections of unworn lenses of a monomeric composition identical to that of the discoloured lenses were subjected to staining procedures undergone by sections of discoloured lenses, in order to assess the specificity of the stain, that is the degree to which it reacts with the lens polymer. The

stain recipes and procedures are those laid down in Pearse and are not recorded here [237]. All reagents were freshly prepared prior to the commencement of staining. The histochemistry of the stain methods is discussed below.

The Masson-Fontana method depends on the characteristic of melanin to reduce solutions of ammonial silver nitrate to metallic silver. If melanin is present, then black particles of reduced silver will become evident. In the case of the sections of discoloured lenses examined in this study, no black precipitate was evident after staining the sections for 24 hours. This finding held true for all lenses studied and confirms the data of Kleist who also found a negative reaction with this method [181]. Because of the sensitivity of silver reduction to pH, it is possible that the hydrogel matrix does not provide a suitable environment for this reaction. To the best of the knowledge of the author this reaction has not been seen to occur in such an environment.

As a result of the inconclusiveness of the above reaction further work was undertaken with the Schmorl, ferric ferricyanide reaction. This reaction is sensitive to the presence of sulphydryl groups and depends on the reduction of a freshly prepared solution of ferricyanide, in acid solution of pH 2.4 by sulphydryl groups.

$$2\left[Fe(CN)_6\right]^{3+} + 2RSH \longrightarrow 2\left[Fe(CN)_6\right]^{4+} + RS - SR$$

The resulting ferricyanide combines with ferric iron

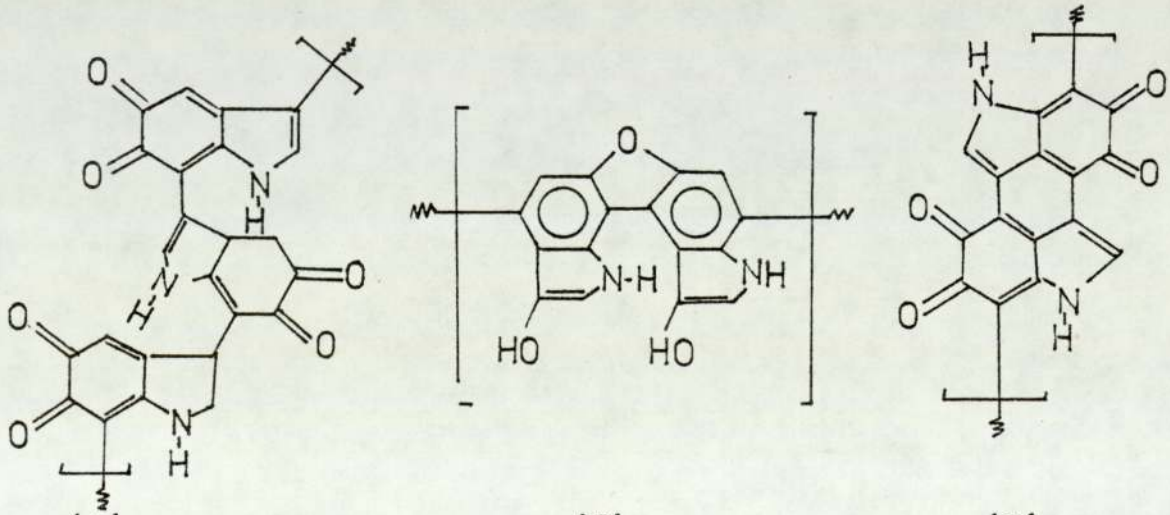
(provided by ferric sulphate) to give a precipitate of insoluble Prussian blue:

$$4Fe^{3+} + 3K_4[Fe(CN)_6] \longrightarrow Fe_4[Fe(CN)_6]_3 + 12K^+$$

The results of discoloured lens sections stained by this method are shown in plates 7.13 - 16. From these it is evident that the matrix-bound particulate deposits stain Prussian blue, indicating a positive reaction. This was found to be the case in all lenses examined by this method. This stain indicates that the particulate deposits response for discolouration of a lens, are sulphydryl containing melanin precusors.

7.3 HYPOTHESIS FOR DISCOLOURATION OF HYDROGEL LENSES

In order ot relate the findings of this study to the discolouration of hydrogel lenses, it is first necessary to consider the biochemistry of melanin synthesis. The various structures that have been proposed for melanin are outlined below



[A] [B] [C]

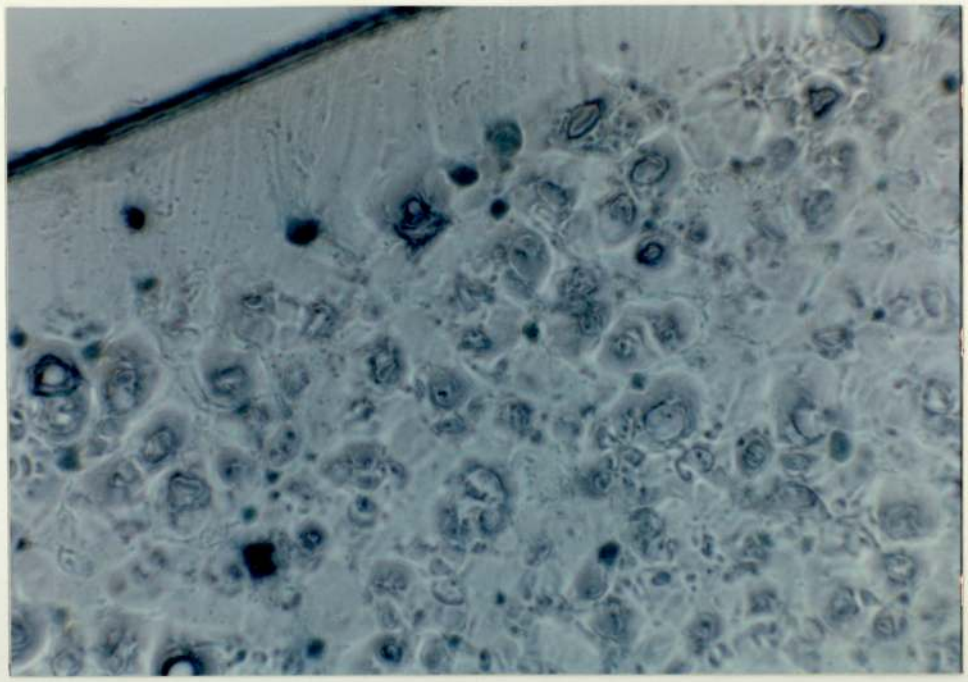


Plate 7.13 Schmorl Reaction, Permalens, Phase Contrast, X 240

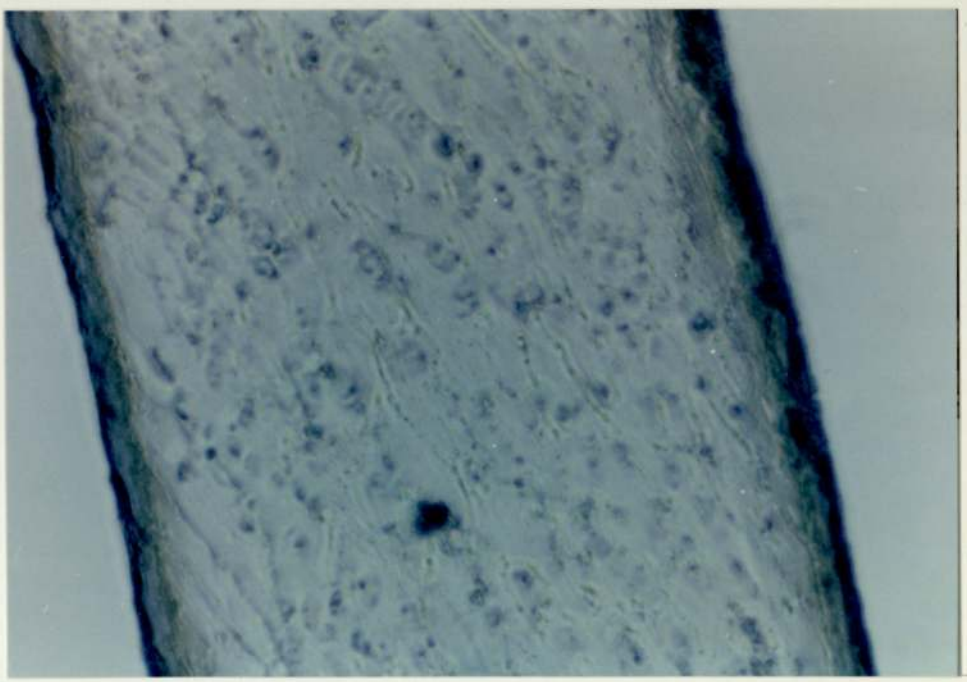


Plate 7.14 Schmorl Reaction, Hydron Lens, Phase Contrast, X 160

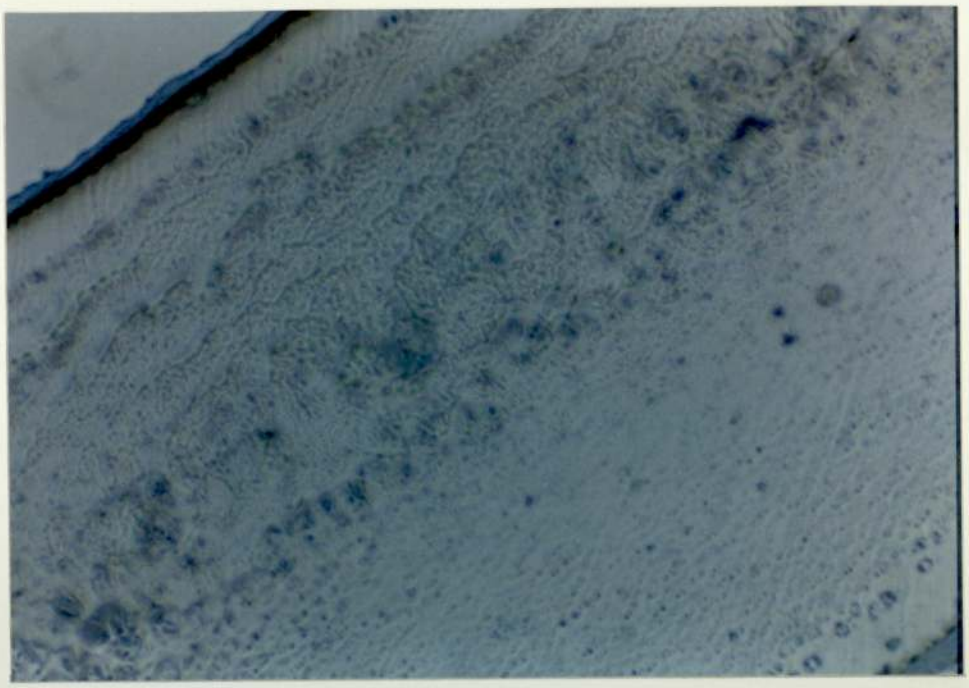


Plate 7.15 Schmorl Reaction, X-TEN Lens, Phase Contrast, X 240

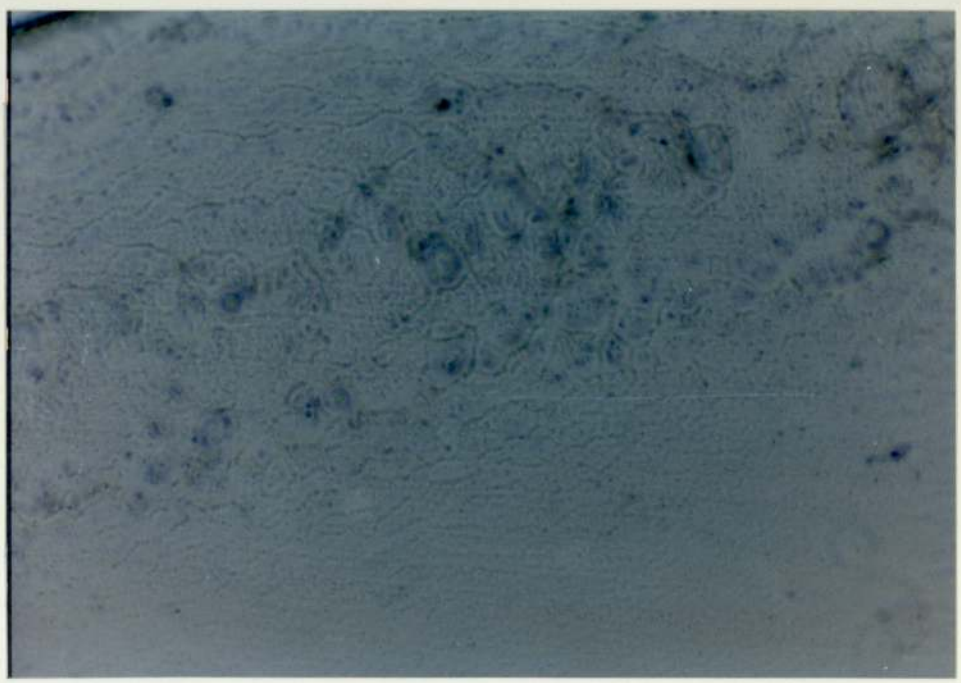


Plate 7.16 Schmorl Reaction, X-TEN Lens, Phase Contrast, X 240

Of the above (A) is considered to be one of the most likely structures produced by workers, however in any case, melanin is described as inhomogeneous, so precise repeat structures are thought not to exist [243]. The most important feature of the above structures is that all are highly conjugated and therefore marry well with U.V.spectra obtained for discoloured lenses.

The exact details of melanin biosynthesis still remain unclear, although there is almost general agreement about the key points of synthesis. The basic biosynthetic pathway is shown in Figure 7.1. The amino acid tyrosine is enzymatically oxidised to dihydroxyphenylalanine (DOPA) which undergoes further enzymatic and non enzymatic oxidation, decarboxylation and coupling reactions to form the melanin pigment. More precisely oxidation of DOPA to dopaquinone is followed by an intramolecular addition reaction, together with tautomerisation to the indole derivative leucodopachrome. A second oxidation by the enzyme tyrosinase is followed by decarboxylation and tautomerisation to 5,6,dihydroxyindole. The latter may undergo a third oxidation step to indole 5,6 quinone. Coupling of these last two products yields a dimer which is able to continue the addition of dihydroxyindole units with oxidation to form a high molecular weight pigmented compound, melanin. Although the above mechanism normally occurs in melanosomes, DOPA undergoes rapid darkening when exposed to oxygen and U.V.radiation, specifically at the wavelengths 290 - 320 nm [115]. It is possible that yellow-brown

FIGURE 7.1 BIOSYNTHETIC PATHWAY OF MELANIN SYNTHESIS

discolouration of hydrogel lenses results from the polymerisation of low molecular weight melanin precusors that form matrix-imbided particulate deposits. This reaction may well proceed by way of direct oxidation or U.V.photocatalysis.

7.4 GEOGRAPHICAL DISTRIBUTION OF PARTICULATE DEPOSITS

Microscopical studies of stained and unstained sections of discoloured lenses have revealed that the geographical distribution of pigmented deposits noted to occur in lenses worn on a daily basis (plate 7.4), differs from that observed in the case of extended wear lenses that are worn continuously. (plates 7.11, 7.14-16). It is considered that the formation of matrix bound particulate deposits, that are responsible for the discolouration of the lens, results from the diffusion of low molecular weight precusors of the melanin-like structures into the water swollen matrix whereupon they undergo polymerisation to form insoluble deposits. Since daily wear lenses are removed prior to sleep and stored in preserved saline overnight, it is possible that partial solubilization of the particulate deposits occurs. These solubilized products then move out of the matrix by passive diffusion. When the lens is placed in the tear environment the following morning, relative to tear fluid concentrations, there will be a high concentration of soluble matrix-located melanin precusors at both the anterior and posterior lens surface. Therefore it is in these regions of the matrix that further

polymerisation of the precursors would commence, leading to the observed particulate density. In the case of the extended wear lenses that are worn on a continuous basis (plates 7.15-16), the observed asymmetrical distribution of pigmented particles results from the fact that dissolution and diffusion of the particulated deposits will only occur at the anterior lens surface tear interface.

7.5 CONCLUSIONS

This study has shown that lens discolouration results from the presence of matrix-bound insoluble particulate deposits. The nature of the particulate deposit is independent of the bulk and surface properties of the hydrogel lens. The exact chemical composition of the pigmented deposits remains unclear, however, spectral and histological analysis suggest that the deposits consist of highly conjugated aromatic, sulphydryl containing, melanin-like structures. This work has further demonstrated that more darkly stained lenses are caused by an increase in the population density of particles and a gradual increase in size. Furthermore the distribution of these particulate deposits is not restricted to the lens surface but occur throughout the lens matrix. It is considered that the distribution is however, a function of the wear protocol of the lens.

CHAPTER EIGHT

CHAPTER VIII

LIGHT AND SCANNING ELECTRON MICROSCOPICAL STUDIES OF DISCRETE ELEVATED DEPOSITS

8.1 INTRODUCTION

Of the observed clinical manifestations of hydrogel lens spoilation, the most morpholically complex are discrete elevated structures, commonly referred to as 'white spots'. These deposits lead to a decrease in visual acuity and may give rise to injury of the cornea and palbebral conjunctiva. Patients with such deposits on their lenses often show clinical conditions such as giant, papillary, follicular and other allergic conjunctivitis. The nature and incidence of occurrence of these structures has been considered in Chapter 4. However, it is evident from this review, that the chemical composition and structure of these deposits still remains unclear.

Previous research conducted in this area has concentrated on the chemical characterisation of these structures[156,167,176], whilst little effort has been made in attempting to correlate the nature and incidence of occurrence of deposits with the bulk and surface properties of spoiled hydrogel lenses. In addition, little is known whether there is any variation in the deposit structure, composition and incidence of occurrence with alterations in the wear protocol of the lens. As a result of the development of suitable analytical

techniques in this study, the widespread supply of spoiled lenses and the availability of a clinical trial group of lens wearers, this project is uniquely positioned in order to attempt to establish whether a relationship exists between the deposit structure and composition, and the bulk and surface properties of the hydrogel lens.

This chapter is concerned with the various light and scanning electron microscopical studies carried out on discrete elevated deposits, whilst Chapter 9 deals with the various histological and elemental dispersive analytical studies employed in the analysis of these deposits. The various types of lenses examined and light microscopical techniques employed in this study are outlined below.

8.2 HYDROGEL LENS SOURCES AND WEAR PROTOCOLS

A total of 52 hydrogel lenses, contaminated with discrete, elevated deposits, were examined in this study. The commercial identity, wear programmes and applications of hydrogel lenses bearing deposits are outlined below. Whilst the bulk and surface properties of these lenses have been considered in Chapter 5

Table 8.1

LENS MANUFACTURER	MANUFACTURING PRODUCT	WEAR PROGRAMME	LENS APPLICATION
Kelvin Lens Ltd	X-TEN X-TEN	Continuous Extended	Aphakia Cosmetic
Cooper-Vision Optics	Permalens	Extended	Cosmetic
Scanlens	Scanlens	Continuous	Aphakia

Hydrogel lenses analysed in this study were primarily worn in extended wear applications, although a small number of spoilt daily wear lenses were examined. A significant proportion of spoilt lenses were supplied to these laboratories by Kelvin Lenses Ltd., although a number of spoilt Permalenses were also donated by a large, London based opthalmic practice. During the course of this study several returned X-TEN lenses used in the treatment of aphakia were also examined, together with a small number of other lens types.

The primary reason for the removal of lenses examined in this chapter, was a decrease in visual acuity and an increase in patient discomfort. However, in the majority of cases, little information was available on the length of wear and wear protocol of these devices. As a consequence, these lenses were analysed in order to establish whether the composition and structure of deposits varied with differing bulk and surface properties of the hydrogel lenses on which they were located.

During this study a number of spoilt X-TEN and Permalenses, worn by patients involved in comparative extended wear trials, were examined. Patients who participated in these studies were prescribed and fitted with an X-TEN lens in one eye and a Permalens in the other. In one study, lenses were worn for a period of three months, during which the lenses were removed on a weekly basis for cleaning. In another trial, lenses were worn continuously for the whole

trial period. The Septicon (Allergan Ltd.) cold cleaning disinfection regime, based upon 3 percent hydrogen peroxide, was employed in the treatment of weekly wear lenses, since no adverse effects on the lens parameters of either the X-TEN or Permalens have been reported during its use [244].

In these studies, all possible steps were taken to eliminate ocular responses to the lens that result as a function of the fitting technique employed, rather than those that occur as a function of the bulk and surface properties of the lens. No attempt was made to characterise the tears of patients who participated in either of these studies, although it was recognised from an early stage in this project that certain patients displayed a greater propensity to lay down deposits.

As has already been outlined the above trials were initiated prior to the commencement of this project by Kelvin Lenses Ltd., in order to compare the wear tolerance of the X-TEN lens against that of an established and successful extended wear device (Permalens). However, spoilt lenses derived from these trials, provide a rare opportunity, not only to study the effect of alterations in the bulk and surface properties of the lenses on the structure and composition of discrete elevated deposits, but also the incidence of deposit occurrence.

8.3 MORPHOLOGICAL STUDIES AND THE GEOGRAPHICAL DISTRIBUTION OF DISCRETE ELEVATED DEPOSITS

The morphology and geographical distribution of discrete elevated deposits was examined by various light and electron microscopical techniques. The methods employed to mount and photodocument lenses in light microscopical examinations, allied with the preparative techniques developed for S.E.M. studies have been considered in Chapter 6. The findings from these studies are discussed below.

8.3.1 Discrete Elevated Deposit Morphology

Phase contrast, light, darkfield and S.E.M. studies of discrete elevated deposits are displayed in plates 8.1-10. Phase contrast studies revealed a complex deposit morphology. The primary layer of the deposit, that which is most adjacent to the lens surface, appears to be an elevated disc-shaped plateau. Superimposed on this layer is an elipsoid, dome-shaped structure that significantly contributes to the physical dimensions of the deposit. Studies of these deposits with darkfield microscopy has displayed numerous lobular deposit sub units, that are arranged in a circular fashion at the base of the deposit (plate 8.11-12). However, it should be noted that these structures are more evident in some deposits than others. Such a deposit morphology has been reinforced by S.E.M. studies (plates 8.5-10). These studies indicate that deposits are primarily circular in nature, with diameters in the range of 160-

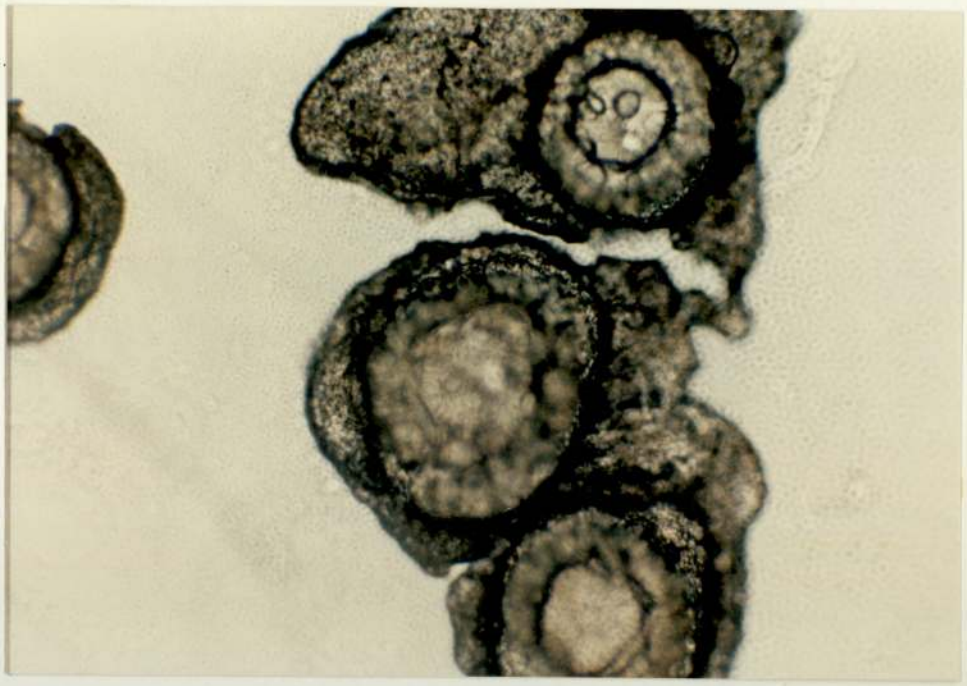


Plate 8.1 Discrete Elevated Deposit Morphology,X-TEN Lens,Phase Contrast, X 65

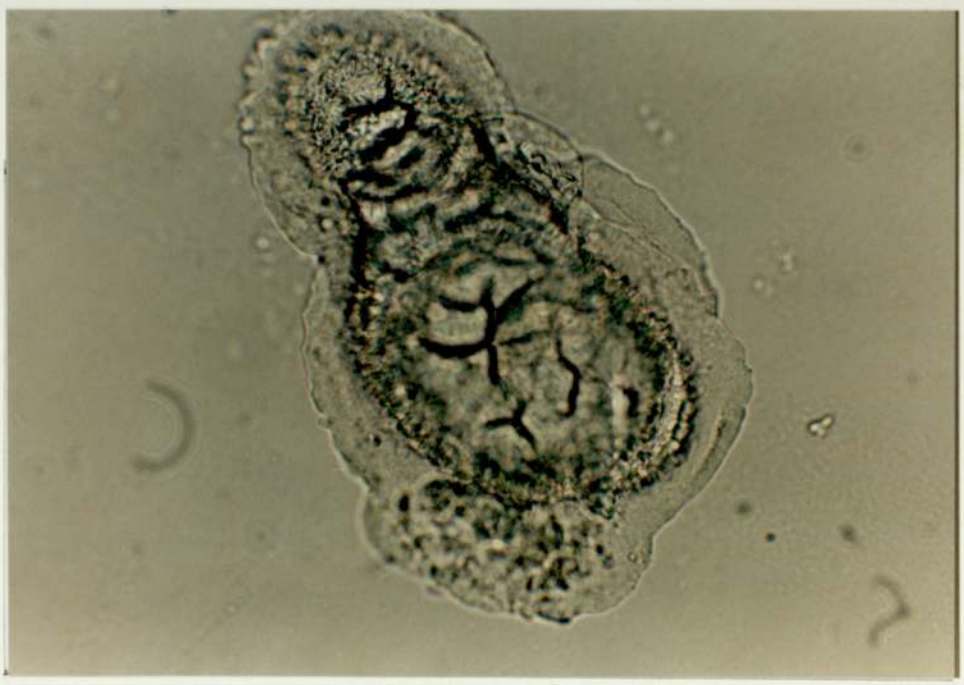


Plate 8.2 Discrete Elevated Deposit Morphology, X-TEN Lens,Phase Contrast, X 160

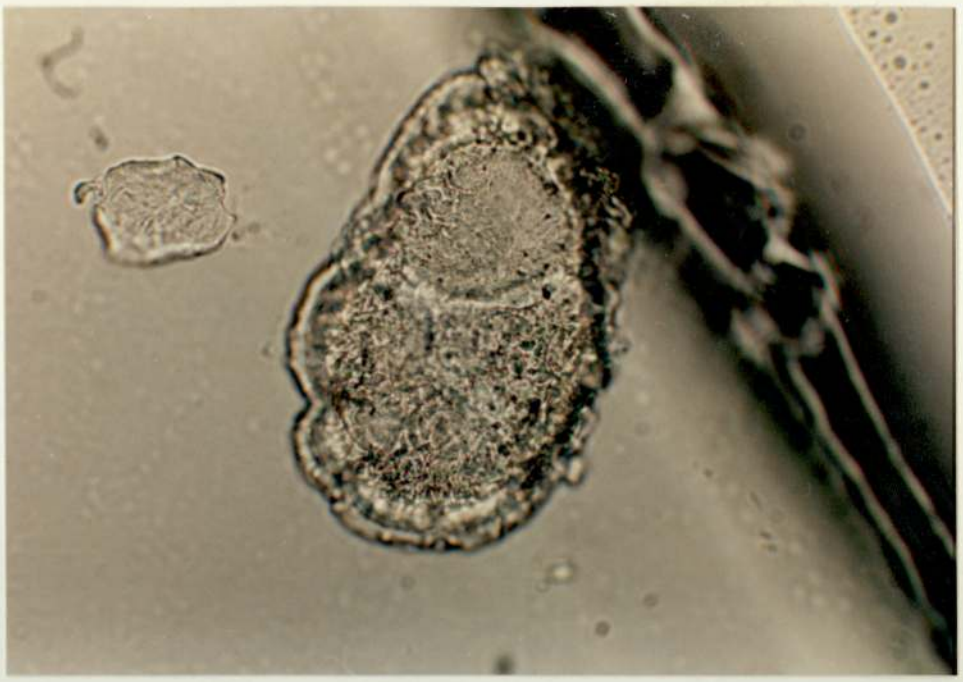


Plate 8.3 Discrete Elevated Deposit Morphology, Permalens, Phase Contrast, X 240

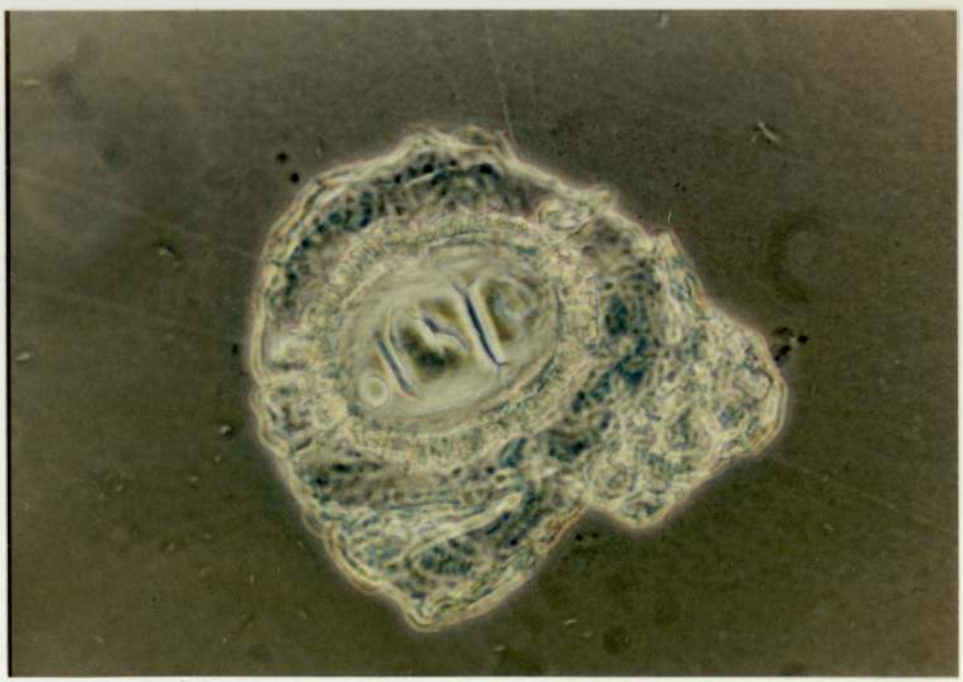


Plate 8.4 Discrete Elevated Deposit Morphology, X-TEN Lens, Phase Contrast, X 240

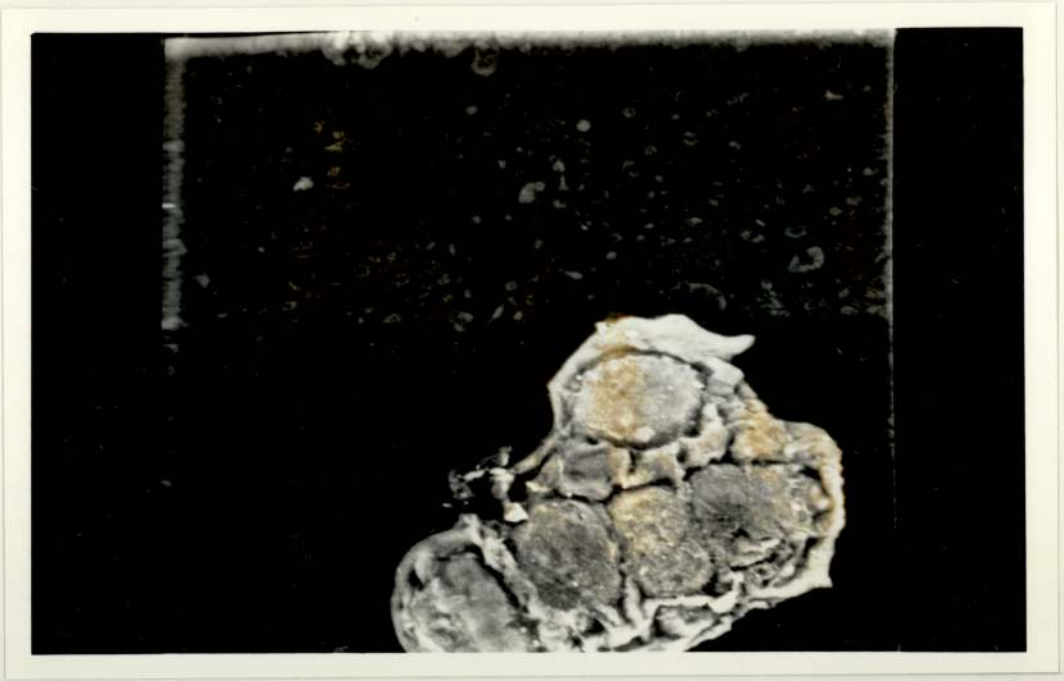


Plate 8.5 Discrete Elevated Deposit Morphology, X-TEN Lens, S.E.M., X 500

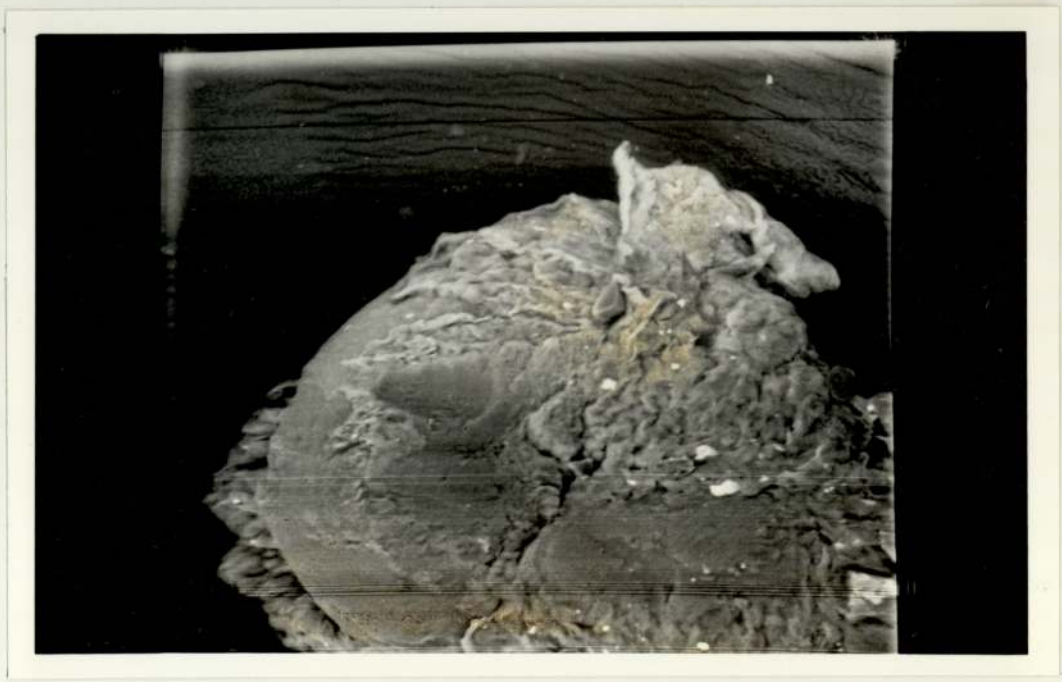


Plate 8.6 Discrete Elevated Deposit Morphology, Permalens, S.E.M., X 500

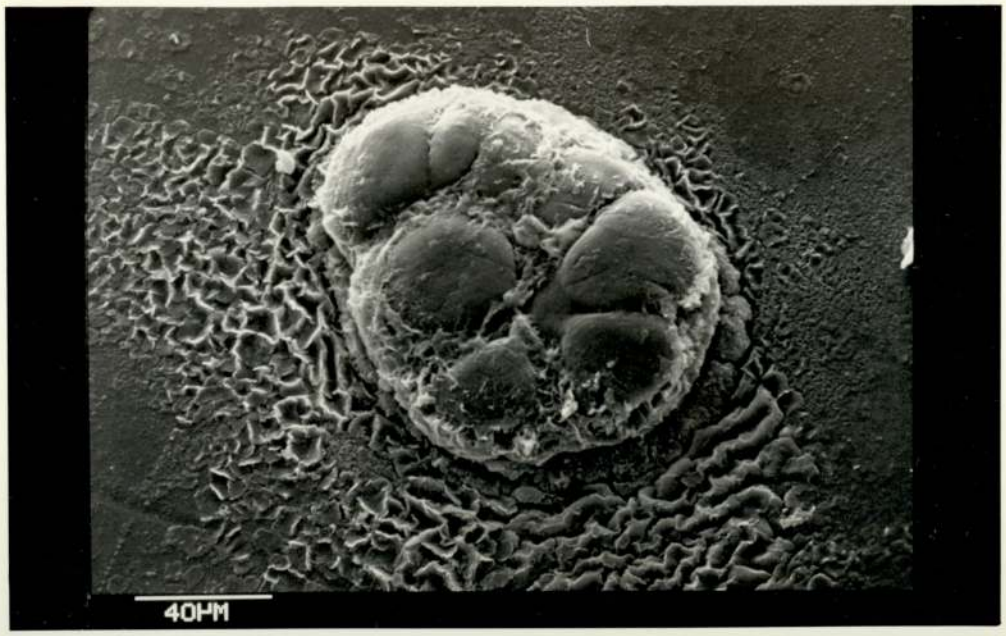


Plate 8.7 Discrete Elevated Deposit Morphology, Permalens, S.E.M., X 500

Plate 8.8 Discrete Elevated Deposit Morphology, Permalens, S.E.M., X 500

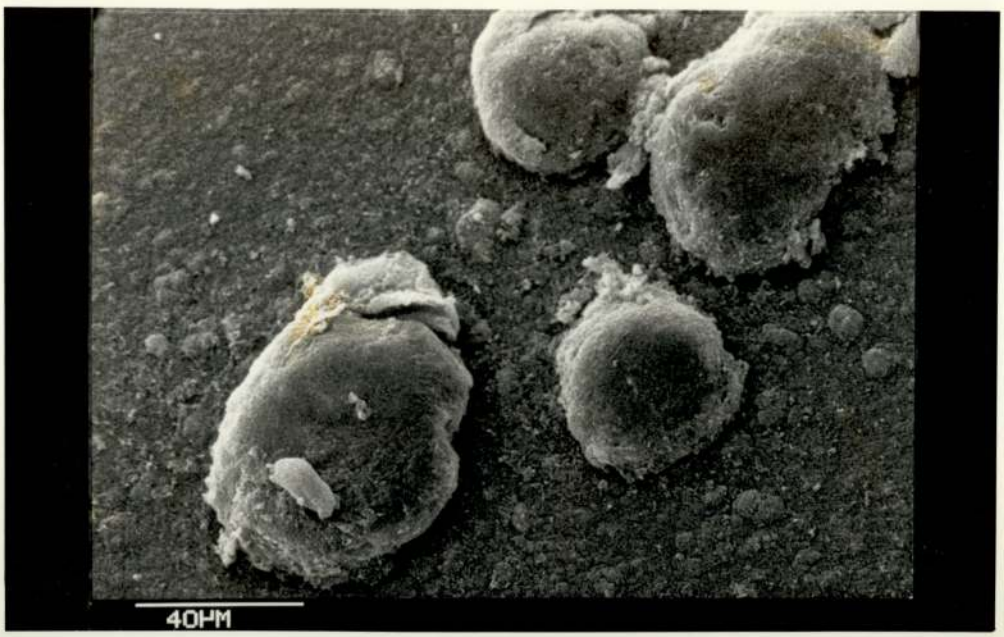


Plate 8.9 Discrete Elevated Deposit Morphology, X-TEN Lens, S.E.M., X 500

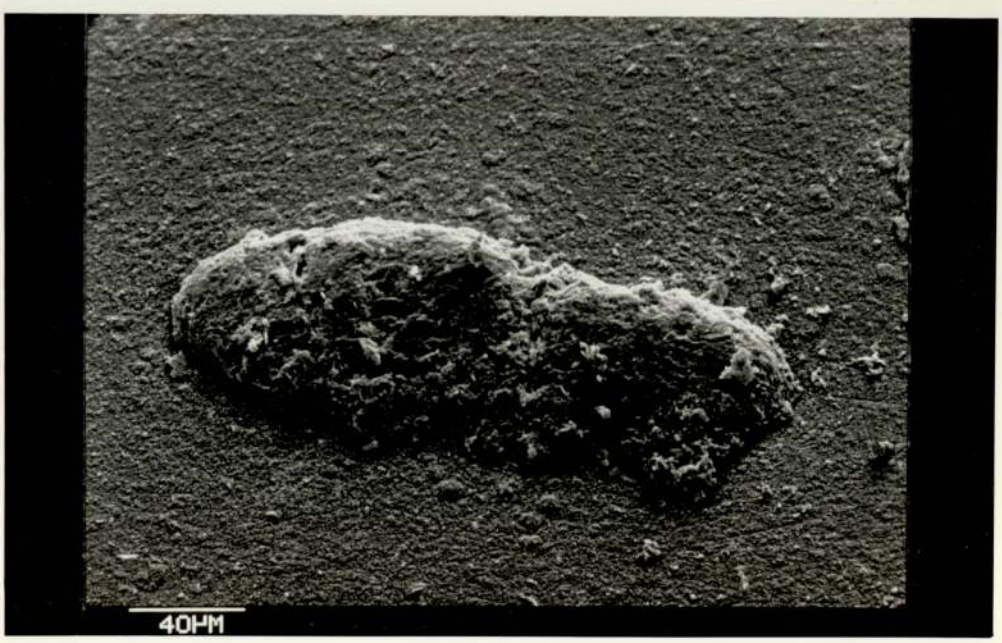


Plate 8.10 Discrete Elevated Deposit Morphology, X-TEN Lens, S.E.M., X 500

Plate 8.11 Discrete Elevated Deposit Morphology, Lobular Component, X-TEN Lens Darkfield, X160

Plate 8.12 Discrete Elevated Deposit Morphology, Lobular Component, X-TEN Lens, Darkfield, X65

200 μm. In several cases, circular deposits have developed in close proximity, thus giving the impression of double-dome shaped structures.

By comparison of spoiled Permalens and X-TEN lenses worn by patients during clinical trials, it was evident that there is little variation in deposit morphology from one lens to another, when worn by a particular patient, or from one patient to another. It was also apparent from this study that neither the wear protocol of the lens, nor the application of the device alters the deposit morphology. Although similar deposit morphologies have been described by other workers[156], to date no comment has been made concerning the effect of the bulk and surface properties of hydrogel lenses on deposit morphology. From this study it is plausible to suggest that the morphology, as distinct from the occurrence, of discrete elevated deposits is little affected by the bulk and surface properties, wear protocols or application of hydrogel lenses.

Light and electron microscopy studies have revealed the presence of discrete elevated plateau-like structures on spoilt lenses, that display a complex morphology. (Plates 8.13-17). In a number of studies discrete elevated deposits were found to be in close proximity of, or superimposed upon, these structures. Light and electron microscopy examinations revealed a circular shaped structure with the bulk of the deposit mass positioned at the periphery of the deposit structure. As a result of their morphology it

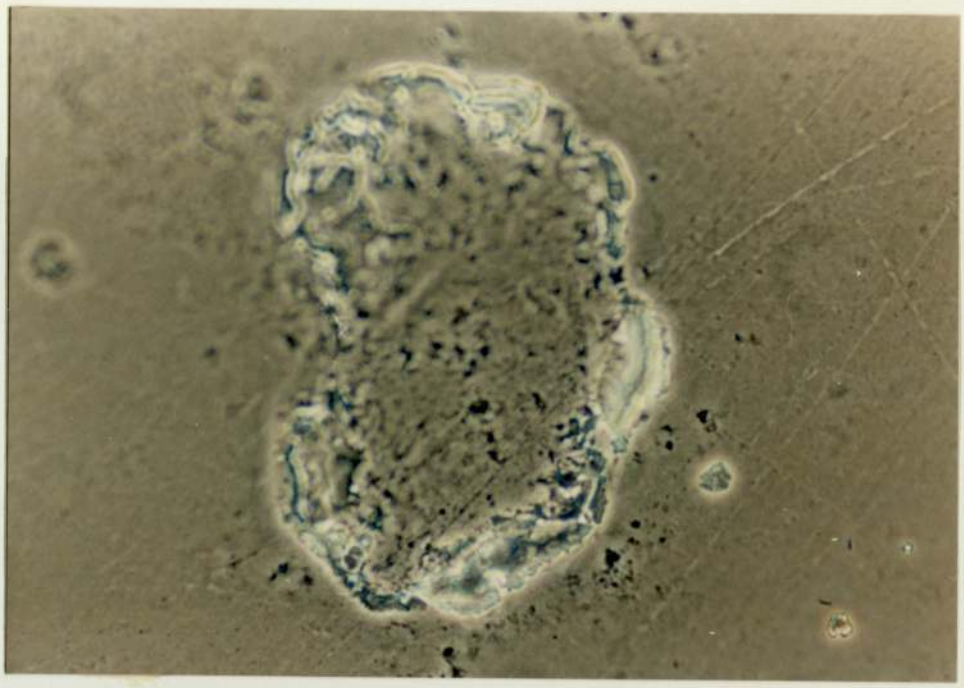


Plate 8.13 Morphology of Elevated Plateau Structure, X-TEN Lens, Phase Contrast, X 240

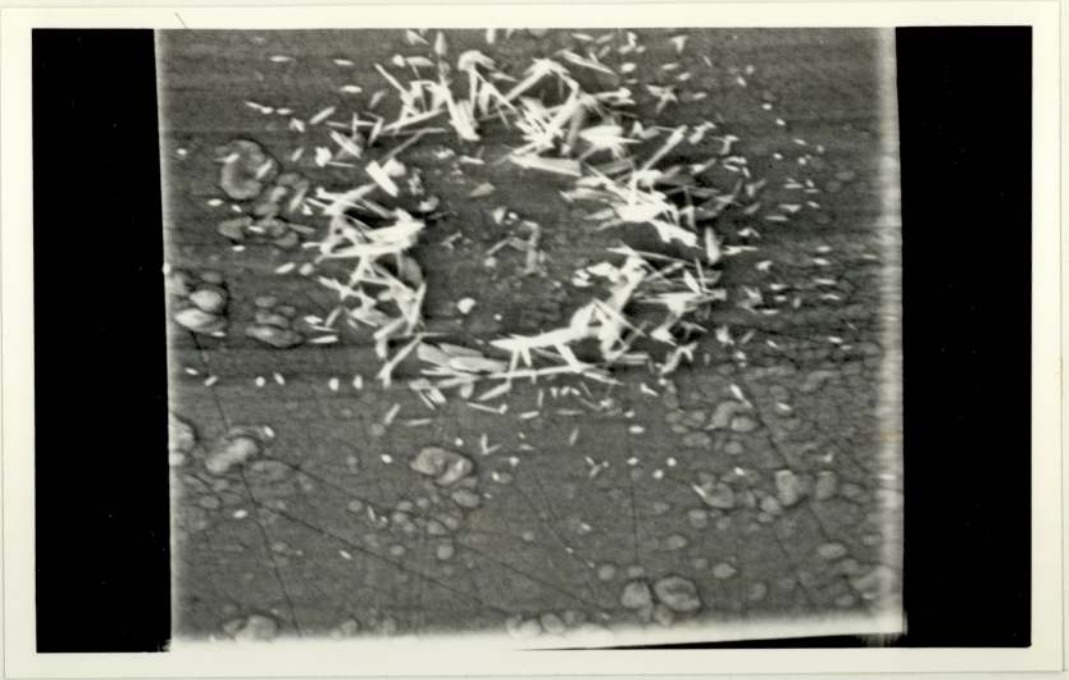


Plate 8.14 Morphology of Elevated Plateau Structure, X-TEN Lens, S.E.M., X 500

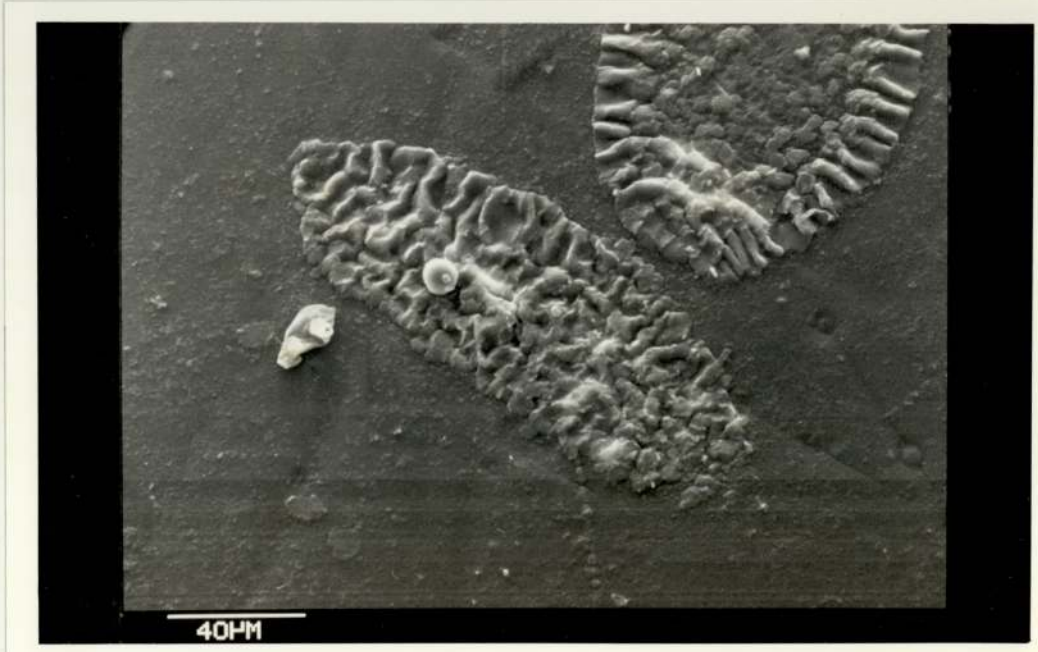


Plate 8.15 Morphology of Elevated Plateau Structure, X-TEN Lens, S.E.M., X 500

Plate 8.16 Morphology of Elevated Plateau Structure, Permalens, S.E.M., X 500

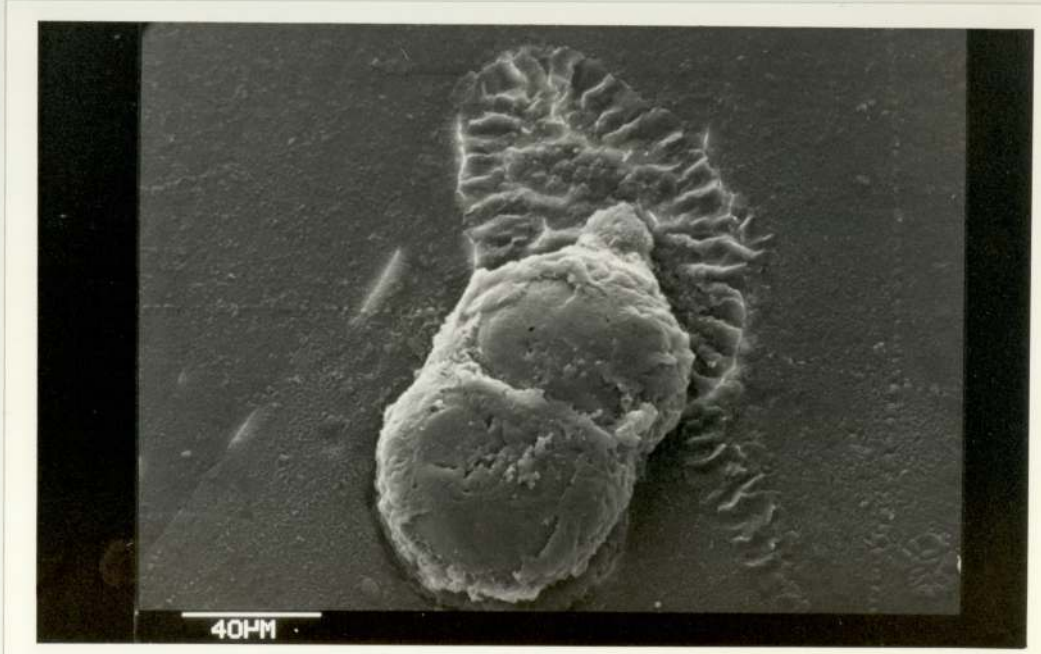


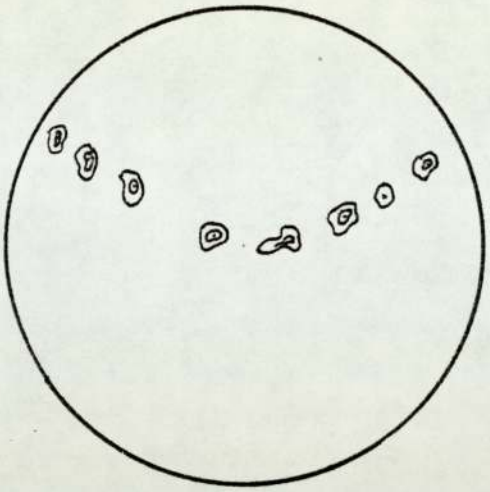
Plate 8.17 Morphology of Elevated Plateau Structure, X-TEN Lens, S.E.M., X 500

is tempting to equate these structures with the so-called 'dry-spots' or localised areas of hydrophobicity. Prior to this study the existence of these structures had not been reported. This work however, not only provides evidence of their occurrence but also suggests that they may play a role in the formation of discrete elevated deposits, or represent an early stage in their structural development.

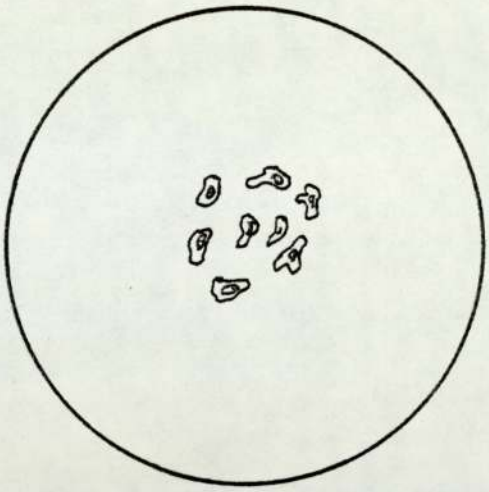
8.3.2 Geographical Distibution of Deposits

Initial investigations of the geological distribution and morphology of lens deposits were conducted with the aid of various techniques employed in deposit morphology studies. Microscopical studies revealed that discrete elevated deposits were located on the anterior lens surface only. This finding is in accordance with other workers who have considered this problem. However, as a result of this study the geographical distribution of discrete elevated deposits on spoilt lenses examined during this project, may be classified into four groups as shown in Figure 8.1.

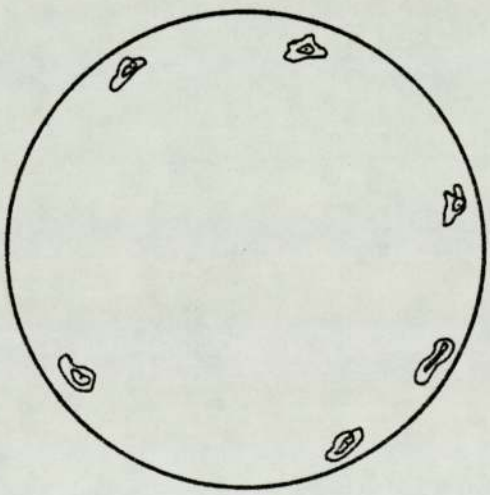
Spoilt lenses that are typical of a Group 1 type distribution, are characterised by the presence of deposits positioned on contour lines that arc across and bisect the epicentre of the lens. A most likely explanation of this phenomenon is that this type of distribution results from a decentering of the lens whereby, on blinking, the upper eyelid only partly traverses the lens surface. This



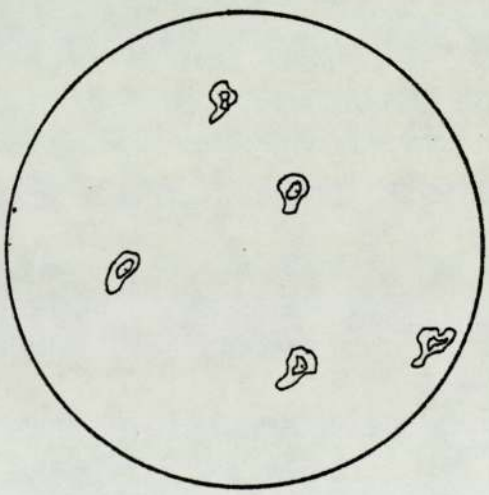
Group 1 Distribution



Group 2 Distribution



Group 3 Distribution



Group 4 Distribution

FIGURE 8.1 SCHEMATIC GEOGRAPHICAL DISTRIBUTION OF DISCRETE ELEVATED DEPOSITS

causes a compression of the layered structure of the tear film on such an arc and the deposition of tear components thereon.

Deposits detected on the anterior surface of aphakic lenses were found to be particularly prevalent at, or in the near vicinity of, the epicentral region of the lens. This type of distribution is typical of Group 2 lenses. Group 3 lenses are characterised by a peripheral distribution of deposits whereby discrete elevated structures were only located towards the outer lens edge whilst the central region of the lens is devoid of deposits. Such a distribution is thought to arise from the type of fitting technique employed by the prescribing optician. Indeed this type of distribution was almost uniquely observed to occur on X-TEN and Permalenses that had been fitted to clinical trial patients by the resident optician at Kelvin Lenses Ltd. The fourth type of deposit distribution is displayed by lenses whereby the geographical location of deposits on the lens surface is purely random.

It is evident from this study that the distribution of discrete elevated deposits may be significantly affected by several factors such as; the method of fitting technique employed and the application of the hydrogel lens device. From studies of spoilt X-TEN, Permalens,left eye, right eye combinations, in which the devices were fitted by the same optician, no obvious differences in the geographical distribution of deposits found on the

X-TEN lens as compared to that of the Permalens, were noted. This finding suggests that lens bulk and surface properties do not directly determine lens deposit distribution.

8.4 DEPOSIT INCIDENCE OF OCCURRENCE

A number of studies were undertaken in order to determine the effect of the bulk and surface properties of hydrogel lenses on the incidence of occurrence of discrete elevated deposits on spoiled lenses. As previously mentioned, it was noted from an early stage that certain patient members of the clinical trial study group, displayed a greater propensity to lay down discrete elevated deposits, than other patients. As a result of the construction of patient lens banks, a comparison of the incidence of occurrence with alterations in the surface energy of lenses was made possible.

A custom-designed circular grating that split the microscopic field of view into equal quarters was manufactured from Melinex (polyethylene terephthalate) film and placed in the eye piece of a Leitz stereomicroscope. Spoilt lenses were then flattened and examined by this technique. Stereomicroscopy was employed since this technique allows immediate access to the entire lens surface. The number of discrete elevated deposits on each lens was then recorded. Lenses examined during the course of this study had been worn by the same patient with the employment of identical wear and cleaning protocols. It is reported that

the method employed to fit hydrogel contact lenses affects the incidence of lens spoilation since a loose fitted lens appears to be coated with adsorbed tear species at a greater rate than a tightly fitted lens [245]. It is suggested that the mechanism seems to be that the slipping of the lens causes it to be slightly coated, the upper lid then does not slide so easily over the lens surface and increases the amount by which the lens slips, thus accelerating the spoilation process. In this project however, all lenses were fitted by the same optician and it is highly probable that a standard technique was employed to fit all the lenses examined in this study. Therefore, in consideration of the latter,it is plausible to suggest that lens responses that might have arisen due to variations in the method of fit were minimised and those that were observed, resulted from the interaction of the lens hydrogel with tear components. Thus, apart from a significant deviation in the tear chemistry of the lens wearer, the only variable in these studies was the bulk and surface properties of the hydrogel lens. These studies indicated that there was a significant reduction in the incidence of occurrence of discrete elevated deposits when X-TEN lenses of reduced surface energy were employed. Plates 8.18-19 depict the lens surfaces of a non-fluorinated (plate 8.18) and fluorinated X-TEN lens (plate 8.19) that were worn by the same patient for an identical period of time. It is evident that although discrete elevated deposits are present on both lenses, the incidence of occurrence of deposits is significantly less on the fluorinated lens. Further studies with

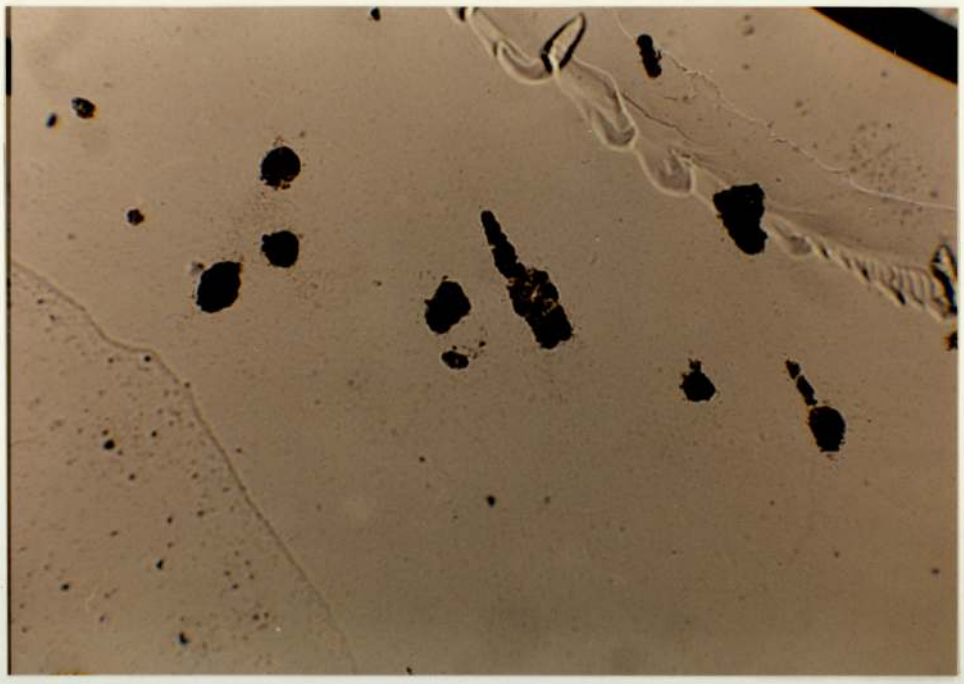


Plate 8.18 Discrete Elevated Deposits, X-TEN Lens, (non fluorinated), Brightfield, X 25

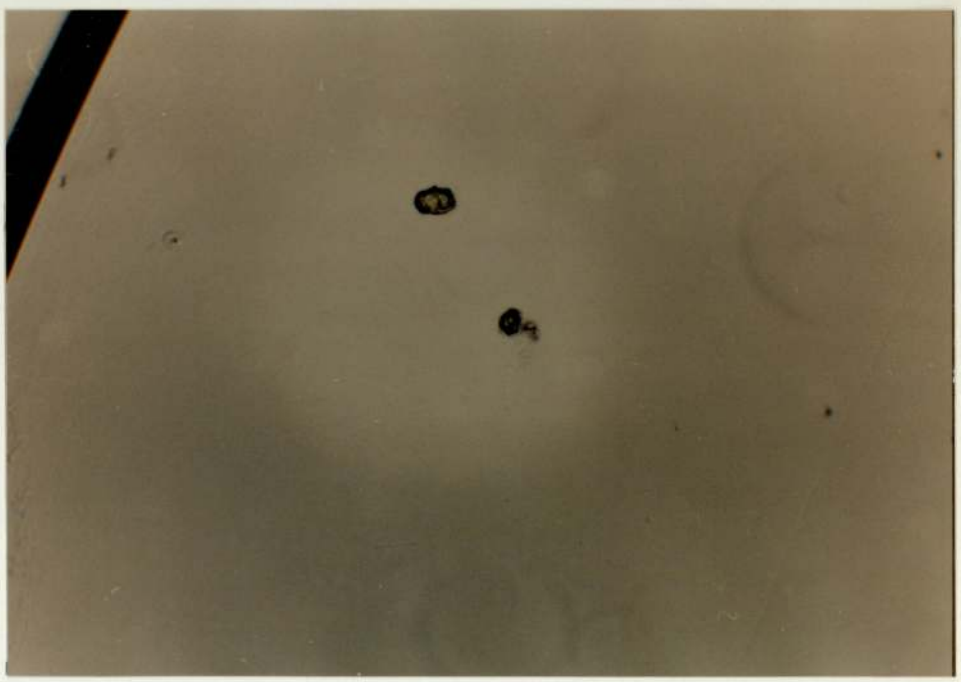


Plate 8.19 Discrete Elevated Deposits, X-TEN Lens, (fluorinated), Brightfield, X 25

other trial patients yielded similar results. Indeed when spoiled Permalenses of these patients were compared with their X-TEN counterparts, the fluorinated X-TEN lenses displayed a lower incidence of deposit occurrence. Although these studies employed patients who were known to be heavy spoilers, by using the incidence of deposit occurrence as a measure of lens biocompatibility, X-TEN lenses of a reduced surface, and thus minimal interfacial tension with tears (see Chapter 5), energy appear to be more biocompatible with the ocular environment than their non-fluorinated and Permalens counterparts.

8.5 FLUORESCENCE AND POLARISATION MICROSCOPY

Spoiled lenses were mounted and photodocumented by the techniques previously described. When examined with fluorescence and polarisation microscopy, discrete elevated structures revealed a distinctive birefringence. The central elevated component of the deposit structure was found to display a maltese-cross type of birefringence. This property may be equated with the presence of lipid spherocytes, such as cholesterol esters.[237] Further studies of the deposit structure with fluorescence microscopy, revealed distinctive autofluorescence patterns. Studies indicated that it is the lobulated components positioned at the base of the deposit structure, that are the primary autofluorescent sites. (Plates 8.20-22). From plates 8.20-22 it can be seen that the anterior layers of the deposit display negligible autofluorescence. The property of auto-

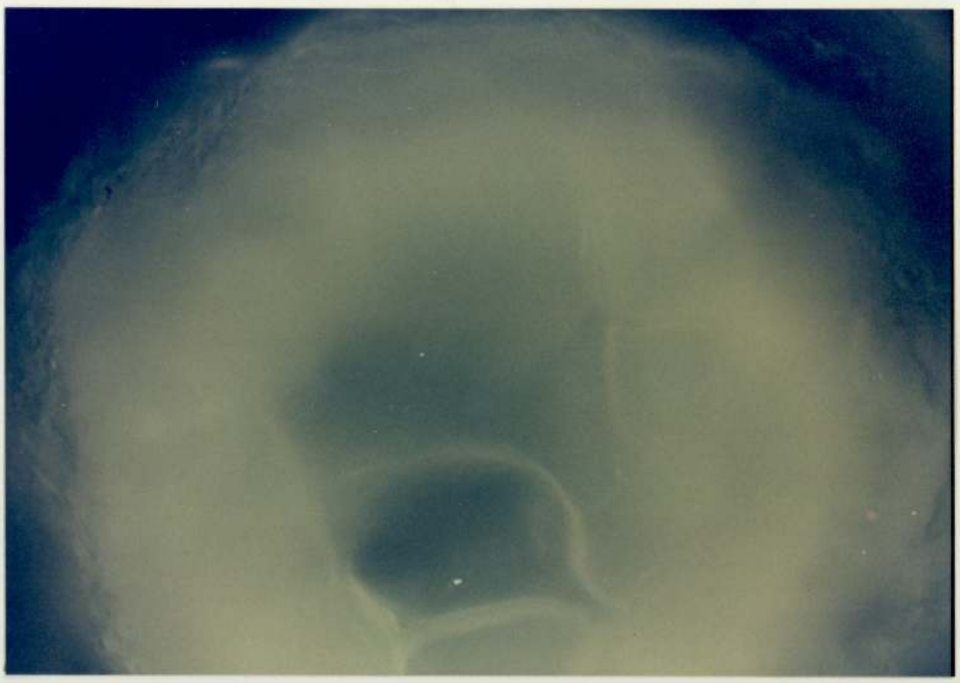


Plate 8.20 Discrete Elevated Deposit, X-TEN Lens, Autofluorescence, X 240

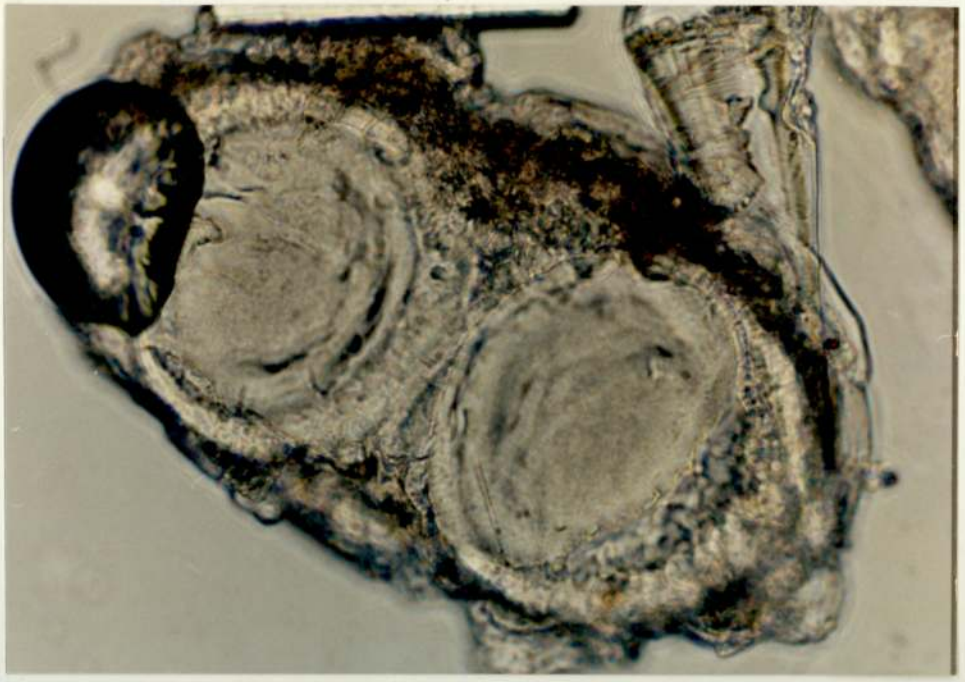


Plate 8.21 Discrete Elevated Deposit, Permalens, Phase Contrast, X 240

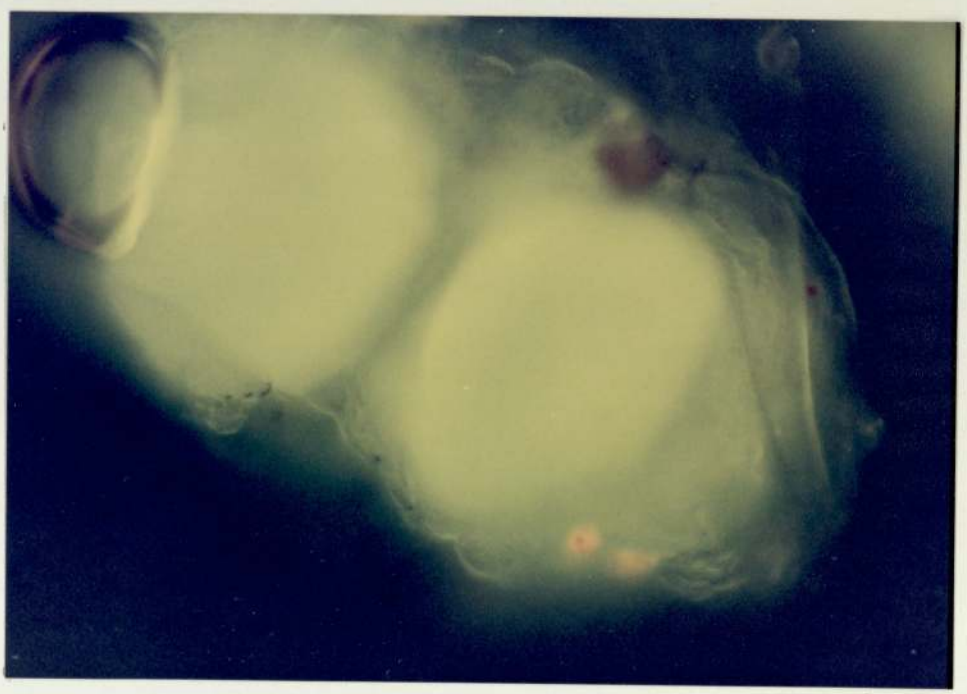


Plate 8.22 Discrete Elevated Deposit, Permalens, Autofluorescence, X 240

fluorescence has been equated with the presence of cholesterol esters and squalene, the highly conjugated precursor of cholesterol. This was confirmed by evaporating a series of lipoidal components on clean glass slides and studying the autofluorescence properties of these species under identical conditions in which deposit structures were examined. Negligible fluorescence was observed with unsaturated fatty acids, whilst autofluorescence of an identical colouration to that of deposit structures was observed with cholesterol esters. Similar findings have been reported by other workers[176], however, this study indicates that the fluorescence and polarisation patterns of discrete elevated deposits remain constant, regardless of the bulk and surface properties of the hydrogel lens matrix. Differences were however, noted to occur in the fluorescence intensity of deposits, from differing lens types and patients. Fluorescence and deposit polarisation were particularly prevalent with patients known to produce heavy lipid coating on lenses and patients who wore lenses in aphakic applications. It is evident from fluorescence and polarisation studies that discrete elevated deposits contain cholesterol derivative species that are asymmetrically distributed within the deposit structure.

CHAPTER NINE

CHAPTER IX

HISTOLOGICAL AND ELEMENTAL DISPERSIVE ANALYSIS OF DISCRETE ELEVATED DEPOSITS

9.1 INTRODUCTION

This chapter is concerned with the histological and elemental dispersive analysis of discrete elevated deposits. Particular emphasis was placed on the chemical characteristics of the deposit-hydrogel matrix interfacial region. As a result of these studies a working hypothesis concerned with the etiology of discrete elevated deposits will be presented. The various histological techniques employed in this study are outlined below.

9.2 HISTOLOGICAL STUDIES

Histological studies were conducted on discrete elevated structures, in order to establish the geology and principal bio-organic components of this type of deposit, together with the structural relationship between the deposit and hydrcgel lens matrix. Significant emphasis was placed on the characteristics of the bio-organic components of the deposit-hydrogel matrix interfacial region. From the analysis of this area of the deposit, it was hoped to identify those components of the tear film that play a role in the initiation of discrete elevated deposit formation.

The techniques employed to fix, embed and section lenses have been considered in Chapter 6. The respective fixatives and choice of embedding media are discussed more fully below. However, prior to the commencement of the various staining procedures employed in the detection of deposit imbibed bio-organic species, unstained sections of deposits were examined by various light and S.E.M. techniques, in order to establish the internal structure of discrete elevated deposits and the structural relationship between the deposit material and the hydrogel lens matrix.

The geology of discrete elevated deposits is complex, with the deposit structure consisting of a number of lobular sub units that are superimposed on each other, to give the deposit a multi-layered structure. Plates 9.1 and 9.2 are representative of longitudinal sections through deposits found on X-TEN lenses, Plate 9.1 was produced by cryomicrotomy whilst Plate 9.2 resulted from resin embedding. It is interesting to note that the preservation of the deposit structure appears to be significantly greater with resin-embedded sections. However, it is evident that the deposit geologies are very similar. Longitudinal sections through a discrete elevated deposit on a Permalens (Plate 9.2) demonstrates a similar geology to that of deposits found on X-TEN lenses. Thus it would appear that the geology of the deposit remains relatively constant, regardless of the bulk and surface properties of the hydrogel lens and, the application of the device. The findings of sections studied by light microscopy, were reinforced by S.E.M.

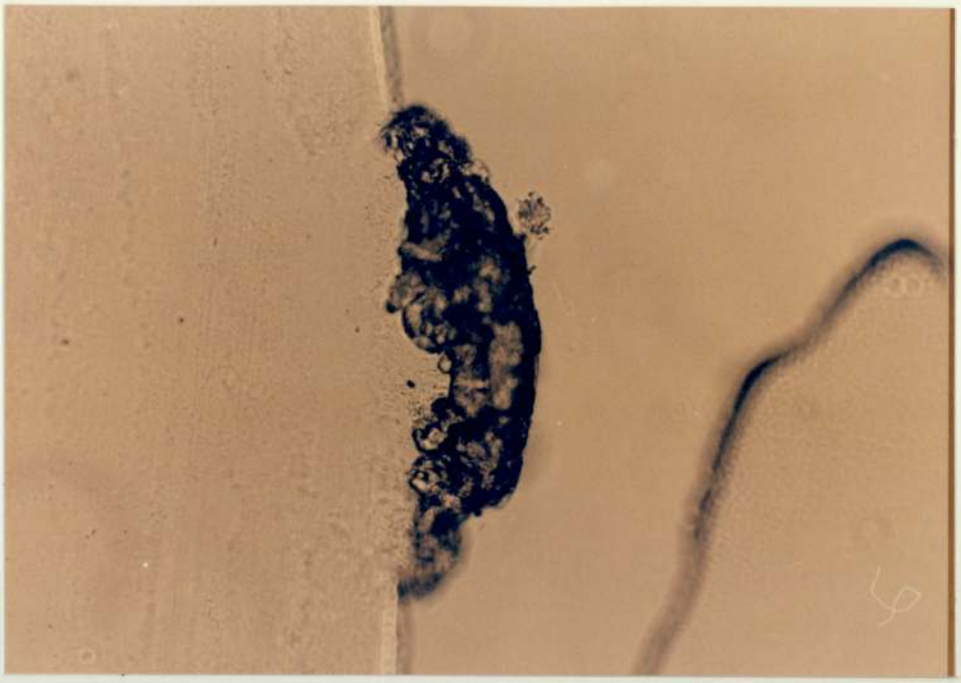


Plate 9.1 Longitudinal Section, Discrete Elevated Deposit, X-TEN Lens, Cryosection, Phase Contrast, X 65

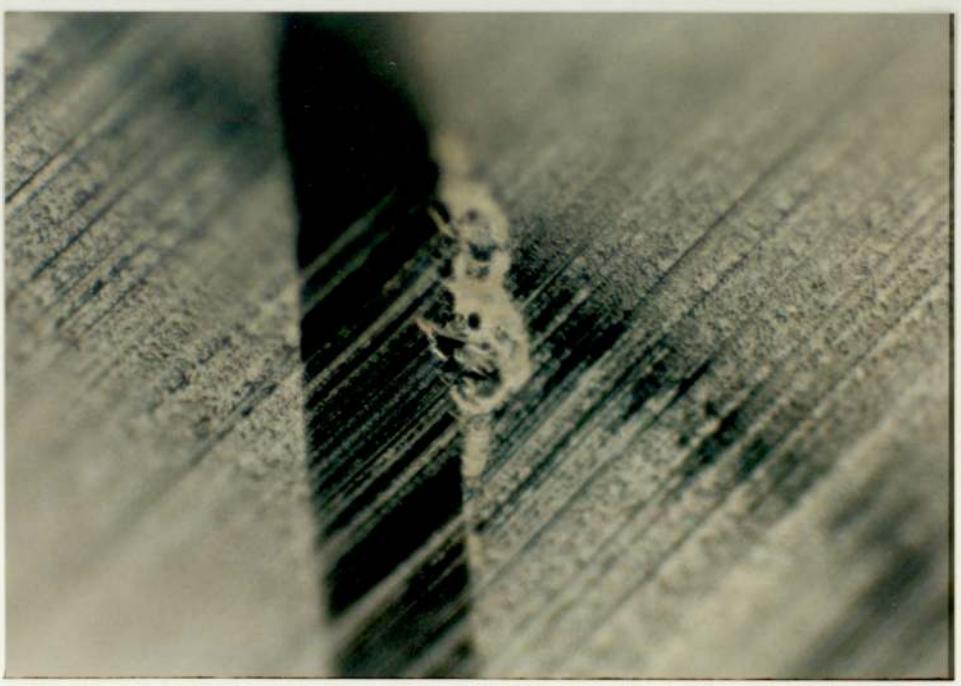


Plate 9.2 Longitudinal Section, Discrete Elevated Deposit, X-TEN Lens, Resin Section, Phase Contrast, X 65

analysis (Plates 9.3-6). It is evident that the electron-micrographs confirm the lobulated, multi-layered structure of these deposits. Analysis of deposit depth indicates these structures are approximately 30-60 μm in height. Furthermore these studies revealed that the deposit material is not solely limited to the hydrogel matrix tear interface but occasionally penetrates the expanded gel network. A possible explanation of the reasons why these deposits cause a puckering of the lens matrix will be presented at a later stage in this chapter.

9.2.1 Histological Studies - Autofluorescence and Polarisation Studies of Unstained Sections

When unstained sections of discrete elevated deposits were examined with fluorescence and polarisation microscopical techniques, a distinctive and specific autofluorescence and birefringence was noted in all sections studied. Plate 9.7 depicts a longitudinal section through a discrete elevated deposit found on an X-TEN lens worn continuously in the treatment of aphakia. The multi-layered structuring of lobulated components detected with phase contrast and darkfield microscopy is evident. The specific autofluorescence pattern of the deposit section is shown in Plate 9.8. From this photomicrograph it is evident that it is the primary layer, that which is most adjacent to the deposit matrix, that displays autofluorescence, whilst the overlying deposit layers do not display this property. This type of fluorescence pattern was found to be typical of all

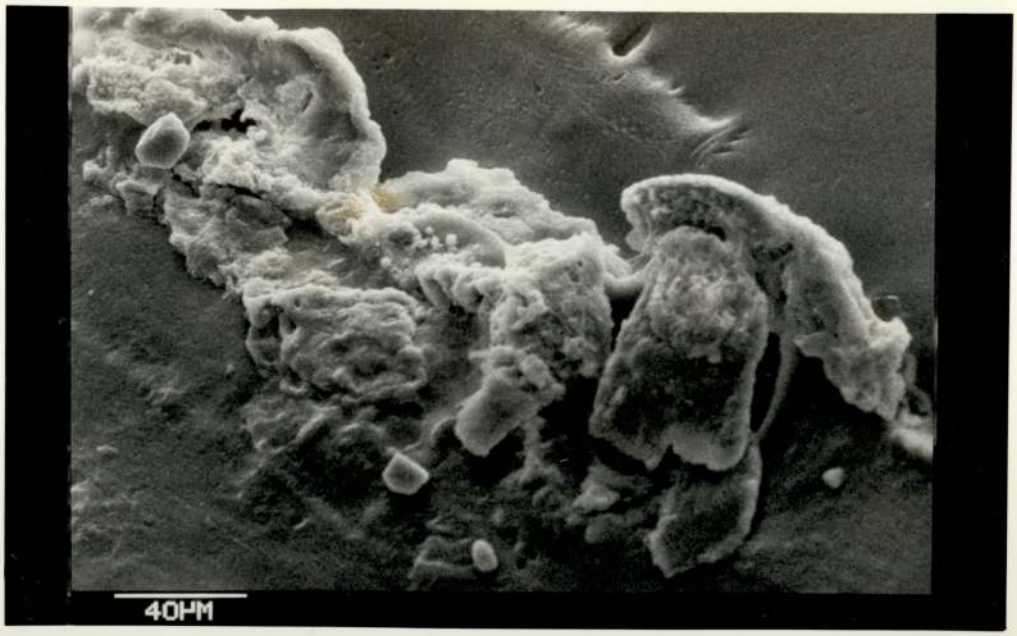


Plate 9.3 Longitudinal Section, Discrete Elevated Deposit, X-TEN Lens, S.E.M., X 500

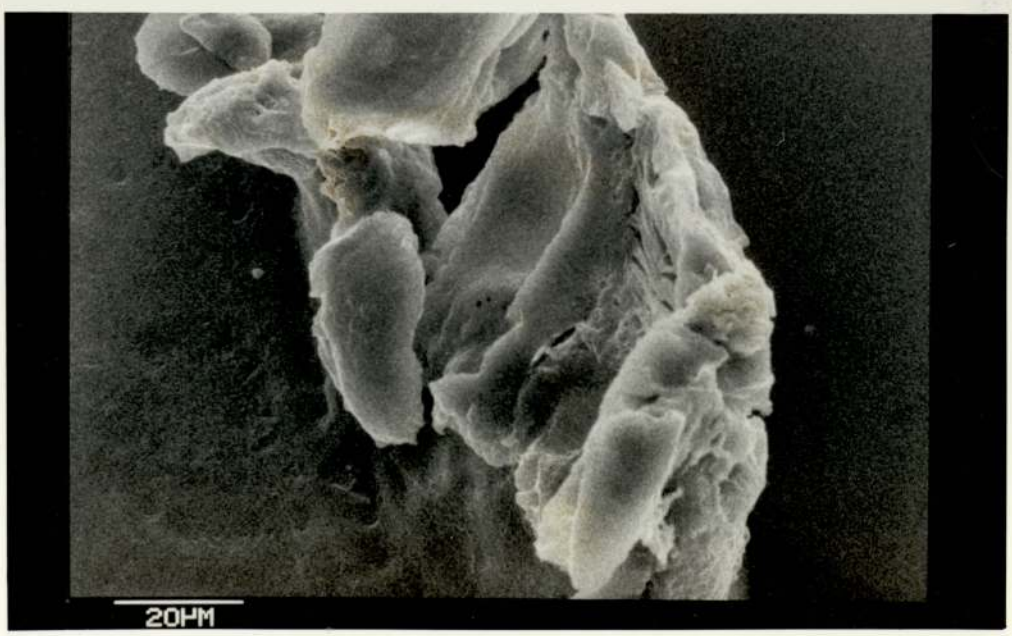


Plate 9.4 Longitudinal Section, Discrete Elevated Deposit Permalens, S.E.M., X 500

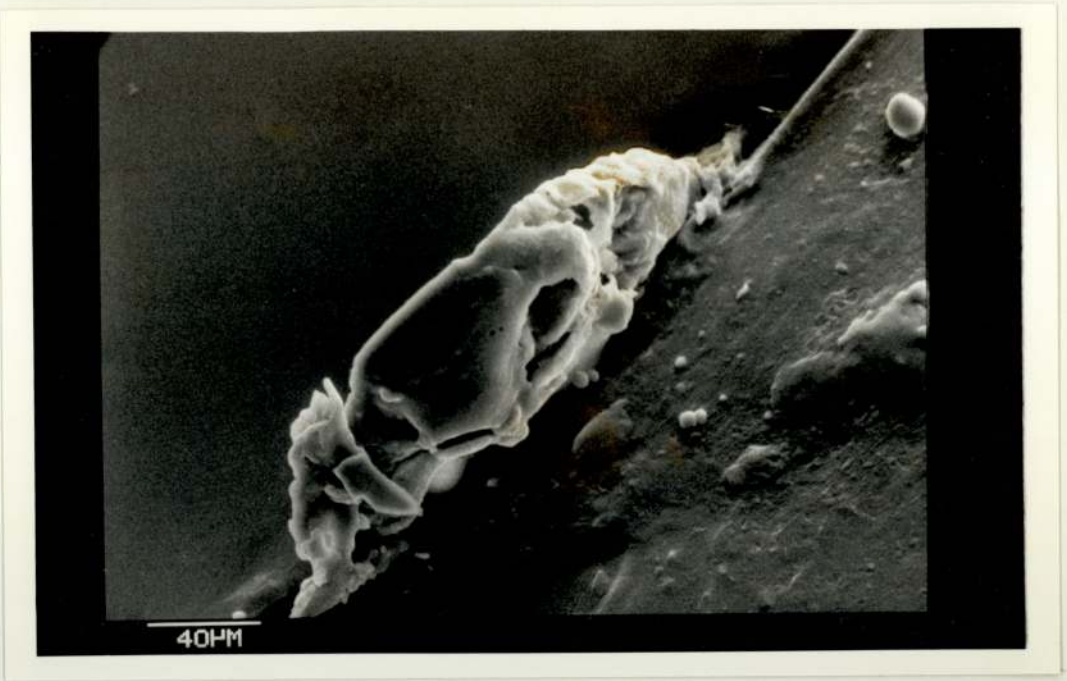


Plate 9.5 Longitudinal Section, Discrete Elevated Deposit X-TEN Lens, S.E.M., X 500

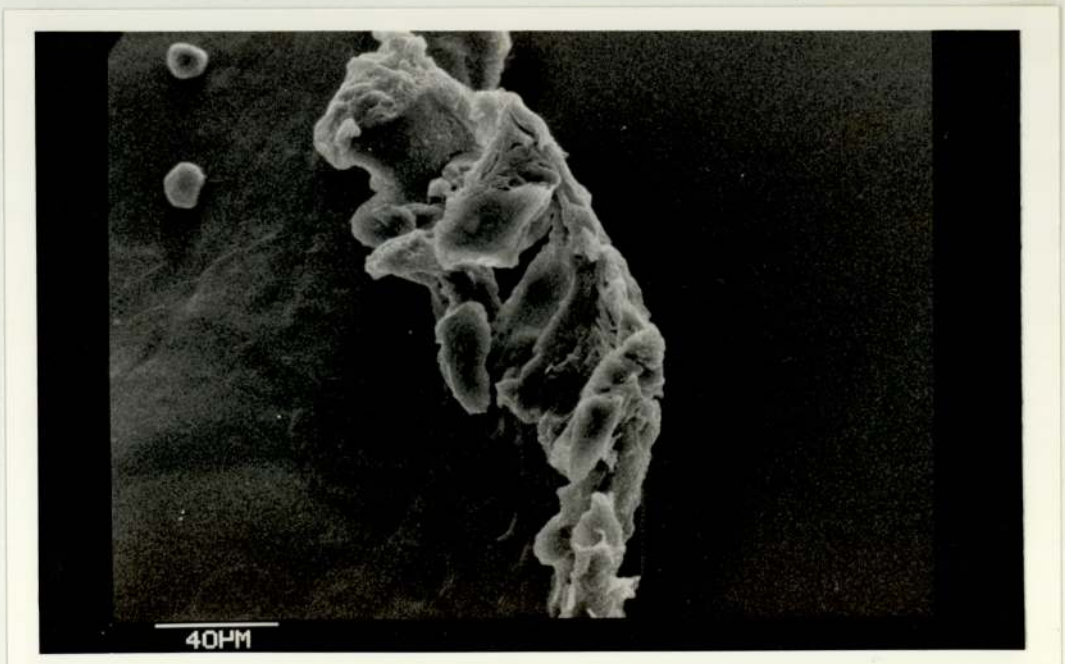


Plate 9.6 Longitudinal Section, Discrete Elevated Deposit, Permalens, S.E.M., X 500

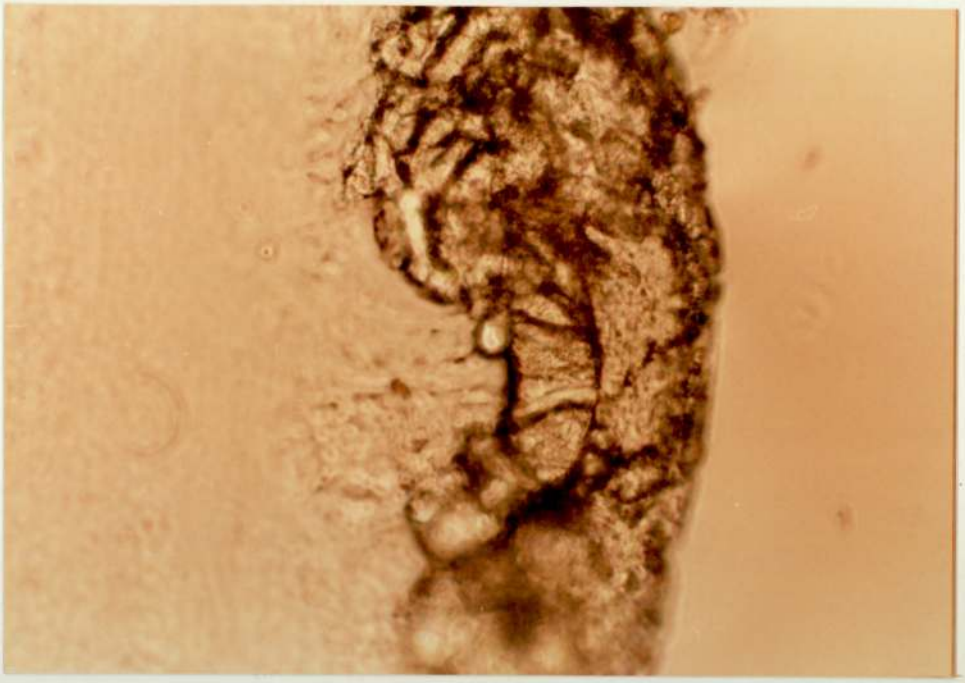


Plate 9.7 Discrete Elevated Deposit, Longitudinal Section, X-TEN Lens, X 65

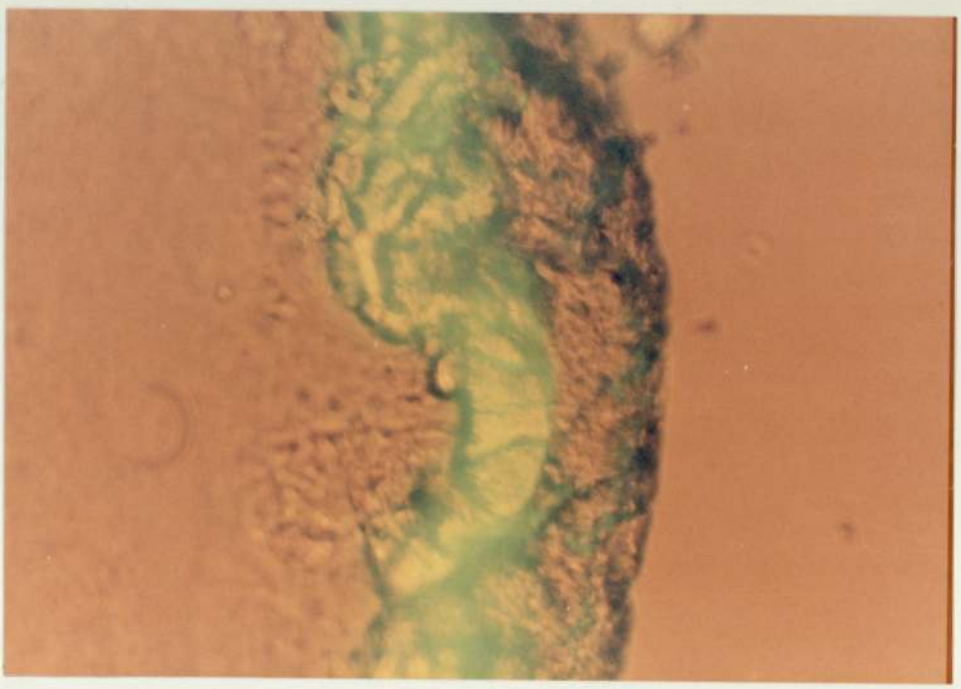


Plate 9.8 Autofluorescence of Discrete Elevated Deposit, Longitudinal Section, X-TEN Lens, X 65

discrete elevated structures examined and appears regardless of the bulk and surface properties of the hydrogel matrix upon which they are found. These findings correlate well with the fluorescence patterns that were noted in the previous chapter for unsectioned discrete elevated deposits. Therefore it would appear from fluorescence and polarisation studies, that the primary layer of the deposit contains a significant level of cholesterol precursors and cholesterol esters. The asymmetric distribution of the cholesterol species suggests that these lipoidal components may play a major role in the initiation of such deposits.

9.3 THE ANALYSIS OF THE LIPID COMPONENT OF DEPOSITS

From polarisation and fluorescence studies it was evident that deposit structures contain an appreciable lipid component. In order to detect the geological location and identify this component within the deposit structure, lenses contaminated with discrete elevated deposits were fixed in a 10 percent formaldehyde-calcium solution for 12 hours. Fixatives that are based solely on the presence of formaldehyde are ineffective as lipid preservative agents, since many lipoidal components, especially phospholipids, are soluble in formaldehyde and are therefore lost during fixation [238]. After washing repeatedly in distilled water, lenses were either frozen or resin embedded and sectioned by the techniques previously outlined in Chapter 6. Cut sections were then examined with a stereomicroscope and those bearing deposit material were removed for staining.

The stain recipes and procedures employed in this study are those laid down in Pearse [237] and are not reproduced here, however their respective histochemistries are discussed below.

9.3.1 Oil Red O

This stain was employed in order to assess the extent of the deposit lipid component and geological location within the deposit structure. The demonstration of lipids using lipid soluble dyes, such as Oil Red O, is a physicochemical process in contrast to staining procedures that generally involve chemical mechanisms. This technique depends on the solubility of the fat stain and staining results from the presence of the dye within the lipid substance [237]. A red colouration of stained matter indicates the presence of lipid material. The Oil Red O method was chosen in preference to other fat-soluble dyes employed in the detection of lipids for two reasons. Firstly, the colour is deeper and the staining is therefore intensified and secondly there is less tendency to form dye precipitates [238].

Discrete elevated deposit sections are displayed in Plates 9.9-11. From these photomicrographs it can be seen that the deposit material stained a deep red colour, indicating the presence of lipid species. The deposit appears to consist substantially of lipid since the intensity of colouration is uniform throughout the deposit section.

Plate 9.9 Oil Red O, X-TEN Lens, X65

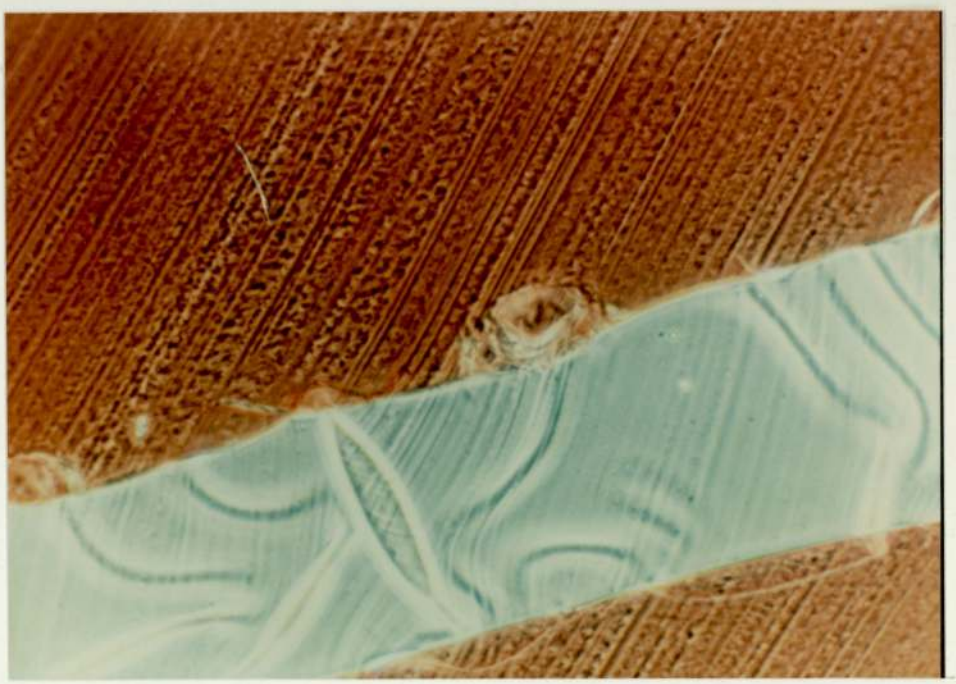


Plate 9.10 Oil Red O, Permalens, X65

Plate 9.11 Oil Red O, X-TEN Lens, X 65

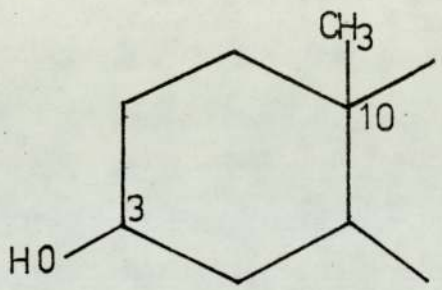
This finding suggests that the lipid component is symmetrically distributed throughout the deposit. From examination of these photomicrographs it is clear that the hydrogel lens matrix displays no evidence of staining, indicating that the development of discrete elevated deposits is a surface-mediated event. Plate 9.11 displays the characteristic matrix stress fractures that occur beneath the deposit material. The interaction of Oil Red O with deposit material in this study confirms the findings of other previous examinations of discrete elevated deposits.

Examination of deposit sections of spoilt X-TEN, Permalens combinations worn by clinical trial patients with Oil Red O revealed similar findings. Deposit material on both X-TEN and Permalenses stained positively with Oil Red O in a consistent and comparable fashion to other previous studies. Indeed there was no appreciable difference between Oil Red O deposit staining patterns from patient to patient, nor was it significantly affected by alterations in the wear protocol of the lens or the application of the device. As a result of these findings, further staining techniques were employed in an attempt to establish the nature of the lipid species.

9.3.2 Digitonin Reaction

Fluorescence and polarisation microscopy indicated the presence of cholesterol precursors, cholesterol and its derivatives asymmetrically distributed within the deposit

structure. In order to confirm these findings a cholesterol specific stain, the digitonin reaction, was therefore employed. The digitonin reaction is based on the work of Windaus [237], who found that non-esterified cholesterol forms a crystalline complex with an alcoholic solution of digitonin that is insoluble in water or acetone. Digitonin precipitates in general those sterols which have a 3B-hydroxyl group as shown below, the hydroxyl group



at C3 being on the same side of the ring system as the methyl group at C10. Although it was originally considered that this reaction was specific for non-esterified cholesterol, this stain has been shown to react with cholesterol esters [237]. When formal-calcium fixed sections of discrete elevated deposits were examined with this stain, the deposit structure displayed a white brilliance when examined with phase contrast microscopy (9.12-13), whilst those areas of the deposit structure corresponding to the white brilliance were found to be birefringent when examined in polarised light. These findings are indicative of the presence of cholesterol and its esters. In Plate 9.12, representative of an X-TEN deposit section, it can be seen that the primary site of the stain reaction is at the

Plate 9.12 Digitonin, X-TEN Lens, X 65

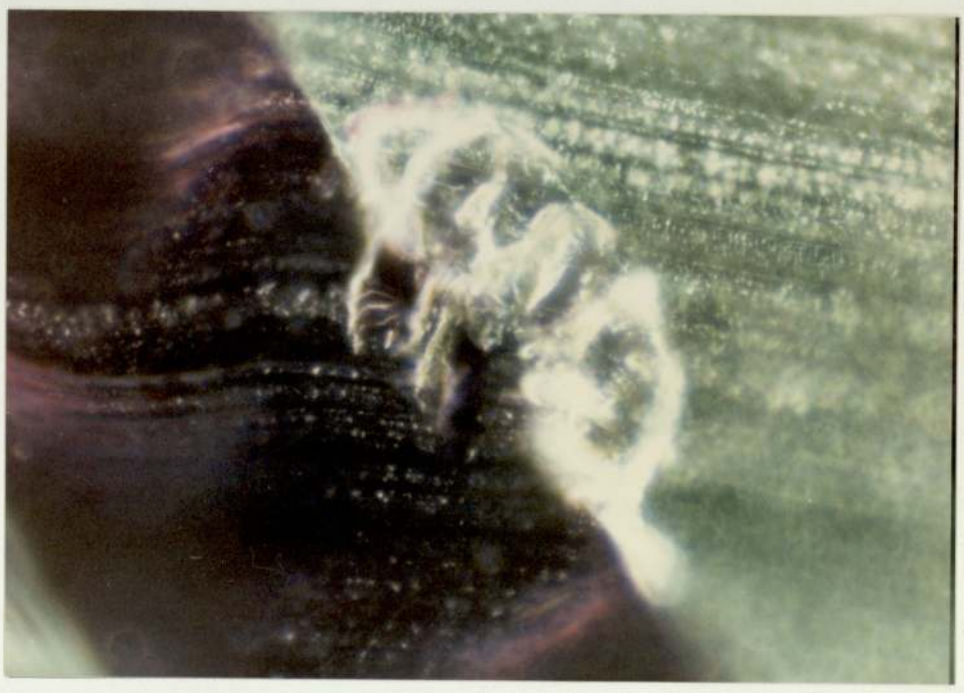


Plate 9.13 Digitonin, X-TEN Lens, X 65

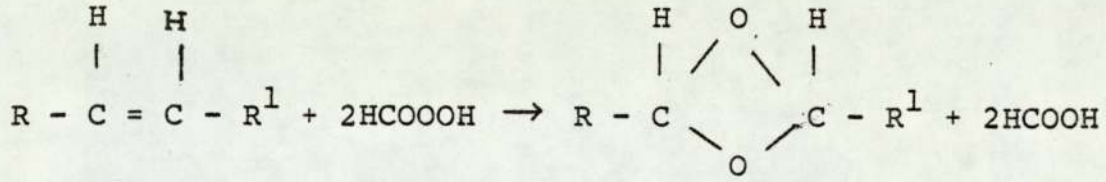
deposit-hydrogel matrix interface. This finding correlates well with the fluorescence studies of unstained sections (Plate 9.8) which tends to confirm the theory that cholesterol precursors and derivatives are species that initially react with the lens polymer. A possible explanation of the differing sites of stain reaction observed in Plates 9.12 and 9.13 lies in the fact that lens deposits were inevitably examined at differing stages of development. Plate 9.12 suggests that there is a repetitive adsorption process occurring, it is plausible to suggest, therefore, that the stained deposit depicted in Plate 9.13 has not yet reached this stage of development.

9.3.3 Unsaturated Lipid Detection

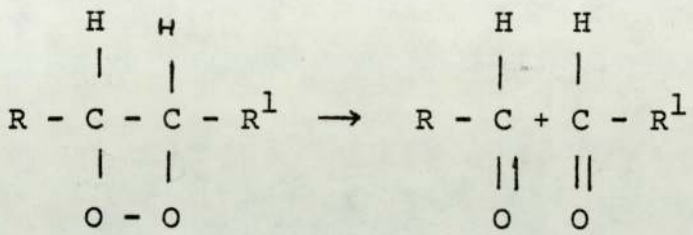
Two staining procedures, the Performic-acid Schiff and silver bromide reactions, were employed in order to establish the presence of, and the geological distribution of unsaturated lipids within the deposit structure. The histochemistries and results of these studies are discussed below.

9.3.3.i Performic Acid-Schiff Reaction

The Performic acid-Schiff's reaction, (PFAS), is based on the oxidation of unsaturated lipids by performic acid. Pearse diagrams the oxidation of ethylene groups by performic acid in the following way.



Molecular rearrangement then produces two aldehyde groups,



The aldehyde component then reacts with Schiff's reagent to give a red colouration, which is indicative of a positive response. In order to establish the specificity of the reaction for ethylene groups control sections were previously treated with bromine water by the method laid down in Barka et al [246]. The ethylene groups were blocked by this procedure and no red colouration was observed with the PFAS reaction. By this latter technique it is possible to differentiate between a positive stain reaction that results from the presence of unsaturated bonds rather than the presence of 1:2 glycol groups. This point is considered more fully at a later stage in this section.

A typical deposit stain response is depicted in Plate 9.14. It is evident that the deposit structure exhibits differential staining, since it is the deposit material-hydrogel matrix interfacial area that stains most strongly. This finding was found to be consistent throughout the analysis

Plate 9.14 Performic Acid-Schiff, Permalens, X 65

of discrete deposits by this method, regardless of the bulk and surface properties of the lens. It is suggested therefore, that in collaboration with cholesterol derivatives, unsaturated lipids primarily interact with the lens polymer.

9.3.3.ii Bromine-Silver Nitrate Method

The bromine silver nitrate method is based on the combination of bromine with unsaturated lipids at the site of the double bonds,

$$- CH = CH - + Br_2 \longrightarrow -CHBr - CHBr -$$

in the presence of alcoholic silver nitrate (as a silver ammonia complex) reacts with the brominated lipid.

$$- CHBr - CHBr - + 2AgNO_3 \longrightarrow CHO + 2AgBr + 2NO_2$$

and

$$- CHO + AgNO_3 \longrightarrow COOH + Ag + NO_2$$

The free aldehyde reduces another molecule of silver nitrate to metallic silver, as indicated. Finally the silver bromide is reduced in situ to metallic silver.

$$2AGBr + HCHO + H_2O \longrightarrow 2Ag + 2HBr + HCOOH$$

Formal calcium fixed discrete elevated deposits stained by this technique, are typified by the section depicted in Plate 9.15. The deposit material is clearly blackened by the deposition of metallic silver indicating the presence of substantial levels of deposit imbibed unsaturated lipids. Similar findings were noted for deposit sections of spoilt

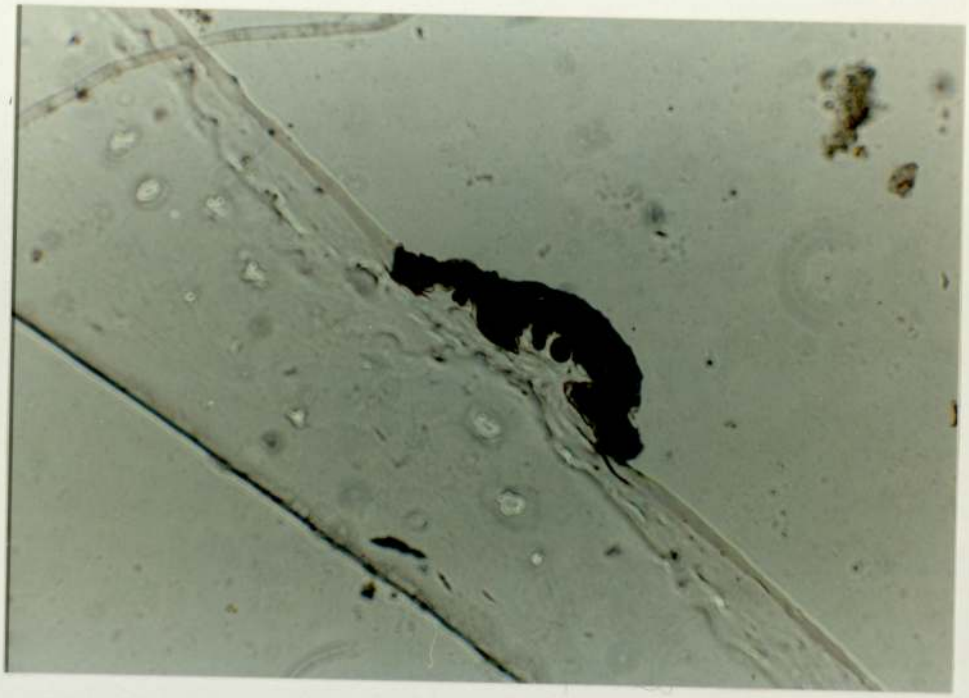


Plate 9.15 Bromine-Silver Nitrate,
X-TEN Lens, X 65

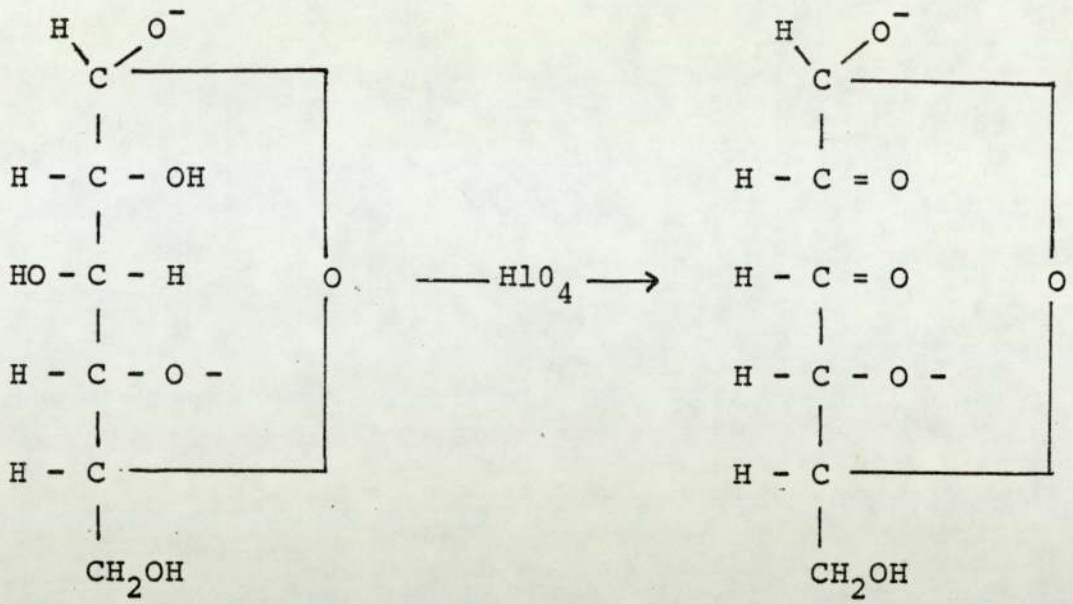
X-TEN, Permalens clinical trial patient combinations. This staining procedure clearly reinforces the role and presence of unsaturated lipid species within discrete elevated deposits. Furthermore it is evident from this photomicrograph that the development of discrete elevated deposits is a surface limited event.

9.4 THE DETECTION OF CARBOHYDRATE COMPONENTS OF DEPOSITS

The periodic acid schiff (PAS) reaction, was employed in order to detect the presence and localisation of carbohydrate components within the deposit structure. Periodic acid, an oxidising agent, cleaves the C-C bonds in various structures such as those outlined below.

CHOH	CHOH	CHOH	CHOH
|	|	|	|
CHOH	$CHNH_2$	CHN-H	C = O
|	|	\ R	|
1,2 glycol	1, hydroxy 2 amino	1 hydroxy 2 alkylamino	1 hydroxy 2 keto

As a result of oxidation at least one aldehyde group is formed and there is cleavage of a carbon-carbon bond as illustrated.



In the case of 1,2 glycols, two aldehyde groups are formed. The aldehyde groups are subsequently visualised by Schiff's reagent to give a characteristic red colouration. PAS is reported to react with unsaturated lipids. The mechanism of unsaturated lipids differs from that of carbohydrates in that it involves the oxidation of double bonds through the addition of two hydroxyl groups. These are further oxidised to produce aldehyde groups which are then demonstrated with Schiff's reagent. This reaction may be blocked by prior treatment with bromine water. When formaldehyde fixed cryo-sections of deposits were examined with this stain a strong but variable positive reaction was noted, with the red colouration most intense at the lens surface. However when sections were treated with bromine water prior to treatment with PAS there was a very significant decrease in the red colouration of the section. Such a finding was observed with all lens deposit sections that gave an initial PAS positive response. It would seem therefore that the

observed red colour is principally due to the presence of unsaturated lipids and that carbohydrate components contribute a very limited amount to the deposit structure. In a study of discrete deposits Allen et al reported a similar finding with PAS staining to those of this project.[176] Differential staining of the deposit was noted, a considerable proportion of the deposit showed no evidence of staining, whilst the interfacial region was deeply coloured. Taking into consideration the original finding that the entire deposit stained with Oil Red O (general lipid stain) and no mention of bromination studies were made, it is plausible to suggest that the interfacial region is composed of unsaturated lipid species.

9.5 THE DETECTION OF THE PROTEIN COMPONENT OF DEPOSITS

Millon's Reagent

This reagent was employed as an indicator of the presence of deposit imbibed protein. The reaction results from the presence in the protein molecule of the hydroxyphenyl group. It is given by any phenolic compound which is unsubstituted in the position meta to the hydroxyl group, but such compounds are not found free in living tissues and the only known amino acid containing the hydroxyl phenyl group is tyrosine. The reaction proceeds thus; first a nitrosophenol is produced by the substitution of NO for H ortho or meta to the hydroxyl of the phenol. Secondly, mercury (Hg^{2+}) is incorporated into a new ring, by chelation, which includes

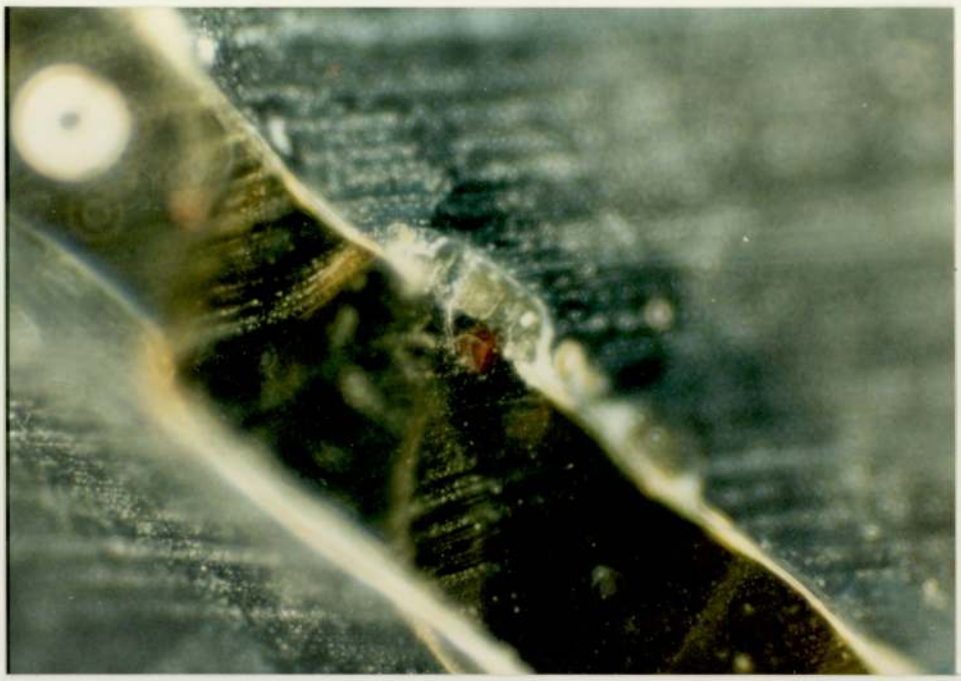
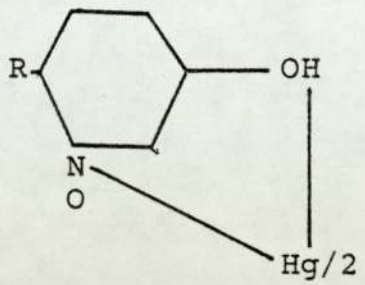


Plate 9.16 Millons Reagent, X-TEN Lens, X 65

the nitrogen of the nitroso group. The new complex is red in colour and is responsible for a positive result.



Deposit sections stained with this reagent displayed a variable response. Plate 9.16 is typical of the reaction between deposit material and the stain. From this it is evident that there is negligible colouration of deposit material. Although a very small area displays a red colour, from other studies it would be implausible to suggest that protein plays a major role in the formation of these deposits. It is interesting to note that Plate 9.16, representative of a worn X-TEN lens, shows no evidence of surface staining and therefore correlates well with previous radio labelling experiments.

9.6 CONCLUSIONS FROM HISTOLOGICAL STUDIES

Histological analysis of discrete elevated deposits was undertaken in order to identify the major organic components and geology of these structures. In Chapter 4 the composition of these deposits was variously ascribed to mucoproteins and lipids. From this study however it is evident that discrete elevated deposits are principally composed of

several lipid species and polysaccharide material to a lesser extent that appear to be asymmetrically distributed within the deposit structure. The various histological stains employed have highlighted the presence of and, as a result of the geological positioning at the deposit-hydrogel matrix interface, a potential role for unsaturated lipid species in deposit formation. As a result of these findings it is plausible to suggest that the primary adsorption of unsaturated lipids initiates and governs any further adsorption events that occur.

By comparison of discrete elevated deposit compositions and geologies on spoilt X-TEN Permalens patient combinations, it is evident that no appreciable differences exist between the composition and geology of deposits on X-TEN lenses and those that occur on Permalenses or Scanlenses. Therefore it is apparent that the bulk and surface properties of these hydrogel lenses play a negligible role in determining the organic composition and geological structure of these deposits. Allied to this is the fact that when a number of spoilt X-TEN lenses were examined no appreciable differences in deposit composition and geology were found from one patient to another. The application of the lens device little affects the nature of the deposit since discrete elevated deposits that were examined from aphakic lenses were similar in composition to those found on cosmetic lenses.

9.7 EDXA OF DISCRETE ELEVATED DEPOSITS

9.7.1 Introduction

Histological studies have revealed that the principal organic components of discrete elevated deposits. These techniques are however, limited in their ability to detect inorganic constituents either on or within the deposit structure or the lens matrix. Therefore discrete elevated deposits were examined by EDXA in order to assess the principal inorganic elemental components of lens deposits and their respective distributions and densities. The preparative techniques and operative conditions employed in this study have been considered in Chapter 4.

9.7.2 Qualitative Elemental Analysis of the Surface of Lens Deposits

Initial studies were conducted in order to identify the principal inorganic components found at the surface of discrete elevated deposits. Elemental analysis spectra of a number of deposits are located in Appendix 3. From these it can be seen that the major elemental peaks may be equated with osmium (Os) and gold (Au) whilst the minor peaks were found to be equated with chlorine, potassium and calcium. This characteristic elemental energy profile was found to be consistent with discrete elevated deposits regardless of the lens properties or application of device on which these structures were located. This finding was reinforced by

comparison of energy profile spectra obtained from spoilt X-TEN, Permalens clinical trial combinations since no discernible difference in the elemental profiles were found.

Studies of the lens surface immediately adjacent to the deposit structure revealed a negligible presence of deposit surface detected elements. Indeed the apparent concentration of these elements on the deposit surface was borne out by the construction of potassium, chlorine and calcium X-ray elemental distribution maps. The electron micrograph depicted in Plate 9.17 is representative of a discrete elevated deposit found on a Permalens that was worn on a weekly basis for 3 months, whilst Plates 9.18-20 are representative of the geographical distribution and density of the respective elements. Although the maps were produced by a slight degree of deposit rotation, it is quite obvious that the X-ray elemental maps correspond almost identically to the outline of the deposit structure. The lens matrix surrounding the deposit structure displayed negligible levels of these elements. Studies of deposits on X-TEN lenses revealed similar findings. The presence of gold on the deposit structures results as a consequence of the sputtering technique that was employed in order to render the lens surface and deposit structure conductive.

9.7.3 Reactions of Osmium Tetroxide

Since elemental analytical studies revealed that osmium is almost exclusively concentrated within the deposit structure

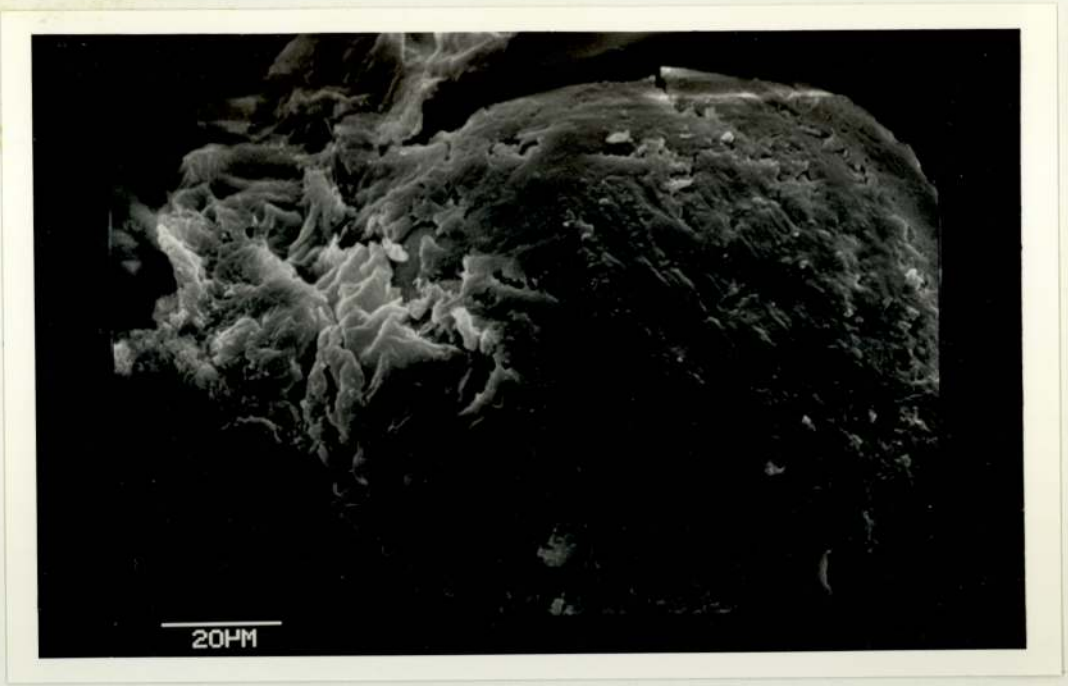


Plate 9.17 Discrete Elevated Deposit, Permalens, S.E.M., X100

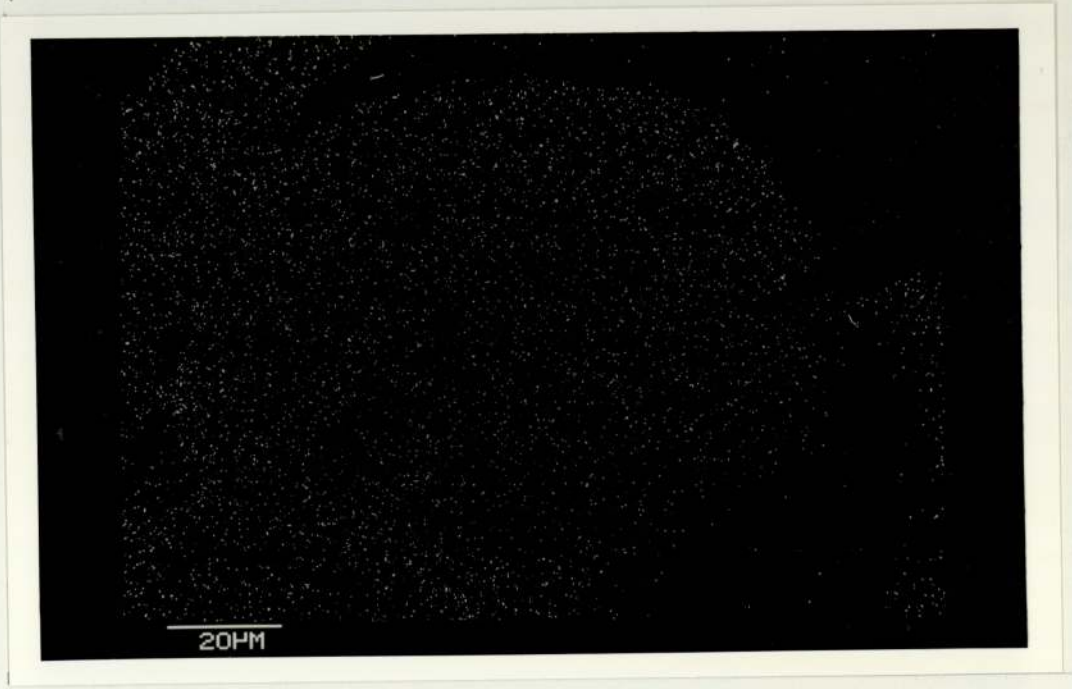


Plate 9.18 Calcium X-ray Distribution Map, Permalens, S.E.M., X1000

Plate 9.19 Potassium X-ray Distribution Map, Permalens, S.E.M., X1000

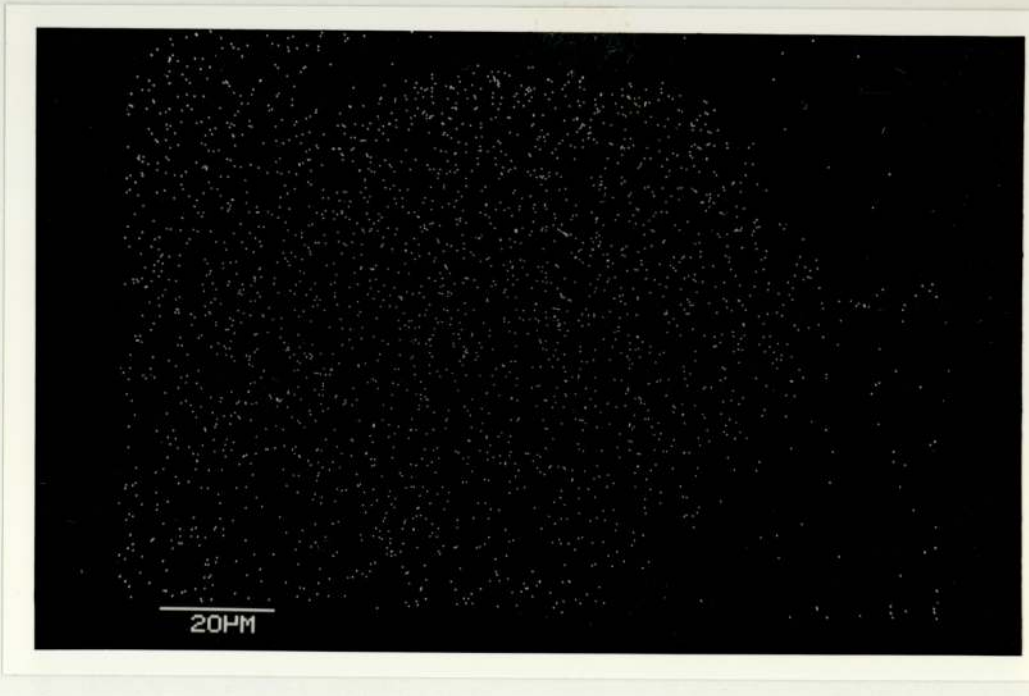
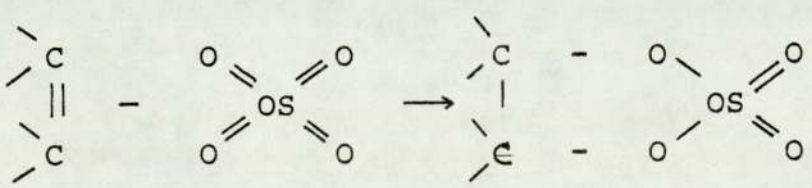


Plate 9.20 Chlorine X-ray Distribution Map, Permalens, S.E.M., X1000

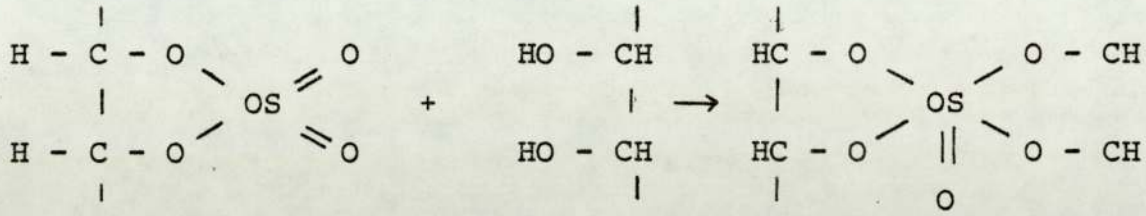
it is plausible therefore to consider the reaction of osmium tetroxide (OsO_4) with organic species. The organic species that react primarily with OsO_4 are unsaturated fatty acids, indeed it is reported that OsO_4 interaction with proteins and carbohydrates is negligible [236]. From experimentation OsO_4 has been shown to reduce oleic and olein whereas palmitic and stearic acids and their triglycerides were found not to react. These findings lead to the conclusion that OsO_4 oxidises olefinic double bonds. Since it has been shown that bromination of double bonds prevents OsO_4 reaction, the presence of a double bond in the lipid molecule is therefore, a prerequisite for reaction with OsO_4 under conditions of preparative E.M, such as those employed in this study. Osmium tetroxide may also react with one or more of the highly unsaturated precursors of cholesterol, especially squalene, a molecule that contains six double bonds [247].

The reaction of OsO_4 is thought to occur in the following way. Studies have indicated that oxidation of a double bond by OsO_4 leads to the formation of monoesters.



The monoester is not very stable and is easily hydrolysed to form a diol and the osmate ion $OsO_2(OH)_4^{2-}$. The diols

then react with another molecule of monoester to form a diester that are stable entities. It has also been proposed that OsO_4 can directly form a double co-ordinate link with the carbons of the double bond.



From the above reaction mechanisms it would be plausible to suggest that OsO_4 attaches inside the apolar lipid layers rather than that at the polar regions. However there is overwhelming evidence to suggest that the build-up of reduced osmium $(OsO_2.nH_2O)^-$ is greater at the polar end of the molecule than the apolar regions, although the primary site of reaction of OsO_4 is still the C=C bond [248]. From studies with phospholipids it has been found that one molecule of OsO_4 reacts with one double bond and for every diester form one atom of osmium in the form of the lower oxide is produced. Thus, as a result of the above considerations it is evident from deposit elemental analysis that these structures contain a substantial unsaturated lipid component.

9.7.4 Geological Distribution Of Elements Within The Deposit Structure

In order to assess elemental geological distribution and densities and specifically that of osmium, a number of

discrete elevated deposits were fixed in BGPA, post-fixed in OsO_4 and sectioned by the techniques previously described. EDXA of the deposit structure revealed the presence of osmium, chlorine, potassium and calcium and silicon. Several studies were undertaken in which semi quantitative estimations were made of elemental concentrations at various levels of the deposit structure. Analysis of the most anterior areas of the deposit material revealed significant levels of osmium, however deposit imbibed osmium was found to be maximal at the deposit material-hydrogel matrix interface. Plate 9.21 depicts a section through a deposit found on an X-TEN lens worn continuously for 3 months. X-ray elemental distribution maps for osmium (Plate 9.22) confirmed these findings since the density of the element is greatest at the deposit matrix interface. From this electron micrograph it is apparent that osmium is located in the hydrogel matrix, however, semi quantitative studies revealed that this was not the case. Indeed osmium was located in deposit material alone. The most plausible explanation of the x-ray distribution map is that the erroneous matrix findings result from the presence of contaminating gold atoms. Calcium, chlorine and potassium were found in both deposit and matrix material although their levels were found to be relatively higher in deposit material. This finding is confirmed by consideration of the x-ray distribution map for calcium (Plate 9.23).

The geological distribution of silicon was found to be the reverse of that of osmium. In all studies, no silicon was

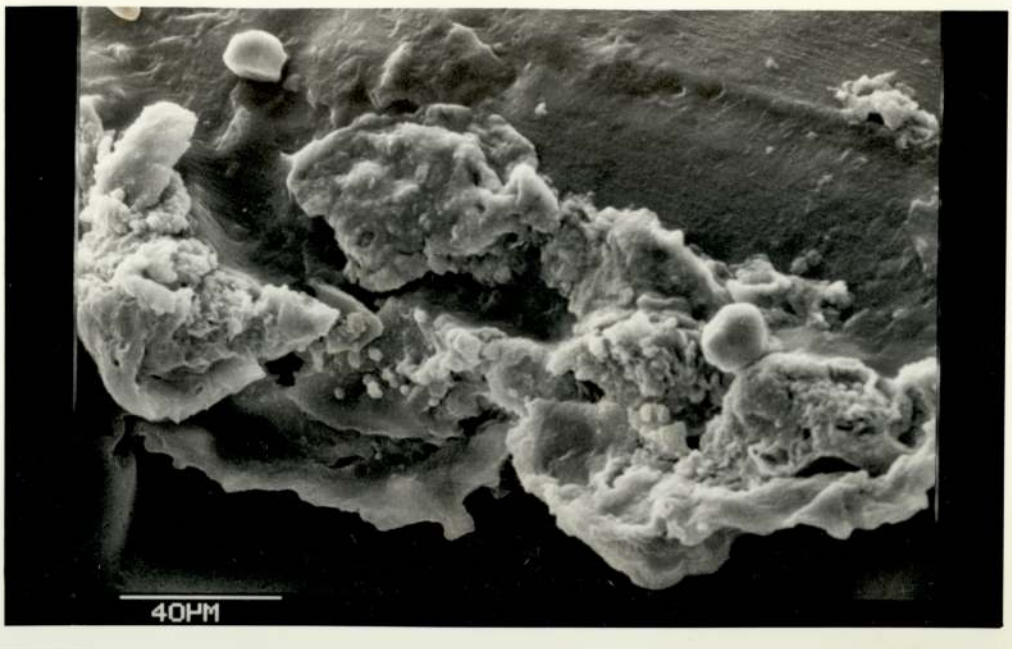


Plate 9.21 Transverse Section Discrete Elevated Deposit, X-TEN Lens, S.E.M., X 500

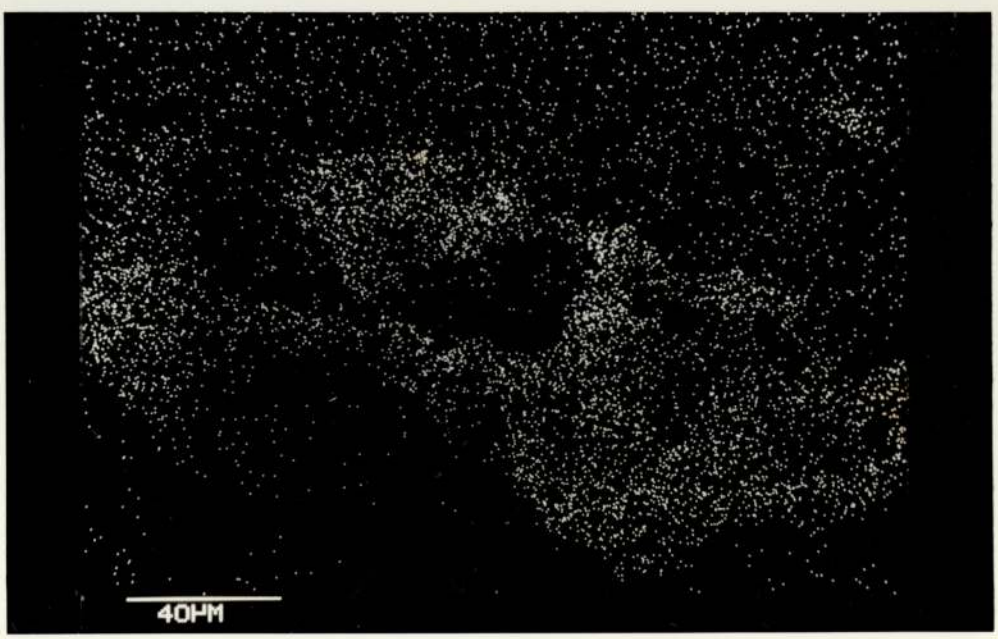


Plate 9.22 Osmium X-ray Distribution Map, X-TEN Lens, S.E.M. X 500

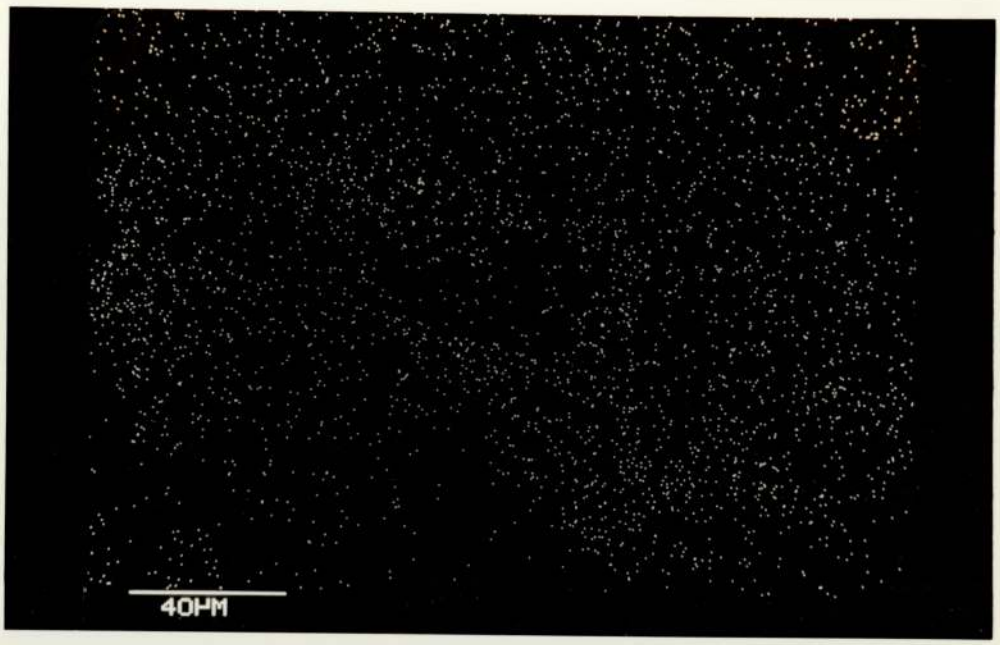


Plate 9.23 Calcium X-ray Distribution Map, X-TEN Lens, S.E.M., X 500

located within the deposit material component whilst maximal levels were located at the hydrogel matrix-deposit interface. Further studies of non-deposit bearing areas of the lens matrix revealed a random distribution of silicon although the element was found to occur at levels considerably lower than deposit areas of the lens. Studies of lenses from clinical trial patients revealed similar findings and silicon was found to be present in a significant number of lenses contaminated with discrete elevated deposits. However contamination of the lens matrix with silicon was found to be particularly prevalent in lenses worn in the treatment of aphakia, a point which will be enlarged upon at a later stage. EDXA analysis of the discrete elevated plateau structures previously detected by S.E.M. revealed a similar elemental profile to that of the discrete elevated deposit.

9.8 SUMMARY OF HISTOLOGICAL AND EDXA STUDIES

Previous discussions in Chapter 1 cited the significance of protein adsorption in the initiation of thrombus formation, marine fouling, dental plaque build-up and hydrogel contact lens deposit formation. However by employment of the various analytical techniques developed in this project it is evident that discrete elevated deposits are primarily composed of lipoidal species and, polysaccharides to a much lesser extent. Both histological and EDXA studies of the deposit geology revealed the presence of polar unsaturated lipid species that are asymmetrically distributed within

the deposit structure. The density of unsaturated lipids was found to be maximal at the deposit material-hydrogel matrix interface. As a result of these findings it is plausible to suggest that it is polar unsaturated tear lipids that initially adsorb onto the hydrogel lens surface.

This study has revealed the presence of variable concentrations of silicon in a significant number of extended wear lenses examined in this project. The presence of localised areas of silicon within the hydrogel lens matrix probably results as a function of the lens fabrication technique. The most common technique of hydrogel soft lens fabrication, and the method of choice for lenses examined in this study, is by the lathe cutting of dehydrated xerogels in rod form to give the necessary optical radii, followed by a polishing procedure to remove surface irregularities. During this latter procedure a 'lens' is affixed to a lathe chuck by wax and polished by way of particulate polishing compounds based on aluminium oxide lubricated with silicone oil. The oil is usually removed by washing with an appropriate solvent, such as chlorinated hydrocarbons and surfactants. It is considered however, that the presence of silicon contamination of the lens results from the inefficiency of the silicone oil extraction protocol. It is evident from elemental analysis that the extraction process is diffusion controlled since maximal silicon contamination was found at the hydrogel-tear interface. Silicon contamination was found to vary from one lens to another and variations between commerical lens types were also noted, the X-TEN

lens generally displayed a higher level of contamination than the Permalens. However, continuously worn aphakic lenses were found to display the highest level of contamination particularly concentrated at the optic zone of the lens. As a result of the lens thickness it is highly probable that silicone oil extraction protocols are least efficient with this type of lens.

9.9 A POSSIBLE HYPOTHESIS FOR DISCRETE ELEVATED DEPOSIT FORMATION ON HYDROGEL LENSES

Previously a major obstacle in the construction of a hypothesis concerned with the initiation and growth of discrete elevated deposits has lain in producing an explanation for the fact that these deposits are not uniformly distributed over the entire lens surface. However, it would seem less than speculative after considering the composition and structure of the normal tear film to assume that the introduction of a hydrophilic material into the environment may produce a disruption in tear film stability. Work by Zisman et al [249], in another context have shown that thin aqueous film coating a hydrophilic surface can be made to rupture and recede by the migration of polar lipid-like compounds placed on the water surface. Even if these compounds are relatively immiscible with water eventually sufficient migrate to the interface and absorb there, giving rise to localised areas of hydrophobicity and thus increasing the interfacial tension at these points. On the basis of this model, it is plausible to suggest that polar unsaturated

lipids of the tear lipid phase migrate and adsorb onto the surface of the hydrophilic soft lens (Fig 9.1). Maintenance of a stable film over a surface is known to be dependent on film thickness and the nature of the surface and tear film thickness is much less than the critical film thickness of liquids over hydrophobic surfaces [250]. It is possible therefore that the observed discrete elevated deposit plateaux or 'dry spots' may result from a combination of the above factors. However it is possible that the blink-reflex will re-establish the tear film and these abnormal areas will either be washed off or rewetted by mucin of the tertiary phase. It is considered that, as a result of the analysis of the organic composition of discrete elevated deposits, that although the rate of formation of these structures is undoubtedly affected by the patient tear composition, the genesis of these deposits is governed by two independent but synergistic properties of the lens, the critical surface tension of the device and the presence of hydrogel matrix imbibed contaminating agents such as silcone oil.

It is plausible to suggest that the adsorption of polar lipids would be expected to be more prevalent with lenses displaying a relatively high interfacial tension with tears. Indeed studies of the incidence of deposit occurrence revealed that the incorporation of fluorinated monomers into the X-TEN lens composition lead to a significant reduction in the number of deposits. Recent clinical evidence confirms these findings since it has been reported that

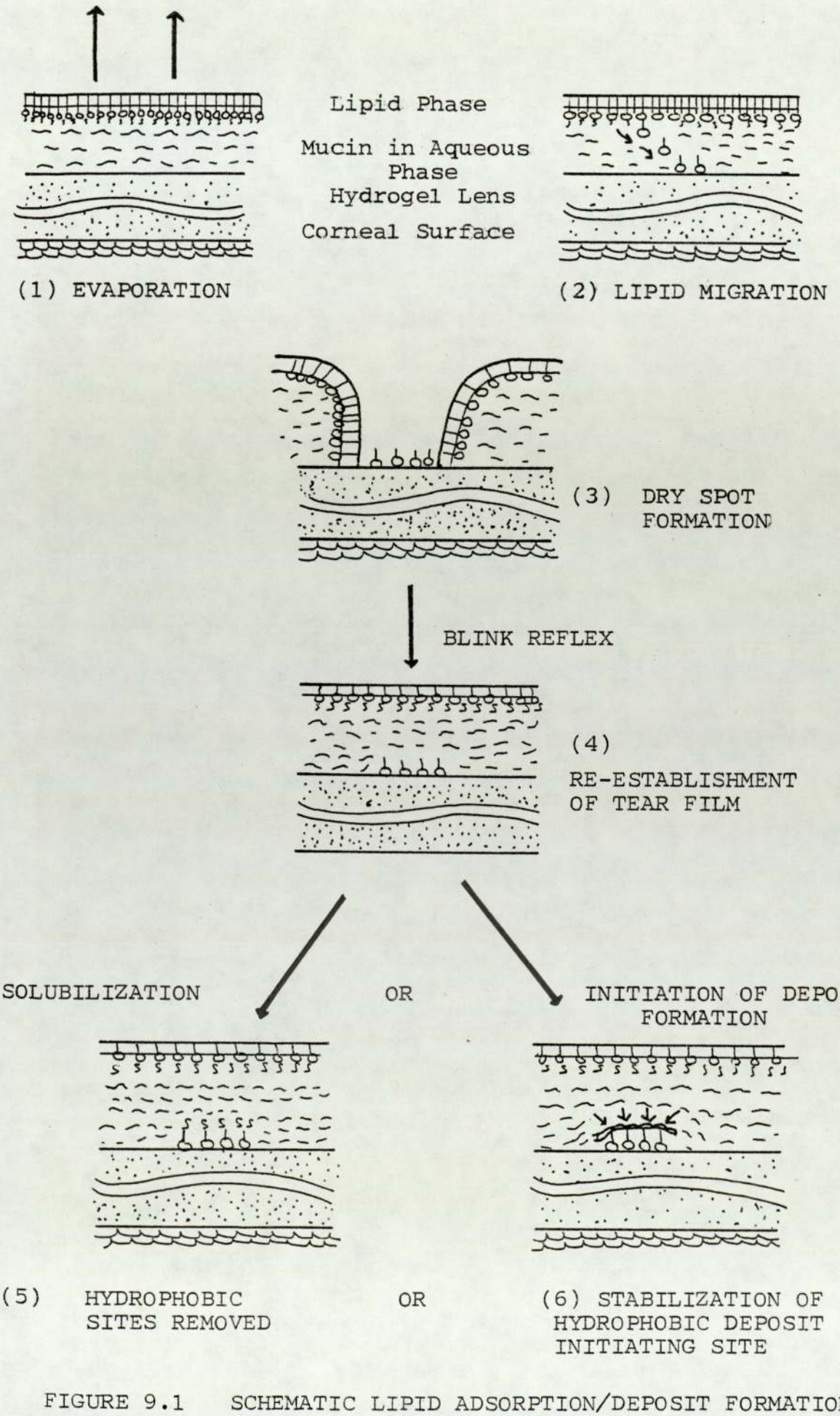


FIGURE 9.1 SCHEMATIC LIPID ADSORPTION/DEPOSIT FORMATION

removal of the fluorinated X-TEN lens and substitution of non fluorinated lenses, with a consequential rise in surface energy has led to an increase in the incidence of deposits [251]. Lipid adsorption probably occurs by way of the polar head groups. The hydrophobic tails of the adsorbed lipids then present a hydrophobic interface to the aqueous phase and a site for further adsorption. The adsorption of these lipids thus results in the modification of the lens surface. The adsorbed lipid complexes may well be stabilised by the complexing of calcium ions, thus explaining the consistent finding of the presence of deposit imbibed calcium. These stabilised hydrophobic 'dry spots' or embryonic deposits, would initiate the premature rupturing of the tear film and thus expose the areas of the lens to a surface drying effect. This concept of surface-drying marries well with the presence of deposit imbibed tear electrolytes. Indeed once the deposit structure has attained a height exceeding the thickness of the tear film (10 μm) it is highly unlikely that the tear film is able to wet these structures and as a result they are exposed to a continuous air interface. The movement of the upper eyelid over the deposit structure during blinking would inevitably lead to the coating of the deposit with meibomian gland lipid products that would bind tenaciously to the deposit.

The presence of hydrogel matrix imbibed silicon is thought to accelerate and stabilise lipid adsorption. However from elemental analysis it is evident that there appears to be a critical threshold of silicon density that must be exceeded

before deposit structures are stabilised. The presence of contaminating silicon and subsequent lipid adsorption and deposit formation in hydrogel contact lenses displays a number of similarities with the formation of calcified lipid deposits on left ventricular assist devices and heart devices that are composed of Biomer, a poly (urethane co ether) [252]. In vitro studies of the deposition of lipid species revealed that Biomer displayed a selective and rapid adsorption of polar aliphatic unsaturated lipids, cholesterol and to a lesser extent cholesteryl esters. The adsorbed lipids were found to complex calcium, and such a calcification phenomenon led to the stabilisation of the deposit structure. Lipid adsorption was found to be particularly prominent where the concentration of contaminating hydrophobic residual fabrication monomers was maximal.

9.10 CONCLUSION

Discrete elevated deposits are primarily composed of tear derived lipid species. The geology of these deposits is complex although the primary layer is composed of unsaturated polar lipids. It is considered that the development of these structures results from the modification of the hydrogel lens surface by the adsorption of this primary lipoidal layer. Studies have indicated that neither the deposit morphology nor composition is affected by the bulk and surface properties of the hydrogel lens. The incidence of deposit occurrence has however been shown to be dependent on the critical surface tension of the hydrogel lens. A

reduction in the interfacial tension between tears and the hydrogel lens has resulted in a decrease in deposit occurrence. This finding further reinforces the application of the Minimal Interfacial Energy hypothesis in the design of hydrogels with suitable ocular compatibility for extended wear. It is considered that the presence of fabrication residues, such as silicon, adversely and significantly affects the ocular biocompatibility of hydrogel lenses.

CHAPTER TEN

CHAPTER X

ANALYSIS OF HYDROGEL LENS SURFACE COATINGS AND PLAQUES

10.1 INTRODUCTION

This chapter is concerned with the analysis of coatings and plaques that contaminate the surface of commercially available hydrogel contact lenses. The nature and occurrence of these types of deposits has been considered in Chapter 4. Although several studies have been concerned with the chemical composition of these deposits little is known about the causative mechanism or mechanism of deposit formation or, whether the development of these particular classes of lens spoilation is governed by the bulk and surface properties of the hydrogel lenses. Of particular interest was the identification of the composition and geological structure of surface coatings, since this type of deposit is rarely seen to occur on extended wear hydrogel lenses and appears to be limited in its incidence of occurrence to daily wear poly(HEMA) devices. This chapter will therefore, attempt to correlate the composition of this type of deposit to the surface properties and methods of lens fabrication of daily wear poly(HEMA) hydrogel lenses.

10.1.1 Sources of Hydrogel Lenses

The commercial identity, number and wear programmes of

hydrogel lenses examined in this study are outlined below.

Table 10.1

Clinical Manifestation	Lens Manufacturer	Commercial Identity	No.	Wear Protocol
Surface Haziness	Kelvin Lens Ltd	X-TEN	35	Extended
		Eurolens	10	Extended
Surface Plaques	Cooper-Vision	O_2T	12	Daily

10.2 EXPERIMENTAL TECHNIQUES

10.2.1 Microscopical Analysis

Spoilt hydrogel lenses were examined by darkfield, phase contrast and fluorescence microscopical techniques. The methods employed to mount and photodocument the lenses have been outlined. Prior to microscopical studies, examination of spoilt X-TEN lenses with the naked eye revealed a milky-white opaque coating that covered the entire lens surface. However phase contrast studies revealed that the coating was composed of discrete elevated crystalline structures that eminated from the anterior lens surface superseded by an overlying transparent film (plate 10.1). This type of deposit morphology was found to be constant from one lens to another and independent of patient identity, bulk and surface properties or the duration of lens wear. Fluorescence studies indicated that neither the crystalline structures nor the overlying organic film displayed autofluorescence.

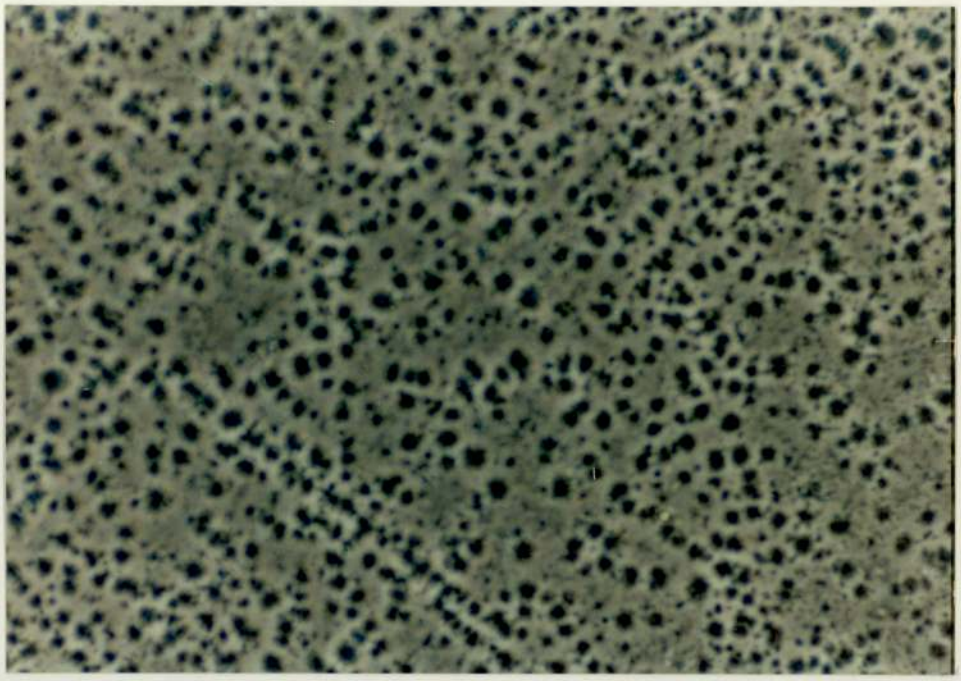


Plate 10.1 Crystalline Surface Coating,
X-TEN Lens, Phase Contrast, X 65

Microscopical studies of lens plaques found on daily wear poly(HEMA), O_2T lenses are displayed in plates 10.2-4. From these it is evident that the lens plaque is characterised by a surface orientated, morphologically complex, highly rugose deposit material. Plaques cover almost the entire lens surface, although the thickness of the deposit material varies across the lens surface. Phase contrast studies revealed the plaque to be a multilayered construction. As can be seen from plate 10.3 where the outer anterior layer of the deposit structure has disengaged from the lens surface, beneath it is found an underlying adsorbed layer. Lens plaques were found to display a characteristic autofluorescence (plate 10.5). The most striking feature appears to be the autofluorescence 'edge effect' and it can be seen that the most intense autofluorescence is to be found at the periphery of each plaque rectangular component. From these observations it appears that the most autofluorescent component lies beneath the outer deposit layers and is exposed only when these outer layers crack. It is evident from this study that this type of autofluorescence is consistent with that observed with the lipid component of discrete elevated deposits. Therefore it is plausible to suggest that the primary layer of the lens plaque deposit is composed of unsaturated lipid.

10.2.2 Lens Deposit Dissolution Studies

Various combinations of reagents known to disrupt different

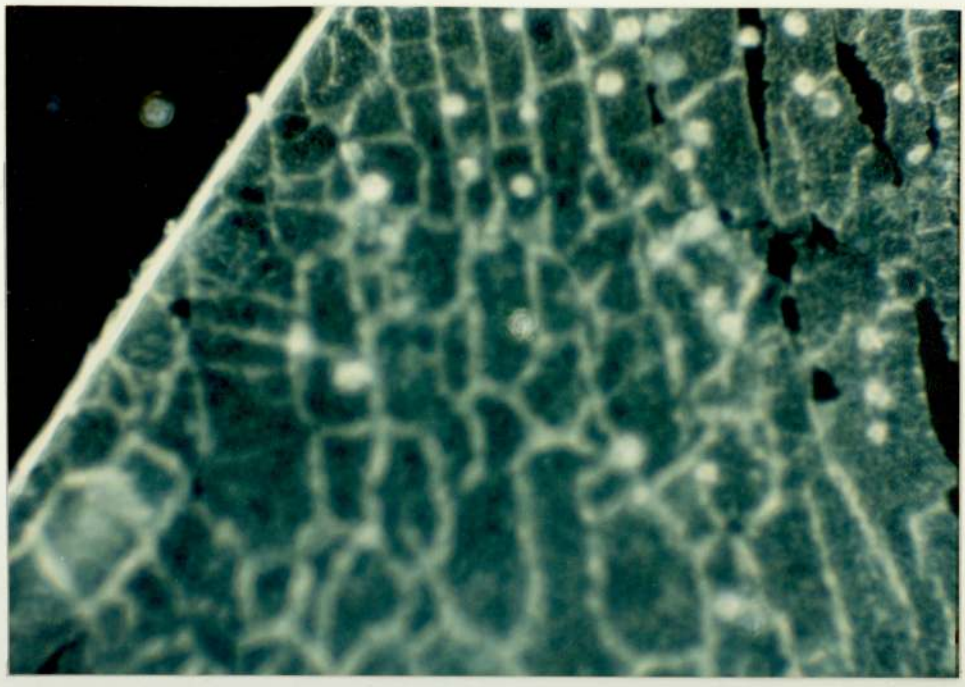


Plate 10.2 Lens Plaque, O_2T Lens,
Darkfield, X 65

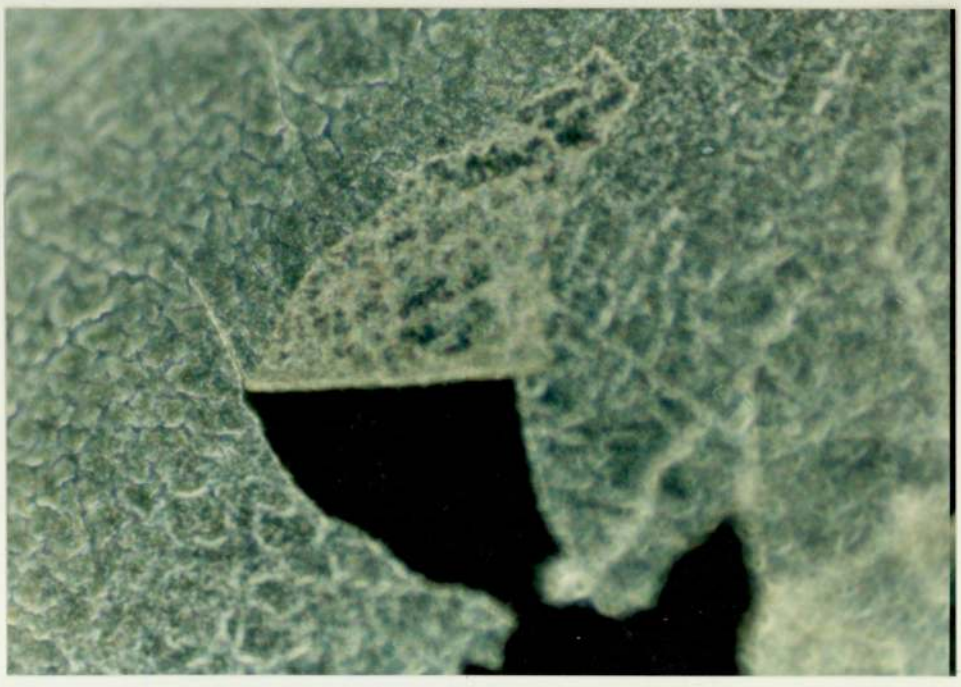


Plate 10.3 Lens Plaque, O_2T Lens,
Phase Contrast, X 65

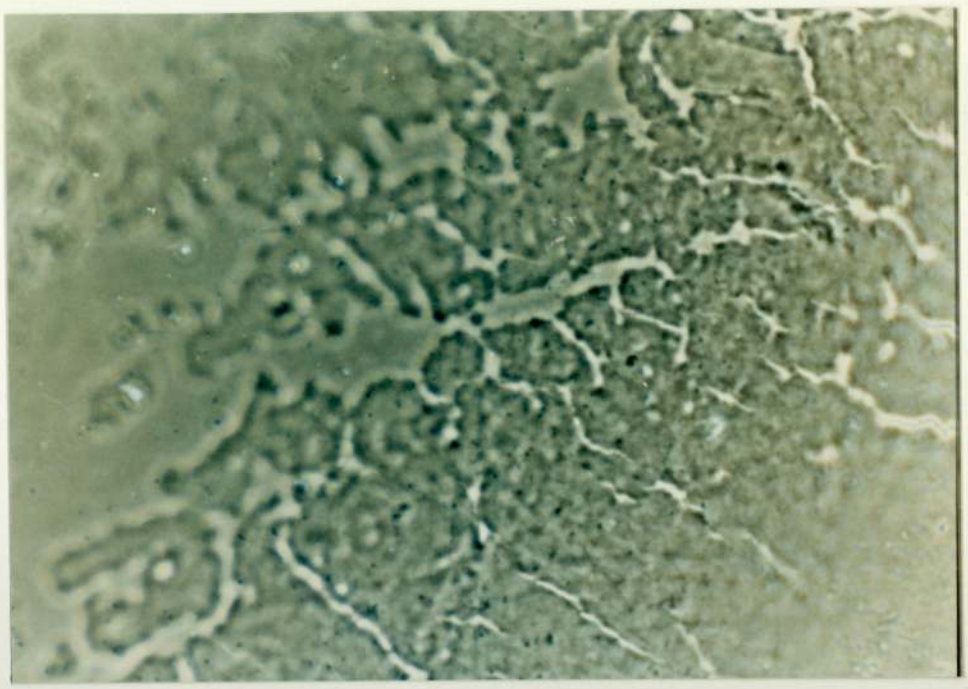


Plate 10.4 Lens Plaque, O_2T Lens, Phase Contrast, X 65

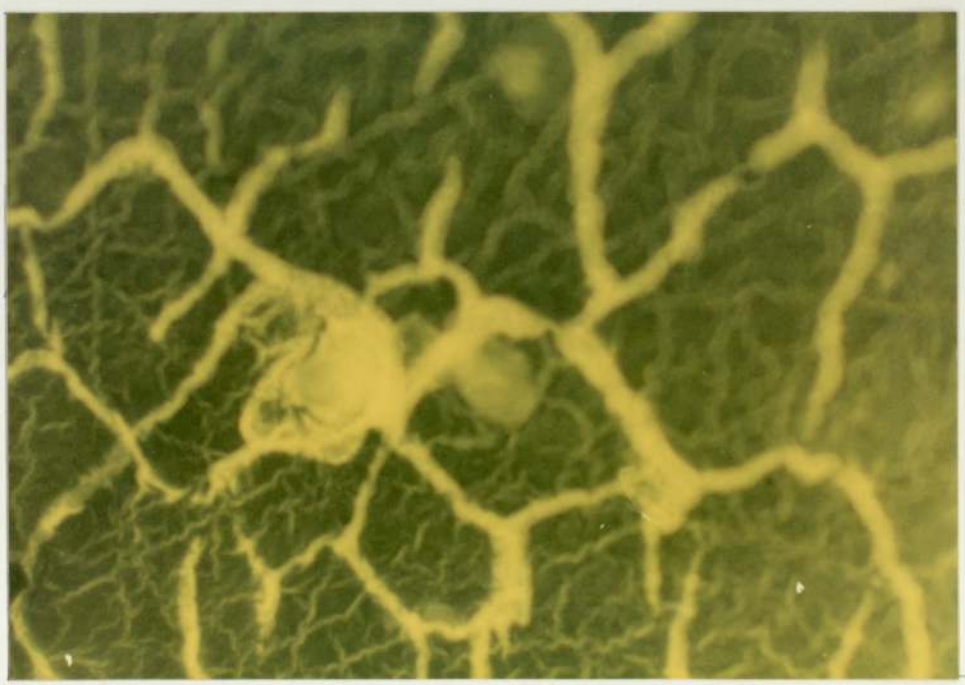


Plate 10.5 Autofluorescence of Lens Plaque, O_2T Lens, X 65

types of biological structures were employed in order to initiate the dissolution of surface coatings and plaques and therefore provide information on the composition of these deposits. The dissolution regime, based upon that of Wedler, involved sectioning of the spoiled lenses into quarters and exposing them to the various reagents in turn. Areas of lenses previously examined by light microscopy were removed by the use of a custom designed drill rig. They were then placed in sealed glass vials that had previously been cleaned with concentrated nitric acid in order to remove all contaminating organic matter, into which was placed 5 mls of the respective dissolution reagents. Lenses were then mechanically shaken overnight.

All reagents employed in the dissolution regime were obtained from B.D.H.Ltd. and were analytical grade. The following solutions were made up; 0.1 M potassium perchlorate, 5 M urea, 1 percent sodium dodecyl sulphate (SDS), 5 mM dithiothreitol (DDT), 50 mM ethylenediamine tetra-acetic acid (EDTA), and 1 M hydroxylamine. The first two reagents are considered to be 'chaotropic' or protein denaturing agents, SDS is a strong detergent that is routinely used to unfold proteins and dissolve lipids. DDT is a thiol reagent used to cleave or exchange disulphide bonds, EDTA is a divalent cationic chelating agent, and hydroxylamine is a very strong nucleophilic agent, specific for labile derivatives of carboxylate groups. The reagents were used singly and in pairs. After treatment the resulting morphology of lens coatings and plaques was

further investigated by phase contrast and fluorescence microscopy.

In the case of the X-TEN and Eurolenses coated with milky-white surface films, the most efficient deposit dissolution regime was found to be a combination of SDS and EDTA. However, when a lens section was initially treated with EDTA there was very little morphological alteration or decrease in deposit density, although after prior treatment of the lens with SDS almost complete dissolution and removal of the lens coating was noted. Analysis of the SDS eluent with a colourimetric protein determination system (Sigma Ltd) revealed the presence of variable levels of protein. From these results it is evident that the milky-lens coating is composed of discrete densely packed elevated calcium deposits that are covered by a proteinaceous layer. The presence of the superficial protein layer would explain the observed initial ineffectiveness of EDTA treatment.

The most efficient solvent system that initiated a decrease in the structural integrity of lens surface plaques was found to be a 1 percent SDS solution. Indeed combinations of the other reagents were found to have a negligible effect on the integrity of the plaque-like deposit. The negligible alteration in deposit morphology after treatment with EDTA suggests that calcium does not play a major role in the stabilisation of the plaque structure, nor is it a principal deposit component. After SDS treatment there

was a significant reduction in the intensity of plaque deposit autofluorescence, suggesting that this deposit is partially composed of a primarily adsorbed cholesterol lipid layer. Protein analysis of the SDS eluents revealed the presence of proteinaceous components, in the range of 3-5 mg/100 ml. Further experiments with hexane as the primary solvent lead to a total extinction of plaque autofluorescence and a destabilisation of plaque structure. Indeed continuous treatment of the plaque with hot SDS ($70^{o}C$) was found to precipitate the complete removal of the plaque.

10.2.3 Histological Studies

In order to confirm the findings of the dissolution studies on surface coatings, a number of lenses were fixed in formal saline, frozen and sectioned. Sections bearing deposit material were then stained with the silver method of Von Kossa as laid down in Pearse[237] for the demonstration of calcium deposits. This staining mechanism does not depend on the presence of calcium but on the presence of phosphate and carbonate. In animal tissues however, insoluble phosphates and carbonates are nearly always those of calcium and the test is therefore, considered to be specific for the detection of calcium. The presence of this element is indicated by the identification of black, particulate silver deposits located within the deposit.

Plates 10.6-7 depict the reaction of the stain with sections of various hydrogel lenses coated with the crystalline deposits. From these it is evident that inorganic calcium salts are located throughout the hydrogel matrix, although the density of black particulate deposits is greatest at the anterior surface (i.e. the tear interface surface of the lens) (plate 10.6). Similar findings were noted with all sections studied by this method regardless of the patient identity or the length of lens wear.

A number of lenses contaminated with surface plaques were also fixed in formal saline, frozen, sectioned and stained with a number of histological techniques. These included Oil Red O, for the detection of lipids, PAS for the detection of polysaccharides and Millon's Reagent for the detection of proteins. The histochemistries and specificities of these techniques have been discussed in the previous chapter. The heterogeneous nature of lens plaques was reinforced by these histological studies. The primary layer of the plaques stained strongly with Oil Red O, indicating and confirming the lipoidal nature of this layer. A positive PAS reaction was noted to occur with the upper layers of the deposit. Since prior bromination did not prevent a positive staining response, it is evident that the secondary layer of the plaque is composed of polysaccharide derivatives, probably muco-lipid complexes. A limited positive response was also found with Millon's Reagent, thus confirming the findings of SDS protein

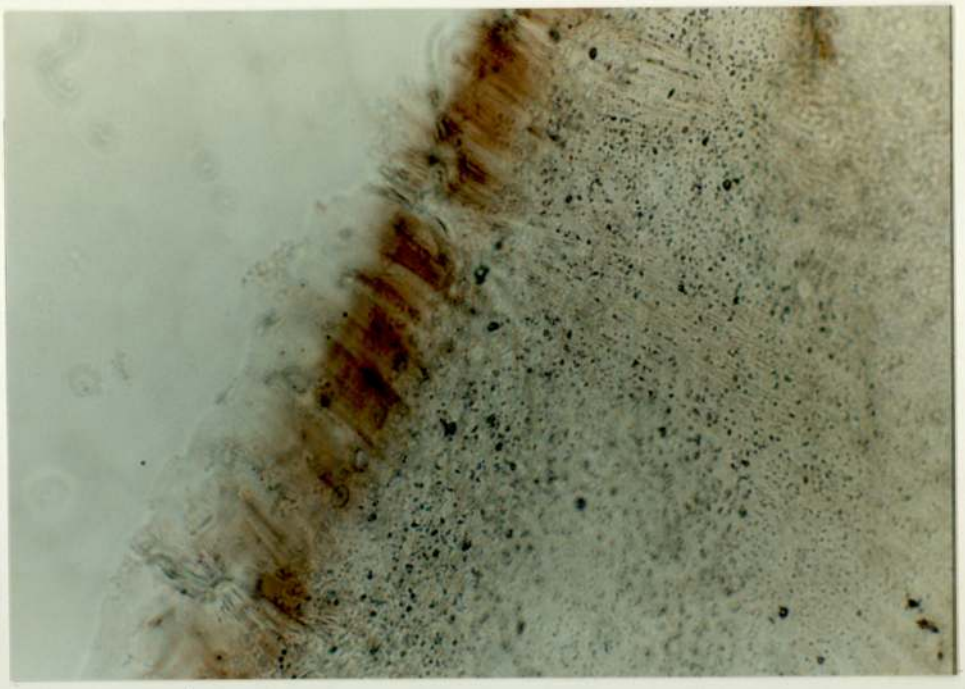


Plate 10.6 Von Kossa, X-TEN Lens, X 65

Plate 10.7 Von Kossa, X-TEN Lens, X 65

determination studies. However from the intensity of the stain colour it was evident that proteins are a minor deposit component and are restricted to the most anterior layers of the plaque structure.

10.2.4 S.E.M. and EDXA Studies

A number of sections of lenses contaminated with the crystalline lens coatings were subjected to both qualitative and semi-quantitative elemental analysis. These studies confirmed the presence of surface located deposit structures that were found to be particularly rich in calcium and phosphorus. These elements were also located in the hydrogel lens matrix of all lenses examined. From semi-quantitative elemental studies it was concluded that surface located, elevated deposits are composed of inorganic calcium phosphate. When areas of lenses coated with surface plaques were treated with a 1 percent osmium solution and subjected to EDXA studies, the primary autofluorescence layer of the plaque structure was found to be equated with areas of elevated osmium levels. From previous discussions in Chapter 9 it is evident that the primary layer of this deposit is composed of unsaturated lipid species.

10.3 POSSIBLE DEPOSIT ETIOLOGIES

From histological and elemental analytical studies it can be concluded that milky-white,opaque lens coatings, observed on X-TEN and Eurolenses, are composed of discrete

elevated inorganic calcium phosphate crystals superceded by a proteinaceous layer. These findings are in accordance with those of others who have considered this problem[181,187-9]. However, since the composition of these deposits was found to remain constant regardless of the lens wearer, bulk and surface properties of the lens or the length of wear, it is considered that the patient's tear components are not directly responsible for the formation of these deposits. Prior to insertion, both X-TEN and Eurolenses were stored for prolonged periods of time in phosphate-buffered saline. Analysis of this buffer medium revealed the presence of contaminating calcium at significant levels. From further investigations it was found that artesian water, high in calcium concentration, was employed in the formulation of the buffer. It is suggested therefore, that the formation of these crystalline deposits results from the following. Whilst stored for prolonged periods of time in the contaminated buffer calcium and phosphate ions would passively diffuse into the expanded gel network of the hydrogel lens. The solubility product of calcium phosphate is very low and once the concentration of matrix imbibed calcium and phosphate ions exceed this value precipitation and nucleation of calcium phosphate would result. This reaction may well be accelerated by the transient decrease in buffer pH due to the presence of carbonic acid, formed as a result of the solubilisation of atmospheric CO_2 when the seal of the vial is broken. On insertion of these ion saturated lenses into the ocular environment it is highly probable that these nucleation sites would further develop

from the supersaturated tears. The presence of elevated calcium and phosphorous levels and the location of crystalline deposits at the anterior lens surface, results from the fact that it is in this area of the hydrogel lens that the diffusion of tear electrolytes and therefore, precipitation and growth, is maximal.

In order to assess the validity of this hypothesis unworn X-TEN lenses were repeatedly rinsed in freshly distilled deionised water and then placed in glass vials containing the contaminated phosphate buffered saline. The lenses were mechanically shaken and periodically CO_2 was bubbled through the buffer and the pH was accordingly lowered. After several days the lenses were noted to exhibit the characteristic milky-white haze. Further SEM and EDXA studies revealed the presence of anterior surface located discrete elevated crystalline deposits that were composed of calcium phosphate. It is plausible to suggest that, from these findings, the above working hypothesis may be employed in accounting for the formation of this type of deposit. Indeed as a result of the findings of this study a borate buffer was substituted for the phosphate buffer and as a result the incidence of this type of deposit was significantly decreased.

Histological studies indicate that surface plaque deposits are both heterogeneous in composition and geological structure. The primary layer of the deposit is thought to be composed of unsaturated lipid species. It would appear

therefore, that this type of deposit displays similarities in its geological structure to the discrete elevated 'white spot'. However, there is a major disparity between the geographical location of these types of deposits in that the plaques were found to cover the majority of the lens surface.

Poly(HEMA), O_2T lenses, are manufactured by a static cast polymerisation process. This technique involves the direct conversion of monomer mixture into lens form. In principle, a two part mould, fabricated from poly(propylene) is filled with monomer mixture which subsequently polymerises. During the polymerisation process the orientation of the lens surface functional groups is governed to a significant extent by those of the mould material that acts as a functional group template. Since poly(propylene) is non polar and hydrophobic, dispersive functional groups will preferentially orientate at the lens surface. However, on release from the mould, the xerogel lenses are hydrated. During this process a reorientation of surface groups will occur so that the more polar, hydrophilic groups are preferentially orientated towards the aqueous interface. When the hydrogel lens is placed in the eye, the lens surface is subjected to an intermittent surface drying effect and as a result of break up of the tear film, to an air interface. As a result the surface functional groups will revert to the mould derived 'hydrophobic memory'. It is plausible to suggest that lipid polar species would then adsorb at the lens surface by way of hydrophobic tail ends,

as opposed to the polar group attachment in the formation of discrete elevated deposits, and as a result form a 'surface modifying' primary coating of the lens surface. The accumulative effect of this phenomenon would be a general modification of the surface properties of the lens polymer that would govern any further adsorption phenomenon. The production of a hydrophobic interface with tears would lead to a premature rupturing of the tear film. Elevated dissolved mucin levels are likely to occur in order to re-wet the hydrophobic lens surface. As a result of the thermal disinfection protocol employed in the treatment of these lenses, it is considered that stabilised muco-lipid complexes would be formed. The formation of such complexes is confirmed by the positive PAS reaction. The plaque morphology probably results from drying of the lens surface during blinking, and from the compression and flexation of the lens matrix during the blink cycle.

10.4 CONCLUSIONS

From this study it can be concluded that the formation of inorganic surface coatings is independent of the bulk and surface properties of hydrogel lenses and the tear chemistry of the lens wearer. It is highly probable that they result as a consequence of the use of non deionised diltilled water in the construction of buffer agents.

The morphology, geology and chemical composition of surface 'plaques' is extremely complex. Indeed it is

evident that these structures display many similarities in the presence of organic components and structure with discrete elevated deposits. It is highly probable that the 'plaque' is representative of a more geographically diffuse discrete elevated deposit. It is considered that the disparity in deposit geographical distribution results not only from the difference in the type of lens manufacturing techniques, but more significantly it is dependent on the high interfacial tension of poly(HEMA) lenses compared to that of the fluorinated surface modified X-TEN lenses. Thus this study enforces the view that the various manifestations of hydrogel contact lens spoilation, such as the rate of formation of discrete elevated deposits and the formation of surface 'plaques' are resultant and dependent upon the bulk and surface properties of hydrogel lenses and the method employed in the fabrication of hydrogel contact lenses.

CHAPTER ELEVEN

CHAPTER XI

CONCLUDING DISCUSSIONS AND SUGGESTIONS FOR FURTHER WORK

The outstanding conclusion to be drawn from this research is that the main objectives of the identification of the chemical composition, geological structure and the deposit-hydrogel matrix structural relationship of various deposits found on and within commercially available hydrogel contact lenses have been achieved.

Several factors place this research in a unique position to comment critically on the phenomenon of hydrogel ocular-biocompatibility. The problem of hydrogel contact lens spoilation has previously been considered by a number of workers. The majority of these studies were undertaken by clinicians who empirically observed this phenomenon and as a result evolved a classification of hydrogel lens spoilation based principally on the morphology of these deposits. However, the limited scope of analysis suggests that this empirical classification of the manifestations of hydrogel contact spoilation cannot be correct.

The few studies in which chemical analysis was employed, were extensively limited to the detection of the principal organic deposit components. Furthermore negligible information has previously been provided on the geological structure of deposits or the role of the lens chemistry, wear protocol and application of the device on the formation

of lens deposits. This is partially due to the lack of a suitably extensive range of analytical and histological techniques, but in addition is a reflection of the fact that lenses subjected to analysis were uncontrolled in their chemistries and wear protocols.

This project however, has devised and assembled a wide range of analytical and histological techniques and, has been able to exercise some control over the lens materials worn and wear protocols followed. It has been noted elsewhere that this aspect of biomaterials studies needs to be carefully controlled and as a consequence, much more detailed attention should be given to this in subsequent work in this area.

Furthermore, this research has resulted in the establishment of deposit morphology, detection of the principal deposit imbibed organic and inorganic species and the geological strata of deposits. In addition, as a result of the development of suitable geological probes, those tear components that constitute the hydrogel polymer interactive primary layer of the deposit structure have been uniquely identified.

A feature that contributed significantly to the relevance of this study was the facility of continued access to contact lenses worn by patients who participated in comparative lens material wear trials. Not only was it possible to determine the role of the lens chemistry in the formation of lens deposits but also to establish the

effect of modifications in lens surface properties on the relative biotolerance of these devices.

The yellow brown discolouration of hydrogel contact lenses is a major and significant problem encountered during wear. This study has shown that lens discolouration results from the presence of matrix-bound insoluble particulate deposits. The nature of the particulate deposit was found to be independent of the bulk and surface properties of the hydrogel lens. The exact chemical composition of the pigment remains unclear, however spectral and histological analysis suggests that the deposits consist of highly conjugated aromatic sulphydryl containing, melanin-like structures.

This work has further demonstrated that the more darkly stained lenses result from an increase in the population density of particulate deposits and a gradual increase in size. Furthermore, the distribution of these particulate deposits is not restricted to the lens surface but occurs throughout the lens matrix. It is considered however, that the distribution of matrix imbibed deposits is a function of the wear protocol of the lens.

Hydrogel lens surface coatings were found to be composed of discrete elevated inorganic calcium phosphate crystals, overlain with a proteinaceous coating. The formation of this type of deposit is considered to be independent of the bulk and surface properties of the hydrogel lens. It is

considered that this type of deposit results as a consequence of the employment of inadequately de-ionised water in the formulation of lens buffer solutions.

Hydrogel lens surface plaques and discrete elevated deposits are the most morphologically complex forms of deposits found on daily and extended wear lenses respectively. It was originally considered that tear protein complexes were the major structural constituent of these deposits. However, histological studies undertaken in this project found both the plaque and elevated deposit to consist substantially of, but by no means solely, tear derived lipoidal species. Furthermore, studies of the deposit geological strata indicate that the primary deposit layer is composed of unsaturated lipids.

In the case of discrete elevated deposits, the adsorbed deposit component and attendent hydrogel matrix demonstrated elevated levels of deposit and matrix imbibed calcium. The presence of this cation was found to be independent of the hydrogel lens chemistry. It is considered that calcium functions as a hydrogel matrix-deposit component stabilising species. Further studies should be undertaken to reduce the level of matrix imbibed calcium by modifications in the hydrogel lens chemistry, since it is considered that by this action, the formation of this type of deposit may be substantially reduced.

From chemical analysis it is considered that the formation

of discrete elevated deposits and surface plaques result from the adsorption of tear lipid species. The insertion of a hydrogel contact lens into the ocular environment results in the competitive adsorption of protein and lipid species. The rate of adsorption is undoubtedly a function of the lens surface chemistry and the tear composition of the individual. However, as a result of the ampipathic nature of tear lipids, the adsorption of these tear species results in the biological interfacial conversion of the lens surface properties. Any further adsorption results as a function of the primary adsorption of tear lipids.

Although the ocular environment may be considered to be simplistic in composition to that of plasma, the adsorption of lipids, with an increase in the interfacial tension of the hydrogel lens with tears, would result in the destabilisation of the tear film structure and consequential collapse in the tear film. The modified lens surface is then exposed to an air interface which is considered to accelerate the formation of these deposits. The adsorption and surface modifying properties of lipids may be used to explain the finding that the respective compositions and geologies of the two types of deposits remain constant regardless of the lens chemistry, wear protocol or application of the device since this process of biological interfacial conversion renders otherwise biocompatible materials non-compatible with the ocular environment.

From this project it is considered that the rate and

therefore, incidence of, deposit occurrence is a function of the lens chemistry, method of lens fabrication and type of patient tear chemistry. Surface modified X-TEN lenses, that display a low interfacial tension, were found to exhibit a decrease in the incidence and therefore, rate of, discrete elevated deposit occurrence, relative to other non-modified commercial hydrogel lenses. It seems reasonable to suggest therefore that the Minimal Interfacial Energy hypothesis, a theory specifically pertaining to hydrogel blood compatibility, is equally applicable in the design of hydrogels with suitable ocular compatibility. It is one of the important findings of this project that modifications in the surface chemistry of hydrogel lenses has been shown to alter the rate of discrete elevated deposit formation. However the morphology, composition and geological structure of deposits found on surface modified lenses remained unchanged. Such a finding is consistent with the hypothesis that this type of deposit results from the general modification of the hydrogel lens surface properties by the adsorption of tear lipids.

Elemental analysis of the hydrogel lens matrix immediately beneath the discrete elevated deposit structure has shown the presence of significant levels of contaminating silicon, that was found to be independent of the respective bulk and surface properties of the hydrogel lens. It is highly probable that the existance of discrete elevated matrix imbibed silicon levels may well be responsible for, and would inevitably greatly accelerate, the formation and

stabilisation of discrete elevated deposits. The finding that the presence of silicon is independent of the lens chemistry is particularly significant since it further reinforces the contention that discrete elevated deposits result from the modification of the lens chemistry by tear lipids. The presence of silicon would accelerate the adsorption and consequential hydrogel surface modification by lipid species.

Further studies should be conducted in order to improve the efficiency, or the development of more appropriate lubricative and solvent extraction protocols in the case of lathe cut extended wear lenses. Furthermore since this project has indicated a potential role for the mould material, employed in the manufacture of mould cast hydrogel lenses, in determining the surface chemistry of these devices, much more detailed attention should be given to the selection and development of these materials in further studies.

This research has been primarily concerned with the analysis of pre-formed deposits on hydrogel contact lenses. As a result of the existance of a clinical trial group of lens wearers, further studies should be undertaken in order to identify and characterise more precisely, those tear components, specifically unsaturated lipids, that initially adsorb onto the lens surface. This could be achieved by implementing lens wear protocols of an extremely short duration, in the order of several minutes. The lenses would

then be removed and subjected to a suitable rinsing procedure in order to remove adsorbed lipids and proteinaceous components, the eluent would then be examined by High Pressure Liquid Chromatography.

In tandem, research should be undertaken in order to characterise more fully the functional surface chemistry and resultant surface free energies of these devices prior to insertion into the ocular environment. In this way the profile of adsorbed bio-organic species could be correlated more precisely with the functional characteristics and modifications of, the surface chemistry of hydrogel lenses.

By closely controlling the fitting technique and cleaning regime, further studies should be undertaken to establish the effect of surface modifications on the tear adsorption profile and ultimately the biocompatibility of the modified hydrogel lenses. It is considered that studies should be undertaken to characterise the tear component of those patients who participated in the clinical wear trials. It is envisaged that particular emphasis would be placed on the qualification and quantification of the patient tear lipid component.

Although this study has strongly implicated the role of lipid adsorption in the surface modification and consequential deposit formation, further immunohistochemical studies employing fluorescently labelled human protein antibodies should be carried out on the geology of deposits

in order to characterise more fully the role and composition of deposit imbibed proteinaceous components.

APPENDICES

APPENDIX I

FIXATIVES

10 PERCENT FORMALIN

100 mls concentrated formaldehyde solution (37-40 percent, B.D.H), 900 mls of freshly distilled water

FORMAL-CALCIUM

1 percent (w/v) $CaCl_2$ in neutral 10 percent formaldehyde

NEUTRAL BUFFERED FORMALDEHYDE SOLUTION

100 mls 37 percent formaldehyde, 900 mls distilled H_2O, 4 gm acid sodium phosphate monohydrate ($Na_2H_2PO_4H_2O$), 6.5 gm anhydrous disodium phosphate (Na_2HPO_4)

NEUTRAL 10 PERCENT FORMALDEHYDE

100 mls formaldehyde solution (37-40 percent)
900 mls distilled water, calcium carbonate (chips) in excess

BUFFERS - PHOSPHATE BUFFER SALINE

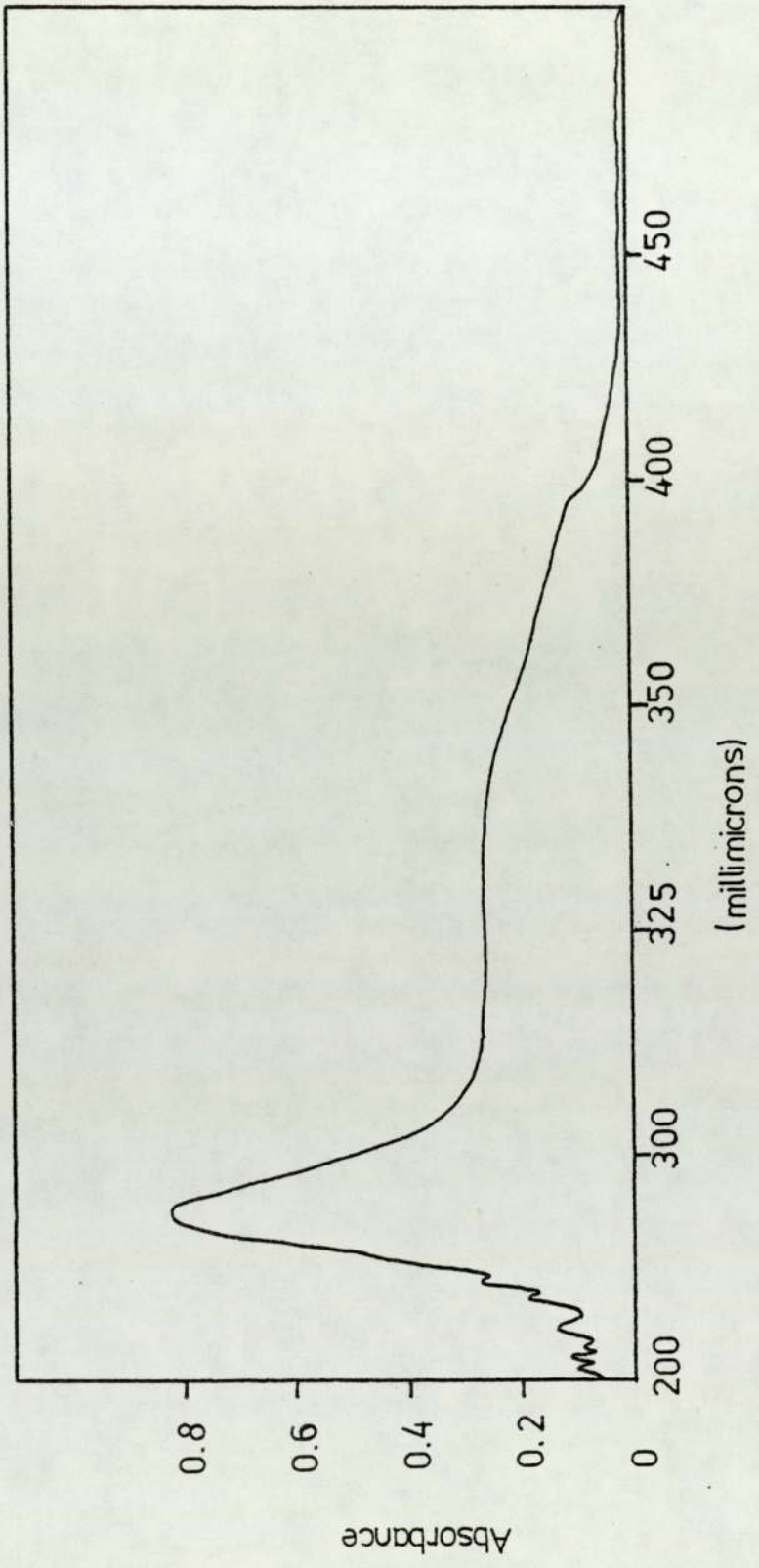
Stock Solution

A - 0.2 m solution of monobasic sodium phosphate, $Na_2H_2PO_4$ (27.58 gm/100 ml)

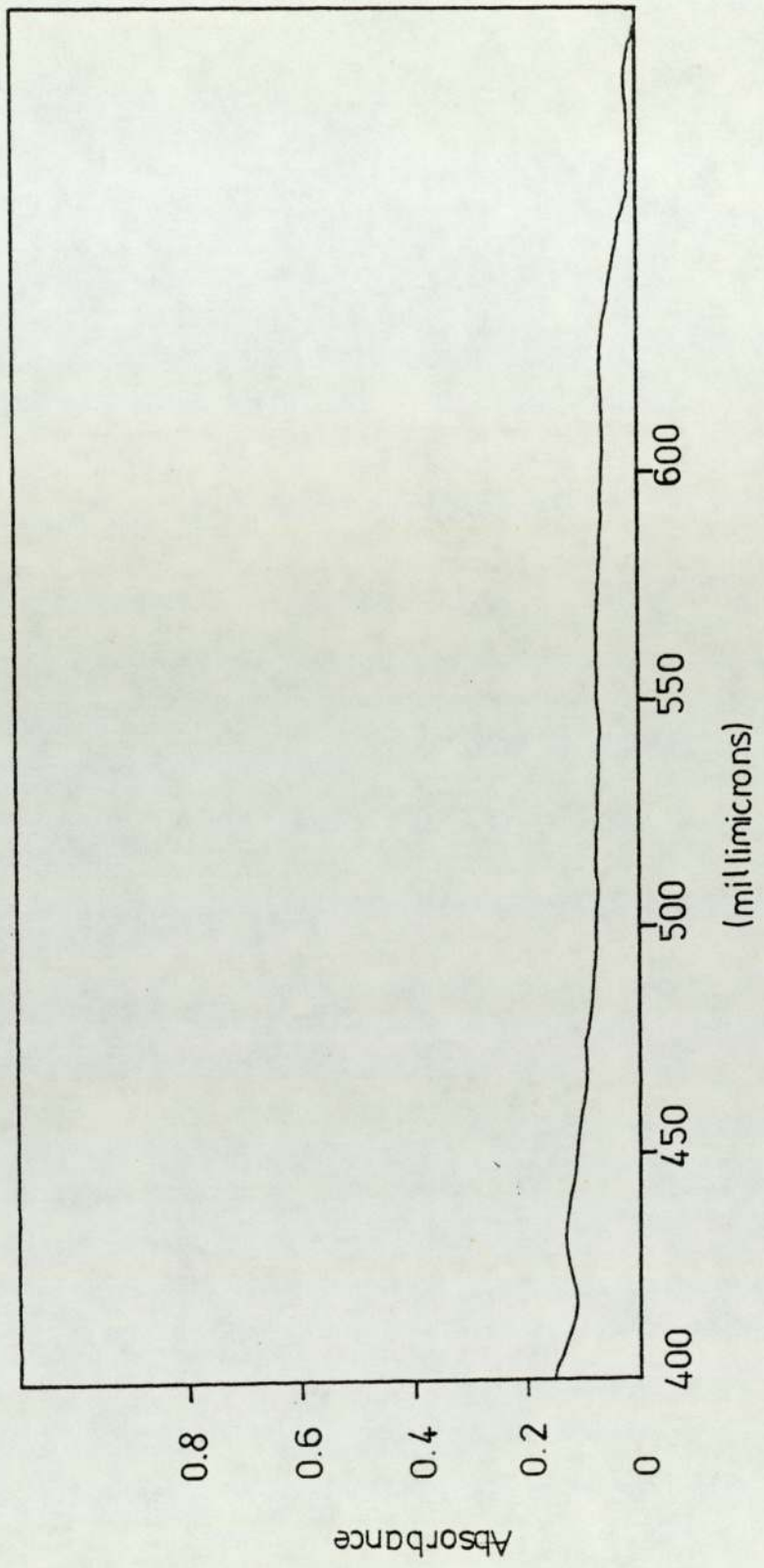
B - 0.2 m solution of dibasic sodium phosphate, Na_2HPO_4 (28.38 gm/100 ml)

pH 7.4 95 mls of solution A + 405 mls of solution B

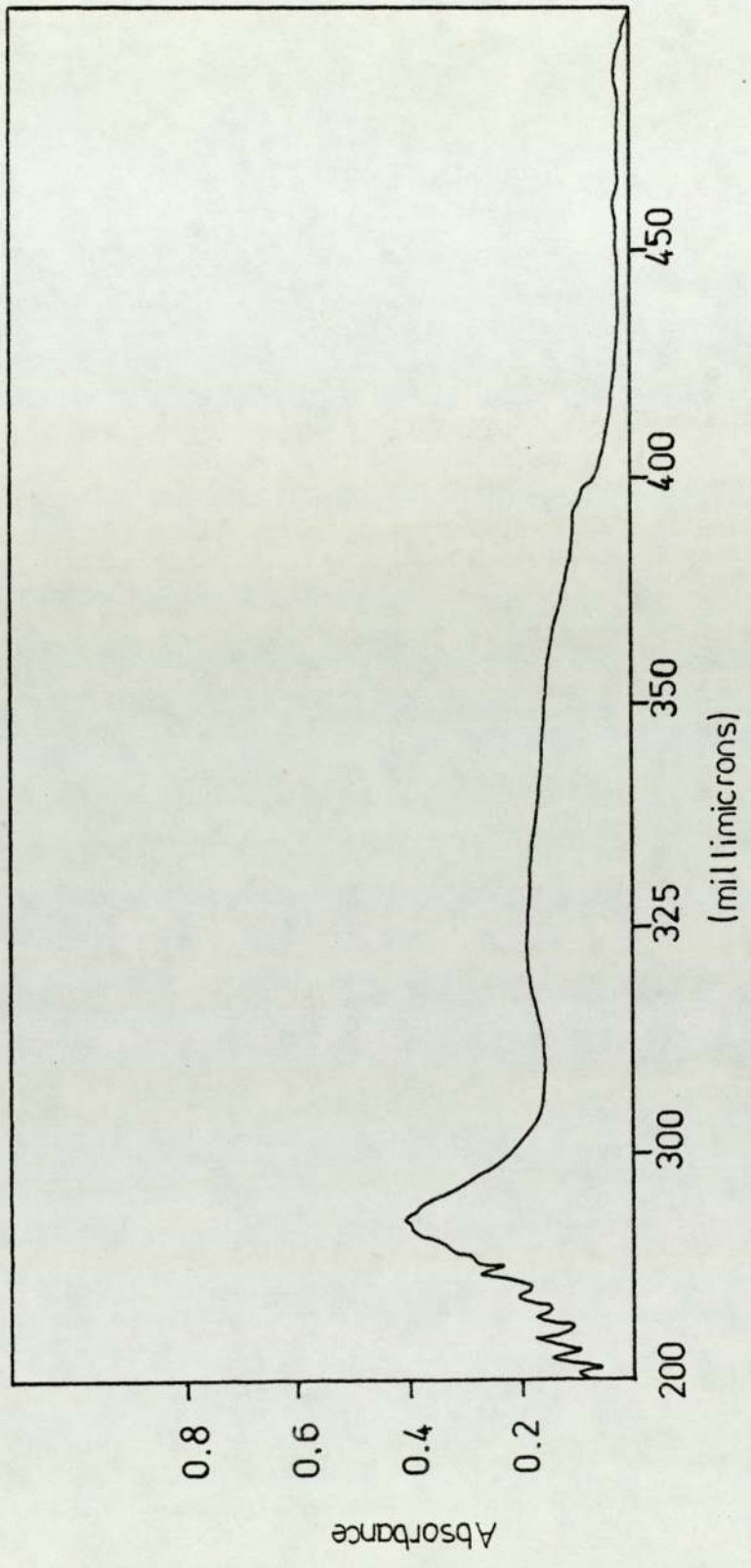
add 500 mls of distilled water + 9.5 gms of NaCl



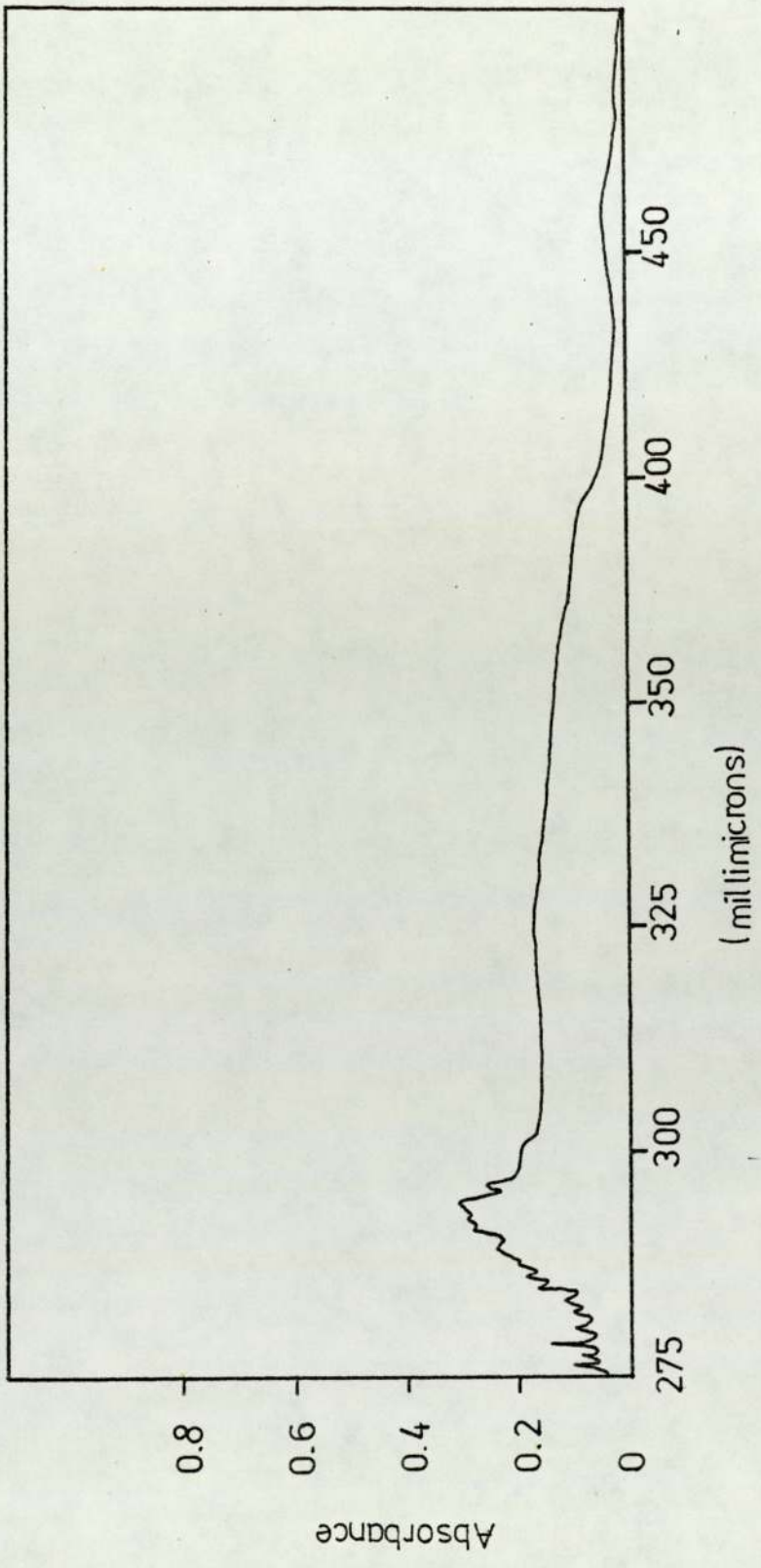
APPENDIX 2 U.V. SPECTRUM OF DISCOLOURED PERMALENS



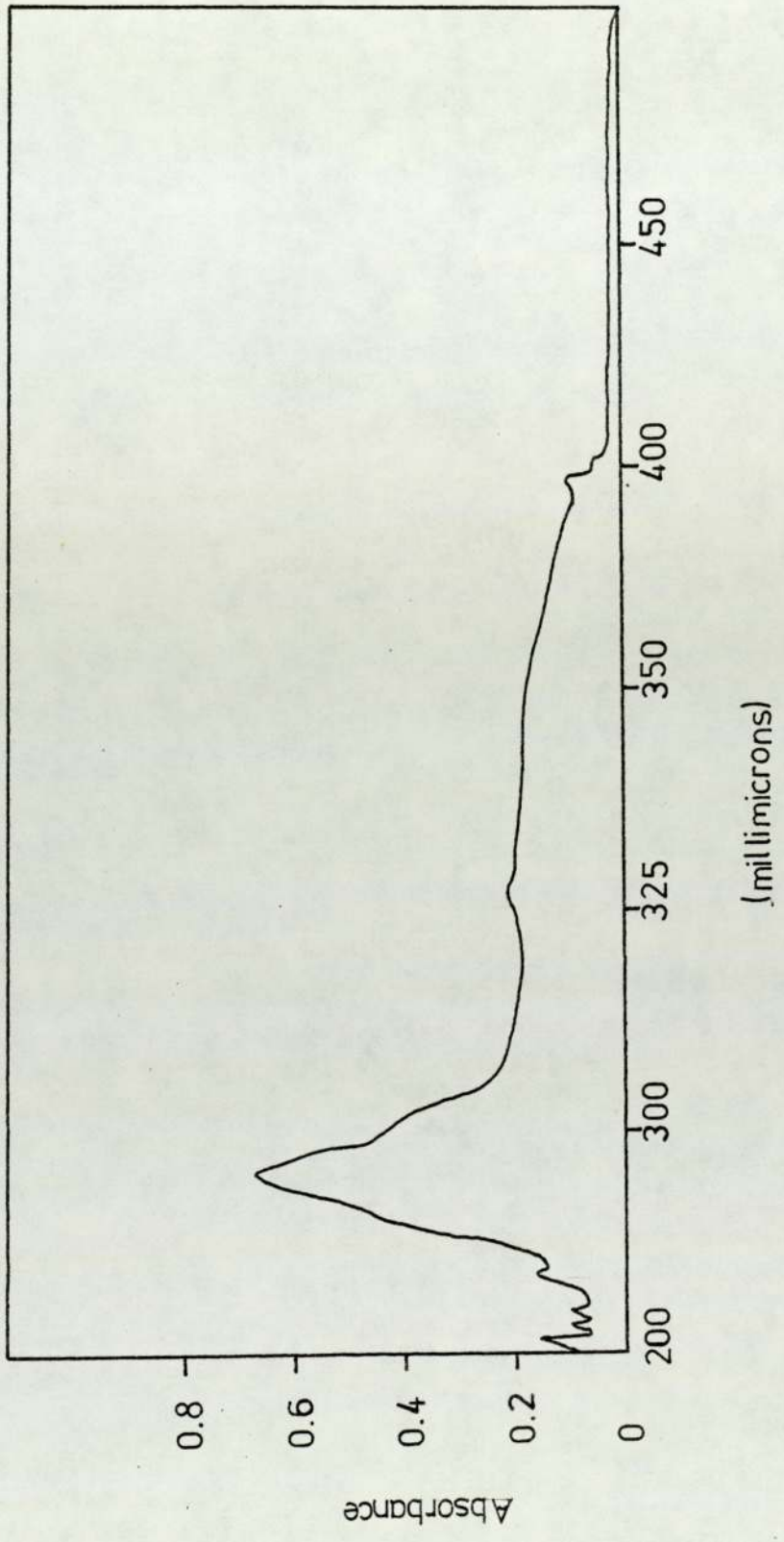
VISIBLE SPECTRUM OF DISCOLOURED PERMALENS



U.V. SPECTRUM OF DISCOLOURED X-TEN LENS



U.V. SPECTRUM OF DISCOLOURED
HYDRON LENS



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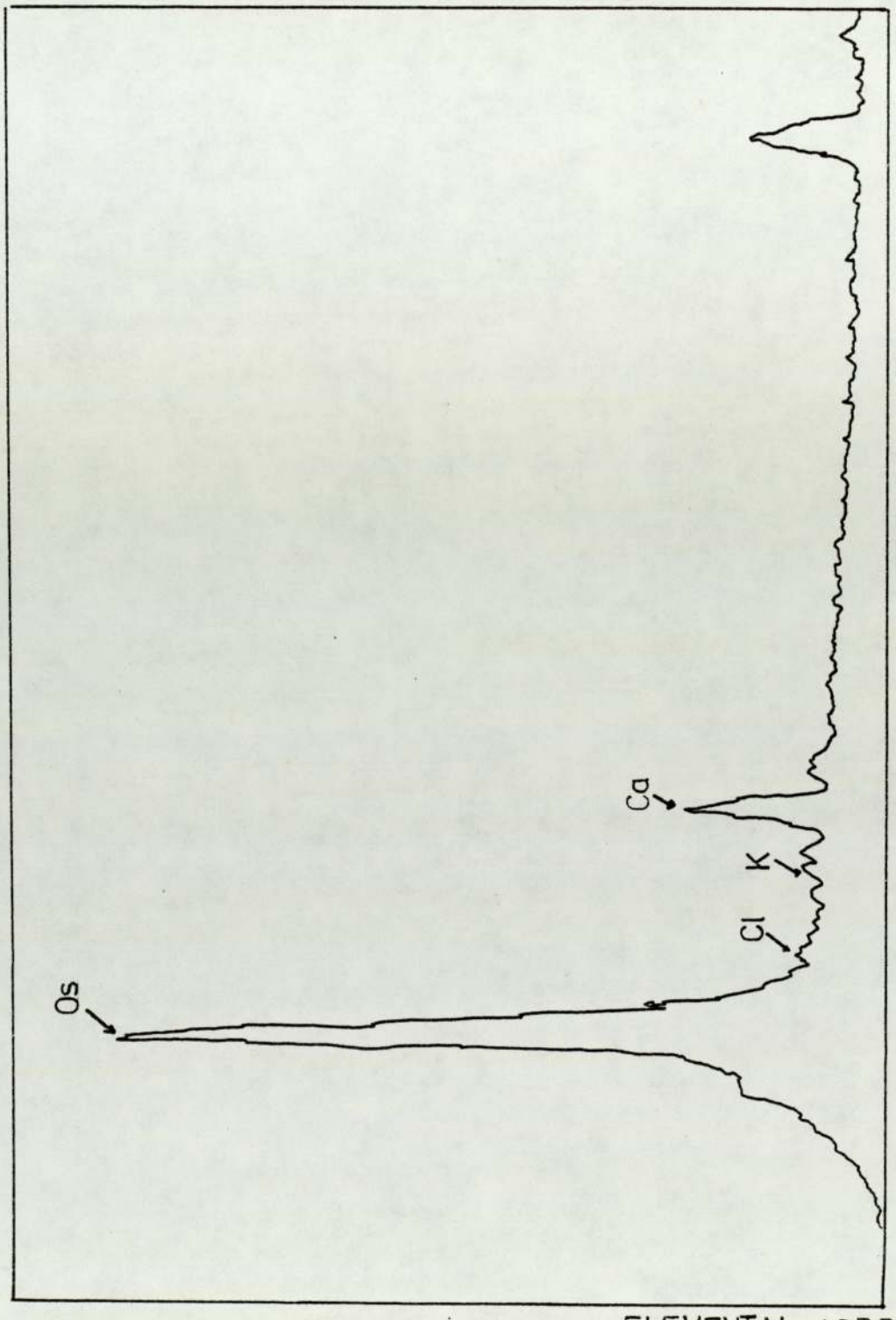
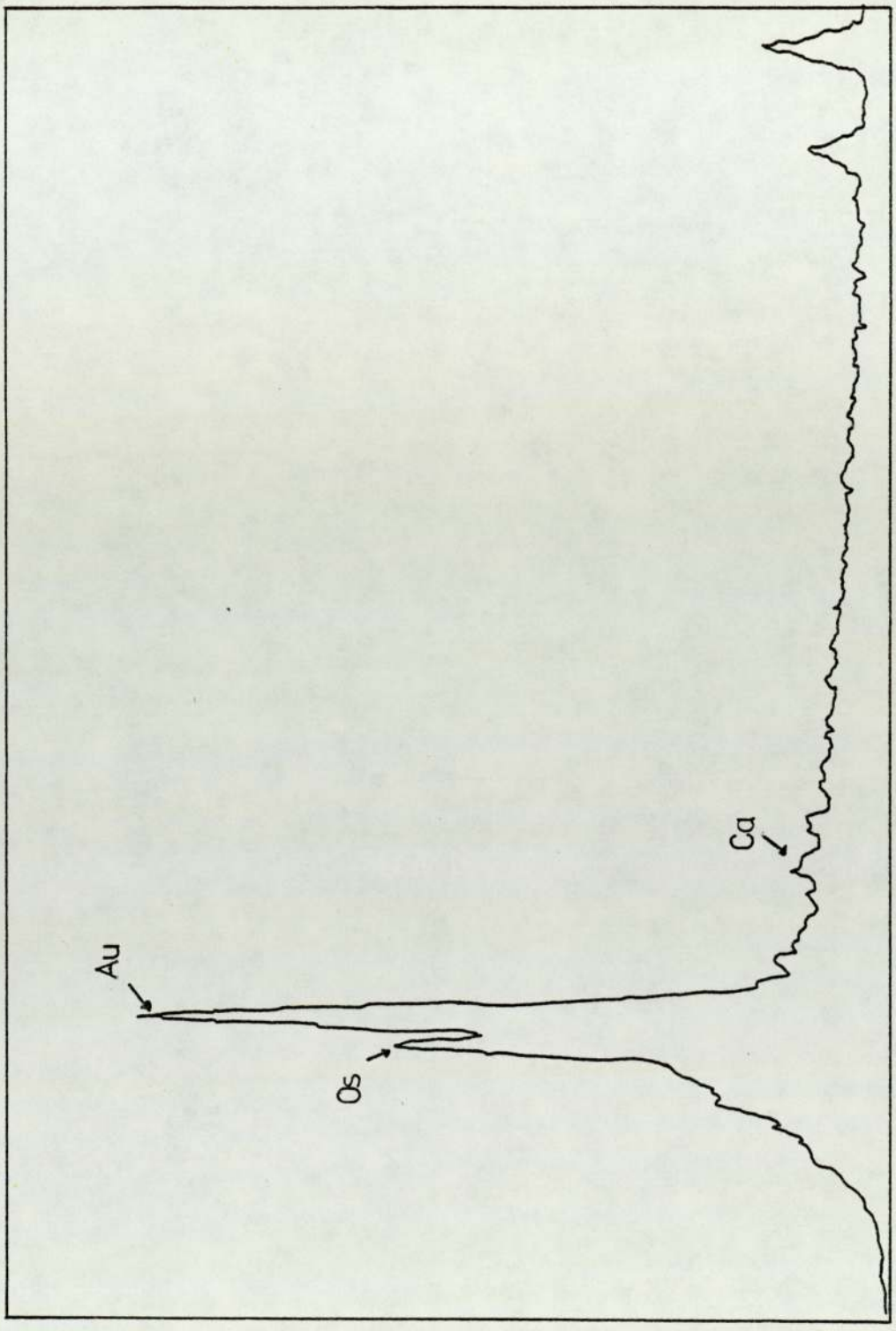


ELEMENTAL ABBREVIATIONS

Au - Gold
Os - Osmium
Ca - Calcium
K - Potassium
Cl - Chlorine
Si - Silicon
Al - Aluminium

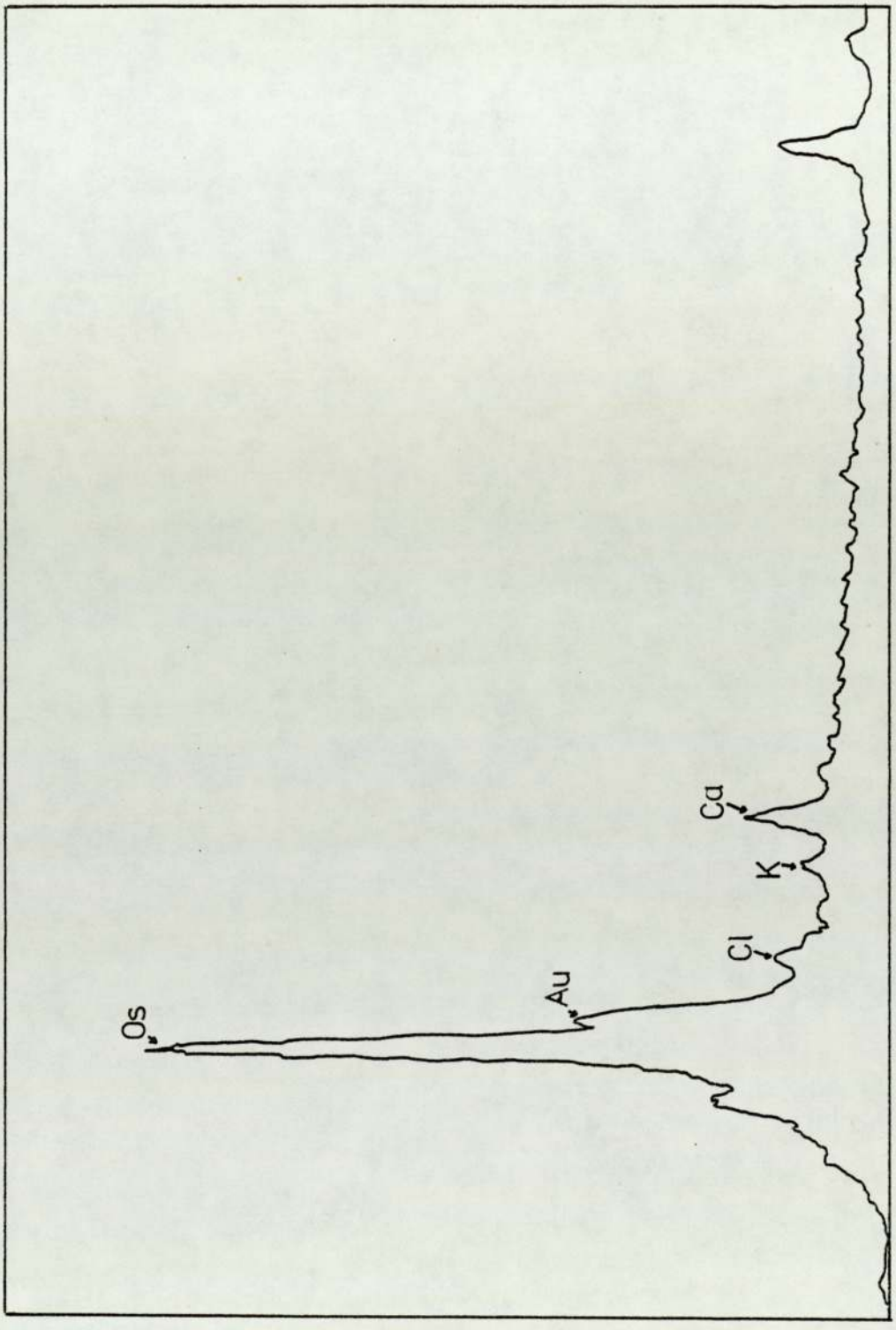
APPENDIX 3

EDXA SPECTRUM OF PLATEAU DEPOSIT



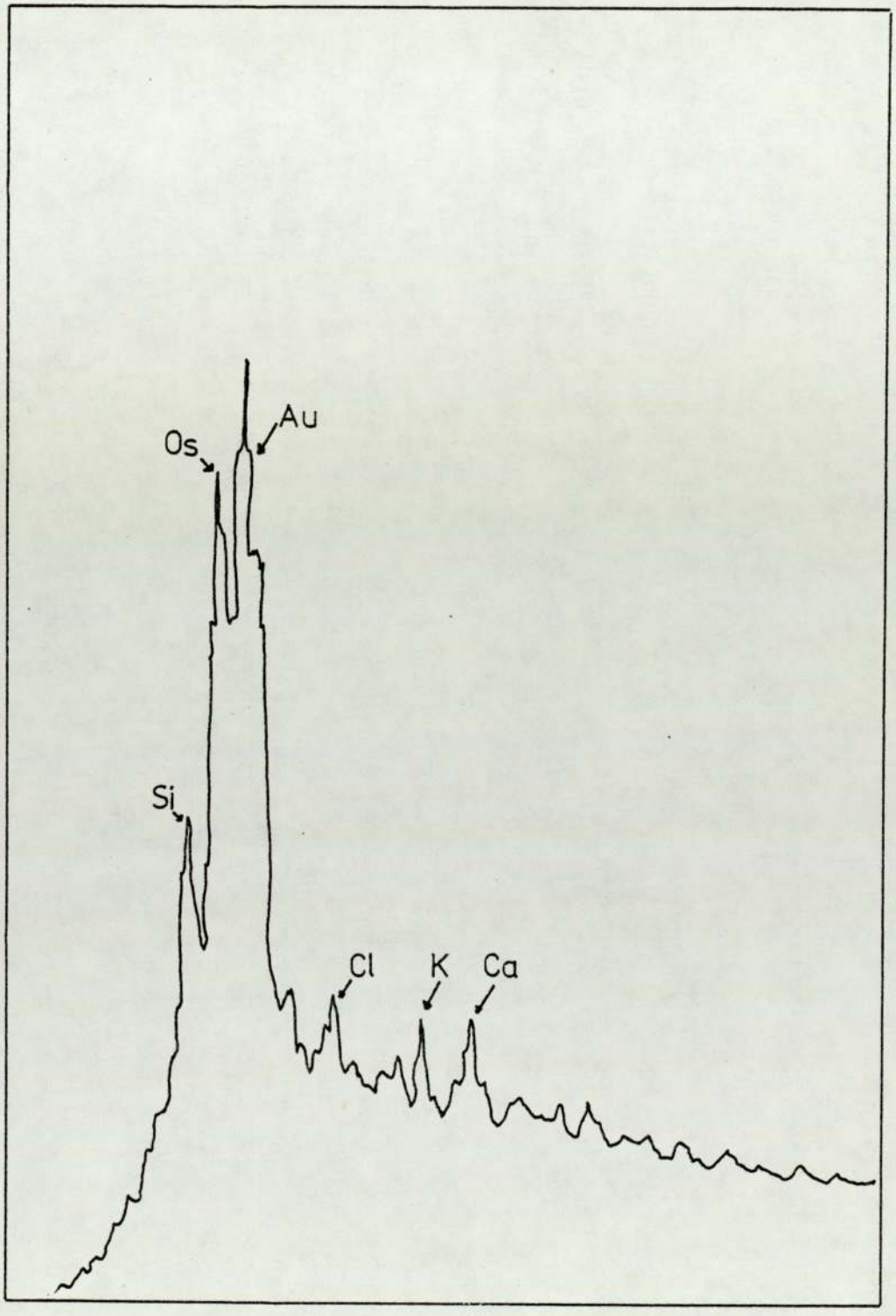
EDXA SPECTRUM OF DISCRETE ELEVATED DEPOSITS,

X-TEN LENS



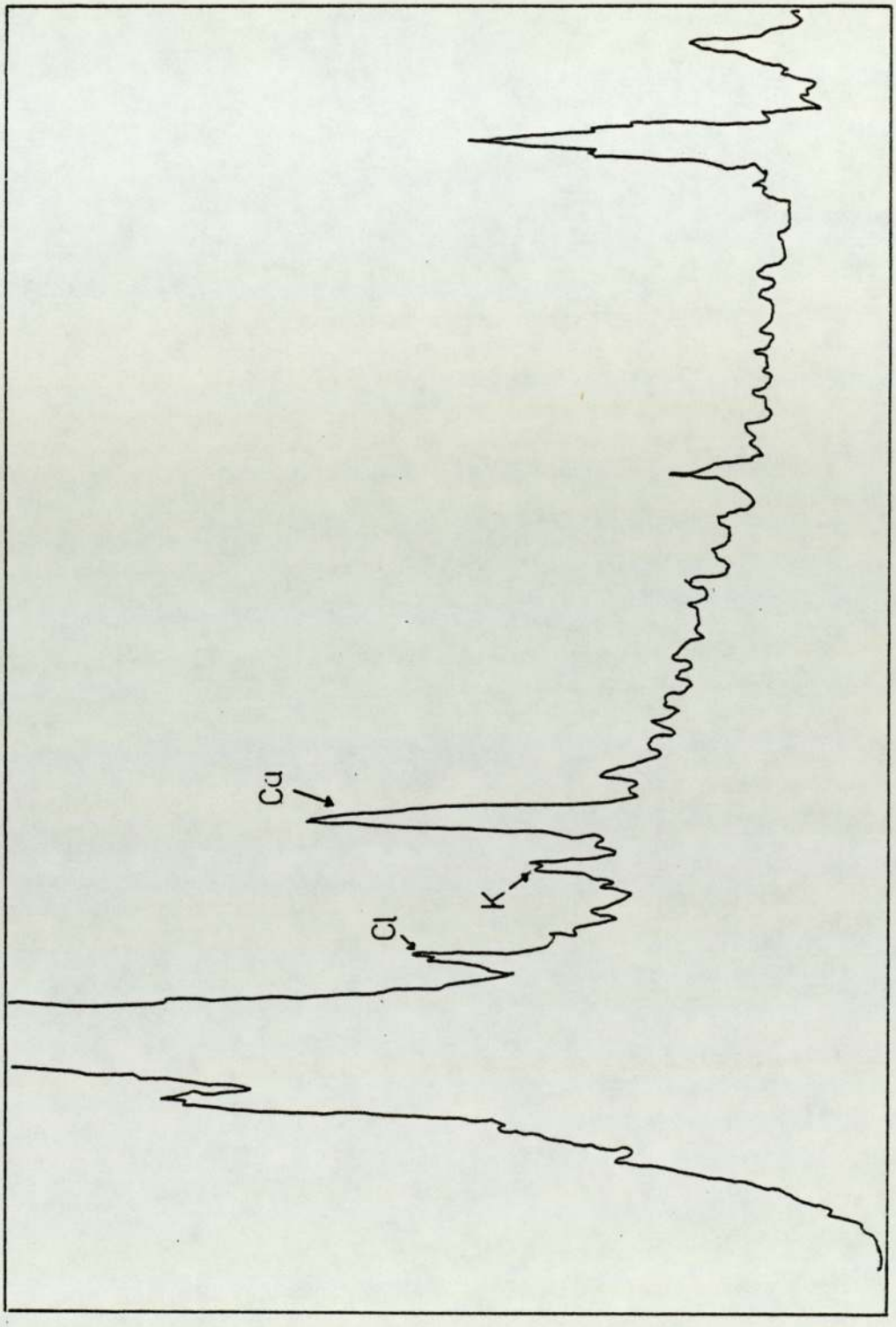
EDXA SPECTRUM OF DISCRETE ELEVATED DEPOSIT,

X-TEN LENS



EDXA SPECTRUM OF DEPOSIT GEOLOGY AND

ATTENDENT HYDROGEL MATRIX



EDXA SPECTRUM OF DISCRETE ELEVATED DEPOSIT-

PERMALENS - TO SHOW Ca, Cl, K PEAKS

REFERENCES

REFERENCES

1 Williams, D.F., Biomaterials and Biocompatibility in Fundamental Aspects of Biocompatibility (ed. Williams,D.F.) CRC Press, Florida (1981)

2 Williams,D.F., Biomaterials and Biocompatibility In Fundamental Aspects of Biocompatibility (ed. Williams D.F.) CRC Press, Florida (1981)

3 Bruck,S.D., Biomat., Med. Dev., Artif. Organs., (1979), 1, 79

4 Milligan,H.L., Cleminshaw,W.T., Edmark,K.W., J.Biomed. Mater. Res., (1973), 7, 445

5 Murphy,P.V., La Croix,A., Merchant,S., Bernhard,W., J.Biomed. Mater. Res. Symp., (1971), 1, 59

6 Mattson,J.S., Smith,C.A., Science, (1973), 181, 1055

7 Sawyer,P.N., Stanczewski,B., "Blood Vessels", (ed. S.Effent and J.D.Meyer-Erkelenz), (1976), 143, Springer-Verlag, Berlin, Germany

8 Sawyer,P.N., Burrows,C., Ogoniak,J., Smith,A.O., Wesolowski,S.A., Trans. Amer. Soc. Artif. Int. Organs, (1964), 10, 316

9 Gott,V.L., Furuse,A., Fed. Proc., (1971), 30, 1679

10 Lyman,D.J., Klein,K.C., Brash,J.L., Fritzinger,B.K., Tromb. Diath. Haemorrh., (1970), 23,120

11 Baier,R.E., Shafrin,E.G., Zisman,W.A., Science, (1968), 162, 1360

12 Baier,R.E., "Adhesion in Biological Systems" (ed. R.S.Manley), Ch.2, Academic Press, Ney York, (1970)

13 Baier,R.E., Dutton,R.C., Gott,V.L., Adv. Exptl. Med., (1970), 7, (Surf. Chem. Biol. Surfaces), 235

14 Kim,S.W., Lee,R.G., Lyman,D.L., Proc. Utah. Acad. Sci. Arts. Lett., (1971), 48 (1), 56

15 Levine,S.N., J. Biomed. Mater. Res. (1969), 3, 83

16 Neumann,A.W., Hope,C.J., Word,C.A., Herbert,M.A., Dunn,G.W., Znigg,W., J. Biomed. Mater. Res., (1975), 9 (2), 127

17 Lyman,D.J., Muir,W.M., Lee,I.J., Trans. Amer. Soc. Artif. Int. Organs, (1965), 11, 301

18 Ratner,B.D., "Biomaterials Interfacial Phenomena and Applications" (ed. S.L.Cooper, N.A.peppas) Am. Chem Soc. Adv. Chem. (1982), 199

19 Andrade,J.D., Lee,H.B., Jhon,M.S., Kim,S.W., Hibbs,J.B.(Jr), Trans. Amer. Soc. Artif. Int. Organs, (1973), 19, 1

20 Jhon,M.S., Andrade,J.D., J. Biomed. Mater. Res. (1973), 7, 509

21 Andrade,J.D., Med. Instrum. (1973), 7,(2), 110

22 Ratner,B.D., Hoffman,A.S., Amer. Chem. Soc. Symp. Ser., (1976), 31 (Hydrogels. Med. Relat. Appl. Symp. 1977), 1

23 Ratner,B.D., Hoffman,A.S., J. Appl. Polym. Sci., (1974), 18, 3183

24 Limber,G.K., Mason,R.G., Thromb. Res. (1975), 6, 421

25 Lyman,D.J., Metcalf,L.C., Albo,D., Richardo,K.F., Lamb,J., Trans.Am. Soc. Artif. Intern. Organs (1974), 208, 474

26 Weatherby,P.K., Horbett,T.A., Hoffman,A.S., Trans Am. Soc. Artif. Intern. Organs, (1976), 22, 242

27 Zucker,M.B., Vroman,L., Proc.Soc. Exptl. Biol. Med. (1969), 131, 318

28 Vroman,L., Adams,A.L., Surface Sci., (1969), 16, 438

29 Vroman,L., Thrombo. Diath. Haem. (1964), 10, 455

30 Vroman,L., Adams,A.L., J. Polym. Sci. Part C, (1971), 34, 159

31 Vroman,L., Adams,A.L., Klings,M., Fischer,G., Adv. Chem. Soc. (1975), 145 (Appl. Chem. Protein Int. Symp., 1973), 255

32 Vroman,L., Adams,A.L., Klings,M., Fed. Proc., (1971), 30(5), 1494

33 Lee,H.B., Shim,H.S., Andrade,J.D., A.C.S. Polym. Preprints, (1972), 13(2), 729

* 34 Ratner,B.D., Hurbett,T.A., Shuttleworth,D., Thomas,H.R., J. Colloid. Interface Sci. (1981). 83, 630-642

35 Hoffman,A.S., J. Biomed. Mater. Res. Symp., (1974), 5(1), 77

36 Morrissey,B.W., Stromberg,R.R., J. Coll. Int. Sci., (1974), 46(1), 152

37 Chattoraj,D.K., Bull,H.B., J. Amer. Chem. Soc., (1959), 81, 5128

38 Morrissey,B.W., Stromberg,R.R., Polym. Sci. Technol., (1975), 7 (Biomed. App. Polym.), 57

39 Kochwa,S., Litwak,R.S., Rosenfield,R.E., Leonard,E.F., Ann. N.Y. Acad. Sci., (1977), 283, 37

40 Morrissey,B.W., Ann. N.Y. Acad. Sci., (1977), 283, 50

41 Morrissey,B.W., Smith,L.E., Fenstermaker,C.A., Stromberg,R.R., Grant,W.H., Natl. Bur. Srand. (U.S.) Spec. Publ., (1978), 415, 83

42 Grant,W.H., Morrissey,B.W., Stromberg,R.R., Polym. Preprints, Amer. Chem. Soc., Div. Polym. Chem., (1978), 16(2), 163

43 Morrissey,B.W., Han,C.C., J. Coll. Int. Sci., (1978), 65(3), 423

44 Brash,J.L., Brophy,J.M., Faverstein,I.A., J. Biomed. Mater. Res., (1976), 10, 429

45 Kronick,P.L., Rembaum,A., Polym. Preprints, Amer. Chem. Soc., Div. Polym. Chem., (1975), 16(2), 157

46 Neumann,A.W., Moscarello,M.A., Singg,W., Hum,D.S., Chang,S.K., J. Polym. Sci., Polym. Symp., (1979), 66, 391

47 Packham,M.A., Evans,G., Glynn,M.F., Mustard,J.F., J. Lab. Clin. Med., (1969), 73(4), 686

48 Roohk,H.V., Pick,J., Hill,R., Hung,E., Bartlett,R.H., Trans. Amer. Soc. Artif. Int. Organs, (1976), 22, 1

49 Salzman,E.W., Fed. Proc., (1971), 30(5), 1503

50 Bruck,P.O., J. Biomed. Mater. Res., (1973), 7, 387

51 Lyman,D.J., Knulson,K., McNeill,B., Shibatani,K., Trans. Amer. Soc. Artif. Int. Organs, (1975), 21, 49

52 Kim,S.W., Peterson,R.V., Lee,E.S., J. Pharm. Sci., (1976), 65(5), 670

53 Bakan,J.A., Polym. Sci. Technol., (1975), 8, 213

54 Graham,N.B., McNeill,M.E., Biomaterials, (1984), 5, 27

55 Tighe,B.J., Br. Polym. J., (1964), 177, 678

56 Graham,R., Amer. J. Optom. Arch. Amer. Acad. Optom., (1970), 47, 50

57 Tighe,B.J., Br. Polym. J. (1976), 81, 71

58 Hill,R.M., Fatt,I., Amer. J. Optom. Arch. Amer. Acad. Optom., (1970), 47, 50

59 Hill,R.M., Fatt,I., Amer. J. Optom. Arch. Amer. Acad. Optom. (1964), 41, 678

60 Pedley,D.G., Tighe,B.J., J. Br. Contact Lens. Assoc., (1978), 2, 12

61 Pedley,D.G., Skelly,P.J., Tighe,B.J., Br. Polym. J. (1980), 12, 99

62 Dreifus,D., "Soft Contact Lenses. Clinical and Applied Technology" (ed. M.Ruben) Wiley, New York 1978, Chpt.2

63 Ruben,M., "The Cornea in Health and Disease" (Ed. P.D.Trevor-Roper. Royal Society of Medicine (1981)

64 Aichmair,H., "The Cornea in Health and Disease" (Ed. P.D.Trevor-Roper), Royal Society of Medicine (1981)

65 Callender,M.G., Woog,G., Int. Cont. Clinic, (1977), 4, 57

66 Barber,J.C., Cont. and Intraoc. Lens. Med. J., (1970), 3, 10

67 Woodward,E.G., "The Cornea in Health and Disease" (ed. P.D.Trevor-Roper), Royal Society of Medicine, (1981)

68 Ruben,M., Cont. and Intraoc. Lens. Med. J., (1976), 2, 18

69 Kenyon,K.R., Int. Ophthalmol. Clin. (1978), 19, 3

70 Isto,S., J. Philos. Trans. R. Soc. Lond. (Biol. Sci.), 1974, 208, 55

71 Mooq,F., Sci. Am., (1981), 245, 154

72 Peachey,L.D., Radmussen,H., J. Biophys. Biochem. Cytol. (1961), 10, 529

73 Dohlman,G.H., Invest. Ophthalnol., (1971), 10, 383

74 Mishima,S., Arch. Ophthalmol., (1967), 73, 233

75 Holly,F.J., Lemp,M.A., Exp. Eye Res., (1971), 11, 239

76 Mooseker,M.S., J. Cell Biol., (1976), 71, 417

77 Rodenwald,R., Newman,S.B., Karnvosky,M.J., J. Cell. Biol., (1976), 70, 541

78 Gipson,I.K., Anderson,R.A., Invest. Ophthalmol. Vis. Sci., (1977), 16, 161

79 Luft,J.H., Int. Rev. Cytol., (1976), 45, 291

80 Clamp,J.R., Br. Med. Bull., (1978), 34, 25

81 Wilson,L.A., Bacterial Conjunctivitus. External Diseases of the Eye, Wilson L.A., ed. Hagerstown, Harper & Row, 1979, pp 31-46

82 Wilson,L.A., Bacterial Corneal Ulcers, External Diseases of the Eye, Wilson,L.A., ed. Hagerstown, Harper & Row, 1979, 215-233

83 Feldman,G.L., Contacto., (1972), 16, 25

84 Jackus,M.N., in 'The Cornea in Health and Disease' (ed. Trevor-Roper,P.D.) Royal Society of Medicine, (1981)

85 Douliques,A., in 'The Cornea in Health and Disease', (ed. Trevor-Roper,P.D.), Royal Society of Medicine (1981)

86 Maurice,M., in 'The Cornea in Health and Disease', (ed. Trevor-Roper,P.D.), Royal Society of Medicine (1981)

87 Francois,J., Victoria-Troncoso,V., 'The Cornea in Health and Disease', (ed. Trevor-Roper,P.D.), Royal Society of Medicine, (1981)

88 Johnson,D.H., Bourne,W.M., Campbell,R.J., Arch. Ophthalmol. 81:214

89 Cogan,D.G., Kowabara,T., Trans. Ophthalmol. Soc. U.K. (1971), 91, 875

90 Sperling,D., Acta. Ophthalmol., (1980), 58, 96

91 Sherrard,E.S., Invest. Ophthal., (1978), 17, 322

92 Hedbys,B., Corneal Odema in "The Cornea in Health and Disease" ed. P.D.Trevor-Roper, Royal Society of Medicine (1971)

93 Harris,J.E., Nordquist,L.T., Am. J. Ophthal. (1955), 40, 100

94 Edelmann,I.S., Am. Rev. Physiol. (1961), 23, 37

95 Donn,A., Maurice,D.M., Mills,N.L., Arch. Ophthal. (1959). 62, 748

96 Freidman,E., Kupfer,C., Arch. Ophthal., (1960), 64, 892

97 Hodson,S., J. Physiol., (1974), 236, 271

98 Hodson,S., Miller,F., J. Physiol., (1976), 263, 563

99 Holly,F.J., Int. Ophthalmol. Clin., (1973), 13, 73

100 Maurice,D.M., Int. Ophthalmol. Clin., (1973), 13, 103

101 Holly,F.J., Am. J. Optom., (1980), 57, 252

102 Ehlers,N., Acta Ophthalmol. Suppl., (1965), 81, 136

103 Futukama,R.E., Poloe,K.A., Am. J. Optom., (1978), 55, 69

104 Larke,J., (in press) 1985

105 Nicolaides,N., Kaitaranta,J.K., Rawdah,T.N., Macy,J.I., Boswell,F.M., Smith,R.E., Invest. Ophthalmol,. (1981), 20, 522

106 Tiffany,J.M., Exp. Eye Res., (1978), 27, 289

107 Pez,O., Arch. Ophtahl., (1897), 5, 82

108 Linton,R.G., Cumow,D.A., Riley,W.J., Brit. J. Ophthal. (1961), 45, 718

109 Keith,C.G., Trans. Ophthalmol. Soc. U.K., (1967), 87, 85

110 Andrews,J.S., Exp. Eye Res., (1970), 10, 223

111 Gachon,A.M., Verehe,P., Betail,G., Dastugue,B., Exp. Eye. Res., (1979), 29, 539

112 Janssen,T., Van Bijsterueld,O.P., Invest. Ophthalmol. Vis. Sci., (1983), 24, 623

113 Chao,C.W., Vergnes,J.P., Freeman,I.L., Brown,S.I., Exp. Eye. Res., (1980), 30, 411

114 Ridley,F., Proc. Roy. Soc. Med., (1928), 21, 1495

115 Bowman,W.C., Rand,M.J., Textbook of Pharmacology, Blackwell, London, 1980

116 Haggerty,C., Doctoral Thesis, University of Aston in Birmingham, 1979

117 Gillette,T.E., Allansmith,M.R., Greiner,J.V., Jansoz,M., Amer. J. Ophthalmol., (1980), 89, 724

118 Barnett,E.V., J. Immunol., (1968), 100, 1093

119 Sapse,A.T., Bonavida,B., Stone,W., Sercarz,F.E., Arch. Ophthalmol. (1969), 81, 815

120 Thompson,R., Gallardo,E., Am. J. Ophthalmol., (1941), 24, 635

121 Freidland,B.R., Anderson,D.R., Foster,R.K., Amer. J. Ophthalmol., (1972), 74, 52

122 Tiffany,J.M., Bron,A.J., Trans. Ophthal. Soc. U.K., (1978), 98, 335

123 Ford,L.C., Delange,R.J., Petty,R.W., Amer. J. Ophthal., (1976), 81, 30

124 Friedland,B.R., Anderson,D.R., Foster,R.K., Ibid., (1972), 74, 52

125 Broekhuyse,R.M., Invest. Ophthalmol., (1974), 13, 550

126 Franklin,R.M., Kenyon,K.R., Tomasi,T.B., J. Immunol., (1973), 110, 984

127 Kijlstra,A., Jeurissen,S.H.M., Konig,K.M., Brit. J. Ophthalmol., (1983), 67, 199

128 Magii,J., Liakos,G.M., Goulding,N., Seal,D.V., Br. J. Ophthalmol. (1984), 68, 316

129 Veerhuis,R., Kijlstra,A., Exp. Eye. Res., (1982), 34, 257

130 Kijlstra,A., Jevrissen,S.M.H., Immunology (1982), 47, 263

131 Arnold,R.R., Cole,M.F., McGhee,J.R., Science, (1977), 197, 263

132 Bullen,J.J., Armstrong,J.D., Immunology, (1979), 36, 781

133 Arnold,R.R., Russell,J.E., Champion,W.J., Brewer,M., Gathier,J.J., Infect. Immun. (1982), 35, 792

134 Broxmeyer,H.E., Smithyman,A., Eger,R.R., Meyer,P.A., de Soussa,M., J. Exp. Med., (1978), 148, 1052

135 Badgy,G.C., Rigas,V.D., Bennett,R.M., Vanderbark,D.A., Garewell,H.S., J. Clin. Invest., (1981), 68, 56

136 Bonavida,B., Sapse,A.T., Am. J. Ophthalmol. (1968), 66, 70

137 Josephson,A.S., Weiner,R.S., J. Immunol. (1968), 100, 1080

138 Sapse,A., Bonavida,B., Stone,W., Sercarz,E.E., Am. J. Ophthalmol., (1968), 66, 76

139 Renner,O.M., Kaiser,D., Sollberger,H., Joller-Jemelka,S., Human. Generic., (1974), 23, 73

140 Allansmith,M.R., Int. Ophthalmol. Clin., (1973), 13, 193

141 Brauninger,G.E., Centifanto,Y.M., Am. J. Ophthalmol. (1971), 72, 558

142 McCellan,B.H., Whitney,C.R., Newman,P.L.P., Allansmith,M.R., Am. J. Ophthalmol., (1973), 76, 89

143 Bluestone,R., Easty,D.L., Goldberg,L.S., Jones,B.R., Pettit,T.H., Brit. J. Ophthalmol., (1975), 59, 279

144 Azen,E.A., Biochem. Genet. (1978), 14, 225

145 Josephson,A.S., Wald,A., Proc. Soc. Exp. Biol. Med., (1969), 131, 677

146 Dhir,S.P., Gars,S.K., Sharma,Y.R., Lath,N.K., Am. J. Ophthalmol., (1979), 87, 403

147 Abelson,M.B., Sorer,N.A., Simon,M.A., Am. J. Ophthal. (1977), 83, 417

148 Thaysen,J.H., Thorn,N.A., Am. J. Physiol., (1954), 178, 60

149 Huth,S.W., Hirano,P., Leopold,I.H., Arch. Ophthalmol. (1980), 98, 122

150 Bothelho,S.Y., Sci. Am., (1964), 211, 78

151 Terry,J.E., Hill,R.M., Arch. Ophthalmol., (1978), 96, 120

152 Carney,L.G., Hill,R.M., Arch. Ophthalmol., (1978), 94, 821

153 Fatt,I., Exp. Eye. Res., (1968), 7, 413

154 Chao,C.C., Vergnes,J.P., Brown,S.I., Exp. Eye. Res., (1983), 36, 139

155 Adams,A.D., Arch. Ophthalmol., (1979), 97, 730

156 Tripathi,R.C., Tripathi,B.J., Ruben,M., Ophthalmology, (1980), 87, 365

157 Korb,D.R., Henriquez,A.S., J. Am. Optom. Assoc., (1980), 51, 243

158 Lowther,G.E., Hilbert,J.A., Am. J. Optom. Physiol. Optics, (1975), 52, 687

159 Tripathi,R.C., Ruben,M., Tripathi,B.J., Soft Lens Spoilation in Soft Contact Lenses Clinical and Applied Technology (ed.Ruben,M.), Wiley, New York, 1978

160 Stewart,B.V., Int. Contact Lens Clinic, (1978), 5, 269

161 Dohlman,C.H., Borvchoff,S.A., Mobilia,E.F., Arch. Ophthalmol., (1973), 90, 367

162 Freeman,M.I., Bull. Mason. Clin. (1976-77), 30, 141

163 Binder,P.S., Worthen,D.M., Am. J. Ophthalmol., (1977), 83, 549

164 Kersley,H.J., Kerr,C., Pierse,D., Br. J. Ophthalmol., (1979), 61, 38

165 Stein,H.A., Slatt,B.J., Int. Contact Lens Clin. (1977), 14, 35

166 Dahl,A.A., Brocko,E.R., Am. J. Ophthalmol., (1978), 85, 454

167 Doughman,D.J., Mobilia,E., Drago,D., Ann. Ophthalmol. (1975), 7, 345

168 Eriksen,S., Ann. Ophthalmol., (1975), 7, 1223

169 Karageozian,H.L., Contacto., (1976), 20, 5

170 Ganjo,S.N., Cordrey,P., Optician, (1977), 173, 8

171 Tripathi,R.C., Tripathi,B.J., Applied Physiology and Anatomy. Tears, Cornea, Conjunctiva and Ocular Adnexa. in Contact Lens Practice (ed. Ruben,M.), Balliere Tindall, London, 1975

172 Shively,C.P., Accessory Solutions Utilised in Contact Lens Care and Practice. In Soft Contact Lenses. Clinical and Applied Technology, (ed. Ruben,M.), Wiley New York, (1978)

173 Spring,T.F., Med. J. Aust., (1974), 1, 449

174 Allansmith,M.R., Korb,D.R., Greiner,J.V., Am. J. Ophthalmol. (1977), 83, 697

175 Refojo,M.F., Holly,F.J., Contact. Intraocular. Lens Med. J., (1977), 3, 23

176 Allen,J., Botting,R., Sharp,A., Tuffery,A.A., Optician. (1978), Jan.6,8

177 Bailey,N.J., J. Am. Optom. Ass., (1975), 46, 214

178 Wedler,F.C., J. Biomed. Mater. Res. (1977), 11, 525

179 Hathaway,R.A., Lowther,G.E., Int. Contact Lens Clin. (1976), 3, 27

180 Kleist,F.D., Int. Contact Lens Clin., (1979), 6, 120

181 Kleist,F.D., Int. Contact Lens Clin., (1979), 6, 177

182 Morgan,J.F., Titus,L.O., Mills,J.A., Blaine,J.E., Cont. Intraocular Lens Med. J., (1977), 3, 16

183 Hathaway,R.A., Lowther,G.E., Aust. J. Optom., (1978), 61, 92

184 Koetting,R.A., Contacto., (1976), 20, 20

185 Eriksen,S., J. Br. Contact Lens Assoc., (1981), 4, 36

186 Hathaway,R.A., Lowther,G.E., J. Am. Optom. Assoc., (1978), 9, 266

187 Ruben,M., Tripathi,R.C., Winder,A.F., Br. J. Opthalmol. (1975), 59, 141

188 Gassett,A.R., Lobo,L., Hoode,W., Am. J. Ophthalmol. (1977), 83, 115

189 Klintworth,G.K., Reed,J.W., Hawkins,H.K., Invest. Ophthalmol. Vis. Sci. (1977), 16, 158

190 Bloomfield,S.E., David,S.D., Rubin,A.L., Annal. Ophthalmol., (1978), 10, 355

191 Koetting,R.A., Boyd,N.A., Int. Contact Lens Clin., (1976), 3, 37

192 Hilbert,J., Lowther,G.E., King,J., J. Am. Optom. Physiol. Opt., (1976), 53, 51

193 Gassett,A.R., Lobo,L., Houde,W., Int. Contact Lens Clin., (1978), 2, 64

194 Winder,A., Tripathi,R.C., Proc. Royal Soc. Med., (1975), 68, 57

195 Freiberg,J., Int. Contact Lens Clin., (1977), 4, 63

196 Ruben,M., Ocular Pathogens and Contact Lnes Hygeine. In Soft Contact Lenses. Clinical and Applied Technology, (ed. Ruben,M.), Wiley, (1978)

197 Roth,H.W., Contact Intraoc. Lens Med. J., (1978), 4, 38

198 Eriksen,S.P., Hydrophilic Lens Sterility and Disinfection. In Soft Contact Lenses. Clinical and Applied Technology. (ed. Ruben,M.), Wiley, (1978)

199 Bernstein,H.N., Am. Ophthalmol. (1973), 5, 317

200 Vannas,A., Rubsuvvaana,P., Klin,M Augenhelk (1977), 170, 873

201 Filppi,J.A., Pfister,R.M., Hill,R.M., Am. J. Optom. (1973), 50, 553

202 Sagan,W., Arch. Ophthalmol., (1976), 94, 168

203 Shapiro,N., Minn. Med., (1974), 57, 943

204 Yamamoto,G.K., PavannLangston,D.P., Cleveland-Stowe,G., Albert,D.M., Annal. Ophthalmol., (1979), 11, 1731

205 Matas,B.R., Spencer,W.H., Hayes,T.L., Arch. Ophthalmol., (1972), 88, 287

206 Fowler,S.A., Greiner,J.V., Allansmith,M.R., Arch. Ophthalmol., (1979), 97, 659

207 Stewart,G., Int. Contact Lens Clin., (1979), 6, 54

208 Ruben,M., Contact Lens Med. J., (1976), 2, 39

209 Walaas,E., Wataas,S.O., Arch. Biochem. Biophys., (1961), 95, 151

210 Du'lock,J.D., Arch. Biochem. Biophys., (1960), 91, 189

211 Hedin,C.A., Arch. Dermatol., (1977), 113, 1533

212 Hubbard,W.L., Contact Lens J., (1975), 9, 39

213 Mackeen,D.L., Green,K., J. Pharm. Pharmac., (1978), 30, 678

214 Plaut,B.S., Meakin,B.J., Davies,D.J.G., J. Pharm. Pharmacol., (1980), 32, 453

215 Petricciani,R., Krezanoski,J., Contacto., (1977), 21, 6

216 Ganju,S.N., Gordrey,P., Eye (1977), 17, May/June

217 Loran,D.F.C., Contact Lens J., (1973), 4, 3

218 Du Pont and Co. Ltd., Ind. Chem Eng., (1936), 28, 1160

219 Wichterle,O., Lim,D., Nature, (1960), 185, 117

220 Sevcik,S., Stramberg,J., Schmidt,P., J. Polym. Sci. (1967), 16, 821

221 Stramberg,J., Sevick,S., Coll. Czech. Chem. Comm., (1966), 31, 1009

222 Wichterle,O., Lim,P., U.S.Pat. 3,2220,960, (1965)

223 Andrade,J.D., Konitomo,K., Van Wagener,R., Kastigir,B., Cough,D., Kolff,W.J., Trans. Amer. Soc. Artif. Int. Organs. (1971), 17, 222

224 Spacek,P., Kubin,M., J. Biomed. Mater. Res., (1973), 7, 201

225 Ratner,B.D., Miller,I.F., J. Biomed. Mater. Res., (1973), 7, 353

226 Middleton,I.P., 'Acrylamide based Hydrogels for for Continuous Wear Contact Lenses, Ph.D. Thesis, University of Aston, (1981)

227 Jenkins,L.B., Kredal,F.E., McCord,W.M., A.M.A. Arch. Surg., (1956), 72, 612

228 Gee,H., Tighe,B.J. U.S. Patent 4,430,458 (1984)

229 Ng.C.O., Synthetic Hydrogels in Contact Lens Applications, Ph.D. Thesis, University of Aston, (1974)

230 Baker,D., Protein Adsorption of Polymer Surfaces, Ph.D. Thesis, University of Aston, (1982).

231 Jensson,O.A., Personal Communication

232 Tuffery,A.A., Personal Communication

233 Walker,J.F., Formaldehyde 3rd ed. Vans Nostrand-Reershold, New York, (1964)

234 Wolman,M., Greco,J., Stain Technol. (1952), 29, 317

235 Wolman,M., Int. Rev. Cytol., (1955), 4, 79

236 Hayat,M.A., Fixation for Electron Microscopy, Academic Press, New York, (1981)

237 Pearse,A.G.E., Histochemistry Theoretical and Applied Vols.I and II 3rd ed., Churchill, London, (1968)

238 Bancroft,J.D., Histochemistry, 3rd ed. Churchill, (1968)

239 Levy,W.A., Herzog,I., Sozuki,K., Katzman,R., Scheinberg,L., Cell. Biol., (1967), 27, 119

240 Boyde,A., Wood,C., J. Microsoc., (1969), 90, 221

241 Anderson,T.F., Trans. N.Y. Acad. Sci. Ser. II., (1951), 13, 130

242 Hearing,V.J., Ekel,T.M., Montague,P.M., Biochem. Biophys. Acta., (1980), 611, 251

243 Miranda,M., Urbani,G., DiVito,L., Biochem. Biophys. Acta., (1979), 585

244 Skelly,P.J.S., Bowers,R.W.J., Raistrick,R., Internal Report, Lens Discolouration, (1982)

245 de Carle,J., Hydrophilic Lenses for Continuous or Extended Wear. In Soft Contact Lenses Clinical and Applied Technology, (ed. Ruben,M.), Wiley, New York, (1978)

246 Barka,T., Anderson,P.J., Histochemistry. Theory Practice and Bibliography, Harper Row, New York, (1965)

247 Wollman,M., J. Cell. Biol., (1972), 55, 272

248 Khan,A.A., Riemersma,J.C., Booiji,H.L., J. Histochem. Cytochem., (1961), 9, 560

249 Baker,H.R., Leach,P.B., Singleterry,C.R., Zisman,W.A., Ind. Eng. Chem., (1967), 59, 29

250 Holly,F.J., Int. Ophthalmol. Clinics, (1973), 13,1

251 Brown,E., Personal Communication

252 Owen,D.R., Zone,R., Armer,T., Kilpatrick, 'Biomaterials Interfacial Phenomena and Applications', (ed. S.L.Cooper, N.A.Peppas), Am. Chem. Soc. Adv. Chem., (1982), 199