BIOCHEMICAL MARKERS AND CONTACT LENS WEAR

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Summary

The project objective was to develop a reliable selection procedure to match contact lens materials with individual wearers by the identification of a biochemical marker for assessment of in-eye performance of contact lenses. There is a need for such a procedure as one of the main reasons for contact lens wearers ceasing wearing contact lenses is poor end of day comfort i.e. the lenses become intolerable to the wearer as the day progresses. The selection of an optimal material for individual wearers has the potential benefit to reduce drop out, hence increasing the overall contact lens population, and to improve contact lens comfort for established wearers.

Using novel analytical methods and statistical techniques, we were able to investigate the interactions between the composition of the tear film and of the biofilm deposited on the contact lenses and contact lens performance. The investigations were limited to studying the lipid components of the tear film; the lipid layer, which plays a key role in preventing evaporation and stabilising the tear film, has been reported to be significantly thinner and of different mixing characteristics during contact lens wear.

Different lipid families were found to influence symptomatology, in vivo tear film structure and stability as well as ocular integrity. Whereas the symptomatology was affected by both the tear film lipid composition and the nature of the lipid deposition, the structure of the tear film and its stability were mainly influenced by the tear film lipid composition. The ocular integrity also appeared to be influenced by the nature of the lipid deposition.

Potential markers within the lipid species have been identified and could be applied as follows:

- When required in order to identify a problematic wearer or to match the contact lens material to the contact lens wearer, tear samples collected by the clinician could be dispatched to an analytical laboratory where lipid analysis could be carried out by HPLC.
- A colorimetric kit based on the lipid markers could also be developed and used by clinician directly in the practice; such a kit would involve tear sampling and classification according to the colour into " Problem", " Border line " and "Good" contact lens wearers groups. A test kit would also have wider scope for marketing in other areas such as general dry-eye pathology.

To my family

Acknowledgements

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List of abbreviations

AMU	Atomic Mass Unit
ANOVA	Analysis of variance
ASYMPT.	Asymptomatic
CHAID	Chi Square Automated Interaction Detector
C&RT	Classification and Regression Tree
CL	Contact Lens
CLRC	Contact Lens Research Consultants
DAA	diacetone acrylamide
Disp	Dispensing Visit
Dk	Oxygen permeability
ELISA	Enzyme Linked Immuno Assay
ESCA	Electron Spectroscopy for Chemical Analysis
FDA	Food and Drug Administration
GAGs	Glycoaminoglycans
GC	Gas Chromatography
GMA	Glyceryl methacrylate
HEMA	2-hydroxyethyl methacrylate
HPLC	High Performance Liquid Chromatography
IEF	Iso Electro Focusing
IgA	Immunoglobulin A
IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IR	Infrared
ISO	International Standards Organisation
KCS	Kerato Conjunctivitis Sicca
LSD	Least Significant Difference
LHYPMAX	Limbal hyperaemia - maximum value
Max	Maximum
MAA	Methacrylic acid
MMA	Methyl methacrylate
MeOH	Methanol
Min	Minimum
MTR-FTIR	Multiple Total Reflectance-Fourier Transform Infrared Spectroscopy
MS	Mass Spectroscopy
NIBMED	Non Invasive Break Up Time - median
NIBMIN	Non Invasive Break Up Time - minimum
NIBUT	Non Invasive Break Up Time
NS	Not Significant
NVP	N Vinyl-Pyrrolidone
ph/CE	Ratio phospholipids vs. cholesterol ester
POTF	Pre Ocular Tear Film

POTFPAT	Pre Ocular Tear Film lipid layer mixing Pattern
PLTF	Pre Lens Tear Film
PLTFPAT	Pre Lens Tear Film lipid layer mixing pattern
PLTFTYP	Pre Lens Tear Film Break up type
Quart.	Quartile
QUEST	Quick, Unbiased Efficient Statistical Tree
RGP	Rigid Gas Permeable
RIA	Radio Immuno Assay
SDS PAGE	Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis
SEM	Scanning Electron Microscopy
SIMS	Secondary Ion Mass Spectroscopy
SNK	Student - Newman - Keuls
STD	Standard Deviation
SYMPT.	Symptomatic
TEM	Transmission Electron Microscopy
TLC	Thin layer chromatography
TSPA	Tear Specific Pre Albumin
UV	Ultraviolet
VPC	Vapor Phase Chromatography

1/52	1 week visit
2/52	2 week visit
1/12	1 month visit

Å	Angstrom
cm	centimetre
cp	centipoise
D	dalton
kD	kilo dalton
dyn	dyne
mEq	molar equivalent
mg	milligram
ml	millilitre
mm	millimetre
µl	microliter
µm	micrometer
mN	milli newton
nm	nanometre
s	second

CHAPTER 1

INTRODUCTION

1.1. Contact lens types

1.1.1. Rigid gas permeable contact lenses

Contemporary contact lenses are of two types: rigid gas permeable (RGP) and hydrogel contact lenses. RGP contact lenses evolved from hard lenses made of a copolymer of methyl methacrylate and silicone (siloxane-acrylate polymer)[1] in 1979 to fluorosilico-acrylate today[2,3]. Most commercial RGP materials, some of which are described in details in Table 5.1, are made up of siloxyderivatives of methacrylate monomers (e.g. tris(trimethyl-siloxy)-γ-methacryloxypropylsilane) or fluoroalkyl methacrylate monomers (e.g. 1,1,9-trihydroperfluorononyl methacrylate). These rigid contact lenses keep their shape while in the eye and are often characterised by their level of oxygen permeability[4].

1.1.2. Hydrogel contact lenses

Hydrogel contact lenses are the most commonly used. Hydrogels are water-swollen cross-linked networks of polymers based on hydrophilic monomers. Hydrogel contact lenses were invented by Wichterle and Lim[5] in 1960 and consisted originally of poly(2-hydroxyethylmethacrylate) or poly(HEMA). Hydrogels result from the copolymerization of three components: a main hydrophilic monomer, one or more hydrophobic monomers for mechanical strength and a cross-linking agent for stability. A large variety of hydrogel contact lens materials IS now available, ranging from 30 to 85% water content; they are 0.5 to 1.0% cross-linker and based on various combinations of monomers[2,6].

These monomers are either non-ionic (e.g. HEMA, GMA (glyceryl methacrylate), DAA (diacetone acrylamide), NVP (N-vinyl-pyrrolidone)), or ionic (e.g. MAA (methacrylic acid)) at physiological pH. The chemical structures of these monomers are represented in Figure 1.1. The copolymerization of the main hydrophilic monomer with variable concentration of hydrophobic monomers provides a range of water contents and thus a range of mechanical and surface properties. Hydrogel contact lenses do not maintain their shape unsupported and therefore are classified as soft contact lenses[7]. At times the two terms are used as synonymous although it is not strictly correct, as some soft contact lenses are not hydrogels.

1.1.3. Classification of contact lens materials

Soft contact lens materials are classified according to the material type (monomer composition), water content and surface ionicity. These characteristics determine the interaction of the lens material with the tear film, the in vivo wettability and as a result, the amount and nature of lens spoilation[8]. The International Standards Organisation (ISO) and Food and Drug Administration (FDA) classifications are reported in Tables 1.2 and 1.3.

Table 1.1. Some commercial RGP materials

Lens Name & manufacturer	Material	Oxygen permeability (Dk X 10^{-11})
Aquila (CIBAVision)	Fluoromethacrylate siloxy copolymer	143
Boston (Polymer Technology)	Siloxymethacrylate itaconate copolymer	10/12-14/14,19,24,26
Polycon I (Wesley-Jessen)	Siloxymethacrylate copolymer	3.1,5
Quantum I (Bausch & Lomb)	Fluoromethacrylate siloxy copolymer	92

Table 1.2. ISO categories

Group	Characteristics
1a	essentially pure HEMA (2-hydroxyethylmethacrylate) with not more than 0.2% weight of ionisable component
1b	essentially pure HEMA (2-hydroxyethylmethacrylate) with more than 0.2% weight of ionisable component
2a	Copolymer of HEMA and/or hydroxyalkylmethacrylates, dihydroxyalkylmethacrylates, alkylmethacrylates with not more than 0.2% weight of ionisable component
2b	Copolymer of HEMA and/or hydroxyalkylmethacrylates, dihydroxyalkylmethacrylate, alkylmethacrylates with more than 0.2% weight of ionisable component
3a	copolymer of HEMA and/or N-vinyl lactam, alkyl acrylamide with not more than 0.2% weight of ionisable component
3b	copolymer of HEMA and/or N-vinyl lactam, alkyl acrylamide with more than 0.2% weight of ionisable component
4a	copolymer of alkyl methacrylate and/or N-vinyl lactam, alkyl acrylamide, with not more than 0.2% weight of ionisable component
4b	copolymer of alkyl methacrylate and/or N-vinyl lactam, alkyl acrylamide, with more than 0.2% weight of ionisable component
5	Polysiloxanes

Table 1.3. FDA groups

Group	Characteristics (water content[a], ionicity[b])
I	low water content , non ionic
II	high water content , non ionic
III	low water content , ionic
IV	high water content , ionic

[a] high water content > 50%, low water content < 50%
[b] presence or not of ionisable chemical groups at the surface

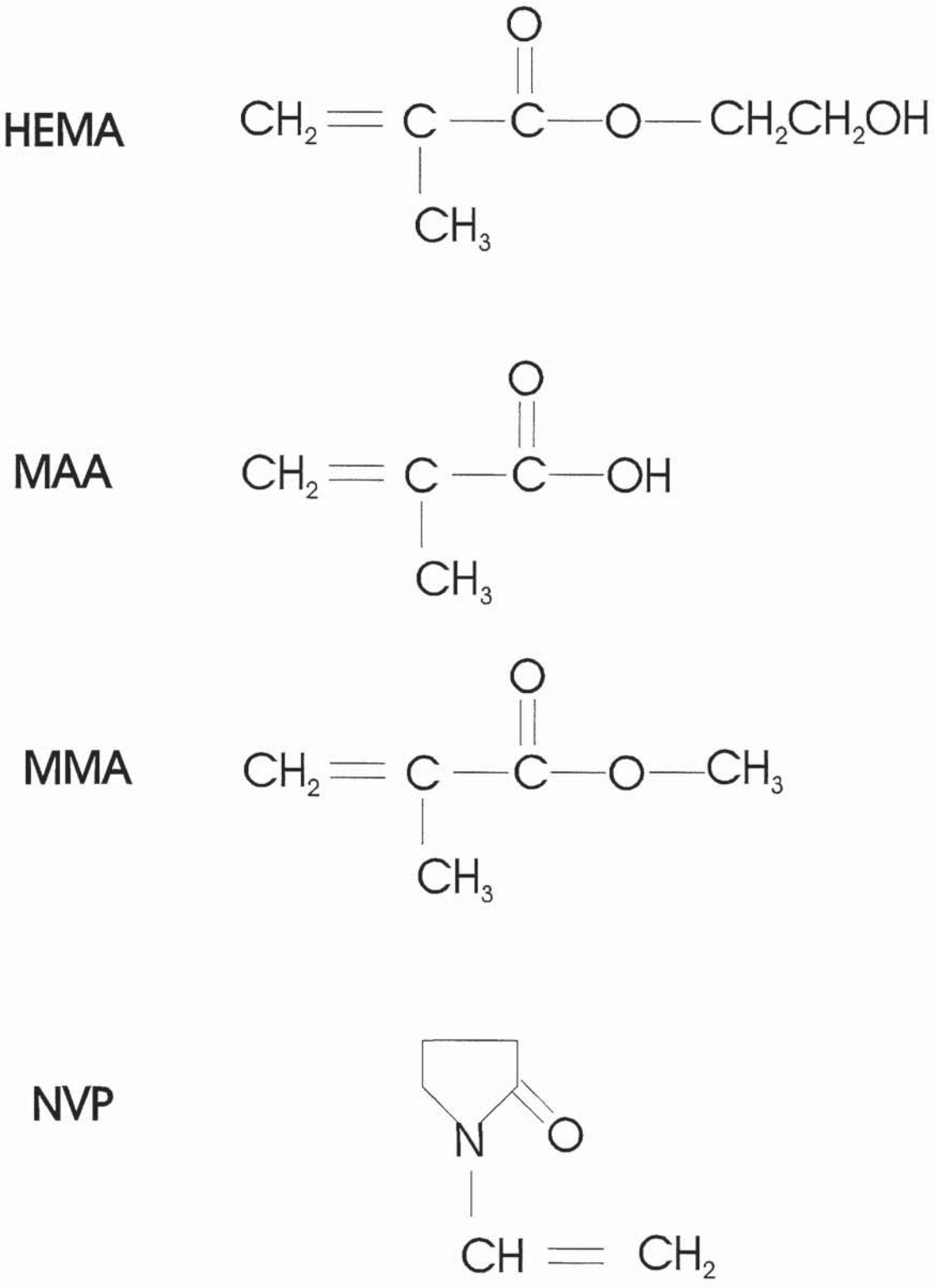


Figure 1.1. Principal monomers and abbreviations used in contact lens materials.

1.2. The Tear Film

1.2.1. Role of the tear film

The tear film is a complex liquid structure covering the anterior surface of the eye. The main functions of the tear film can be summarised by the three general headings: optical, protective and lubricative[9]. The tear film ensures the high optical property of the eye by smoothing the irregularities of the corneal surface and the contact lens surfaces[10]. The tear film assures ocular integrity by providing the anterior cornea with oxygen and removing carbon dioxide from it, by washing away debris and removing foreign bodies from the front surface of the eye, by inhibiting microbiological[11] contamination and by maintaining hydration. It also contributes to the mechanical[12,13] protection of the corneal epithelium. Any tear film anomaly, either inherent to the pre-ocular tear film or induced by the contact lens, may have significant consequences ranging from decreased comfort to serious adverse events.

1.2.2. Tear film structure

1.2.2.1. General description

The three-layered structure of the tear film, first reported by Wolff[14] in 1954, consisting of a superficial lipid layer, an aqueous phase rich in proteins and mucins and an underlying layer of mucus[12,15], is generally accepted[16]. Recent research has put forward opposing data regarding the total thickness of the tear film and particularly the thickness of the mucus layer. The structure reported by Holly and Lemp (1971)[12,13], shown in Figure 1.2, quoted a total tear film thickness of 10 μm with a monocular mucus layer less than 1 μm thick. But Prydal[17,18] has reported in 1992 a 30 μm mucus layer and a 10 μm aqueous phase as shown in

Figure 1.3[19]. The latter structure is now accepted as the correct description by many researchers in the field[19].

Figure 1.2. Schematic structure of tear film - Holly and Lemp model

Figure 1.3. Schematic structure of the tear film - Dilly model

1.2.2.2. Lipid layer

The outermost lipid layer is made up of oily secretions from the meibomian glands and the glands of Moll and Zeiss (Figure 1.4). The thickness, which has been reported between 0.1 to 1.75 μm, varies considerably between individuals[16,20,21].

The main function of the lipid layer is to stabilise the tear film by providing a hydrophobic barrier at the outer surface. The lipid layer prevents the evaporation of the aqueous phase[22,23] and its spreading over neighbouring areas. In the absence of this outer oily layer, the tear film evaporation rate has been reported to be 10 to 20 times faster[23]. The lipid layer also protects the tear film against contamination from destabilising agents such as highly polar skin lipids[24] or lid margin sebum of higher spreading pressure than meibomian oils that can provoke a rapid break-up of the tear film. Finally, due to the action of fatty acids complexing with mucins, the lipid layer also contributes to prevent microbial invasion[25].

Figure 2.4. Cross-section of an eye showing the location of the main tear secreting glands. Taken from 'The eye in contact lens wear'. Ed. Larke JR 1996

1.2.2.3. Aqueous layer

The aqueous layer with an average thickness of 7 to 10 μm is made up of 98% water and 2% of solutes. The solute phase is made up of a wide variety of organic and inorganic substances originating from the main and accessory lachrymal glands (Figure 1.4). The aqueous is particularly rich in soluble proteins, which nurture the corneal epithelium. The aqueous layer plays a lubricating role by providing a layer that mechanically protects the cornea and facilitates the removal of foreign materials from the surface of the eye.

1.2.2.4. Mucus layer

The mucus layer is a dual structure. The bulk of the mucus layer is a sponge-like material with fluid in a meshwork of glycoproteins[18]. It is covered by a thin layer of soluble mucus that freely exchanges with the aqueous phase. The mucus is mainly secreted by the conjunctival goblet cells but recent work has shown that the entire ocular surface epithelium including the corneal epithelium produces mucins[26]. Additionally, some glycoproteins, found in the lipid and aqueous layers, originate from the lachrymal glands and the serum[27]. The ocular mucin is at the interface between the corneal epithelium and the aqueous tears or dissolved in the aqueous phase of the tear film.

The shear action of the lids contributes to the distribution of glycoproteins on the corneal and conjunctival surface[28]. It is now believed that only the soluble layer and not the sponge-like structure is redistributed at each blink. This mucin layer acts as a viscoelastic buffer and protects the epithelial layer from any mechanical damage. It assures the wettability of the epithelial surface[12,13] by acting as a

biological lubricant and plays a part in the stability of the tear film by trapping destabilising lipids within its structure.

Under severe pathological conditions such as vitamin A deficiency, ocular pemphigoid (conjunctival alteration) or Stevens-Johnson syndrome, which is associated with the extinction of the goblet cells, the concentration of mucus in the tear film may decrease severely which results in a rapid break up of the tear film[29]. The micellar structure of the mucus layer also acts like a reservoir for immunoglobulins and for many biologically active substances[18,30].

Prydal and Campbell[17] suggested that mucus is present throughout the tear film and that there is no distinct free aqueous layer but rather free fluid space within a loose network of dilute mucus which may form a protective barrier between living cells and environment, lubricate the ocular tissues during lid and eye movements and help maintain an optically smooth surface and therefore clear vision. The mechanism of interactions of the mucus layer with the surface as well as its own structure are still subject to discussion[18].

1.2.3. Tear film characteristics [11,31]

The tear film is a clear fluid with the following characteristics:

- Temperature: 30-35°C,
- pH ~ 7.4,
- Refractive index: 1.336-1.337,
- Surface tension: 43.6 mN/m,
- Viscosity: 1.26-1.32 cp
- Freezing point (Fp) : -0.56°C
- Osmotic pressure: 304 mOsm/kg

The mean volume of tears covering the ocular surface is between 5 and 10 μl, most of it is found not over the cornea and conjunctiva but along the lid margin where it acts as reservoir. The average rate of tear renewal (tear turn over rate) is 1.2 μl/min[11].

1.2.4. Tear film composition

1.2.4.1. The lipid layer

1.2.4.1.a. Brief review of lipids [32]

i. General description

Lipids are groups of compounds that are water insoluble but soluble in a range of polar and non-polar organic solvents such as methanol, benzene, chloroform, hexane. Lipids can be divided in two broad categories: the simple lipids, non-polar and neutral, and the complex or ampiphilic lipids, which contain both hydrophobic and hydrophilic regions. The latter lipids are structural components of cell membranes and are used for storage and transport of metabolic fuels. The main lipid classes are fatty acids, triacylglycerols, phospholipids, isoprenoids, esters, eicosanoids and glycolipids.

ii. Fatty acids

Fatty acids consist of varying chain lengths (C3 to C30) of hydrocarbons with a carboxylic group at one end (Figure 1.5). The properties of each fatty acid are dependent on its chain length and its degree of unsaturation. The longer the chain the more hydrophobic and the higher the melting point. The more double

bonds present the lower the melting point and the higher the degree of fluidity it imparts to a cell membrane.

iii. Triacylglycerols

Triacylglycerols are storage forms of fatty acids. One molecule of triacylglycerol is made up of three fatty acids covalently bonded by ester bonds to a glycerol molecule (Figure 1.6).

Figure 1.5. Fatty acids. Taken from Biochemistry of the eye. Whikehart DR. Ed. Butterworth-Heinemann

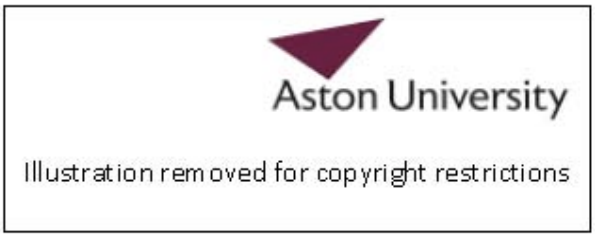


Figure 1.6. Triglycerides. Taken from Biochemistry of the eye. Whikehart DR. Ed. Butterworth-Heinemann

Fatty acids on each glycerol molecule are often of different types with various chain lengths and levels of unsaturation to assure fluidity. Most triacylglycerols are kept in fat cells as a large depot of stored energy. In the eye, only a limited amount of triglyceride is kept in storage to maintain cellular membranes.

iv. Phospholipids

Phospholipids are the most important lipid class for the formation and maintenance of cellular membranes. Similar in structure to triacylglycerols, phospholipids consist of a glycerol molecule bonded to two fatty acids and a polar head group. There are four possible polar head groups: ethanolamine, choline, serine and inositol (Figure 1.7) which bond to the free carbon of the glycerol molecule via a phosphate bridge. The fatty acids go into the interior of

the cell membrane whereas the polar head group stays at the membrane–water interface.

v. Isoprenoids

The isoprenoid family consists of molecules metabolically built up from the five-carbon molecule isoprene. The main members are cholesterol and its steroids, vitamin A and coenzyme Q.

Cholesterol is a molecule made up of 27 carbons arranged in four fused isoprene rings, two methyl groups, a hydrocarbon branch and a single hydroxy group (Figure 1.8). The cholesterol molecule is flat when on its side but bulky and rigid otherwise. Cholesterol is a highly apolar lipid that fits into the cell membrane structure and imparts its rigidity.

The hydroxy group of cholesterol may form cholesteryl esters when associated with fatty acids (Figure 1.9). The latter is an important component of the pre ocular tear film and the precursor of steroid hormones that can affect ocular functions and dysfunctions.

vi. Waxes

Waxes are esters of long chain fatty acids (C14 to C36) and long chain alcohols (C16 to C20) which derived from fatty acids (Figure 1.10). In the eye, waxes have been reported to be one of the major components of the pre ocular tear film (POTF) lipid layer.

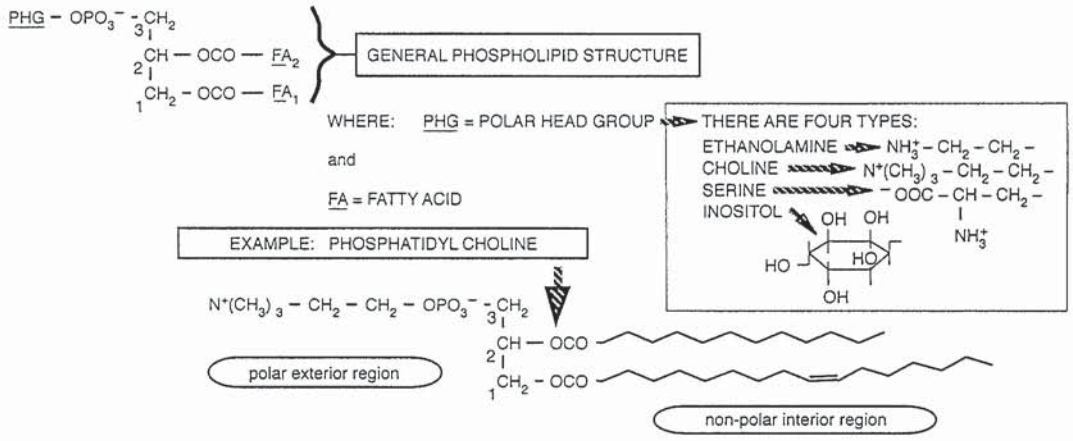


Figure 1.7. Phospholipids. Reproduced from Biochemistry of the eye. Whikehart DR. Ed. Butterworth-Heinemann

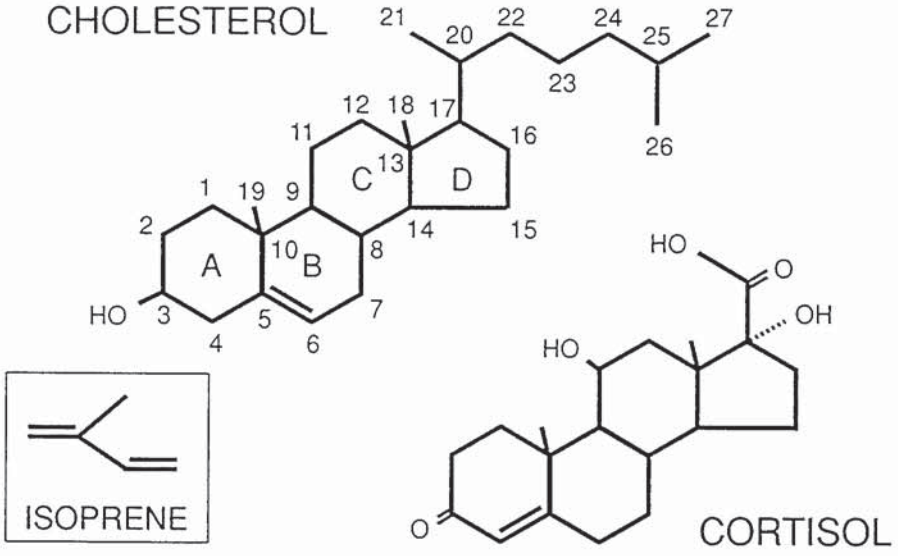


Figure 1.8. Cholesterol and other isoprenoids. Reproduced from Biochemistry of the eye. Whikehart DR. Ed. Butterworth-Heinemann

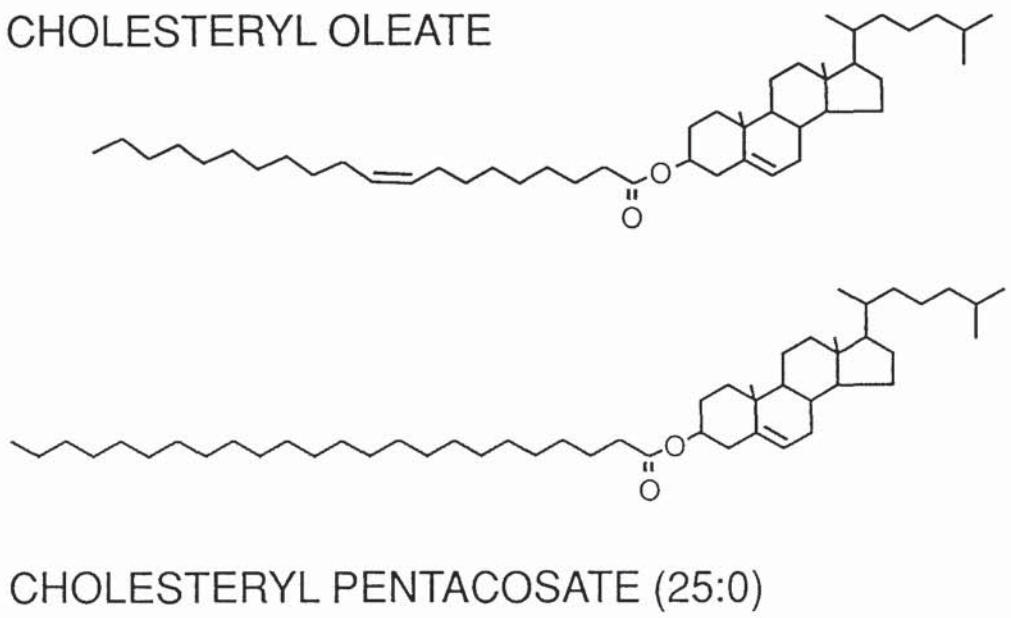


Figure 1.9. Cholesterol esters molecules. Reproduced from Biochemistry of the eye. Whikehart DR. Ed. Butterworth-Heinemann

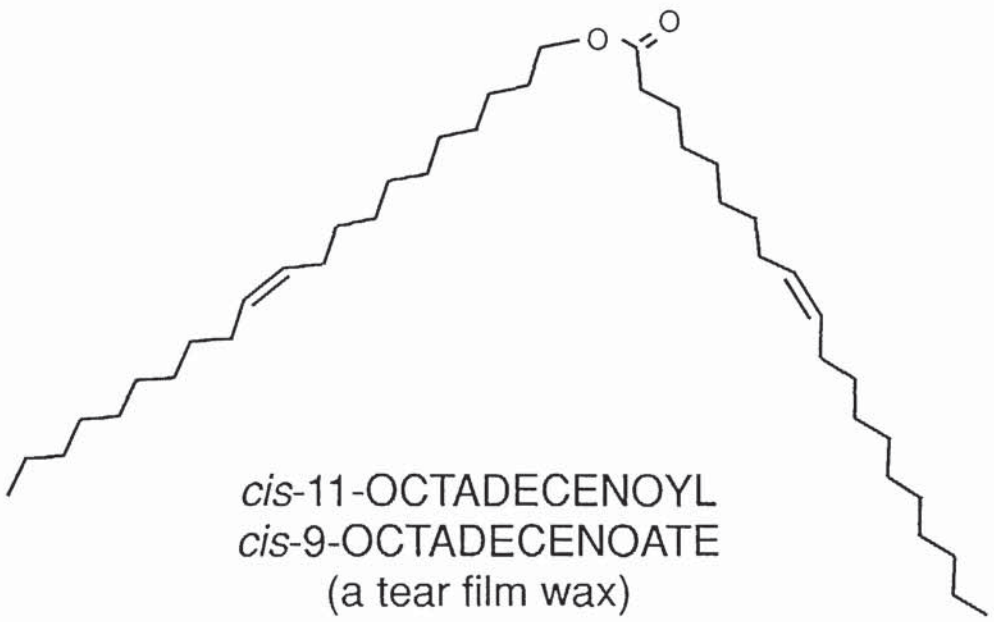


Figure 1.10. Wax. Reproduced from Biochemistry of the eye. Whikehart DR. Ed. Butterworth-Heinemann

1.2.4.1.b. Tear lipids

The lipid layer was first described in 1897 as a mixture of 'cholesterol, fatty acids and fat'[33], but consists in fact of a more complex mixture of various lipids[34,35,36]. The principal non-polar component has been identified as a mixture of waxes[37] and sterol esters. Further analysis showed that all lipid classes were present, in particular, hydrocarbon, wax esters, cholesterol esters, triglycerides as well as diglycerides, monoglycerides, fatty acids, cholesterol and phospholipids[20,38] . More than 25% of the lipid types within the tear film have not yet been identified. The lipid layer has been described as a duplex structure[39] in which the outer layer consists of cholesterol esters and the inner layer of a monomolecular film of phospholipids, fatty acids and free cholesterol. Important variations in composition between individuals have been reported[20]. The principal sources of tear lipids are the meibomian glands whose orifices are situated along the upper and lower lid margins[40,41]. The meibomian secretion forms the anterior layer of the pre ocular / pre lens tear film and is reconstituted entirely with each blink[25].

1.2.4.1.c. Tear lipid analysis

In contrast to the exhaustive publication list on tear proteins, only a limited number of studies dealing with tear lipid composition are found in the literature[20,34]. Further, in most cases, the samples analysed were not collected from the tear film but directly extracted from the meibomian glands. The main techniques used to study lipids have been chromatographic techniques such as Thin Layer Chromatography (TLC)[20,36,42], Gas chromatography (GC) alone[43] or in conjunction with Mass Spectroscopy (MS)[42], Vapor Phase Chromatography (VPC)[38] and High Performance Liquid Chromatography (HPLC)[6].

1.2.4.2. The aqueous phase

The aqueous phase is made of 98% of water. The 2% of solutes present are made up of electrolytes and organic substances.

The electrolytes are both cations and anions. The principal cations present in the tear film are sodium (140-150 mEq) in concentration similar to that in serum and potassium in concentration 4 to 6 times (20-30 mEq) greater than in serum[9]. Magnesium and calcium (1.5±0.5 mEq) are other cations present in the tears but in lower concentrations. Iron (16μg/100ml), copper (135μg/100ml) as well as traces of zinc and manganese can also be found[9]. The main anions found in the tear film are chloride and bicarbonate anions. The three main tear electrolytes, sodium, potassium and bicarbonate, contribute to the tear film osmotic pressure and act as a buffering agent to regulate the tear film pH[9,31].

The organic substances which have been found in tears are urea, uric acid, ammonia, nitrogen, glucose, organic acids (pyruvic or citric acids), vitamins (C ascorbic acid, B12 cyanocobalamin, B2 lactoflavin) and free amino acids[9]. In addition to these simple organics, the aqueous phase contains a remarkably complex mixture of proteins, secreted locally or serum derived.

1.2.4.2.a. Brief review of proteins [32]

Proteins are large molecules of molecular weight ranging from thousands to millions Daltons (D); they are polymers of amino acids linked together by peptide bonds as shown in Figure 1.11. The amino acids molecules that make up proteins contain at least one carboxyl group (-COOH) and one amino group (-NH_3) attached to the adjacent carbon atom (alpha carbon). Twenty different amino acids are commonly found in proteins (Table 1.4) (Figure1.12); they are

differentiated from each other by the nature of the group attached to the alpha carbon. The amino acids, through their individual chemical properties (Table 1.4), contribute to the functional properties of protein. Charged or polar amino acids may contribute to the water solubility of a protein whereas aromatic amino acids contribute to its hydrophobicity.

The primary structure of a protein is defined by the sequence of amino acids that constitute the protein. The secondary structure describes the shape taken by each sequence of amino acids. Four types of secondary structure are found in proteins: α-helix, β-sheets, β-turns, random coil. All the secondary structures are determined by hydrogen and disulphide bonding within and between amino acid chains. Finally the tertiary structure defines the entire shape of the polypeptide chain with its different domains.

Proteins are usually classified according to different criteria, such as function, solubility, size, and biological locations. Changes in environmental conditions such as temperature or pH may alter the conformation of the protein and result in the protein becoming non-functional or 'denatured'.

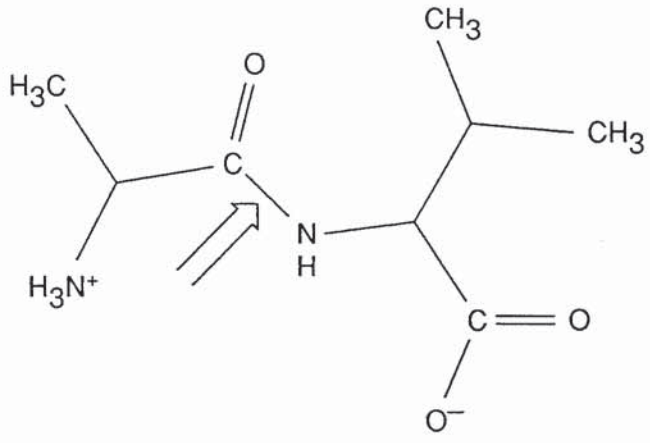


Figure 1.11. Amino acid formula. Reproduced from Biochemistry of the eye. Whikehart DR. Ed. Butterworth – Heinemann.

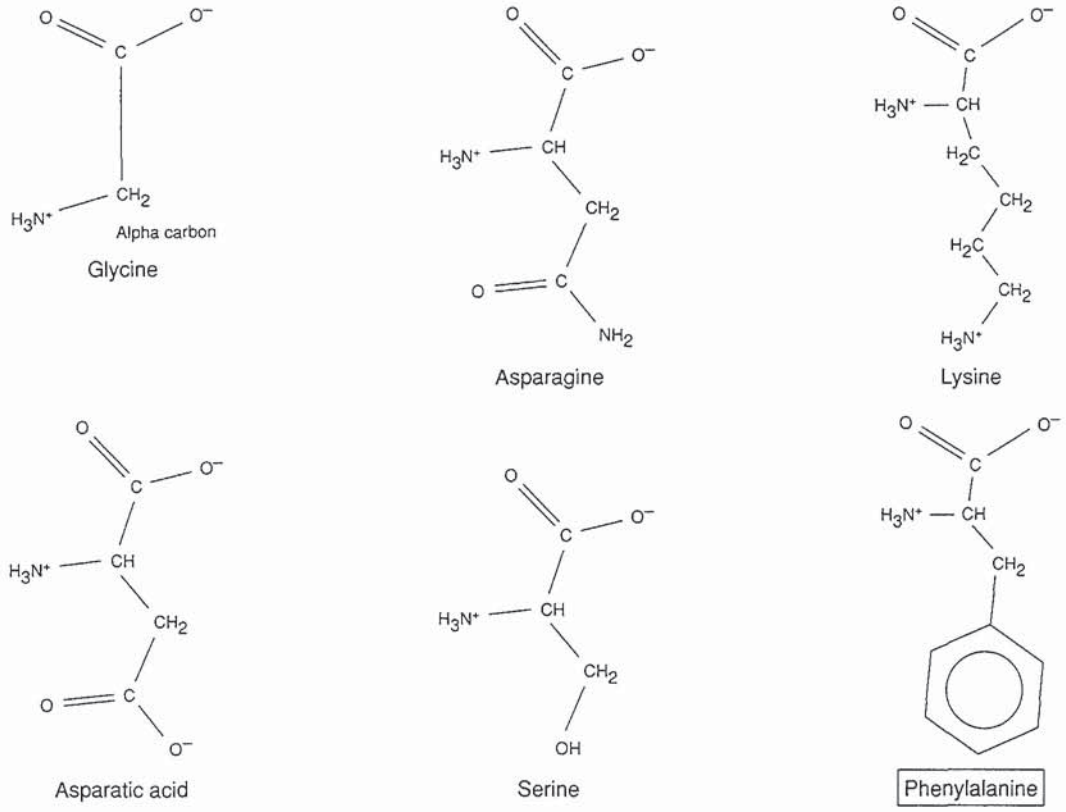


Figure 1.12. Various amino acids. Reproduced from Biochemistry of the eye. Whikehart DR. Ed. Butterworth – Heinemann.

Table 1.4. Amino acids most commonly found in proteins

Name	Symbol	Nature of R group
Alanine	Ala	
Valine	Val	
Leucine	Leu	
Isoleucine	Ile	
Proline	Pro	Non Polar
Phenylalanine	Phe	
Tryptophan	Try	
Methionine	Met	
Glycine	Gly	Uncharged polar
Serine	Ser	
Threonine	Thr	
Cysteine	Cys	
Tyrosine	Tyr	
Aspargine	Asn	
Glutamine	Gln	
Aspartic acid	Asp	Polar Negatively charged
Glutamic acid	Glu	
Lysine	Lys	Polar Positively charged
Arginine	Arg	
Histidine	His	

1.2.4.2.b. Tear proteins

i. General description

Among the 60 proteins found by Gachon *et al.* (Table 1.5)[44] while studying the tear film, lysozyme, lactoferrin, sIgA and tear specific pre albumin (TSPA) make up the major part of the proteins in the tear film. sIgA, lysozyme and lactoferrin are known to play a protective role against microbial invasion of the external ocular surfaces.

ii. Lysozyme

Discovered in 1922 by Fleming, this lachrymal gland secreted protein shows bacteriolytic properties by its capacity to hydrolyse the β1,4 glucosidic linkage between N-acetylmuramic acid and N-acetylglucosamine that occurs in the mucopeptide cell wall of Gram+ bacteria[45]. Lysozyme, which is involved in the defence mechanism of the eye, is also found in other bodily secretions such as urine, saliva and milk. Lysozyme is a small and compact protein of 14.6 kD (pI =11.1) with highly positively charged domains due to the cluster of basic amino acids (Arginine). The concentration of lysozyme in the tear film has been reported to decrease with patient age[46,47] and in patients suffering from dry eye states (decrease of lysozyme in aqueous tear deficiency states)[48], ocular diseases or health[49-55]. Lysozyme concentration is also affected by patient gender: a significantly lower concentration has been found in female subjects[56]. However the concentration is not influenced by tear flow, lysozyme is present in similar levels in stimulated and non stimulated tears[47,49,57,58]. Similarly the concentration of lysozyme in the tear film is not affected by contact lens wear[37,51,59].

iii. Lactoferrin

The presence of lactoferrin in tears was first detected by Mason et al [60](1966) and confirmed later by Broekhuyse[61] (1974). Secreted by the main and accessory lachrymal glands, lactoferrin accounts for 25% of the total protein content [62] . The concentration of lactoferrin which is usually around 1 to 2 g/l[49] may vary largely in human tears[62]; it decreases with age and in patients with keratoconjunctivitis sicca, Sjogren syndrome, viral conjuntivitis[49-55] or giant papillae conjunctivitis (GPC)[63]. Lactoferrin is present in similar concentrations in stimulated or non stimulated tears and does not seem affected by contact lens wear[37,52,59]. Lactoferrin is a 82 kD iron binding protein (pI=5.5-5.8) which belongs to the transferrin family and helps control iron levels in bodily fluids. Two atoms of iron can attach reversibly to one molecule of lactoferrin. The amino acids involved in the process/binding are histidine, cystine, threonine and tyrosine. By depriving the ocular mucosa of free iron[45], an essential factor in bacterial growth, lactoferrin plays a protective role against bacterial colonisation of the surface of the eye[49]. Lactoferrin has also been suspected to play a part in the defense of the outer eye[64,65] and in the regulation of inflammatory disorders[49]. It possesses properties to alter the external membrane of Gram negative bacteria and allow lysozyme to lyse the inner peptidoglycan cell walls.

iv. Immunoglobulins

Immunoglobulins are large proteins (160-900 kD) which are found in tears and in many body secretions such as saliva, nasal fluid, breast milk, perspiration and gastric secretion. Several immunoglobulins including IgA, IgG, IgM, IgD and IgE are present in tears[66,67] and are involved in the defence mechanism of the eye[68].

IgA is present in the tears in two forms, serum IgA and secretory IgA (sIgA). sIgA is secreted by the main and accessory lachrymal glands and is the predominant antibody in tears[49]. It protects the mucosal surface from microbial infection by creating an immunological barrier to the adherence of microbial organisms[69]. sIgA is involved in the prevention of bacterial attachment, the reduction of antigen absorption, the neutralisation of viruses and toxins and the elimination of plasmids[66].

The level of sIgA in the tear film decreases after tear stimulation and increased tear flow[47,49] but becomes the main protein present at the surface of the eye during closed eye (8.4 g/l)[70].

A decrease in the concentration of sIgA during contact lens wear has been observed by some researchers[59,71] but Mannucci et al.[72] and Temel et al.[73] reported an increase during extended wear.

v. Serum albumin

Albumin, which is the main protein in the blood serum, is also found in tears. It has a molecular weight of 68 kD and is negatively charged at tear pH (pI=4.9 at pH=7). The concentration of serum albumin in tears is, similar to sIgA, significantly higher during closed-eye than open eye situation[70] and it also increases with any trauma to the ocular surface[74,75]. In addition to being part of the defence mechanism of the outer eye and because of its affinity for cations and anions, albumin has a physiological function and transports inorganic ions such as Zn^{2+}, Ca^{2+} or Cu^{2+}.

vi. Tear Specific Pre Albumin (TSPA)

TSPA, which was so called because of its molecular weight lower than that of albumin, was discovered by Bonavida and is unique to tears[76,77]; its function remains so far speculative. TSPA has, however, been reported to play a role in the defence mechanism of the eye; in association with lysozyme and a glycoprotein, tear albumin has been suggested to have a bactericidal activity[31,78].

It is secreted by the main and accessory lachrymal glands and accounts for 10 to 20% of the total protein content. Similar in its sequence of amino acids to the human Von Ebner gland protein[79] found in saliva, it belongs to the lipocalins family. TSPA exists in multiple isoforms[80,81] of molecular weight ranging from 15 to 20 kD and has been reported to aggregate into a 31 kD protein known as protein G[81,82,83]. Finally, its concentration in tears is reported not to be affected by contact lens wear[37].

1.2.4.2.c. Tear protein analysis

i. General description of techniques used

Various techniques have been developed or applied to the analysis of human tear proteins. The techniques for characterisation of proteins in tears are gel filtration and anion chromatography, high performance (or high pressure) liquid chromatography (HPLC)[46,47,57,84,85,86,87,88,89] and various electrophoresis techniques such as SDS PAGE, native electrophoresis[44,70,86,90] , immuno electrophoresis, isoelectric focusing (IEF), two dimensional electrophoresis[91] and capillary electrophoresis technique for the evaluation of tear proteins[44,54,89,90]. The analysis of individual proteins or enzymes in tears can also be carried out by using

Table 1.5. Some proteins identified in tears (Gachon *et al.*)[44]

I. Principal tear proteins	
Secretory immunoglobulinA	
Lysozyme	
Lactoferrin	
Albumin	
Specific tear albumin	
I.Further proteins	
Immunoglobulins G, M, D, E	Anti chimotrypsin
Complement component C3,C4	Anti trypsin
Histamine	Prostagladins
Beta Lysin	Zinc Alpha II glycoprotein
Transferrrin	Ceruloplasmin
II.Enzymes from the lachrymal glands	
Alpha galactosidase	Amalysase
Beta hexoaminidase	Hexokinase
Beta glucurodinase	Glutamate pyruvate
Acid phosphatase	Transaminase
Alkaline phosphatase	
III. Enzymes from the corneal and conjunctival epithelium	
Lactate dehydrogenase	Glucose-6-phosphase
Malate dehydrogenase	Dehydrogenase
Pyruvate kinase	Sorbitol dehydrogenase
Isocitrate dehydrogenase	Glutamate dehydrogenase
Aldolase	Glutamate oxalacetate

immunological techniques such as ELISA (enzyme linked immunoassay)[46,47,85], RIA (radio immunoassay), immunodiffusion[70] and various assays.

ii. HPLC

HPLC is a technique that separates solutes from a mixture by the differential movement of the individual solutes through a porous medium under the influence of a moving solvent, pumped under high pressure.

iii. Electrophoresis

The principle of electrophoresis is to transport particles through a solvent by applying an electric field. The charged particles migrate towards the electrode with a charge opposite to its own. The particle movement is dependent on the charge, size and shape of the particle, the strength of the applied field, the buffer and the composition of the supporting medium. Electrophoresis techniques utilise the property conferred on proteins by their ionisable groups; proteins can be made to exist in solution as electrically charged species depending upon the pH of the environment. Since the molecules which have similar charges also have a different charge to mass ratio, a protein mixture such as tears show therefore enough differences for differential migration when subjected to an electric field [92].

1.2.4.2.c. Concentration of proteins in tears

Published concentrations of tear proteins are highly variable. The average value and the range of the concentrations of major tear proteins found over hundred

publications, which have been reported by Bright et al.[56], are reproduced in Table 1.6.

The high variability in concentrations reported can be explained by different factors such as the following:

- method of tear fluid collection[84]: filter paper[46], Schirmer[84,85], glass capillaries[44,70,89], sponge[93]
- condition of tear fluid collection: In order to increase the volume of tears available, the collection is often performed under stimulating conditions (ethanol vapour, air flow, onions). This can result in a dilution of proteins and/or an increased ratio of lachrymal gland secreted proteins in comparison to serum proteins. Similarly the environmental conditions, temperature and humidity ratio, as well as the time of day when the collection is made can affect the tear protein concentrations.
- Patient demographics[54,92], age[46,47], health[54], contact lens wear [37];
- Patient ocular status: contact lens wearer, non contact lens wearer, dry eye symptomatology or any ocular pathology such as keratoconjunctivitis sicca (KCS), Sjogren syndrome;
- Analytical technique used to assess the protein concentrations.

Table 1.6. Reported concentrations of major tear components in mg/100ml - Reproduced from Bright et al.[56]

Component	Average	Minimum	Maximum
IgE	0.01	0.003	0.02
IgM	0.86	0	5.0
Calcium	3.6	1.2	8.0
Ascorbic acid	6.8	0.14	2327
Glucose	11.5	0	65
IgG	12.6	trace	79
IgA	30	7.0	85
Potassium	82.7	58.7	137
Tear specific prealbumin	123	52	184
Albumin	130	1.03	390
Lactoferrin	184	81	286
Lysozyme	236	65	555
Sodium	337.7	326.6	354
Chloride	469.6	85	512.9
Total protein	751	652	800

1.2.4.3. The mucus layer

The mucus layer is mainly of a gel of mucins/glycoproteins complexed with salt, plasma proteins, lipids, carbohydrates, glycoamoniglycans (GAGs) and water[94]. It resembles mucus of other body organs such as gastrointestinal tract, nasal passage and trachea[94].

Glycoproteins are the main organic component of mucous secretions; they consist of large polypeptide chains to which carbohydrates side chains attach by covalent bond with a O-glycosidic linkage of N-acetylglucosamine to hydroxy groups of amino acid[95]. Tear glycoproteins are composed of 55% of

carbohydrates which is a significantly higher ratio of carbohydrates than serum or plasma glycoproteins. All polypeptide chains with a branched carbohydrate are separated by a short length peptide chain with no attached carbohydrates[27]: 1 attached chain for 4 amino acid residus.

Different types of glycoproteins, neutral or acidic, exist within the tear film[27]. They originate principally from the conjunctival goblet cells but also from the lachrymal gland, the entire ocular surface epithelium[26] and the serum. Berta & Torok[27] have shown that the relative ratio of different types of glycoproteins within tear film varies with different pathological status. The concentration of mucus decreases for contact lens wearers, for patients with dry eye syndromes and for glaucoma patients over 60 years of age[96].

1.2.5. Clinical evaluation of the tear film

1.2.5.1. Introductory remarks

The stability of the tear film is crucial to the health and transparency of the cornea. Tear film stability depends upon the interaction of the three layers of the tear film but particularly upon the superficial lipid layer. Using a slit lamp based non invasive technique [97,98], the lipid layers of the pre ocular tear film (POTF) and of the pre lens tear film (PLTF) can be visualised.

1.2.5.2. Lipid layer assessment

The lipid layer can be classified according to its appearance. Four clinically significant types of patterns have been reported [99]: the marmoreal, amorphous, flow and the colour-fringe patterns.

Marmoreal patterns are found in 60% of patients; they correspond to the normal lipid layer with a thickness ranging from 13 to 70 nm. The grey marbled appearance is due to some thicker lipid areas visible against a thinner and so lighter lipid background. Contamination by mucus or debris is visible in 10% of marmoreal patterns; it is associated with a decreased stability.

Amorphous patterns are found in 15% of the patients. They have a grey-bluish appearance, which is due to a more even thickness than the marmoreal one and ranging from 70 to 90 nm thick. This pattern assures a highly stable tear film.

Flow patterns are encountered in 10% of cases, shows a wavy appearance resulting from a heterogeneous spreading of lipids at various thickness (10 to 90 nm) with poor mixing properties.

Colour fringe patterns, found in less than 5% of cases, are often contaminated. Characteristics of a much higher lipid layer thickness (86 to 170 nm), they are the result of excessive secretion from the meibomian glands.

Combinations of the four types of patterns described are also observed; the most common ones are combinations of marmoreal and flow or amorphous and colours fringes patterns.

In the presence of contact lenses, the appearance of the lipid layer in the pre-lens tear film is highly dependent upon the nature of the contact lens material and its wettability[100,101].

1.2.5.3. Aqueous layer assessment

In the majority of cases, when the pre soft lens lipid layer is similar to the POTF lipid layer, the aqueous layer lying underneath is invisible. When the lipid layer becomes thinner and not fully reflective, it is possible to see the underlying aqueous layer. The aqueous layer thickness is such that the interference fringes of increasing order can form. With the clinical observation technique available the normal aqueous layer is too thick to produce interference fringes. The visualisation of fringes is indicative of an aqueous layer of reduced thickness. The number of fringes, if visible, increases with an increase of the aqueous thickness.

1.2.5.4. Tear film stability and Non Invasive Break Up Time (NIBUT)

The tear film stability can be directly assessed by the measurement of the pre ocular tear film non invasive break up time (POTF NIBUT), which is the time

elapsed between eye opening after a blink and the destabilisation of the film. It is characterised by the appearance of the first dark spot within the tear film under wide diffuse light observation with the Tearscope. The NIBUT measurement is recorded in seconds on a continuous scale from 0 to 45. The average value for a normal subject is around 16-17 seconds. Values below 10 seconds are characteristic of poor POTF stability. A significantly lower stability is recorded when the subjects were suffering from dry eye[102]. The stability of the POTF has also been reported to be influenced by the characteristics of the lipid layer present; greatest stability was associated with a thick and homogeneous lipid layer, characterised by an amorphous pattern during slit-lamp examination[102].

In addition to the measurement of the NIBUT, the type (spots/line/surface) and the position (central/nasal/temporal/inferior/superior) of the break in the tear film can be recorded.

In the presence of contact lenses, the stability of the tear is assessed in a similar way to the pre ocular tear film. The PLTF stability is lower than that of POTF: Guillon & Guillon[103] reported that whereas 60% of the POTF NIBUT was equal or superior to 45 seconds only 2 % of the PLTF NIBUT values recorded on same population reached the same value. Guillon et al.[104] reported an average PLTF NIBUT with high and low water soft contact lenses between 5 and 10 seconds and between 4 and 6 for RGP contact lenses.

1.2.5.5. Tear film contamination

Contamination of the tear film may also be evaluated during the clinical examination and is characterised by the presence of particulate matter such as

atmospheric contaminants, mucus particles floating at the surface of the lipid layer.

1.3. Contact lens spoilation

1.3.1. Tear film-contact lens interaction[105]

The presence of a contact lens disrupts the stability of the tear film and changes its salt and protein concentrations[31]. Some components of the tear film are associated with contact lens surface deposits[106]. The coating of contact lenses by a proteino-lipidic film takes place immediately upon insertion of a new contact lens and continues throughout wear. Material characteristics influence the pellicle formation by producing specific biofilm of both proteins and lipids during contact lens wear. This biofilm is essential to ensure contact lens clinical acceptance and contact lens biocompatibility. It is the degeneration of the biofilm that affects the performance of the contact lens. This phenomenon remained a significant problem for practitioners and contact lens wearers until the early 90's with soft contact lenses. Polymer degradation together with presence of deposits induced changes in surface physical and chemical characteristics of contact lens materials that generated wearer intolerance. The consequences ranged from degradation of the visual performance[107-110], discoloration of the lens or minor to more severe discomfort associated with abnormal immunological-like reactions (contact lens induced papillary conjunctivitis, giant papillary conjunctivitis)[111,112], pain, intolerance to the lens, corneal ulcer. The trend in contact lens wear developed towards more convenient wear regimen such as planned replacement and daily disposable soft contact lenses which in 1999 accounted for 29% of soft contact lens market[113], contact lens spoilation has greatly diminished as an issue

for non tolerance. The magnitude of the problem may rise again with the development of 30 day continuous wear modality with the new high Dk materials. Spoilation was reported in 10 to 80% of extended wear hydrogel lenses with conventional materials[114]. The new materials do not address the question of improved surface properties.

Up to 50% of the surface of the hydrogel lenses has been estimated to become covered by a biofilm within 30 minutes of wear which may produce contact lens intolerance within 6 months [115]. Several favourable conditions for lens deposition and spoilation in the tear contact lens interaction have been reported during wear:

- both lipid and proteins have been shown to deposit on synthetic surfaces like polymers or hydrogels, characterised by a high polar surface and a porous structure allowing water soluble components to diffuse into them [116],
- Langmuir-Blodgett type of deposition occurred when the lens passed through the air-liquid interface during insertion, blinking or removal[105],
- the tear fluid is a nutrient rich medium (glucose, proteins, physiological pH, body temperature) appropriate to nurture the microflora and microfauna[105].

1.3.2. Protein deposition [105,117]

When protein solutions are in contact with another phase with which it is unmiscible, protein molecules tend to accumulate at the interface. Thereby when a solid surface such as a contact lens comes into contact with a biological fluid like the tear film, adsorption of proteins takes place almost immediately. The surface of a protein is a complex arrangement of surface groups with differing properties: hydrophilic or hydrophobic, positively or negatively charged. These

groups can participate in the interaction with other surfaces[105]. The main factor influencing the protein deposition is its surface energy; thereby hydrophobic surfaces attract more proteins than hydrophilic surfaces. The net surface charge of materials may also play a part. Proteins with a positive charge will bond strongly with negatively charged surfaces. The protein surface groups are the most likely to interact with any solid surface with which they come into contact. Internal groups may also be exposed through conformational changes and play a part in the adsorption mechanism. Therefore for proteins with low structural stability, adsorption can occur even under unfavourable conditions these being overcome by structural rearrangements. Depending on the surface properties of the contact lens material, protein molecules are able to reorient themselves and favour the interaction with the surface.

Small proteins such as lysozyme have been reported[117] to enter the matrix of hydrogel materials in particular ionic materials with high water content.

The level of protein deposition has been reported to vary significantly between the four FDA Groups since the protein deposition is highly dependent upon the ionicity of the material. *In vitro* protein deposition has been described as a function of the FDA classification by Minarik and Rapp (1989)[8]. Group IV lenses are reported to exhibit the greatest amount of protein deposition and Group I lenses the least. Group II materials attract more proteins than Group I or III but significantly less than Group IV. High water content materials, characterised by a porous network, allow the diffusion of low molecular weight proteins. Protein deposition occurs in the first seconds of wear[118] and increases with the time of wear until it reaches a plateau[119,120,121]. The level of protein accumulation at which a plateau occurred is material[121] and subject dependent[120].

The interaction of proteins with biomaterials usually appears as a thin protein film of 100-500 ng/cm^2 of proteins[122]. Initially the proteinaceaous film in its natural state maintains biocompatibility; with time, if the film becomes degraded it acts as a primary layer for further deposition of other tear components, bacteria and micro-organisms[120]. This long term effect may result in decreased visual performance, in modification of lens movement and may trigger red eye reaction. In the ocular environment, some macroscopic deposits may be found as a result of the simultaneous deposition of lipids and other non-proteinaceous components as well as denatured proteins. A type of deposit called white spot is described in details in Section 1.3.3.

1.3.3. Lipid deposition[105,117]

The dominant factor in the interaction between lipids and contact lens materials is the hydrophobicity of the material[122]. Lipid adsorption is therefore particularly a problem for RGP materials[123,124]. RGP contact lenses deposit 2 to 3 times more lipids than soft contact lenses and the deposition is proportional to their silicone content[123]. All hydrogels are based on a carbon backbone with hydrophobic segments and therefore the partitioning of lipids into the contact lens matrix is common to all materials. Higher water content materials attract more lipids than lower water content materials[123] and whereas the ionicity of contact lens does not seem to directly affect the lipid deposition, it may still play a part as deposition may be affected by the presence of proteins[125,126]. A Langmuir-Blodgett type of deposition at the contact lens surface has also been suggested when the contact lens passes through the air liquid interface.

In addition to the most frequently encountered thin film lipid deposition, lipids are also highly involved in the formation of discrete elevated deposits called 'white spots'. The composition of these elevated deposits, also 'jelly bumps', 'lens calculi' has been subject to discussions and attributed in part to calcium, protein, mucus, lipid, bacteria and fungi [127,128,129,130,131]. It has now been recognised that these mucoprotein-lipid deposits are predominantly (90%) made up of lipoidal species (cholesterol, cholesterol ester, unsaturated lipids) [127]. A distinct internal stratification with a flat basal layer at the interface with the lens matrix has been reported[123,131,132,133,134]. Unsaturated lipids form the base structure for the deposit formation as revealed by the high concentration of unsaturated fatty acids found by HPLC at the hydrogel-deposit interface. Cholesterol-based lipids seem to contribute significantly to the deposit mass[123,131,132,133,134]. A complex mechanism of formation of these three dimensional aggregates has been proposed by Tighe and Bowers in 1990[133,134]. The basal layer probably results from the interaction of the carboxyl group of the fatty acids with hydrogen bonding sites of the hydrogel.

1.3.4. Inorganic deposits

Inorganic deposits which contain calcium, magnesium, or sodium are a result of mineral accumulation from tears whereas those containing iron, mercury, silica, chromium and copper are due to extrinsic factors [135]. Among these inorganics deposits, the most common ones are those which involve calcium. Calcification has been reported for various biomaterial devices and usually involves deposition of calcium phosphate (hydroxyapatite). In the case of contact lenses [132,133,134], the calcification penetrates the lens matrix and is composed of a co-precipitation of proteins and calcium phosphate. Bowers and Tighe [134] suggested

that protein deposition was a factor that favoured the mineralisation of the calcium phosphate present in tears, with a mechanism similar to cornea calcification where the release of calcium and phosphorus as well as enzymes (adenosine triphosphatase, alkaline phosphatase) that hydrolyse pyrophosphatases, inhibitors of calcification of soft tissues have been reported.

1.3.5. Others [31,105]

A wide range of other types of spoilation have been reported such as pigment deposits, fungal and yeast deposits, mercurial deposits and rust coloured spots (Table 1.7). These deposits have been encountered by researchers and practitioners, especially in the early days of contact lenses but are uncommon today with the increase of daily wear planned replacement contact lenses and disposable contact lenses. Microbial spoilation of the contact lens surface is still common and produces adverse ocular effects before being visible.

Table 1.7. Contact lens deposits- Variations in appearance and nature[105]

Appearance	Nature
Uniform films Grainy films Hazy films Jelly bumps Stringy material Non-wetting patches Lens discoloration Discrete elevated white spots Crystalline deposits Particles	**Organic** Proteins Lipids Mucin Polysaccharide Drugs Cosmetics Organic pigments **Inorganic** Calcium salts Mercury and iron compounds **Mixed** Complex lipid-mucoprotein deposits, with or without other organic or inorganic component **Microorganisms** Bacteria Fungi Amoeba

1.3.6. Evaluation of contact lens spoilation

1.3.6.1. Biochemical methods

A number of biochemical techniques can provide information on the species involved in the lens deposits. Methods that have been applied to contact lens deposit analysis are electrophoresis [8,136,137,138,139], liquid chromatography, immunologic studies, BioRad assay [5] and Lowry assay [137,140]. The first two techniques, described in detail in Section 1.2.5, require extraction of the components from the contact lens surface for analysis. Immunological techniques may provide a positive identification of specific proteins from the outermost area of the deposit if no denaturation had occurred. The BioRad protein assay and Lowry assay are dye binding assays relying on the differential

colour change of the dyes in response to different protein concentrations. Finally the radiolabelling method, limited to *in vitro* experiments, enables us to study one protein at a time and to monitor its interaction with the contact lens material. Lipid deposition on contact lenses is usually detected by fluorescence assays[141,142,143,144] and liquid chromatography[144].

1.3.6.2. Microscopic techniques

1.3.6.2.a. General

The microscopic techniques, which are mainly qualitative and non-destructive, allow the examination of the morphological aspects of the deposits. Slit lamp biomicroscopy, specular microscopy, light microscopy can easily be performed by contact lens practitioners, especially as they can be performed without contact lens removal. Since these are exclusively qualitative techniques, the literature reports several attempts to classify contact lens coatings and deposition according to the clinical observations.

1.3.6.2.b. Clinical techniques and classifications

The first classification was developed in 1974 by Rudko and Proby[145]. This classification developed *in vitro,* differentiated the following four types of spoilation:

(1) = no deposit on wet or dry lens with 15X magnification,

(2) = deposits visible on wet lens with 15X magnification,

(3) = deposits visible on dry lens without magnification, and

(4) = deposits visible on wet lens without magnification.

Tripathi and Tripathi (1984)[135] classified the contact lens deposits according to their appearance:

- uniform films,
- grainy films,
- hazy films,
- jelly bumps,
- stringy material,
- non-wetting patches,
- elevated deposits and
- crystalline.

For the slit lamp biomicroscopy examination, which is most frequently used by the clinical practitioners, a classification was proposed in 1989 by Josephson and Caffery[146] . They rated the level of contact lens spoilation as follows;

(0) = smooth, uniformly reflecting surface,

(1) = coarse, hazy surface,

(2) = a stable non wetting area,

(3) = gross crystalline or amorphous deposits.

Finally Hart recently proposed an *in situ* biomicroscopy classification method grading the lens spoilation as follows:

(1) = no tear break-up > 10 seconds withheld blinking,

(2) = tear film break -up on lens between 5 and 9 seconds,

(3) = tear film break-up on lens between 2 and 4 seconds,

(4) = protuberant deposit, unwettable, instantaneous film break up.

1.3.6.2.c. In vitro techniques

In addition to these techniques and classifications dedicated to the clinical observation of the contact lens spoilation, some newly developed techniques using electron microscopy (SEM Scanning Electron Microscopy[133,147,148], TEM Transmission Electron Microscopy) have been applied to the study of contact lens spoilation. They may offer quantitative information but can only be performed outside of the eye.

1.3.6.3. Spectroscopic techniques

1.3.6.3.a. Principle

The principle of these techniques is to measure the energy absorbed or emitted by the deposit on the contact lens surface after bombarding it with energy.

1.3.6.3.b. Multiple total reflectance Fourier transform infrared spectroscopy

Multiple total reflectance Fourier transform infrared spectroscopy (MTR-FTIR) is one of the most widely used spectroscopic techniques for contact lens deposit characterisation. It involves the collection of an infrared spectrum from the surface of the sample (1 to 5 μm). Some of the molecules present at the surface (proteins, lipids, carbohydrate, and mineralised material) are vibrationally excited by the infrared radiation, this generates an IR absorption spectrum. This spectrum is made of the absorption bands of the excited molecules. The literature has indicated that it might be possible to apply this technique to the analysis of soft contact lenses deposition. Castillo & co-workers[149,150,151] have

reported several studies on the infrared properties of spoiled soft contact lenses and on the adsorption of lysozyme to contact lenses in an *in vitro* experiment.

1.3.6.3.c. Electron spectroscopy for Chemical Analysis

In Electron Spectroscopy for Chemical Analysis (ESCA)[152,153], the samples are bombarded with photons which emit electrons from the upper 100 Å. The resulting spectrum, after detection by an electron spectrometer, allows qualitative (position of the peaks) and quantitative (peaks area) analysis of contact lens deposits.

1.3.6.3.d. Secondary Ion Mass Spectroscopy

In Secondary Ion Mass Spectroscopy (SIMS), the upper layer (10 Å) of the contact lens is bombarded by a beam of accelerated ions which causes an emission of anionic and cationic components from the surface. The emitted species are then analysed by mass spectroscopy [154,155,156,157].

1.3.6.3.e. EDXA

In EDXA, electrons bombard the upper few microns of the contact lens and cause X-ray to be emitted. This produces an X-ray spatial map with qualitative element analysis.

1.3.6.3.f. Spectrophotometry

UV-visible spectrometry has been used to measure the absorption of light in the visible or near UV region and to produce a spectrum indicative of the concentrations of components with typical functional groups.

Fluorescence spectrophotometry, by measuring the photons emitted by excited molecules yield information about concentration, local polarity and mobility of these molecules. These two techniques have been applied to contact lens spoilation studies [135,158]. They are described in detail in Section 2.

1.4. Scope and aims of this project

The purpose of this project is to increase our knowledge of the contact lens spoilation phenomenon and to investigate its relation to the *in vivo* clinical performance of the contact lens and to the pre-ocular tear film characteristics of the subject.

The biochemical components in the tear film which include proteins and lipids have been reported to play a part in the suitability of a particular lens material for an individual. The establishment of a significant biochemical marker within the tear film and of an effective procedure of measurement will in principle allow an appropriate selection of a contact lens material for the wearer and is the ultimate goal of the work. Being able to match closely the lens material to the individual wearer in this way will ensure longevity of lens use and patient maximum comfort: 'turning drop outs into success stories'.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

The contact lens materials used consisted exclusively of unworn and worn soft contact lenses of various types, currently available on the British market and CE marked.

2.2. Analytical techniques

2.2.1.General description

The analysis of the contact lens spoilation was carried out by UV and fluorescence spectrophotometry. The total amount of protein found either on the surface or in the lens matrix was determined by UV spectrophotometry with analysis of both the contact lens itself and the solution in which it has been stored since its removal from the eye. The analysis of the storage solution and of the lens residual protein allowed a better understanding of the behaviour of the contact lens in the eye and of the capacity of protein to exchange between the tear film and the lens.

The level of protein and lipid adsorbed or deposited on both the anterior and posterior surfaces of the contact lens was assessed by surface fluorescence spectrophotometry.

The lipid composition of the tear film as well as the composition of lipids deposits found on contact lens were determined by High Performance Liquid Chromatography (HPLC).

The dynamic surface behaviour of thin lipid layers, similar to those found at the surface of the tear film, was assessed in vitro by using a Langmuir Trough system.

2.2.2. UV spectrophotometry [159,160,161]

2.2.2.1. Principle

All biochemical compounds absorb energy from at least one region of the electromagnetic radiation spectrum. The wavelength absorbed and the efficiency of absorption depends on both the structure and the environment of the molecule.

In the ground state of a molecule, the electrons occupy the lowest levels of energy as required by the laws of quantum mechanics. Electrons can move to an excited state by absorbing energy; this change between energy levels or electronic transition puts the molecule into an excited state. For most molecules the wavelengths associated with this transition between ground state and first excited state are generally limited to the UV-visible region (200-800 nm).

When light encounters a molecule, it can either be scattered or absorbed. Absorption only occurs when the radiation provides the electrons of the molecule with the exact quantum of energy they require to change energy level. This can be expressed mathematically by the following relationship:

$$\Delta E_{(\text{level2 -level1})} = E_2 - E_1 = E_{\text{radiation}}$$

with $E_{\text{radiation}} = h\nu$ h= Planck's constant = $6.63 * 10^{-34}$ J s

ν=frequency of the radiation= $c/\lambda = 3*10^8 m.s^{-1}/\lambda$ (nm)

E_2 energy level of the electron in the excited state

E_1 energy level of the electron in the initial state

Spectrophotometry involves the measurement of these absorptions and thereby enables the characterisation and quantification of molecules. Proteins can be measured spectrophotometrically using intrinsic chromophores in the UV region such as the aromatic residues that absorb around 280 nm or the peptide bonds that absorb around 215 nm. The amino acids involved in protein absorption of UV light are listed below with their respective wavelength of maximal absorption:

	λmax (nm)
Tryptophan	280
Tyrosine	274
Phenylalanine	257

The transmittance (T) is defined as the ratio of transmitted and incident light:

$$T = I / I_0$$

where I_0 is the intensity of the incident radiation and I the intensity of the transmitted radiation.

A totally opaque sample will have a T value of zero whereas a T value of 1 is characteristic of a totally transparent sample.

The absorbance (A) of a sample is related to its transmittance and is given by the following relationships:

$$A = \log_{10} (1/T) = \log_{10} (I_0 / I)$$

Absorbance values can range from 0 for a totally transparent substance to ∞ for a totally opaque substance.

The absorbance is also stated by the Beer-Lambert law to be proportional to the concentration of the absorbing compound in the solution according to:

$$A = \varepsilon_\lambda * c * L$$

with

ε_λ: molar absorptivity of studied compound ($l.mol^{-1}.cm^{-1}$ or cm^2/mol)

c: concentration of the absorbing compound

L: length of the light path through the sample ~ thickness of the sample ~ size of the cell used

2.2.2.2. Instrumentation

Most spectrophotometers (Fig. 2.1) can be described as follows:

(i)- a **light source** that provides a wide range of wavelengths, usually a tungsten or halogen lamp with an output from 350 to 900 nm (visible range) and deuterium lamp with an output from 200 to 400 nm (UV range).

(ii)- a **monochromator** or optical filter to select the precise wavelength of interest

(iii)- an optical system of mirrors and half mirrors

(iv)- a **compartment** to hold the sample to measure and the reference (sample holder)

(v)- a photosensitive **detector** to measure the amount of light transmitted by the sample.

The multi wavelength radiation emitted by the lamp source is converted into a parallel beam of monochromatic radiation (i.e. radiation of a single wavelength) by the monochromator through refraction by a prism or diffraction by a grating. The beam is then divided between the sample and the reference (or switched from the sample to the reference) so that a light of the same wavelength illuminates both the sample and the reference by an optical system of mirrors and half mirrors. The photosensitive detector then converts the quanta of

radiation transmitted by the sample and the reference into electrical energy that can be amplified, detected and recorded.

The instrument used in this project was a Hitachi U2000 spectrophotometer. It is a double beam spectrophotometer. It has a Seya-Namioka monochromator with an Hitachi high resolution diffraction grating and uses half mirrors to split the incident beam between sample and reference cells. The reference cell consists of a square quartz cuvette (10x10x40mm) and the sample cell consists either of a similar cell or of a cylindrical cell (∅ 5mm, 30 mm).

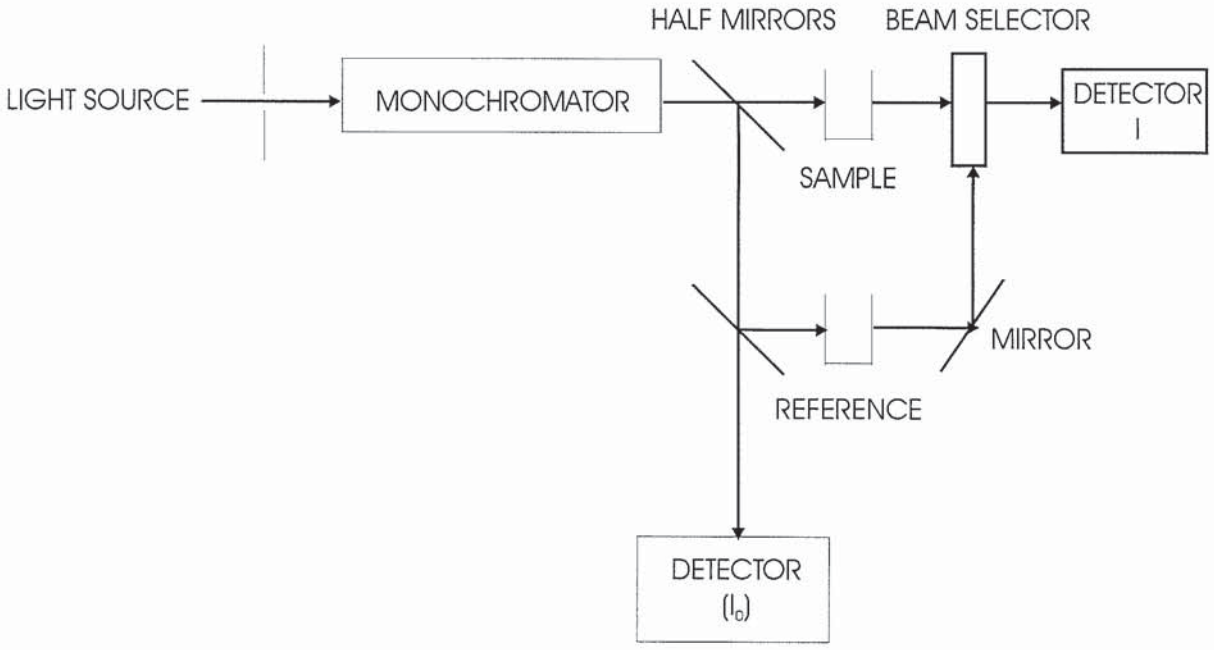


Figure 2.1. Optical arrangement of UV spectrophotometer.

2.2.3. Fluorescence spectrophotometry [159,160,161,162,163,164,165]

2.2.3.1. Principle

Fluorescence is the phenomenon whereby a molecule after absorbing radiation emits radiation of a longer wavelength, a phenomenon described as Stoke's shift.

As described previously a molecule can only absorb discrete amounts of energy that will move the molecule from a lower energy level to a higher energy level. When a molecule is in its ground state S_0, it can absorb amounts of energy greater than the energy of the lowest electronic level of the first excited state S_1 and any excess of energy is absorbed as vibrational energy (S_1V_1). After absorption, this energy is rapidly lost as heat by collision with solvent molecules (relaxation) and the excited molecule will fall to the lowest vibrational level in its new (the lowest) excited state (S_1,V_0 Figure 2.2). This excited molecule eventually regains its ground state (S_0V_0) and the energy emitted as light during this transition gives rise to a fluorescence peak. The fluorescence light emitted thereby will always be of lower energy than the absorbed light.

The determination and comparison of both excitation and fluorescence spectra of a compound may help to identify the different fluorophores involved and when applied to contact lens deposition, enables the monitoring of proteins and lipids present on a contact lens surface. Proteins are characterised by an emission fluorescence peak between 320-350 nm for an excitation wavelength of 280 nm. The lipids are characterised by an emission fluorescence peak around 410-450 nm at an excitation wavelength of 360 nm.

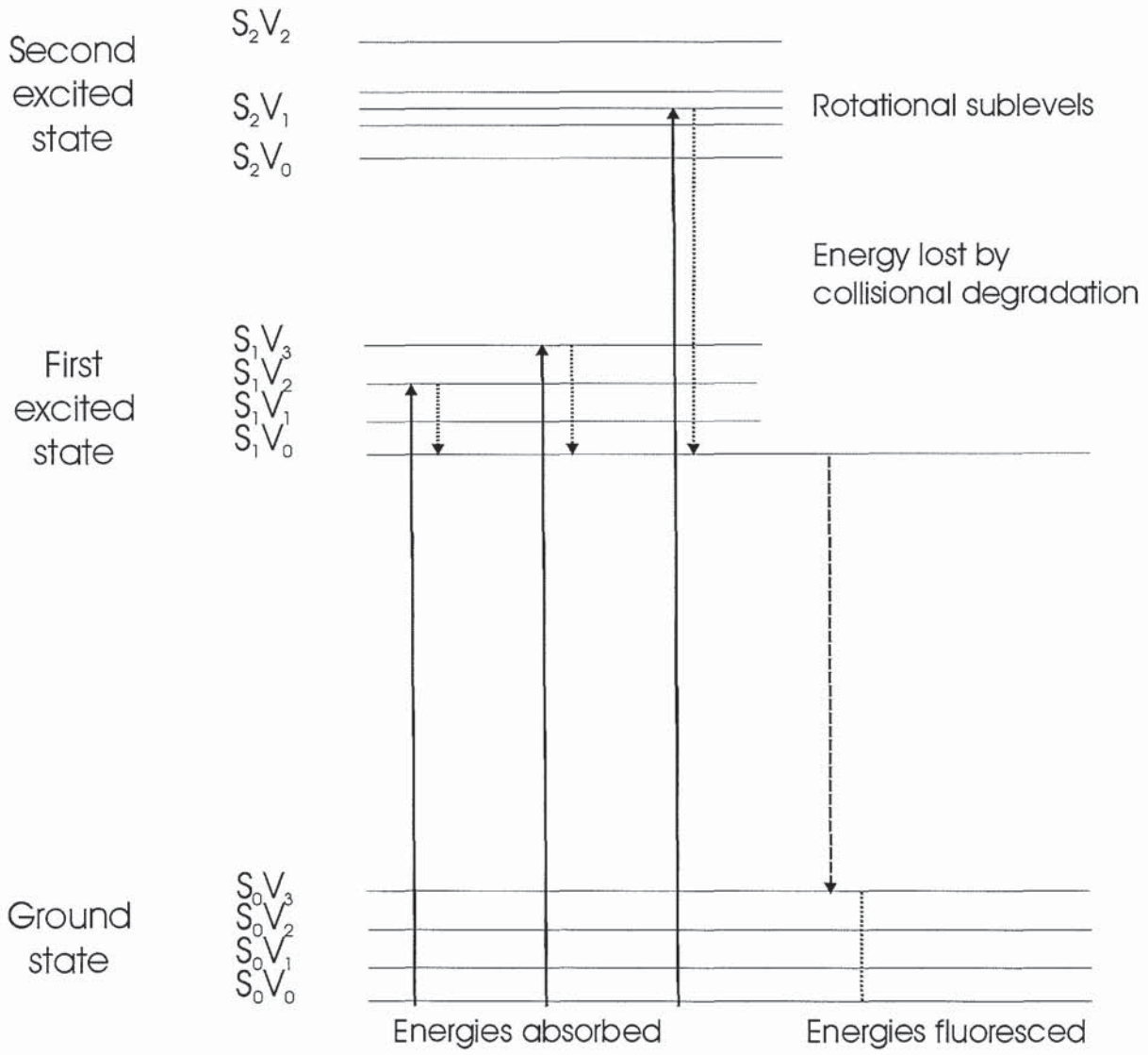


Figure 2.2. Jablonski diagram of energy levels.

The amount of fluorescence emitted by a substance depends upon the intrinsic characteristics of the substance, the characteristics of the excitation radiation but also the environmental conditions such as pH and solutes[166]. 'Quenching' relates to a reduction in fluorescence efficiency due to the absorption of energy by molecules that do not emit radiation. Collisional quenching occurs when a fluorescent molecule in an excited state collides with a quencher, transferring energy[167,168]. The quenching phenomenon is described by the Stein-Volmer equation:

$$\mathbf{F_0/F = 1 + k_q \tau_o [Q] = 1 + k_d [Q]}$$

with F & F_0 = intensities of fluorescence with and without a quencher

k_q = bimolecular quenching constant

τ_o = lifetime of fluorophore in absence of quencher

[Q] = concentration of quencher

$k_d = k_q \tau_o$ = Stern-Volmer quenching constant.

Fluorescence intensities unfortunately reflect the apparatus used to record them as much as the nature of the fluorescent sample. However, the dependence of fluorescence on the chromophore concentration can be derived from the Beer-Lambert law as follows:

(i) $\mathbf{It = Io\ 10^{-\varepsilon cl}}$

where:

Io = incident intensity

It = intensity transmitted

ε = molar absorptivity of studied compound ($l.mol^{-1}.cm^{-1}$ or cm^2/mol)

l = length of the light path through the sample ~ thickness of the sample

c = molar concentration of solute

(ii) $$\mathbf{Ia = Io - It = Io*(1- 10^{-\varepsilon cl})}$$

where:

Ia = intensity absorbed

(iii) $$\mathbf{If = Ia * Qf = Io*Qf*(1- 10^{-\varepsilon cl})}$$

where:

If = intensity of fluorescence

Qf = quantum yield = fraction of photoexcited molecules which lose their excess energy as fluorescence (range from 0 to 1); Qf depends strongly on environmental factors such as temperature, solvent, local polarity, quenching agents)

and $\mathbf{If = 2.303*\varepsilon*c*l*Io*Qf}$ when $\varepsilon cl<0.05$

So, $\mathbf{If \propto c}$ for very low concentrations

Fluorescent molecules or fluorophores are relatively rare and usually involve ring systems. Fluorescence may be expected from aromatic compounds or those having conjugated double bonds.

For proteins there are only three intrinsic fluorophores: tryptophan, tyrosine and phenylalanine each of them containing an indole ring. Their structures are shown in Fig. 2.3. Upon excitation at 280 nm, the fluorescence emission of most proteins around 340 nm is dominated by tryptophan fluorescence (Fig. 2.4).

The fluorescence of lipids is most visible upon excitation at 360nm and the emission is set around 430-440 nm[169].

2.2.3.2. Instrumentation

Spectrophotofluorimeters (Fig. 2.5) are composed of the following elements:

(i) - a **light source**, as fluorescence intensity is proportional to incident intensity, bright continuous source are commonly used, usually mercury or Xenon arcs lamps

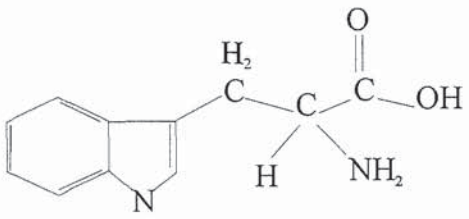
(ii) - a **monochromator** or filter to select the appropriate excitation and emission wavelength

(iii) - a **compartment** to put the sample to measure, with entry and exit ports for the excitation and emission beams and excluding rigorously any environmental light

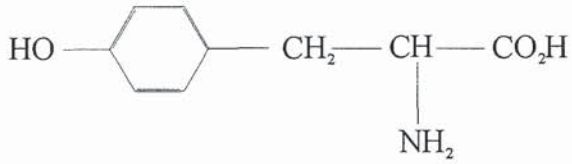
(iv) - a photosensitive **detector** to measure the amount of light emitted by the sample

The instrument used in this project was a Hitachi model F4500 spectrophotometer.

TRYPTOPHAN



TYROSINE



PHENYLALANINE

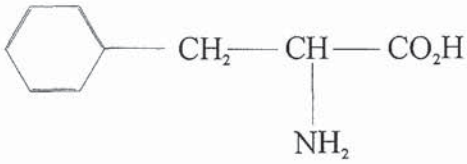


Figure 2.3. Chemical structure of protein intrinsic fluorophores.

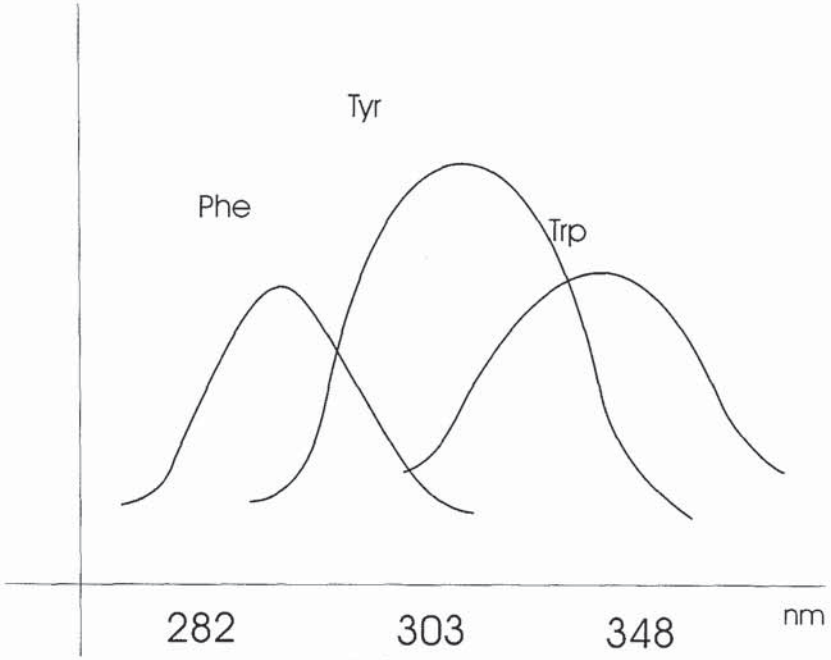


Figure 2.4. Fluorescence emission spectra of phenylalanine, tyrosine and tryptophan.

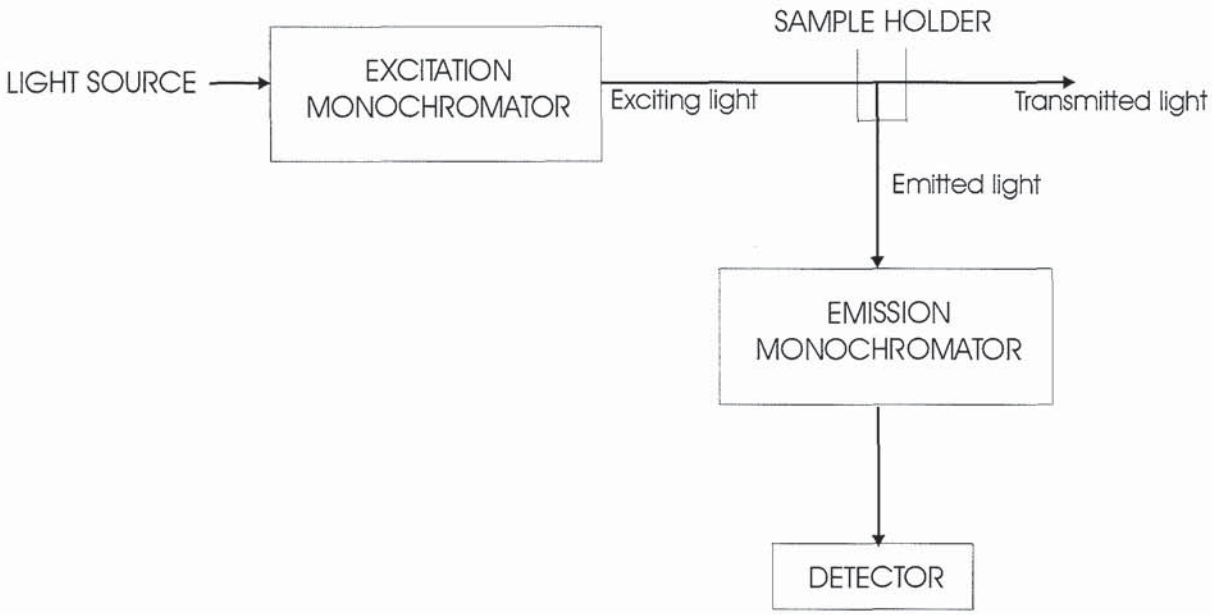


Figure 2.5. Optical principle of spectrophotofluorimeter

2.2.4. High performance liquid chromatography [170,171,172]

2.2.4.1 Principle

Chromatography is one of the most sensitive methods for separating and identifying biochemical compounds.

The substances to be analysed are placed in a system consisting of two physically distinguishable components: a mobile phase and a stationary phase. Because the affinity of the various molecular species differs between the two phases, this property is used to separate them. The movement of each molecule is a result of a balance between a driving force generated by the mobile phase and the retarding effects of partition or adsorption generated by the stationary phase.

There are many modes of separation such as gel permeation, adsorption, partition, ion-exchange or reverse-phase and many specialised techniques based upon the different modes of separation e.g. column, paper, thin layer and gas chromatography. Amongst those High Performance Liquid Chromatography (HPLC) (Fig. 2.6) is an extremely sensitive form of chromatography. It is characterised by a very rapid separation, excellent peak resolution and requires very little sample to test. It is an ideal technique for identification of both proteins and lipids extracted from contact lenses or present in the tear film.

In this project HPLC was used to identify the lipid classes present in tears and/or deposited on contact lenses during wear. The separation between the main lipid classes was done by adsorption chromatography, a technique used mainly for the separation of compounds highly soluble in organic solvents or with low stability and aggregation problems in aqueous mobile phases (eg. phospholipids). In adsorption chromatography, the stationary phase acting, as

an absorbent, is usually made up of silica. The separation process is the result of the competition between the different solute and solvent molecules of the mobile phase for adsorption on the solid surface. Molecules are adsorbed selectively and therefore displaced differently which allows separation of the different components. Solvents are usually organic and non polar. Solutes are eluted in order of increasing polarity and the retention time decreases with the polarity of the solvent.

Five main lipid classes were separated with the following retention times (Table 2.1) and a typical HPLC trace is shown in Appendix A.

Table 2.1. Retention times of various lipids

Lipid type	Retention time range (s)
Cholesterol esters	61-73
Triglycerides	110-163
Diglycerides	202-260
Fatty acids	170-288
Phospholipids part 1	71-118
Phospholipids part 2	127-248
Monoglycerides	507-727
Cholesterol	862-942

2.2.4.2. Instrumentation [173]

In this project HPLC was carried out using a Knauer pump system on a Lichrosorb 5 μm (250mm x 4mm ID) SI 60 normal phase column with UV and fluorescence detectors, respectively Perkin-Elmer LC-75 UV detector and

Perkin-Elmer LS-1 fluorescence detector with a lipid filter set around 330-350 nm. The mobile phase was hexane: propan-2-ol:acetic acid (1000:5:1 v/v) and the flow rate was set up at 2 ml/min.

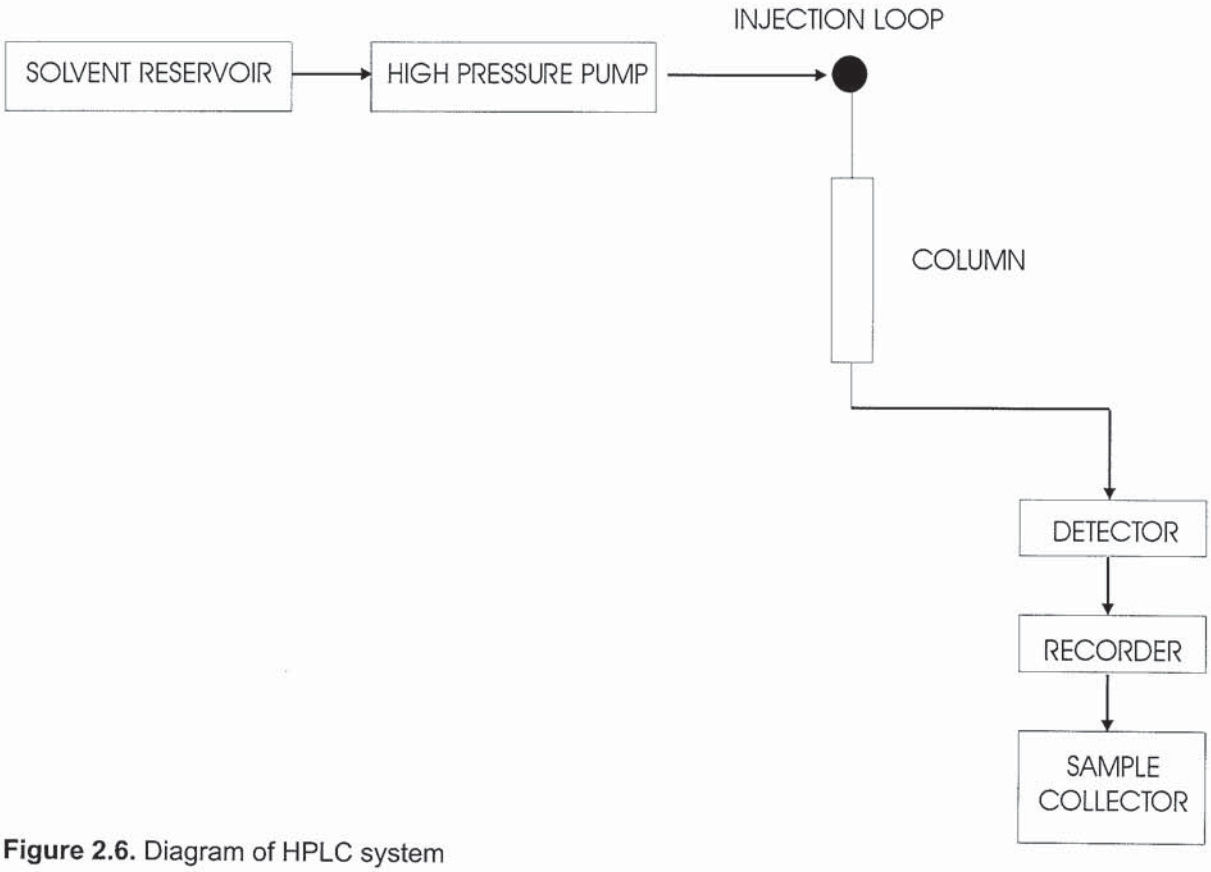


Figure 2.6. Diagram of HPLC system

2.2.4.3. Sample preparation for HPLC

2.2.4.3.a. Extraction procedure from contact lenses

The following steps were followed for each sample:

- The contact lens to be analysed was placed in 2 ml of HPLC grade methanol and left to extract for a minimum of 30 minutes on a low speed shaker;

- After removing the lens, the methanol was evaporated by bubbling nitrogen over the surface of the liquid. The samples were then either analysed straight away or kept refrigerated until analysis.
- A 200 μl mobile phase was added to the sample vial and shaken gently to ensure that any deposit on the walls of the vial was dissolved.

2.2.4.3.b. Extraction procedure for tear analysis

The development of adequate sampling and extraction procedures was part of this research project and is therefore described in details in Chapter 4.

2.2.5. Thin film studies

2.2.5.1. Principle

Langmuir Trough film studies were used to measure the surface pressure of a solution under compression (Fig. 2.7). The technique is usually applied to insoluble monolayers or duplex film spread onto a probe liquid. This technique was used to investigate in vitro the spreading properties of different lipids over an aqueous layer.

2.2.5.2. Instrumentation

The mini trough used, manufactured by NIMA Technology, Coventry, UK, was made of Teflon and could contain between 50 and 60 ml of liquid. 50 ml of HPLC grade water was used as a probe liquid or sublayer. A maximum of 5μl of solution to study was placed on the surface of the probe liquid. The dynamic behaviour under compression of this solution was studied by compressing and expending the layer for 5 to 10 cycles. During the cycles,

the surface pressure, in mN/m, was recorded as a function of the surface area, in cm^2. During a cycle, the total area could be compressed by up to 80% ranging from 89 cm^2 to 15 or 20 cm^2.

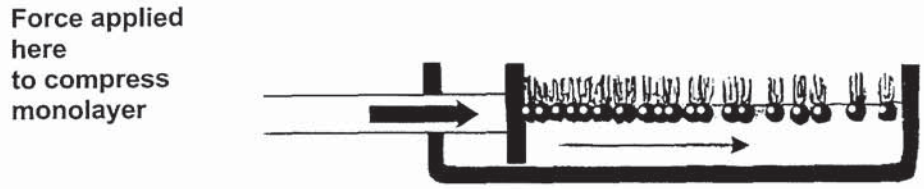


Figure 2.7. Langmuir-Trough system

2.3. Statistical analysis

2.3.1. General procedures [174]

Ordinal data were compared using the following non parametric tests, Wilcoxon and Friedman tests for paired samples and Mann-Whitney test for independent samples. Individual comparisons, for more than two samples/groups, were computed using Friedman two-way ANOVA or Non parametric Tukey test.

Continuous data were compared using parametric tests such as T-test for paired or independent samples. One way analysis of variance (ANOVA) followed by LSD, SNK or Tukey tests was used to carry out individual comparisons. However the continuous data did not always follow a normal distribution; in these cases, the non-parametric tests described previously were selected. For each test, the level of significance was taken as 0.05 at 95% confidence.

2.3.2. Answer Tree / CHAID

Answer Tree™ provides a way to examine data and discover important grouping of cases. It allows the detection of key variables that identify group membership. A software package known as Answer Tree™ is used to generate classification rules from existing data. To do so, three grouping algorithms were available:

- CHAID (Chi Square Automatic Interaction Detector)[175,176]
- C & RT (Classification & Regression Tree)[177]
- QUEST (Quick, Unbiased, Efficient, Statistical Tree)[178].

The algorithms summarised statistically significant patterns or relationships and made decision rules. At each step, the decision rules were used to partition the overall data into subgroups. The same procedure was then repeated for each subgroups until it was not possible to further divide the population. A tree built using the CHAID algorithm was constructed by splitting the subsets into two or more subgroups repeatedly, beginning with the entire data set. To determine the best split CHAID algorithm merges any allowable pair of categories of the predictor variables for which no statistically significant differences are detected. This process is repeated until no non-significant pair is found. The resulting set of categories represents the best split for that predictor variable.

The Exhaustive CHAID algorithm finds the best split by merging similar pairs continuously until only a single pair remains. The set of categories with the largest significance is taken to be the best split for that predictor variable. The process is reiterated for all predictor variables. The predictor that gives the

best prediction is selected and the data set is the divided into subgroups accordingly.

During the C&RT algorithm, for each split each predictor is evaluated the find the best cut point for continuous predictors or grouping of categories, nominal or ordinal predictors, based on improvement score or reduction of impurity, trying to get any subsets as homogeneous as possible. The predictor with best improvement is selected and the data set is split into two subgroups accordingly.

CHAPTER 3

ANALYSIS OF CONTACT LENS DEPOSITION AND ITS EFFECT ON CONTACT LENS PERFORMANCE

3.1. Repeatability of deposition measurements

3.1.1. Repeatability of protein measurements by UV spectrophotometry

3.1.1.1. Objective

The purpose of this study was to evaluate the repeatability of UV spectrophotometry applied to soft contact lenses and to estimate the validity of this technique for the quantification of proteins held by soft contact lenses. This study was also used to determine the number of measurements required of each sample to quantify with 95% confidence the amount of protein present.

3.1.1.2. Test materials

Test materials consisted both of worn and unworn contact lenses. Six types of unworn contact lenses, representatives of the FDA classification, were tested by UV spectroscopy (Table 3.1). The worn lenses tested were provided by CLRC and consisted of 4 contact lens types representative of the FDA classification (Table 3.2). The selection criterion for the worn contact lenses was an average daily wear time of 6 hours.

Table 3.1. Characteristics of unworn SCL types used to assess the repeatability of protein measurements by UV spectrophotometry

Lens Type	Lens characteristic	Power(Diopter)
Acuvue	FDA Group IV, not tinted	-6.00/-5.25/-5.25/ -2.00/ -0.50/+3.75
Acuvue	FDA Group IV, tinted	-2.50/-2.50
Excelens	FDA Group II, tinted	-0.50/ -0.75/ -1.00/ -2.00/ -2.00/ -3.00
NewVue	FDA Group IV, not tinted	-1.25/ -2.00/ -2.75/ -3.00 /-4.00/ -4.75
SeeQuence	FDA Group I, tinted	-0.75/ -1.75/ -2.00/ -2.75/ -3.25 /-3.50
SeeQuence2	FDA Group I, tinted	-2.75/ -3.00/ -4.00

Table 3.2. Characteristics of worn SCL types used to assess the repeatability of protein measurements by UV spectrophotometry

Lens Type	Lens characteristic	Power(Diopter)
Acuvue	FDA Group IV, not tinted	-6.00
Acuvue	FDA Group IV, tinted	-2.50
Acuvue	FDA Group IV, not tinted	-2.00
Excelens	FDA Group II, tinted	-0.50
Excelens	FDA Group II, tinted	-3.00
Excelens	FDA Group II, tinted	-2.00
NewVue	FDA Group IV, not tinted	-2.00
NewVue	FDA Group IV, not tinted	-0.75
NewVue	FDA Group IV, not tinted	-4.00
SeeQuence2	FDA Group I, tinted	-2.00
SeeQuence2	FDA Group I, tinted	-2.75
SeeQuence2	FDA Group I, tinted	-3.50

3.1.1.3. Experimental design and procedure

The experimental design was a ten independent measurement design on both unworn and worn contact lenses with an average of 3 lenses for each type. A total of 12 worn and 32 unworn contact lenses were tested. The advantage of such a design is to permit the determination of the random uncertainties that can affect the instrumental measurements and thus to evaluate the precision (repeatability) of the instrument that indicates its ability to repeat its own results (Hayward, 1977).

The level of proteins on the surface and in the bulk of the contact lenses was assessed non-destructively with a U-2000 spectrometer (Hitachi). The contact lens was placed into a quartz cell with its centre facing the direction of the incident light emitted by a deuterium lamp. The excitation wavelength was set to 280 nm and the absorbance measured in order to calculate the quantity of proteins absorbed on the lens.

The same detailed routine procedure was followed for each sample analysis. After switching the spectrophotometer on and turning on the deuterium lamp currently used for measurements in the UV, the following sequence of procedures was initiated

(i) **-Selection of the appropriate wavelength for the measurements** (280 nm)

(ii) **-Zeroing for absorbance.**

The instrument was zero adjusted using pure distilled water in both quartz cells, the sample and reference cells.

(iii) **-Measurement of the absorbance.**

The contact lens to measure was placed in distilled water in the sample quartz cell and was positioned at the bottom of the cell so that it faced the direction of the incident light.

3.1.1.4. Statistical analysis

The random uncertainty was calculated from the ten absorbance measurements at 280 nm taken for each lens. The measurements were considered independent as the UV spectrophotometer was turned off and re-initialised (zero transmittance, zero absorbance, calibration) between each series of measurements.

For each sample, the half-range random uncertainty **(Ri)** taken at the 95% confidence level was obtained according to statistical laws by multiplying the standard deviation of the ten measurements by the corresponding value (t) of the Student t-function:

$$\mathbf{Ri = \sigma i * t = 2.26 * \sigma i}$$

t= 2.26 for n=10 and α=0.05 (Zar,1974)[174]

The uncertainty for each contact lens type Rmean(%) was then calculated as follows:

$$\mathbf{Rmean(\%)=\sqrt{((Ri\%)^2 / n)}}$$

with n= number of sample of the same type.

The number of measurement required for each lens type was subsequently calculated as follows:

- for a 10% uncertainty with a 95% confidence,

$$\mathbf{n10\%= (Rmean(\%)/10)^2}$$

- for a 20% uncertainty with a 95% confidence,

$$\mathbf{n20\%= (Rmean(\%)/20)^2}$$

Parametric independent T-test and OneWay analysis of variance (ANOVA)[174] were the two main statistical tests used respectively to compare the uncertainty between worn and new contact lenses and between the different lens types.

3.1.1.5. Results

3.1.1.5.a. Unworn contact lenses

The individual uncertainty values for each lens are shown in Appendix B. For the unworn contact lenses, the uncertainty R% is significantly dependent of the lens type (p=0.0012) with a significantly higher uncertainty for SeeQuence and a relatively lower one for Excelens (Tables 3.3 & 3.4.).

The high variability values can be explained by the difficulty in positioning of the contact lens in the quartz cell where there was no system preventing the lens from shifting during the examination. This is particularly noticeable for the

lenses with low modulus of rigidity like the Group IV (high water content) lenses. The inappropriate positioning of the contact lens in the cell can result in various measurement errors such as:

- a bad centration of the beam onto the lens

- a contact lens that is not in a plane perpendicular to the optical beam of the spectrometer, therefore increasing artificially the optical path and provoking an over estimation of the absorbance value of the lens.

Because of the high variability of the unworn contact lenses, ranging from 10.5% uncertainty for Excelens to 31.2% for SeeQuence, for a 20% or less uncertainty on the measurement of the absorbance, 2 measurements are required on average. But up to 10 measurements could be required for a 10% maximum of uncertainty at 95% confidence. A new way of positioning the contact lens before analysis was therefore investigated as described in section 3.1.1.6.

3.1.1.5.b. Worn contact lenses

For the worn lenses, the variability of the UV absorbance measurements, reported for each lens individually in Appendix C, is ranging from 4.5% uncertainty for Acuvue to 13.3% for SeeQuence2. The measurements are statistically significantly ($p=0.044$) more repeatable for the worn contact lenses than for unworn lenses of the same type (Tables 3.5. & 3.6.). This decrease in the variability of the measurements of worn lenses compared to unworn lenses is especially noticeable for Acuvue and NewVue lenses (FDA Group IV) and for SeeQuence 2 (FDA Group I). There was no significant difference in measurement repeatability between worn and unworn Excelens contact lenses.

Therefore for worn contact lenses one measurement of the test sample is sufficient to determine the UV in absorbance with a maximum of uncertainty of 20% and usually at most two measurements to achieve a maximum uncertainty of 10% at 95% of confidence level. The lens type is not a significant factor that modify the technique uncertainty for worn contact lenses.

3.1.1.6. Discussion

The results consolidate the idea that an unworn matching contact lens should not be used as reference while running a UV measurement of the worn lens to analyse in order to minimise the uncertainty of the measurements. The analysis should always be run using distilled water as reference as explained before.

Also, as the variability seems mostly due to the movement of the contact lens in the quartz cell, a new way of positioning the lens for the analysis was tested. The repeatability of the UV technique to measure protein on contact lenses was evaluated in a similar way using a new cylindrical cell sample holder. The results for the different lenses, summarised in Tables 3.7 & 3.8, indicated a noticeable improvement in comparison to the first experiment since a better and easier positioning of the contact lens in the sample holder was achieved. The number of measurements required is cut down for the unworn lenses from 5 to 1 for Group I & II lens materials and from 7 to 3 for Group IV lens materials. The cylindrical quartz cell was therefore used to run all further UV analysis of soft contact lenses.

Table 3.3. UV spectroscopy - Repeatability results for the unworn contact lenses by contact lens type (mean values)

Lens type	R%	n10%	n20%
Acuvue	21.6	5	2
Acuvue(t)	13.9	2	1
NewVue	20.8	5	2
Excelens	10.5	2	1
SeeQuence2	19.8	4	1
SeeQuence	31.2	10	3
Focus	18.1	4	1

Table 3.4. UV spectroscopy - Repeatability results for the unworn contact lenses - Comparison between lens types by Oneway ANOVA with LSD test (* values joined by a line are not significantly different)

(p=0.0012)	Excelens	Acu(t)	Focus	SeeQ2	NVue	Acu	SeeQ
Mean	9.9	13.8	18.0	18.4	19.9	21.0	31.1

LSD(5%)* ——————————————————

Table 3.5. UV spectroscopy - Repeatability results for the worn contact lenses by contact lens type (mean values)

	R%	n10%	n20%
Acuvue	4.5	1	1
Acuvue(t)	13.1	2	1
NewVue	13.5	2	1
Excelens	9.2	1	1
SeeQuence2	13.3	2	1

Table 3.6. UV spectroscopy - Repeatability results for the worn contact lenses - Comparison between lens type by One Way ANOVA with LSD test (* values joined by a line are not significantly different)

(p=0.1822)	Acu	Excelens	NVue	Acu(t)	SeeQ2
Mean	4.5	9.0	12.5	13.1	13.3

LSD(5%)* ——————————————————

Table 3.7 . UV spectroscopy - Repeatability on unworn lenses - Results for both the new and the old sample holder- Individual measurements

Lens Type	Power(D)	Cylindrical cell			Rectangular cell		
		R%	n10%	n20%	R%	n10%	n20%
Acuvue	-6.00	14.9	3	1	25.2	7	2
Acuvue(t)	-2.00	9.9	1	1	14.9	3	1
Acuvue	-2.00	16.9	3	1	15.1	3	1
Excelens	-0.50	3.9	1	1	5.1	1	1
Excelens	-3.00	5.6	1	1	11.3	2	1
Excelens	-2.00	2.6	1	1	13.6	2	1
NewVue	-4.00	11.9	2	1	13.7	2	1
NewVue	-0.75	11.0	2	1	11.3	2	1
NewVue	-2.00	6.0	2	1	19.5	4	1
SeeQuence2	-2.00	12.2	2	1	15.8	3	1
SeeQuence2	-2.75	9.5	1	1	20.3	5	2
SeeQuence2	-3.50	13.3	2	1	14.9	3	1

Table 3.8. UV spectrometry - Repeatability on worn lenses - Results for both the new and the old sample holder- Individual measurements

Lens Type	Power(D)	Cylindrical cell			Rectangular cell		
		R%	n10%	n20%	R%	n10%	n20%
Acuvue	-6.00	-	-	-	3.0	1	1
Acuvue(t)	-2.50	-	-	-	13.1	2	1
Acuvue	-2.00	6.5	1	1	6.0	1	1
Excelens	-0.50	6.5	1	1	11.5	2	1
Excelens	-3.00	5.3	1	1	7.6	1	1
Excelens	-2.00	4.6	1	1	7.9	1	1
NewVue	-4.00	-	-	-	6.5	1	1
NewVue[+]	-0.75	16.7	3	1	11.8	2	1
NewVue	-2.00	-	-	-	19.2	4	1
SeeQuence2	-2.00	11.2	2	1	12.7	2	1
SeeQuence2	-2.75	14.0	2	1	14.9	3	1
SeeQuence2	-3.50	18.1	4	1	12.1	2	1

[+] The scratches on the contact lens can explain the high level of uncertainty.

3.1.2. Repeatability of protein and lipid measurements by fluorescence spectrophotometry

3.1.2.1. Objective

The purpose of this investigation was to evaluate the repeatability of fluorescence spectrophotometry applied to soft contact lenses and to estimate the validity of this technique for the quantification of proteins and the lipids on the surface of soft contact lenses.

This study was also used to determine the number of measurements required of each sample to quantify with 95% confidence the peak intensity, the peak wavelength for both proteins and lipids.

3.1.2.2. Test materials

The experimental material consisted of 12 worn contact lenses representative of the FDA classification (Table 3.9). The selection criterion for the worn contact lenses was an average daily wear time of 6 hours. In addition to these lenses, an identical sample of unworn lenses of the same type and same characteristics was also analysed (Table 3.10).

Table 3.9. Characteristics of worn SCL types used to assess the repeatability of protein and lipid measurements by Fluorescence spectrophotometry.

Sample	Lens Type	Lens characteristic	Power(Diopter)
1	Acuvue	FDA Group IV, not tinted	-5.00
2	Acuvue	FDA Group IV, tinted	-2.50
3	Acuvue	FDA Group IV, not tinted	-3.00
4	Excelens	FDA Group II, tinted	-0.50
5	Excelens	FDA Group II, tinted	-3.00
6	Excelens	FDA Group II, tinted	-2.00
7	NewVue	FDA Group IV, not tinted	-0.75
8	NewVue	FDA Group IV, not tinted	-3.25
9	NewVue	FDA Group IV, not tinted	-4.50
10	SeeQuence2	FDA Group I, tinted	-2.00
11	SeeQuence2	FDA Group I, tinted	-2.75
12	SeeQuence2	FDA Group I, tinted	-3.50

Table 3.10. Characteristics of unworn SCL types used to assess the repeatability of protein and lipid measurements by Fluorescence spectrophotometry.

Sample	Lens Type	Lens characteristic	Power(Diopter)
1	Acuvue	FDA Group IV, not tinted	-5.00
2	Acuvue	FDA Group IV, tinted	-3.00
3	Acuvue	FDA Group IV, not tinted	-3.00
4	Excelens	FDA Group II, tinted	-0.50
5	Excelens	FDA Group II, tinted	-3.00
6	Excelens	FDA Group II, tinted	-2.00
7	NewVue	FDA Group IV, not tinted	-0.75
8	NewVue	FDA Group IV, not tinted	-3.25
9	NewVue	FDA Group IV, not tinted	-4.50
10	SeeQuence2	FDA Group I, tinted	-2.00
11	SeeQuence2	FDA Group I, tinted	-2.75
12	SeeQuence2	FDA Group I, tinted	-3.50

3.1.2.3. Experimental design and routine

The experimental design was based upon the comparison of independent measurements (n=5) on new and worn contact lenses. Three contact lenses of each type were used, making a total of 12 worn and 12 unworn contact lenses tested.

The level of proteins and lipids on the surface of contact lenses was assessed non-destructively with a specially modified fluorescence spectrophotometer (Hitachi).

The lens was placed into a cylindrical quartz cell. The excitation wavelength was set to 280 or 360 nm. A fluorescence emission spectrum, or variation in the fluorescence intensity of the sample when excited at a predetermined and fixed lower wavelength, was recorded from 300 nm to 500 nm.

The detailed routine procedure given below was applied for each sample:

(i) - **Selection of the type of spectrum required (single wavelength scan or 3D scan)**

For this investigation, the measurements were recorded using the single wavelength scan.

(ii) - **Selection of the appropriate excitation wavelength.**

The contact lenses were studied using two different excitation wavelengths: 280 nm for the proteins and 360 nm for the lipids.

(iv) - **Recording of the reference fluorescence spectrum.**

The fluorescence spectrum of cell filled with distilled water was recorded.

(v) - **Recording of the sample fluorescence.**

The contact lens sample was placed into the sample quartz cell and the fluorescence spectrum was recorded for each excitation wavelength.

(vi) - **Measurement of the sample fluorescence.**

The spectrum recorded was then analysed and the peak wavelength and intensity for each fluorescence emission (proteins at 280 nm, lipids at 360 nm = 4 measurements per lens) measured.

3.1.2.4. Statistical analysis

The random uncertainty or precision was calculated for each lens for both the fluorescence emission wavelength and the intensity of emission peak for proteins and lipids. The measurements were considered independent as the fluorescence spectrophotometer was turned off and the samples removed between each set of measurements.

The half-range random uncertainty **(Ri)** taken at the 95% confidence level for each sample (t= 0.376 for n=5 and α=0.05 (Zar, 1974)), the uncertainty for each lens type **Rmean (%)** and finally the number of measurement **(n)** required for each lens type were calculated, as described in section 3.1.1.4 of this chapter.

Parametric independent T-test and One Way analysis of variance were the two main statistical tests used respectively to compare the uncertainty between worn and new contact lenses and between the different lens types.

3.1.2.5. Results

The individual uncertainty values for each contact lens are shown in Appendix D. For all lenses, worn or unworn, the uncertainty (R% ~ 2 in average) on the fluorescence emission wavelength for both proteins and lipids were very low (Tables 3.11 & 3.12). On the other hand, the values of the intensity of the fluorescence peaks showed a high variability, especially for the peak characteristics of lipids for excitation at 360 nm (Tables 3.13 & 3.14).

Because of the high incidence of low emission values producing low mean values, the uncertainty values expressed in percentages were particularly

high. The calculated R values in fluorescence units are therefore more representative of the level of uncertainty to expect for each measurement and the limit of detection of the instrument.

The variability of the intensity of the fluorescence peak around 340 nm for an excitation at 280 nm, characteristic of protein deposition, was high, giving uncertainties ranging from 12% (Acuvue worn lenses) to 59% (SeeQuence 2 unworn lenses). On average, the measurements of worn lenses seemed to be more repeatable than the ones of unworn lenses. For the worn lenses, the range of uncertainty was from 12% to 32% but for the unworn lenses the uncertainty was up to 59%. No statistically significant difference regarding the repeatability was shown between the different lens types (Table 3.17). For a 20% maximum uncertainty with 95% confidence, 1 to 9 measurements of the same sample are required for the unworn lenses but only 1 to 3 measurements of the same sample for the worn lenses (Table 3.16). The numbers of measurements required for a 10% or less uncertainty for proteins are summarised in Table 3.16 for both worn and unworn lenses. As indicated earlier an absolute uncertainty detection is more relevant for this parameter. If we take a threshold value, characteristic of the limit of detection, of 15 for a maximum uncertainty of 10%, the number of measurements required ranged from 1 to 19 for the unworn contact lenses and from 1 to 6 for the worn contact lenses. For a threshold value set at 10 for a maximum uncertainty of 20%, the number of measurements required ranged from 1 to 6 for the unworn contact lenses and was at the most two for the worn contact lenses.

The variability of the measurement of lipid deposition onto the contact lens surface, by using the intensity of the fluorescence peak around 450 nm for an excitation at 360 nm, is high, giving uncertainties ranging from 26% (Excelens worn) to 115% (Acuvue worn). No significant differences in repeatability were

achieved between worn and unworn lenses of the same type. However, significantly higher uncertainty values were reported for Acuvue and SeeQuence2 ($p=0.01$) than for NewVue and Excelens (Table 3.17). For a 20% maximum uncertainty with 95% confidence, 2 to 33 measurements of the same sample were required for the worn lenses and 3 to 17 measurements of the same sample for the unworn lenses. For a 10% or less uncertainty, up to 50 measurements of the same sample were needed. Similarly to protein measurements, the absolute uncertainty when taking a limit of detection of 15, the number of measurements required for a 10% maximum uncertainty was limited to one measurement for the unworn contact lenses and was between 1 and 11 for the worn lenses. With a threshold value of 10 with a 20% maximum uncertainty, the number of measurements required ranged from 1 to 9 for the unworn contact lenses and was at the most two for the worn contact lenses.

3.1.2.6. Discussion

The variability of the measurements affects mainly the intensity of the fluorescence peaks and not their emission wavelength.

The uncertainty appeared higher for the lipid peaks than for the protein ones; this could be explained by the very low intensity of the fluorescence emissions characteristics of the lipids, especially for the FDA group IV lenses such as Acuvue.

The high variability of the fluorescence analysis of deposition on contact lenses can be partly explained either by the hypersensivity of the instrument or the high mobility of protein within the contact lens matrix. Fluorescence is a surface technique and the intensity of the protein peak is dependent on the level of protein on the surface at the time of measurement. Therefore because the level of proteins present at the surface of the contact lens might

not be constant throughout the different measurements, significant variations in fluorescence emission are observed.

Fluorescence measurements were carried out to a 20% or less uncertainty at 95% confidence.

Table 3.11. Repeatability of emission wavelength – Unworn contact lenses (mean values)

Lens type	PROTEIN @280nm			LIPID@360nm		
	R%	n10%	n20%	R%	n10%	n20%
Acuvue	3	1	1	2	1	1
NewVue	1	1	1	2	1	1
SeeQuence2	2	1	1	2	1	1
Excelens	2	1	1	2	1	1

Table 3.12. Repeatability of emission wavelength – Worn contact lenses (mean values)

Lens type	PROTEIN @280nm			LIPID@360nm		
	R%	n10%	n20%	R%	n10%	n20%
Acuvue	2	1	1	4	1	1
NewVue	2	1	1	2	1	1
SeeQuence2	3	1	1	2	1	1
Excelens	1	1	1	1	1	1

Table 3.13. Repeatability of fluorescence intensity – Unworn contact lenses (mean values)

Lens type	PROTEIN @280nm				LIPID@360nm			
	R	R%	n10%	n20%	R	R%	n10%	n20%
Acuvue	18	56	31	8	7	63	40	10
NewVue	55	19	4	1	13	33	11	3
SeeQuence2	57	59	35	9	15	82	67	17
Excelens	38	24	6	1	16	37	14	3

Table 3.14. Repeatability of fluorescence intensity – Worn contact lenses (mean values)

Lens type	PROTEIN @280nm				LIPID@360nm			
	R	R%	n10%	n20%	R	R%	n10%	n20%
Acuvue	32	12	1	1	21	115	132	33
NewVue	57	32	10	3	13	30	9	2
SeeQuence2	14	29	8	2	14	71	50	13
Excelens	28	18	3	1	13	26	7	2

Table 3.15. Number of measurements required for the same sample for 20% maximum uncertainty at 95% confidence.

		PROTEIN	LIPID
Worn Lenses	Acuvue	1	33
	Newvue	3	2
	SeeQuence2	2	13
	Excelens	1	2
Unworn Lenses	Acuvue	8	10
	Newvue	1	3
	SeeQuence2	9	17
	Excelens	1	3

Table 3.16. Number of measurements required for the same sample for 10% maximum uncertainty at 95% confidence.

		PROTEIN	LIPID
Worn Lenses	Acuvue	1	132
	Newvue	10	9
	SeeQuence2	8	50
	Excelens	3	7
Unworn Lenses	Acuvue	31	40
	Newvue	4	11
	SeeQuence2	35	67
	Excelens	6	14

Table 3.17. Repeatability of fluorescence emission intensity - Comparison between contact lens types by One way ANOVA with LSD test (* values joined by a continuous line are not statistically different)

- Unworn contact lenses

Protein: p=0.2278	NewVue	Excelens	Acuvue	SeeQuence2
R%	19.46	23.92	56.20	59.02
LSD(5%)*				
Lipid: p=0.3047	NewVue	Excelens	Acuvue	SeeQuence2
R%	33.49	36.59	63.48	82.18
LSD(5%)*				

- Worn contact lenses

Protein: p=0.3044	Acuvue	Excelens	SeeQuence2	NewVue
R%	12.33	18.27	28.74	31.81
LSD(5%)*				
Lipid: p=0.0333	Excelens	NewVue	SeeQuence2	Acuvue
R%	25.90	30.42	70.60	115.15
LSD(5%)*				

3.2. Analysis of contact lens deposition and its effect on contact lens performance

3.2.1. Introduction

The purpose of this investigation was to evaluate the relationship between the level of protein and lipid deposition and contact lenses replacement modality, patient characteristics (symptomatic or asymptomatic, wearer or non-wearer), comfort and wettability. The experimental material consisted of Focus lenses worn on a daily wear basis for a year with either a daily replacement, a 2 weeks replacement or 4 weeks replacement regimen of the contact lens. For this experiment, the contact lenses to be analysed were limited to the ones worn by the patient at the seven day and one year visit. The total level of protein adsorbed at the contact lens surface or absorbed into lens matrix was measured by UV spectroscopy. The level of surface proteins and lipids was assessed on both front surface and back surface of the contact lenses by fluorescence spectrophotometry.

3.2.2. UV spectroscopy analysis results

The results are summarised in Table 3.18. The level of deposition appeared to be significantly statistically ($p<0.001$) higher for the 2 weeks (0.3110 at 7 day, 0.3576 at 1 year)* and 4 weeks replacement (0.3294 at 7 day and 0.4466 at 1 year) than for the daily disposable one (0.0880 at 7 day and 0.0925 at 1 year) for both the seven day and one year visits (Table 3.19). There was no difference between the 2 weeks and 4 weeks replacement at the 7 day visit but at the one year visit, there was significantly more proteins with the 4 weeks replacement regimen (0.4466) than with the 2 weeks one (0.3576).

* mean values expressed in absorbance units

The wearing time at the time of lens removal appeared to be a significant factor for the daily disposable only. As expected, significantly more protein deposition occurred when the contact lenses were worn for more than 6 hours (Table 3.20). There was no difference between contact lens wearers and non wearers in protein deposition measured by UV spectroscopy independently of wear regimen (Table 3.22). For the 2 and 4 weeks replacement regimen, a trend towards more protein deposition for asymptomatic compared to symptomatic appeared at the 1 year visit (Table 3.21) (0.4642 vs. 0.2165 with $p=0.001$ for 2 weeks replacement and 0.4833 vs. 0.2445 with $p=0.029$ for the 4 week replacement regimen schedule). Finally, there was no significant correlation between the amount of protein deposition and the comfort or wettability.

Table 3.18. Total protein in absorbance units and in mg/ml – Mean, standard deviation and range for each replacement regimen at 7 day and 1 year visits.

	Replacement regimen	Absorbance units	In mg/ml[+]
		Mean ± STD (Min - Max)	Mean ± STD (Min - Max)
7 DAY	1 day (N=43)	0.0880 ± 0.0887 (0.0070 - 0.3910)	0.0425 ± 0.0397 (0.0060 - 0.1780)
	2 weeks (N=54)	0.3294 ± 0.1407 (0.1020 - 0.6710)	0.1496 ± 0.0624 (0.0490 - 0.3030)
	4 weeks (N=50)	0.3110 ± 0.1301 (0.0650 - 0.7580)	0.1423 ± 0.0581 (0.0320 - 0.3420)
1 YEAR	1 day (N=43)	0.0927 ± 0.0922 (0.0070 - 0.4960)	0.0441± 0.0413 (0.0060 - 0.2250)
	2 weeks (N=42)	0.3576 ± 0.2298 (0.0400 - 1.0500)	0.1631 ± 0.1027 (0.0210 - 0.4730)
	4 weeks (N=39)	0.4466 ± 0.2398 (0.0820 - 0.9020)	0.2030 ± 0.1073 (0.0400 - 0.4070)

[+] The absorbance values were converted in mg/ml using a dedicated calibration curve (Appendix E) [179].

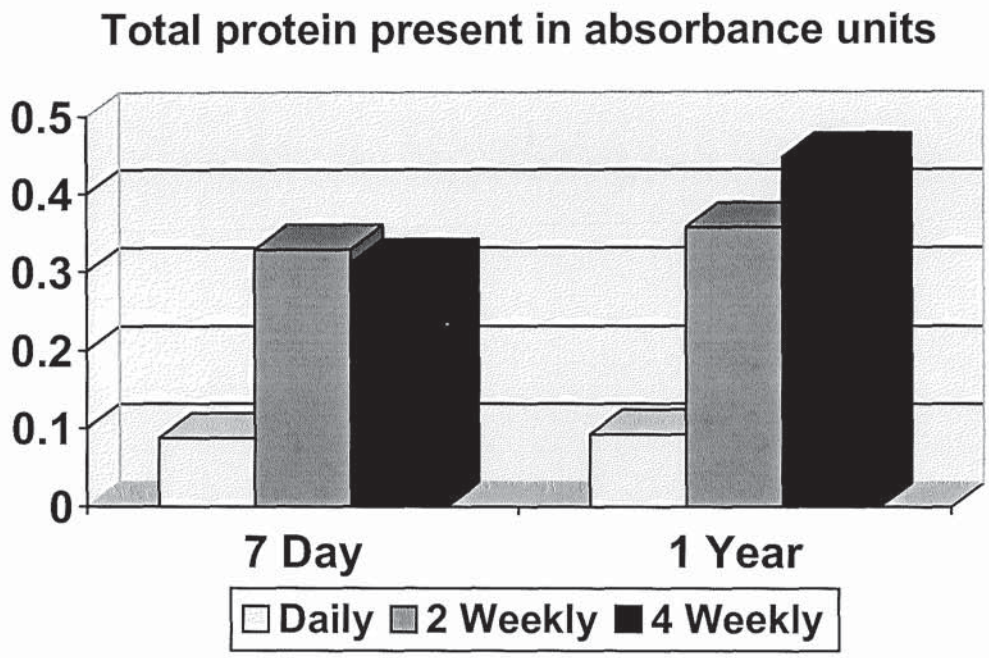


Figure 3.1. Total protein - Mean value for each replacement regimen at the 7 day and 1 year visit (in absorbance units)

Table 3.19. Comparison between different replacement regimen (daily, 2 weeks, 4 weeks) overall and at each visit by One way ANOVA with LSD test (*the values joined by a continuous line are not significantly statistically different) (values in absorbance units).

Overall: p<0.0001	Daily	2 weeks	4 weeks
Mean Abs	0.0903	0.3418	0.3704
LSD(5%)*			
7 Day: p<0.0001	Daily	2 weeks	4 weeks
Mean Abs	0.0880	0.3110	0.3294
LSD(5%)*			
1 Year: p<0.0001	Daily	2 weeks	4 weeks
Mean Abs	0.0927	0.3576	0.4466
LSD(5%)*			

Table 3.20. Comparison between wearing times, overall and for each replacement regimen (daily, 2 weeks, 4 weeks) at each visit by One way ANOVA with LSD test (*the values joined by a continuous line are not significantly statistically different) (values in absorbance units).

- **Daily disposable**

7 Day: p=0.0031	≤ 2 hrs	>2-6 hrs	> 6 hrs
Mean	0.0265	0.0698	0.1447
LSD(5%)*			
1 Year: p=0.0169	≤ 2 hrs	>2-6 hrs	> 6 hrs
Mean	0.0343	0.0774	0.1048
LSD(5%)*			

- **2 weeks replacement**

7 Day: p=0.4580	>2-6 hrs	≤ 2 hrs	> 6 hrs
Mean	0.3061	0.3264	0.3665
LSD(5%)*			
1 Year: p=0.0672	>2-6 hrs	≤ 2 hrs	> 6 hrs
Mean	0.2912	0.2938	0.4789
LSD(5%)*			

- **4 weeks replacement**

7 Day: p=0.8287	>2-6 hrs	≤ 2 hrs	> 6 hrs
Mean	0.3037	0.3041	0.3344
LSD(5%)*			
1 Year: p=0.6659	> 6 hrs	≤ 2 hrs	>2-6 hrs
Mean	0.3638	0.4569	0.4632
LSD(5%)*			

Table 3.21. Comparison between symptomatic and asymptomatic patients, overall and for each replacement regimen (daily, 2 weeks, 4 weeks) by t-test for independent samples (values in absorbance units).

		Asymptomatic (Mean±STD)	Symptomatic (Mean±STD)	p-value
7 DAY	DW	0.0891±0.0695	0.0861±0.1168	0.918
	2W	0.3355±0.1427	0.3131±0.1483	0.593
	4W	0.3334±0.1335	0.2401±0.0907	0.029
1 YEAR	DW	0.0794±0.0569	0.1915±0.1887	0.006
	2W	0.4642±0.2289	0.2165±0.1446	0.001
	4W	0.4833±0.2257	0.2445±0.2297	0.029

Table 3.22. Comparison between wearer and non-wearer, overall and for each replacement regimen (daily, 2 weeks, 4 weeks) by t-test for independent samples (values in absorbance units).

		Non wearer (Mean±STD)	Wearer (Mean±STD)	p-value
7 DAY	DW	0.0753±0.0420	0.0929±0.1014	0.565
	2W	0.2687±0.1225	0.3355±0.1463	0.260
	4W	0.2194±0.0935	0.3466±0.1256	0.001
1 YEAR	DW	0.0857±0.0720	0.1009±0.1040	0.659
	2W	0.2560±0.1405	0.3998±0.2457	0.125
	4W	0.3148±0.1771	0.4706±0.2440	0.146

3.2.3. Fluorescence measurements results

High intensities of protein and lipid fluorescence were recorded at both the front and back surfaces of the contact lenses (Tables 3.23 & 3.25). At both the 7 day and 1 year visits, the intensity of fluorescence emission was significantly ($p<0.0001$) higher for the contact lenses used with a 2 or 4 weeks replacement regimen compared to the daily disposable ones (Tables 3.24 & 3.26). No significant difference was recorded between the 2 and 4 weeks replacement regimen.

One of the advantages of fluorescence spectrophotometry was to provide independent measurements for both contact lens front and back surfaces. No significant differences in protein fluorescence emission were recorded between front surface and back surface for all types of replacement regimen and at both visits. On the contrary, the intensity of lipid fluorescence appeared significantly ($p<0.001$) higher at the front surface than at the back surface.

Analysis of the correlation between subject symptomatology (Tables 3.27 & 3.28) and fluorescence revealed a trend similar to those obtained with UV. For the 2 and 4 weeks replacement regimen at the 1-year visit, the level of proteins at the front surface of the contact lens was higher for the asymptomatic population than symptomatic one. On the other hand, there was no effect of the subject previous contact lens wearing history, (wearer vs. non-wearer), on the protein level measured by fluorescence spectrophotometry (Tables 3.29 & 3.30).

Finally, analysis of the correlation between the clinical parameters (tear film characteristics and stability assessed during the clinical examination by recording the break up time (NIBUT) (Tables 3.31 & 3.32) and type of pattern formed by the mixing lipids at the surface of the tear film (Tables 3.33 & 3.34) and fluorescence data revealed no significance for these factors.

3.2.4. CHAID analysis

The first Chi-square Automated Interaction Detector (CHAID) analysis was between the biochemical data and the clinical data gathered with Focus contact lenses (UV and fluorescence spectrophotometry). CHAID is the analytical tool that makes it possible to quantify the influence of the various clinical parameters (wear regimen, tear film characteristics...) for the protein and lipid deposition. The variables tested were the protein and lipid levels. All continuous variables were transformed into 3 groups to respectively represent the lower quartile (Group 1: Lowest 25%), the central two quartiles (Group 2: Mid 50%) and the upper quartile (Group 3: Highest 25%). CHAID carried out automated chi-square analysis, giving preference to the most discriminant factor. The only significant predictor of the level of spoilation was the type of replacement regimen with a significant shift towards higher deposition for 2 and 4 weeks replacement regimen. The percentage of people showing a high level of total protein measured by UV (high deposition) increases from 1% for daily disposable measurement to 36% for the 2 and 4 weeks replacement regimen. Similarly the percentage of the population with high levels of front surface protein and lipid measured by fluorescence spectrophotometry increased from 5% to 34% for both lipids and proteins when the replacement regimen changes from daily replacement to 2 or 4 weeks replacement schedule (Appendix F).

Using the same technique, the interaction between the two analytical techniques was successfully evaluated since the intensity of protein fluorescence emission detected by fluorescence spectrophotometry was found to be strictly related to the absorbance of the same sample measured by UV spectrophotometry (Appendix G).

Table 3.23. Intensity of protein fluorescence emission - Mean, standard deviation, median and range.

VISIT			Front surface	Back surface	Exact p-value
7 day	Daily	Mean ± STD	314.1 ± 92.7	256.9 ± 74.1	<0.001
		Min - Max	164.1 - 583.2	132.2 - 445.0	
		Median	291.8	247.0	
		N	43	43	
	2 Weeks	Mean ± STD	457.5 ± 149.1	430.5 ± 128.6	0.284
		Min – Max	189.3 - 919.6	213.8 - 769.1	
		Median	428.5	436.0	
		N	55	57	
	4 Weeks	Mean ± STD	442.3 ± 125.2	433.1 ± 181.8	0.154
		Min - Max	209.9 - 765.7	186.0 - 1310.0	
		Median	432.1	388.1	
		N	50	51	
1 year	Daily	Mean ± STD	332.1 ± 90.0	318.7 ± 78.1	0.618
		Min - Max	176.6 - 544.9	181.0 - 522.8	
		Median	298.6	328.2	
		N	40	40	
	2 Weeks	Mean ± STD	528.8 ± 173.3	505.3 ±197.9	0.436
		Min - Max	223 - 945.4	192.9 – 1056.0	
		Median	515.2	575.8	
		N	40	40	
	4 Weeks	Mean ± STD	578.7 ± 278.3	537.0 ± 190.3	1.000
		Min - Max	253.0 – 1441.0	215.2 - 1115.0	
		Median	523.1	505.0	
		N	37	37	

Table 3.24. Protein fluorescence emission - Comparison between different replacement regimen (daily, 2 weeks, 4 weeks) overall and at each visit by One way ANOVA with LSD test (*the values joined by a continuous line are not significantly statistically different).

- **Front surface**

Overall: p<0.0001	Daily	2 weeks	4 weeks
Mean	322.8	487.5	500.3
LSD(5%)*			
7 Day: p<0.0001	Daily	4 weeks	2 weeks
Mean	314.1	442.3	457.5
LSD(5%)*			
1 Year: p<0.0001	Daily	2 weeks	4 weeks
Mean	332.1	528.8	578.7
LSD(5%)*			

- **Back surface**

Overall: p<0.0001	Daily	2 weeks	4 weeks
Mean	286.7	461.4	476.8
LSD(5%)*			
7 Day: p<0.0001	Daily	4 weeks	2 weeks
Mean	256.9	430.5	433.2
LSD(5%)*			
1 Year: p<0.0001	Daily	2 weeks	4 weeks
Mean	318.7	505.3	537.0
LSD(5%)*			

Table 3.25. Intensity of lipid fluorescence emission - Mean, standard deviation, median and range

VISIT			front surface	back surface	Exact p-value
7 day	Daily	Mean ± STD	93.8 ± 54.5	70.9 ± 43.2	<0.001
		Min - Max	38.2 - 299.1	31.5 - 247.9	
		Median	75.4	57.0	
		N	43	43	
	2 Weeks	Mean ± STD	184.4 ± 152.4	164.4 ± 130.7	<0.001
		Min - Max	66.2 - 931.8	51.7 - 710.0	
		Median	146.1	125.3	
		N	55	57	
	4 Weeks	Mean ± STD	194.5 ± 191.3	166.6 ± 219.5	<0.001
		Min - Max	65.4 - 1207.0	49.8 - 1437.0	
		Median	145.5	89.7	
		N	50	51	
1 year	Daily	Mean ± STD	85.7 ± 28.3	60.6 ± 20.4	<0.001
		Min - Max	38.5 - 161.0	38.7 - 133.0	
		Median	78.6	57.3	
		N	40	40	
	2 Weeks	Mean ± STD	255.6 ± 239.6	169.5 ±149.9	<0.001
		Min - Max	40.6 - 1162.0	37.3 - 777.2	
		Median	201.3	132.1	
		N	40	40	
	4 Weeks	Mean ± STD	244.3 ± 167.1	187.6 ± 132.6	<0.001
		Min - Max	57.0 - 791.7	38.3 - 521.5	
		Median	187.8	141.1	
		N	37	37	

Table 3.26. Lipid fluorescence emission - Comparison between different replacement regimen (daily, 2 weeks, 4 weeks) overall and at each visit by One way ANOVA with LSD test (*the values joined by a continuous line are not significantly statistically different).

- **Front surface**

Overall: p<0.001	Daily	2 weeks	4 weeks
Mean	89.9	214.4	215.7
LSD(5%)*			

7 Day: p=0.002	Daily	2 weeks	4 weeks
Mean	93.8	184.4	194.5
LSD(5%)*			

1 Year: p<0.001	Daily	2 weeks	4 weeks
Mean	85.7	244.3	255.6
LSD(5%)*			

- **Back surface**

Overall: p<0.001	Daily	2 weeks	4 weeks
Mean	65.9	166.5	175.4
LSD(5%)*			

7 Day: p=0.003	Daily	4 weeks	2 weeks
Mean	70.9	164.4	166.5
LSD(5%)*			

1 Year: p<0.001	Daily	2 weeks	4 weeks
Mean	60.6	169.5	187.6
LSD(5%)*			

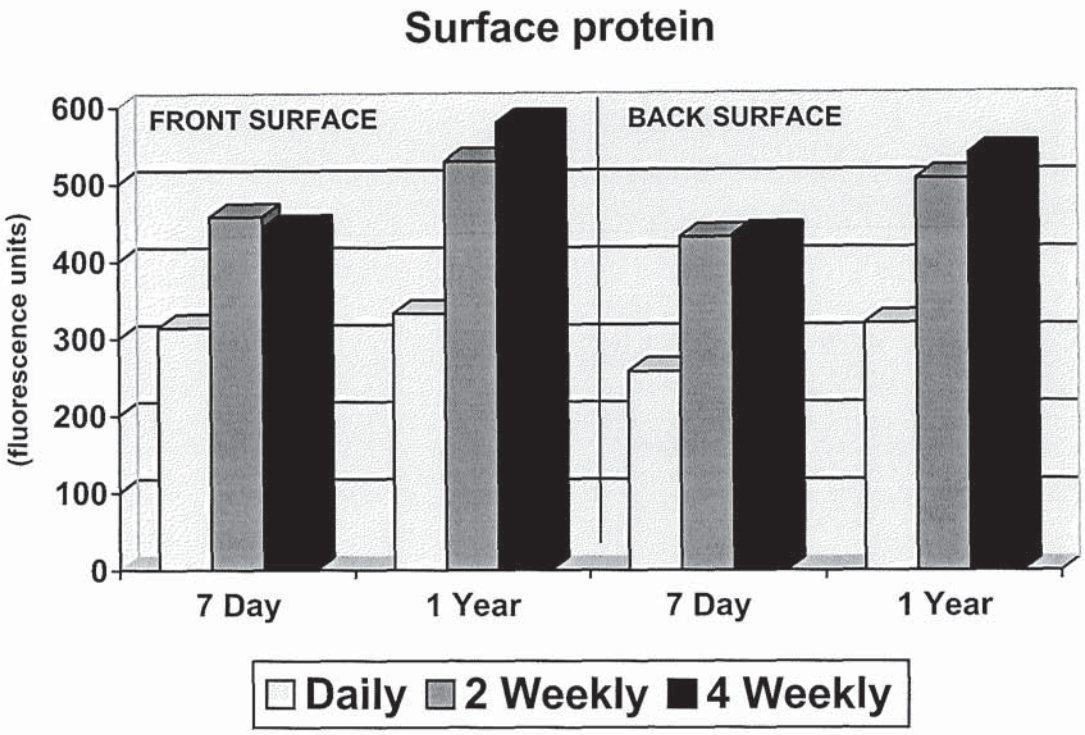


Figure 3.2. Intensity of protein fluorescence emission from contact lens front and back surface – Mean values for each replacement regimen at the 7 day and 1 year visits.

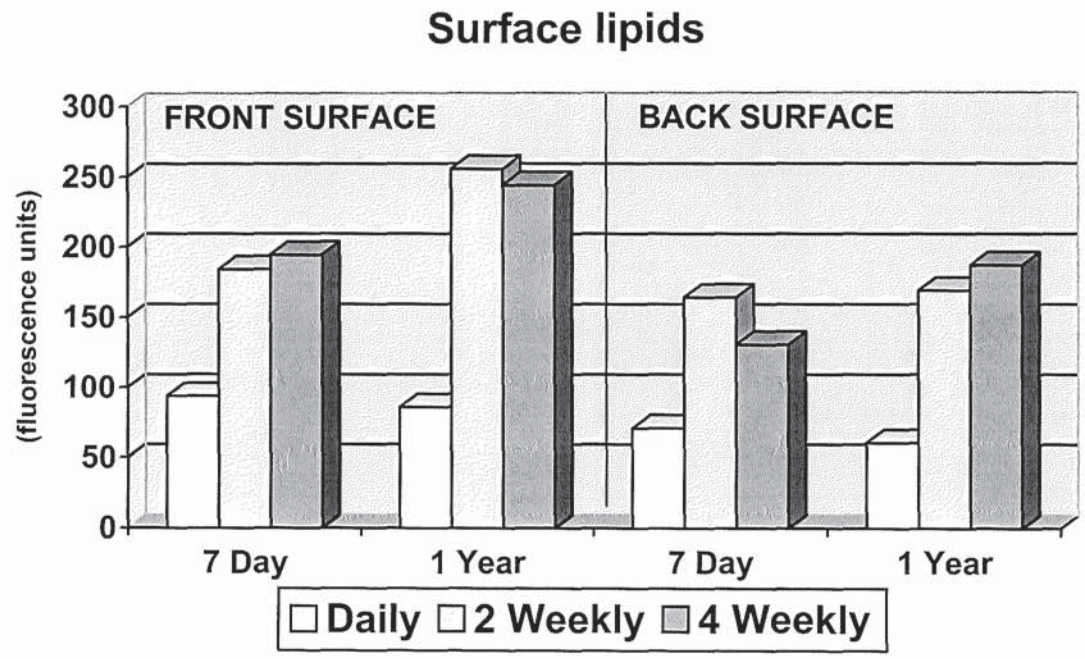


Figure 3.3. Intensity of lipid fluorescence emission from contact lens front and back surface – Mean values for each replacement regimen at the 7 day and 1 year visits.

Table 3.27. Intensity of protein fluorescence emission - Mean, Std Dev, Median and 25% percentile by symptomatology.

				Asymptomatic	Symptomatic	p value
7 day	Daily	FS	Mean± STD Median 25%	320.1±81.7 316.1 251.3	303.4±111.0 274.4 252.8	0.313
		BS	Mean± STD Median 25%	262.2±78.3 254.9 214.7	248.1 ±68.0 232.2 197.7	0.418
	2 weeks	FS	Mean± STD Median 25%	468.0±169.6 449.8 361.9	432.6±122.2 414.8 340.3	0.515
		BS	Mean± STD Median 25%	416.3±123.6 424.7 330.0	450.1±142.7 447.8 338.4	0.425
	4 weeks	FS	Mean± STD Median 25%	460.8±133.4 470.1 389.4	384.0±71.1 392.1 365.1	0.016
		BS	Mean± STD Median 25%	464.2±194.4 422.4 347.0	332.1±71.5 333.3 284.9	0.007
1 year	Daily	FS	Mean± STD Median 25%	323.3±83.9 298.6 261.8	369.7±126.2 316.0 266.8	0.520
		BS	Mean± STD Median 25%	320.1±81.9 336.5 249.4	294.8±66.8 284.7 236.7	0.268
	2 weeks	FS	Mean± STD Median 25%	548.0±161.4 542.1 392.4	442.4±114.5 429.7 362.0	0.061
		BS	Mean± STD Median 25%	560.3±222.8 538.8 366.8	432.2±156.5 405.0 346.7	0.066
	4 weeks	FS	Mean± STD Median 25%	619.3±282.9 531.1 408.5	369.0±119.5 345.5 272.3	0.011
		BS	Mean±STD Median 25%	562.5±182.0 513.1 430.4	405.3±193.1 322.0 262.3	0.080

Table 3.28. Intensity of lipid fluorescence emission - Mean, Std Dev, Median and 25% percentile by symptomatology.

				Asymptomatic	Symptomatic	p value
7 day	Daily	FS	Mean± STD Median 25%	109.0±62.4 79.1 69.1	68.1±21.6 64.5 55.4	0.003
		BS	Mean± STD Median 25%	82.4±50.1 64.8 51.5	51.5 ±15.3 47.3 39.8	0.004
	2 weeks	FS	Mean± STD Median 25%	188.8±174.5 146.9 104.4	190.2±135.2 163.3 99.9	0.873
		BS	Mean± STD Median 25%	153.4±116.7 120.9 86.9	186.6±158.5 125.3 95.0	0.513
	4 weeks	FS	Mean± STD Median 25%	217.0±213.5 155.6 102.0	123.5±49.1 116.2 85.6	0.075
		BS	Mean± STD Median 25%	192.1±245.7 93.9 79.8	83.4±23.3 78.4 68.7	0.016
1 year	Daily	FS	Mean± STD Median 25%	82.7±28.3 77.7 66.9	84.0±30.3 70.5 62.2	0.885
		BS	Mean± STD Median 25%	58.0±15.7 53.7 48.7	52.1±13.1 53.1 38.8	0.442
	2 weeks	FS	Mean± STD Median 25%	214.9±95.1 214.3 118.9	220.9±210.0 149.6 100.2	0.323
		BS	Mean± STD Median 25%	161.1±69.3 142.0 103.1	118.2±72.0 84.1 64.6	0.051
	4 weeks	FS	Mean± STD Median 25%	261.4±176.0 187.8 136.9	156.1±63.7 182.4 77.5	0.363
		BS	Mean± STD Median 25%	198.3±139.7 132.1 97.6	132.0±71.2 142.8 50.9	0.506

Table 3.29. Intensity of protein fluorescence emission - Mean, Std Dev, Median and 25% percentile by wearer/non wearer.

				Non wearer	Wearer	p value
7 day	Daily	FS	Mean± STD Median 25%	300.8±76.5 306.2 248.3	319.3±98.9 281.8 251.6	0.989
		BS	Mean± STD Median 25%	255.2±62.4 243.6 219.3	257.6 ±79.1 247.0 202.0	0.779
	2 weeks	FS	Mean± STD Median 25%	441.0±129.1 423.5 331.7	454.9±155.6 420.4 345.5	0.970
		BS	Mean± STD Median 25%	403.5±121.3 430.4 269.4	435.1±134.1 439.3 343.2	0.670
	4 weeks	FS	Mean± STD Median 25%	379.8±93.9 391.6 338.4	466.7±128.4 470.1 390.4	0.016
		BS	Mean± STD Median 25%	358.5±100.2 341.1 290.5	461.4±198.2 414.0 350.2	0.030
1 year	Daily	FS	Mean± STD Median 25%	317.6±66.9 324.1 244.8	336.1±100.4 292.2 266.0	0.715
		BS	Mean± STD Median 25%	309.4±107.2 268.2 234.2	318.4±68.2 335.0 246.3	0.849
	2 weeks	FS	Mean± STD Median 25%	473.9±171.9 409.5 378.3	513.9±147.0 523.4 405.2	0.460
		BS	Mean± STD Median 25%	496.6±226.3 453.7 301.7	510.9±203.6 475.8 354.7	0.858
	4 weeks	FS	Mean± STD Median 25%	455.8±123.4 476.5 327.9	602.5±294.6 531.1 376.6	0.247
		BS	Mean± STD Median 25%	513.6±140.9 519.7 401.8	541.5±200.1 505.0 215.2	0.763

Table 3.30. Intensity of lipid fluorescence emission - Mean, Std Dev, Median and 25% percentile by wearer/non wearer.

				Non wearer	Wearer	p value
7 day	Daily	FS	Mean± STD Median 25%	105.4±67.5 83.3 72.3	89.3±49.2 69.1 60.9	0.174
		BS	Mean± STD Median 25%	87.9±47.8 77.2 56.5	64.3±40.2 52.0 45.9	0.037
	2 weeks	FS	Mean± STD Median 25%	130.3±51.4 115.6 96.1	200.4±168.0 161.9 105.6	0.204
		BS	Mean± STD Median 25%	99.6±37.0 94.1 73.0	179.2±142.9 130.7 102.0	0.044
	4 weeks	FS	Mean± STD Median 25%	148.3±80.5 107.4 88.4	212.5±218.2 152.9 104.0	0.271
		BS	Mean± STD Median 25%	115.8±63.8 91.0 73.2	185.7±253.1 89.7 77.9	0.654
1 year	Daily	FS	Mean± STD Median 25%	84.4±33.1 79.4 66.1	82.4±26.9 71.2 66.2	0.639
		BS	Mean± STD Median 25%	55.7±10.8 55.6 44.8	57.5±16.9 52.7 46.2	0.849
	2 weeks	FS	Mean± STD Median 25%	250.0±100.4 228.0 201.0	207.3±163.0 155.0 108.3	0.177
		BS	Mean± STD Median 25%	157.3±85.2 135.8 77.1	139.26±69.5 125.4 79.9	0.765
	4 weeks	FS	Mean± STD Median 25%	173.9±108.6 169.2 75.6	258.0±174.3 188.1 136.9	0.302
		BS	Mean± STD Median 25%	132.9±54.4 131.9 83.1	198.1±141.1 141.1 97.6	0.506

Table 3.31a. Intensity of protein fluorescence emission - Median and 25% percentile by NIBUT median.

Visit	Replacement		Median			25 % percentile		
			<4	4-10	>10	<4	4-10	>10
7 day	Daily	FS	291.8	280.9	347.4	164.1	247.4	275.4
		BS	213.0	250.5	214.5	146.1	219.4	194.9
	2 weeks	FS	383.7	420.4	488.3	306.0	390.9	324.5
		BS	439.3	460.7	389.1	443.0	345.7	337.6
	4 weeks	FS	475.5	448.5	392.1	427.2	388.7	266.8
		BS	344.4	422.4	337.4	286.5	369.2	258.7
1 year	Daily	FS	272.3	292.2	364.1	260.5	262.5	341.7
		BS	307.9	324.0	395.1	206.6	249.4	323.2
	2 weeks	FS	599.2	517.1	383.2	407.1	418.2	356.4
		BS	481.9	456.9	477.4	334.2	355.1	297.8
	4 weeks	FS	576.3	525.8	458.1	410.3	362.3	352.8
		BS	505.0	610.0	445.0	440.5	411.6	415.4

Table 3.31b. Intensity of protein fluorescence emission - Comparison between NIBUT groups by Kruskall-Wallis One way ANOVA

		front surface	back surface
7 day	daily	0.1706	0.5616
	2 weeks	0.5512	0.2450
	4 weeks	0.2501	0.0263
1 year	daily	0.1189	0.0495
	2 weeks	0.4048	0.8354
	4 weeks	0.5784	0.7775

Table 3.32a. Intensity of lipid fluorescence emission - Median and 25% percentile by NIBUT median

Visit	Replacement		Median			25 % percentile		
			<4	4-10	>10	<4	4-10	>10
7 day	Daily	FS	61.2	74.6	128.3	55.0	64.6	64.5
		BS	45.9	59.6	89.8	43.2	48.5	38.0
	2 weeks	FS	133.7	138.8	154.1	116.8	104.0	38.0
		BS	103.6	132.8	123.9	72.9	107.6	89.6
	4 weeks	FS	133.1	153.9	163.9	98.4	101.5	101.0
		BS	88.1	93.9	88.8	76.8	77.9	73.3
1 year	Daily	FS	87.7	71.2	84.9	67.7	63.5	78.0
		BS	63.3	53.7	50.8	60.4	44.9	47.1
	2 weeks	FS	201.3	223.3	188.6	122.4	108.7	122.1
		BS	111.3	146.6	113.5	81.3	80.5	76.2
	4 weeks	FS	198.9	187.8	117.9	155.2	125.3	78.9
		BS	165.3	155.9	84.1	112.5	101.5	67.7

Table 3.32b. Intensity of lipid fluorescence emission - Comparison between NIBUT groups by Kruskall-Wallis One way ANOVA

		Front surface	back surface
7 day	daily	0.1790	0.4037
	2 weeks	0.5686	0.0428
	4 weeks	0.5948	0.8749
1 year	daily	0.6282	0.2358
	2 weeks	0.9733	0.5340
	4 weeks	0.1323	0.0637

Table 3.33a. Intensity of protein fluorescence emission - Median and 25% percentile by lipid layer mixing pattern.

Visit				Lipid layer mixing pattern					
				0	1	2	3	4	5
7 day	Daily	FS	Median	284.4	320.9	266.3	343.0	286.1	260.2
			25%	215.6	268.3	247.5	222.5	251.3	-
		BS	Median	221.0	257.1	238.3	262.2	234.2	194.4
			25%	197.7	220.5	203.2	231.0	156.6	-
	2 weeks	FS	Median	488.5	418.3	418.4	324.5	350.3	540.7
			25%	420.1	380.0	322.4	274.9	-	-
		BS	Median	444.8	441.8	436.0	399.4	243.0	585.7
			25%	368.3	336.0	298.4	378.8	-	-
	4 weeks	FS	Median	390.2	437.2	481.9	395.4	448.5	474.9
			25%	381.7	337.2	408.1	266.8	423.6	415.4
		BS	Median	369.7	388.1	407.7	341.3	514.3	369.6
			25%	275.6	340.9	363.2	239.2	502.7	309.7
1 year	Daily	FS	Median	277.3	274.7	318.4	-	414.2	543.3
			25%	275.4	255.4	268.2		-	-
		BS	Median	326.2	334.7	315.2	-	369.0	397.3
			25%	245.4	261.0	241.7		-	-
	2 weeks	FS	Median	662.5	535.3	421.8	365.9	435.8	587.5
			25%	517.1	363.7	378.3	289.1	-	571.8
		BS	Median	535.6	464.6	434.8	513.4	514.4	589.2
			25%	489.5	321.6	329.0	392.9	-	353.6
	4 weeks	FS	Median	506.0	585.2	528.5	450.1	458.1	-
			25%	347.9	350.4	484.5	-	-	
		BS	Median	487.3	483.1	624.5	513.1	445.0	-
			25%	400.4	429.8	489.9	-	-	

Table 3.33b. Intensity of protein fluorescence emission - Comparison between lipid pattern groups by Kruskall-Wallis One way ANOVA

		front surface	back surface
7 day	Daily	0.3933	0.6044
	2 weeks	0.5021	0.4459
	4 weeks	0.3680	0.2673
1 year	Daily	0.2780	0.6557
	2 weeks	0.2353	0.8634
	4 weeks	0.8587	0.7071

Table 3.34a. Intensity of lipid fluorescence emission- Median and 25% percentile by lipid layer mixing pattern.

Visit				Lipid layer mixing pattern					
				0	1	2	3	4	5
7 day	Daily	FS	Median	66.6	78.0	77.1	76.9	106.6	63.6
			25%	57.9	54.6	65.5	67.1	60.9	-
		BS	Median	49.1	60.1	57.6	36.8	91.8	41.5
			25%	43.9	48.0	49.1	34.5	49.6	-
	2 week	FS	Median	172.8	127.7	102.3	176.8	173.0	174.1
			25%	102.3	107.6	89.6	146.9	-	-
		BS	Median	141.1	120.8	103.3	142.6	118.4	235.7
			25%	110.7	96.9	64.6	117.6	-	-
	4 week	FS	Median	150.0	153.9	96.6	191.0	396.5	133.1
			25%	103.4	102.9	77.1	130.1	166.6	108.4
		BS	Median	111.0	115.2	81.6	121.0	357.0	68.8
			25%	86.2	81.5	66.1	69.1	90.2	49.8
1 year	Daily	FS	Median	69.9	99.0	76.2	-	90.3	118.9
			25%	66.9	65.1	66.2		-	-
		BS	Median	53.6	64.0	51.6	-	63.4	65.8
			25%	47.1	49.9	43.7		-	-
	2 weeks	FS	Median	248.2	149.6	214.3	268.6	85.6	350.3
			25%	111.6	89.9	111.3	227.0	-	291.7
		BS	Median	182.0	103.8	139.11	108.2	65.1	231.9
			25%	86.4	74.3	03.2	63.3	-	226.1
	4 weeks	FS	Median	166.4	187.8	230.5	138.2	117.9	-
			25%	79.2	111.9	163.2	-	-	
		BS	Median	118.3	153.2	162.0	109.5	67.7	-
			25%	84.1	90.6	128.2	-	-	

Table 3.34b. Intensity of lipid fluorescence emission - Comparison between lipid layer mixing pattern groups by Kruskall-Wallis One way ANOVA

		front surface	back surface
7 day	Daily	0.7838	0.4973
	2 weeks	0.5367	0.2678
	4 weeks	0.0330	0.0296
1 year	Daily	0.4043	0.2306
	2 weeks	0.2244	0.2385
	4 weeks	0.3767	0.2933

3.2.5. Discussion

The results of the UV analysis confirmed the tendency of Group IV materials to attract high levels of protein in a time dependent fashion. The kinetics of protein deposition on Group IV material has been widely studied. Leahy *et al.*[119] reported a deposition detectable almost upon insertion and increasing over an eight hour period of wear. Lin *et al.*[118] demonstrated the occurrence of a plateau after 7 days of wear. The study confirmed the increase of protein deposition with wear time and demonstrated that this increase was significant for the daily disposable lenses after 6 hours of wear. This rapid build up of positively charged proteins such as lysozyme is characteristic of the Group IV materials: the similar levels of protein deposition found for the 2 weeks and 4 weeks replacement regimen suggested the possible occurrence of a plateau in the protein absorption kinetic.

Amongst the group IV materials, the high intensities found for the protein and lipid fluorescence are characteristics of a subgroup of material such as Focus. Focus contact lenses (FDA Group IV) are high water content and ionic and are based on a copolymer of HEMA and NVP with 1% of methacrylic acid. A high level of protein deposition on FDA Group IV material has been reported by several researchers and is correlated with the water content and the surface ionicity. The material anionic charges both promote a rapid penetration of proteins such as lysozyme into the lens matrix, and contribute to the establishment a higher protein surface concentration than that associated with uncharged materials. The high level of lipids is strictly related to the presence of vinyl-pyrrolidone, known as skin lipid penetration enhancing agent[180], in the chemical composition of the Vifilcon A material from which Focus contact lenses are made. Through vinyl-pyrrolidone, lipids become

associated to the lens matrix itself whereas proteins are taken into the aqueous part of the contact lens.

This study achieved similar findings to those previously reported while analysing the same contact lenses by UV spectrophotometry, and confirms the results reported in the literature of a time dependence for protein and lipid depositions on contact lens materials[121,181]. A progressive accumulation over time of a deposited film was noticeable for the Focus contact lenses. The accumulation over time was however limited by the occurrence of a plateau[118] that led to the similarity in spoilation for the 2 and 4 weeks replacement regimen. The effect of time on deposition was higher for the lipid species due to the process of lipid penetration into the matrix. To date only one study[121] has shown that lipids progressively accumulate on hydrogels with time. The lipids progressively build up and become irreversibly bound to the surface via the hydrophobic backbone of polymer matrix.

The use of surface fluorescence has the advantage of providing non destructive comparative information on the location of proteins and lipids. The lability of the protein associated with the lens matrix and therefore its ready diffusion into the storage solution has been reported as another feature of Group IV contact lens materials[182]. Once placed into the storage solution, the front and back surface of the contact lens are in the same environment contrary to the *in vivo* situation when the front surface interacts with the PLTF and the back surface with the post lens tear film that differ significantly in their composition. The proteins will therefore partition between the front and the back surface of the contact lens which explain the similarity in the protein fluorescence results of each surface. The lipids are more strongly bound to the hydrogel and the difference in the fluorescence emission from the front and back surface was more representative of the *in vivo* situation. The higher

level of deposition on the front surface can be explained by the origin of the lipids itself. Secreted by glands situated in the eyelids they are preferably concentrated in the pre lens tear film than in the post lens tear film, which is more difficult to access.

The analysis of deposition by both UV spectroscopy and fluorescence spectrophotometry for both asymptomatic and symptomatic contact lens wearers seemed to reveal a difference in kinetics of protein uptake for both populations. At one year, a significantly higher level of protein was recorded for asymptomatic than symptomatic wearers using the 2 and 4 weeks replacement schedules (Fig 3.4 & 3.5). For the asymptomatic population, the level of protein increased with replacement regimen. For the symptomatic population, similar levels of protein were found for all types of regimen.

3.2.6. Conclusion

Group IV materials such as Focus are characterised by a high level of protein deposition. The protein deposition increased with time until the occurrence of a plateau.

Similarly for NVP containing contact lens materials, such as Focus, the lipid deposition was high and time dependent. Additionally this deposition was different for the front and back surface of the contact lens.

Finally no correlations were found between the level of both protein and lipid deposition and the clinical performance of the contact lenses.

As no direct correlation was found, the next step in order to elucidate a biochemical marker was to analyse the composition of the deposition in relation to the composition of the tears.

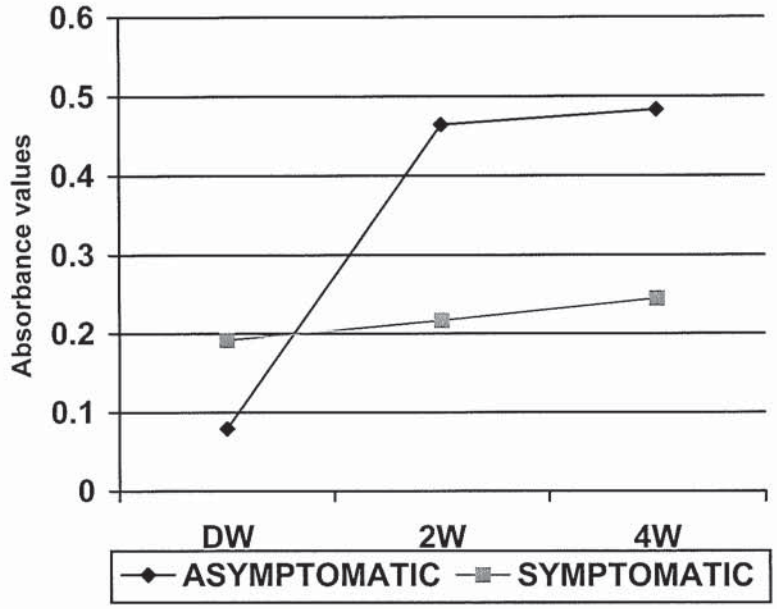


Figure 3.4. Protein level at 1 year measured by UV spectroscopy – Asymptomatic vs. Symptomatic population for each replacement regimen.

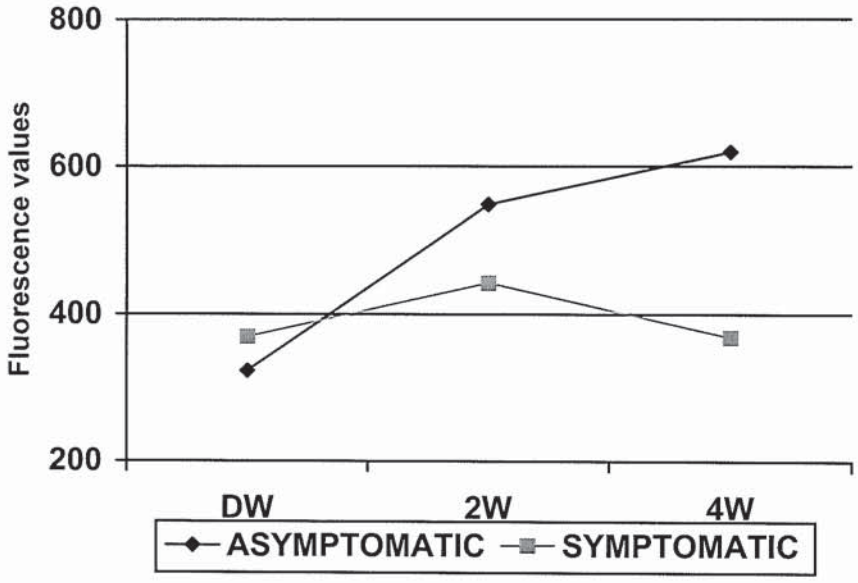


Figure 3.5. Protein level on front surface of soft contact lenses at 1 year visit measured by fluorescence spectrophotometry – Asymptomatic vs. Symptomatic population for each replacement regimen.

CHAPTER 4

TEAR LIPID COMPOSITION – PRELIMINARY STUDIES

4.1. Tear sampling techniques

4.1.1. Rationale

Tear samples are usually collected using glass microcapillaries from the lower tear prism only. This technique harvests low level of lipids, probably due to their affinity for glass surfaces, and is therefore not suitable to study the tear film lipid composition. Hence, prior to carrying out further investigations of the tear film lipid composition, an adequate tear collection sampling method needed to be developed. Also since contact lenses are known to disrupt the tear film, producing changes in concentrations of aqueous components and disruption of the superficial lipid layer, it was important to search for a medium that would enable safe collection from the corneal area in front of a contact lens as well as the lower tear prism. For the lower tear prism collection, the possible media for tear sampling were glass microcapillaries in conjunction with different extraction technique for lipid, threads such as ophthalmic threads used in eye surgery, ophthalmic sponges and finally Schirmer tear test strips. For the corneal area, the methods of collection considered were ophthalmic sponges or specially modified Schirmer tear test strips.

4.1.2. Pilot study

A pilot study was carried out on five subjects to determine the relative feasibility and efficacy of these new sampling techniques. The three collecting media tested were glass microcapillaries, Schirmer tear test strips and ophthalmic sponges.

The Schirmer tear test strip technique was shown to be unsuitable after a small number of subjects. The technique induced discomfort, irritation and reflex tearing and therefore did not allow for the sampling of normal unstimulated tears.

The glass microcapillary technique had two major limitations. Low levels of lipids were recovered after extraction as a result of the propensity for lipids, particularly the hydrophobic lipids, to adhere to glass. Further, samples were difficult to obtain from dry eye subjects without inducing ocular irritation.

In contrast, the ophthalmic sponges offered a faster and easier way of collecting samples, especially from dry eye subjects, while achieving a good recovery of tear lipids and proteins after extraction. Hence, the tear lipid profiles obtained after analysis of samples collected using this method were considered as the most representative of the true tear film composition. The sponge collection technique was therefore chosen for the current investigation.

4.2. Development of extraction method

4.2.1. Rationale

The purpose of this investigation was to optimise the extraction and storage procedures when using Visispear™ ophthalmic sponges (Visitec,Bidford-on-Avon,UK) to collect tear fluid. The three optimisation criteria were:

i. the feasibility of the extraction procedure;

ii. the detection of the highest number of lipid classes present in tears and detectable by the High Performance Liquid Chromatography (HPLC) system currently used at Aston University and,

iii. the avoidance of the conversion or transformation of the different lipids such as the esterification of cholesterol.

4.2.2. Experimental procedure and results

4.2.2.1. Extraction procedure

In the first part of this investigation, two extraction techniques to remove lipids from the sponges were compared: mechanical extraction of the tear fluid (Technique 1) and chemical extraction of the tear fluid (Technique 2).

The samples were collected using Visispear™ ophthalmic cellulose sponges. In Technique 1, the tear fluid was extracted mechanically from the sponge: the cellulose sponge was saturated with saline and then centrifuged to extract the tear fluid and saline. The extract was stored between 0 and 4°C until analysis by HPLC. In Technique 2, the sponge saturated with saline was stored between 0 and 4°C until extraction of the tear fluid with methanol prior to analysis by HPLC.

The results obtained for the two techniques are shown in Table 4.1.

Table 4.1. Two extraction methods

METHOD	RETENTION TIME (s)	AREA	IDENTIFICATION
Method 1 (mechanical)	69.56	1319.24	Cholesterol ester
	78.34	410.18	Phospholipids1
	102.09	162.66	Triglycerides/phospholipids
	561.99	2543.73	Monoglycerides
Method 2 (chemical)	60.29	1588.42	Cholesterol ester
	70.28	810.62	Cholesterol ester/phospholipids1
	78.34	111.96	Phospholipids1
	95.76	362.20	Triglycerides/phospholipids
	103.17	543.73	Triglycerides/phospholipids
	569.04	2792.77	Monoglycerides

The chemical extraction technique with methanol gave the best results. The chemical extraction technique detected six lipid peaks vs. four for the mechanical technique and had overall higher peak intensities for the common lipid classes detected.

4.2.2.2. Effect of storage

This part of the investigation was dealing with the optimisation of the storage process before extraction.

The tear samples (2 μl) were collected with Visispear™ ophthalmic cellulose sponges. Four storage options were tested:

i. Storage dry between 0° and 4°C (right eye sample) (Storage 1),

ii. Storage dry and frozen (left eye sample) (Storage 2),

iii. Storage with a drop of saline between 0° and 4°C (right eye sample) (Storage 3) and,

iv. Storage with a drop of saline frozen (left eye sample) (Storage 4).

Before analysis, the tear samples were extracted from the Visispear™ ophthalmic cellulose sponge using high purity (HPLC grade) methanol following the procedure described in section 2.4. The results reported in Table 4.2. showed Storage 2, that is to store the sponge dry and to keep it frozen until extraction, to be optimal. Storage 2 detected 5 lipid classes vs. 4 for Storage 1 and 3 for Storage 3 and 4.

Table 4.2. Effect of storage

METHOD	RETENTION TIME (s)	AREA	IDENTIFICATION
STORAGE 1	68.87	2129.12	Cholesterol ester
	112.82	542.40	Triglycerides/phospholipids
	248.16	1214.37	Fatty acids
	558.87	2729.12	Monoglycerides
STORAGE 2	67.54	2826.42	Cholesterol ester
	113.18	847.16	Triglycerides/phospholipids
	121.65	969.03	Triglycerides/phospholipids
	247.85	1800.54	Fatty acids
	559.35	3462.50	Monoglycerides
STORAGE 3	66.04	465.74	Cholesterol ester
	111.27	819.91	Triglycerides/phospholipids
	539.24	2108.97	Monoglycerides
STORAGE 4	63.70	853.33	Cholesterol ester
	107.06	942.84	Triglycerides/phospholipids
	550.70	2642.84	Monoglycerides

4.2.2.3. Effect of length of storage

The tear samples collected and analysed with the method described previously failed to show any trace of cholesterol whereas cholesterol had previously been reported in contact lens extracts analysed using the same HPLC system[183,173]. The effect of length of storage before and after extraction on the different lipid species, such as a possible esterification of cholesterol, was investigated.

Samples were collected using Visispear ophthalmic cellulose sponges and were extracted just immediately after collection. The tear samples were then either stored between 0 and 4°C before analysis or analysed straight away. Cholesterol was found in some samples with both routines showing the importance of minimising the time between collection and extraction. Length of storage after extraction did not affect significantly the lipid profile.

4.3. Visispear™ ophthalmic cellulose sponge calibration

4.3.1. Rationale

The purpose of this experiment was to correlate the dimensions of the expanding Visispear™ ophthalmic cellulose sponge with the volume of liquid absorbed.

4.3.2. Material and method

The test material consisted of 10 Visispear ophthalmic cellulose sponges. A total volume of 20 µl of saline solution (Alcon Aerosol saline) was made to be absorbed by each sponge in 1 µl steps. The process was recorded using a COHU High performance colour CCD camera (Fig 4.1a & b).

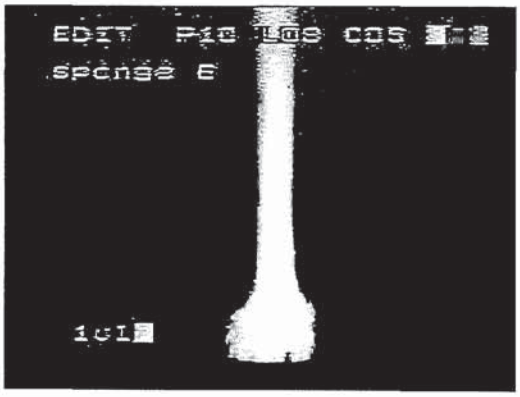


Figure 4.1a. 1µl absorbed

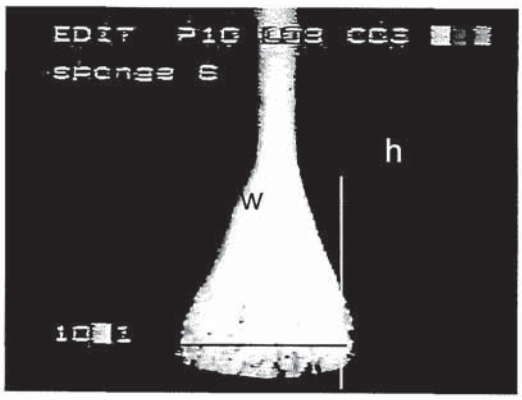


Figure 4.1b. 10 µl absorbed

The width (w) and height (h) of the expanding sponge were measured and reported in millimetres (mm). The measurements were carried out using the image enhancement and analysis software Sigma Scan Pro from Jandel Scientific.

The mean measurements of the 10 sponges for both dimensions are reported in Table 4.3. The individual correlation between the two dimensions measured for each sponge and the volume of liquid absorbed is illustrated on Figures 4.2 & 4.3. From the results obtained, single linear regression lines between the absorbed volume of saline and the height and width of each swollen sponge were calculated:

- Volume (µl) = 2.10* h –5.43 (r^2=0.798) **(Equation 4-1)**
- Volume (µl) = 2.50*w –3.19 (r^2=0.340) **(Equation 4-2)**

The height of the swollen sponge gave a better estimation of the absorbed volume than the width as shown by the higher correlation coefficient (r^2=0.798 vs. 0.340). A multilinear regression between volume absorbed and both the height and width of each swollen sponge gave a slightly better correlation:

- Volume (µl) = -1.60 *w(mm) + 2.80* h(mm) -1.94 (r^2=0.848) **(Equation 4-3)**

The mean variations in height (mm) and in width (mm) for the sponges were plotted in function of the volume of liquid absorbed as shown on Figure 4.4a for all volumes from 1 to 20 µl or on Figure 4.4b for low volumes only. The average regression lines were calculated and whereas the variation in height followed a linear model (Fig. 4.4a & b), the variation in width of the swollen sponge followed

a logarithmic progression. The equations obtained for the trend lines were as follow:

- Volume = 2.5229 x h – 8.5416 (r^2=0.954) **(Equation 4-4)**

and Volume=1.8115 x h –4.7784 (r^2=0.991) for low volumes only **(Equation 4-5)**

- Volume=0.0643 e $^{0.8944w}$ (r^2=0.967) **(Equation 4-6)**

and Volume=0.0967 e ^{7983w} (r^2=0.986) for low volumes only **(Equation 4-7)**

The sponge width followed a similar trend line at low volumes than overall (Equation 4-7 vs. 4-6). On the opposite, the linearity trend for the variation of the sponge height in function of the volume of liquid absorbed was significantly improved for low volumes of liquid (Equation 4-5 vs. 4-4).

The variation of height h (mm) in function of volume of liquid absorbed was taken, because of the good linearity of this correlation for low volumes (Equation 4-5: r^2=0.991), as a calibration curve for volume of tears collected. For example when the sponge height reached 4mm, the volume of sample collected was close to 2 µl, which corresponds to the usual tear volume extracted.

Table 4.3. Sponge dimensions for increasing volume of liquid absorbed. Descriptive statistics.

VOLUME	PARAMETERS	Width(mm)	Height(mm)
1	Mean ± STD	3.16 ± 0.73	2.91 ± 0.65
	Min →Max	1.98→ 4.28	2.21→ 4.31
2	Mean ± STD	3.66 ± 0.78	3.76 ± 0.65
	Min →Max	2.45→ 4.86	2.85→ 4.74
3	Mean ± STD	4.18 ± 0.89	4.36 ± 0.75
	Min →Max	2.65→ 5.37	3.44→ 5.45
4	Mean ± STD	4.58 ± 0.97	5.01 ± 0.60
	Min →Max	2.70→ 5.80	4.19→ 5.97
5	Mean ± STD	4.86 ± 1.05	5.52 ± 0.66
	Min →Max	2.76→ 6.26	4.59→ 6.36
6	Mean ± STD	5.20 ± 0.95	6.04 ± 0.81
	Min →Max	3.66→ 6.89	4.78→ 7.27
7	Mean ± STD	5.40 ± 1.01	6.61 ± 1.08
	Min →Max	3.66→ 7.08	5.34→ 8.74
8	Mean ± STD	5.57 ± 1.08	7.11 ± 1.22
	Min →Max	3.58→ 7.20	5.77→ 9.13
9	Mean ± STD	5.75 ± 1.07	7.56 ± 1.11
	Min →Max	3.89→ 7.47	6.37→ 9.92
10	Mean ± STD	5.82 ± 1.10	7.86 ± 1.30
	Min →Max	3.93→ 7.59	6.68→ 10.29
11	Mean ± STD	5.91 ± 1.06	8.38 ± 1.34
	Min →Max	4.00→ 7.63	7.00→ 10.92
12	Mean ± STD	6.05 ± 1.07	8.72 ± 1.35
	Min →Max	4.10→ 7.67	7.15→ 11.35
13	Mean ± STD	5.98 ± 1.11	8.76 ± 0.91
	Min →Max	4.15→ 7.86	7.83→ 10.28
14	Mean ± STD	6.07 ± 1.13	9.28 ± 1.20
	Min →Max	3.89→ 7.82	8.26→ 11.94
15	Mean ± STD	6.00 ± 1.15	9.06 ± 0.89
	Min →Max	3.98→ 7.94	8.26→ 10.36
16	Mean ± STD	6.10 ± 1.21	9.56 ± 1.31
	Min →Max	3.81→ 7.98	7.94→ 12.17
17	Mean ± STD	6.03 ± 1.14	9.63± 1.02
	Min →Max	4.16→ 8.00	8.58→ 11.03
18	Mean ± STD	6.18 ± 1.11	10.17 ± 1.13
	Min →Max	4.32→ 7.98	8.46→ 12.02
19	Mean ± STD	6.05 ± 1.12	10.09 ± 1.00
	Min →Max	4.20→ 7.90	8.86→ 11.42
20	Mean ± STD	6.17 ± 1.16	10.56 ± 1.12
	Min →Max	4.12→ 7.90	9.25→ 12.21

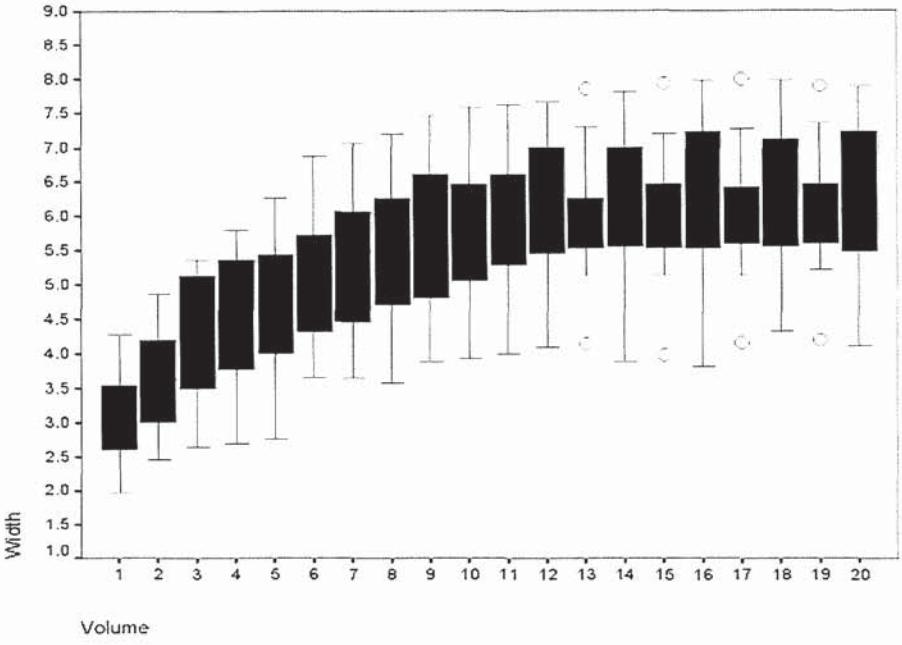


Figure 4.2. Sponge width (mm) vs. volume (µl) - High-Low chart.

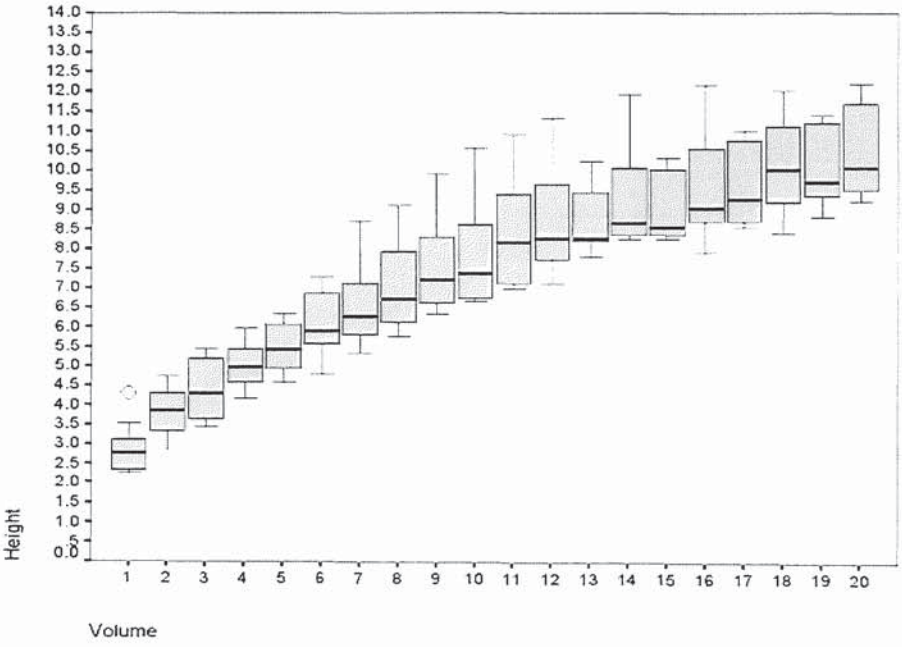


Figure 4.3. Sponge height (mm) vs. volume (µl) - High-Low chart.

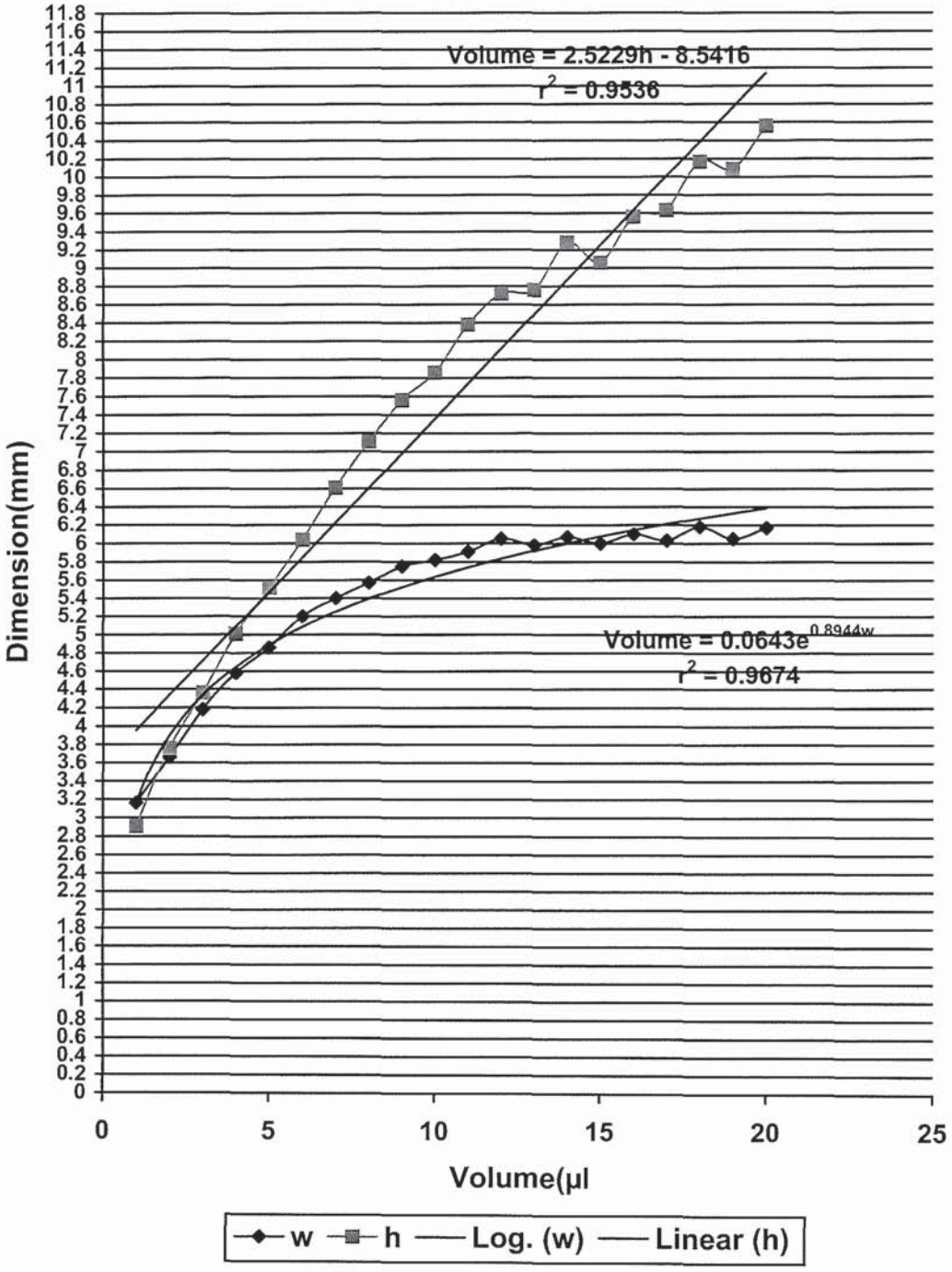


Figure 4.4a. Sponge dimensions (width & height) (in mm) vs. volume of liquid absorbed (in µl) and trend lines

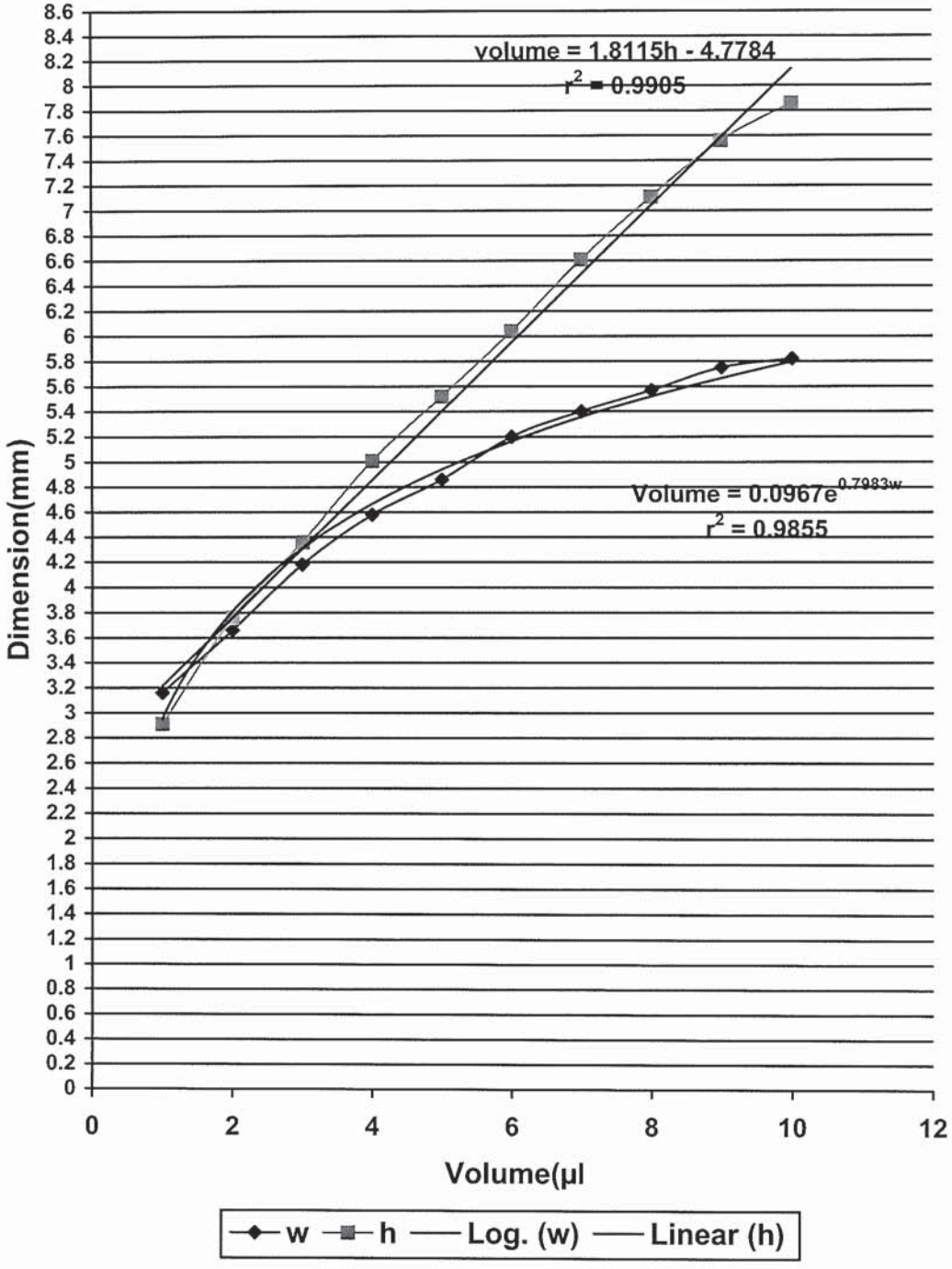


Figure 4.4b. Sponge dimensions (width & height) (in mm) vs. volume of liquid absorbed (in µl) and trend lines for low volumes only.

4.4. Tear analysis of contact lens wearer - Preliminary baseline study

4.4.1. Objective

Whereas the protein composition of the tear film has been widely studied, little information is available on the lipid composition of the tear film of either the normal population or the contact lens wearing population. During the 1996 Dry Eye conference, differences in lipid composition were reported but these differences were limited to comparisons between normal subjects and those suffering from dry eye pathological syndromes[184].

The purpose of this investigation was therefore to i) investigate the lipid composition of tears of contact lens wearers, ii) test for any association between lipid composition and tear film stability and iii) assess the relation between tear lipid composition and dry-eye symptomatology.

4.4.2. Materials and methods

4.4.2.1. Study population

The test population consisted of a group of 33 myopic contact lens wearers, from 20 to 43 years of age, attending Contact Lens Research Consultants (CLRC) for soft contact lens fitting. The population included both asymptomatic and symptomatic contact lens wearers. Diagnosis as to whether the subject was asymptomatic or symptomatic was obtained from their scoring on McMonnies original and modified questionnaires[185,104] (McMonnies & Ho, 1987; Guillon et al. 1992). The subjects were categorised based upon the results they gave for their usual contact lenses. The characteristics of the Pre Lens Tear Film (PLTF) were also determined using

the Tearscope observation system[99,100,101,186]. The population characteristics are summarised in Table 4.4.

Table 4.4. Population characteristics and comparison between asymptomatic and symptomatic groups

		Overall	Asympt.	Sympt.	p value
Sex	**Male**	11	3	8	0.450
	Female	22	9	13	
Age (years)		31.2 ± 6.5	29.2 ± 5.2	32.3 ± 6.5	0.161

4.4.2.2. Methodology

Tears were collected using the Visispear™ ophthalmic cellulose sponges while the subjects wore their usual contact lenses. The tear film lipid composition was determined using High Performance Liquid Chromatography (HPLC) technique on individual tear samples (2 μl), as described in section 2.4.

4.4.3. Results

4.4.3.1. Tear lipid composition

The technique enabled us to identify 5 lipid classes: cholesterol esters, triglycerides/phospholipids, fatty acids, monoglycerides and cholesterol (Table 4.5). The lipid tear composition varied widely between subjects. The distribution of each lipid class identified for individual subjects was not normally distributed but positively skewed. The differences between asymptomatic and symptomatic subjects were limited to two lipid classes, fatty acids and monoglycerides (Table 4.6).

4.4.3.2. Tear film structure and stability

For the same 33 subjects, the characteristics of their pre lens tear film were assessed using a special lighting system, the Tearscope™ in conjunction with a biomicroscope observation system as described in Section 1.2.5.

The tear film clinical evaluation did not identify any differences between the symptomatic and asymptomatic population. Similar types of lipid patterns were observed for both symptomatic and asymptomatic contact lens wearers (Fig. 4.5). For both populations, the most commonly observed pattern during contact lens wear was a meshwork type pattern, which corresponds to a lipid layer thickness ranging from 15 to 70 nm[100].

The vast majority of contact lens wearers tested had a Pre Lens Tear Film Non Invasive Break Up Time (NIBUT) inferior to 15 seconds: 91% of cases for the asymptomatic population and 96% for the symptomatic population (Fig. 4.6). No significant difference in NIBUT was found between asymptomatic and symptomatic contact lens wearers, the two populations achieving a mean median NIBUT of 8.8 and 9.2 seconds respectively.

4.4.3.3. Subjective performance

The subjects were asked to rate their comfort with their usual contact lenses as well as their symptoms of ocular dryness. The ratings were recorded using dedicated 50 point continuous scales with descriptors (Appendix H). A low score corresponded to a poor comfort and high symptomatology and a high score to the opposite.

Statistical comparisons revealed some significant differences between the asymptomatic and symptomatic populations. A statistically significantly lower overall comfort was recorded for the symptomatic contact lens wearers than for the asymptomatic wearers ($p<0.001$) (Fig. 4.7). Clinically the difference between the two populations was also significant. 75% of the asymptomatic population, against only 33% of the symptomatic population, rated their comfort while wearing their own contact lenses as good to excellent. On the other hand, 15% of symptomatic wearers, vs. 0% of asymptomatic wearers rated their comfort during contact lens wearer as bearable or worse.

Similarly, significantly higher dryness symptoms were recorded for the symptomatic population than for the asymptomatic one ($p<0.001$) (Fig. 4.7). When asked to rate their dryness symptoms during contact lens wear, 15% of the symptomatic population reported to suffer "often to constantly" from dryness symptoms whereas no asymptomatic contact lens wearer reported that level of symptomatology. On the other hand, 91% of the asymptomatic population vs. only 20% of the symptomatic contact lens wearers reported to suffer "rarely to never" from dryness symptoms while wearing their contact lenses.

4.4.3.4. Tear lipid composition and PLTF characteristics.

The influence of tear lipid composition on tear film structure was investigated by One way analysis of variance (ANOVA) and Student Newman Keuls (SNK) test. These evaluations revealed the factorial influences listed below.

4.4.3.4.a. Cholesterol esters

The mean concentration of cholesterol esters for the different lipid patterns was ranked in reverse order to the lipid patterns ranking based upon their thickness. A low concentration of cholesterol esters was associated with a thicker lipid layer ($p<0.05$) (Table 4.7). The mean level of cholesterol ester for the amorphous/colours lipid pattern group, characteristic of a thick and stable lipid layer of about 80 nm, was the lowest of all. When the observed lipid pattern was a wave pattern, equivalent to a lipid layer thickness of 30 to 80 nm, the mean level of cholesterol ester level was 97. For a meshwork type of pattern, equivalent to a lipid layer thickness of 15 to 30 nm, the mean cholesterol ester level was 133. Finally, when no pattern was visible during slit-lamp examination, which was characteristic of a very thin lipid layer, the level of cholesterol esters was highest. The difference was statistically significant with the amorphous/colour pattern (Table 4.7. $p<0.05$).

4.4.3.4.b. Ratio phospholipids vs. cholesterol esters

A high ratio of phospholipids vs. cholesterol esters was associated with a higher tear film stability ($p=0.015$) (Tables 4.8). The population was divided into three groups based upon the Non Invasive Break Up Time (NIBUT). The lowest quartile (Lowest 25%) was representative of the quarter of the population with the shortest break-up time and therefore most unstable pre lens tear film. The highest quartile (Highest 25%) was representative of the quarter of the population with the longest break up time and therefore the most stable pre lens tear film. The remaining group was formed by the median quartiles (Mid 50%) and was characteristic of the normal

population. Dividing the population in such a way, which is highly clinically relevant, produced an overall statistically significant difference between the groups (Table 4.8. p=0.015) and a statistically significant difference in phospholipid cholesterol ester ratio was found between the group with most stable tear film (ratio =0.9) and the two other groups (ratio =0.5).

This interaction between median NIBUT and the ratio of phospholipids vs. cholesterol esters was confirmed by the results of the CHAID analysis reported in Table 4.9. The ratio of phospholipids vs. cholesterol esters was assessed as the most influential factor and the population was partitioned accordingly into groups based upon the value of this ratio. For the group with lowest ratio (ratio <0.8), the majority of cases was classified in the lowest 25% or median 50% groups. The group with a ratio superior to 0.8 had a significantly longer break up time: the percentage of cases in the lower quartile decreased from 29% to 20% and the percentage of cases in the higher quartile increased from 15% to 80%.

4.4.3.5. Tear lipid composition and symptomatology.

Differences in the lipid composition of the tear film were associated with different comfort scores and dryness ratings by the subjects during contact lens wear (Tables 4.10 & 4.11). The population was divided into three groups according to their comfort score or their dryness symptoms. For the comfort score, the lowest quartile was associated with poor comfort rating ("bearable or less") and the highest quartile with "good to excellent" comfort during contact lens wear. For the dryness symptoms ratings, the lowest quartile was representative of a population

showing high and frequent symptoms, suffering "often/sometimes" to "constantly" from dryness while wearing their contact lenses. On the contrary, the highest quartile was characteristic of contact lens wearers which "very rarely or never" experienced dryness symptoms during wear. Analysis of the interaction between patients' ratings and tear lipid composition revealed that fatty acids and monoglycerides concentrations influenced the subjective response:

i. A low concentration of monoglycerides was associated with a good comfort and low dryness symptoms;
ii. A high concentration of fatty acids was associated with a good comfort and low dryness symptoms.

Table 4.5. Tear lipid composition for the overall population. Descriptive statistics (Data reported as relative peak intensity).

Lipid classes	Peak	Parameters	Intensity
Cholesterol esters	**Peak 1**	**Mean ± STD**	124 ± 89
		(Min→Max)	(22→ 408)
		1st quart./**Median**/ 2nd quart.	67/**107**/154
	Peak 2	**Mean ± STD**	35 ± 40
		Min→Max	(0→ 134)
		1st quart./**Median**/ 2nd quart.	0/**0**/66
Phospholipids/ triglycerides	**Peak 4**	**Mean ± STD**	86 ± 48
		(Min→Max)	(22→ 244)
		1st quart./**Median**/ 2nd quart.	57/**72**/99
Fatty acids	**Peak 6**	**Mean ± STD**	21 ± 24
		Min→Max	(0→ 76)
		1st quart./**Median**/ 2nd quart.	0/**0**/42
	Peak 7	**Mean ± STD**	8 ± 22
		(Min→Max)	(0→ 74)
		1st quart./**Median**/ 2nd quart.	0/**0**/0
	Peak 8	**Mean ± STD**	30 ± 30
		Min→Max	(0→ 93)
		1st quart./**Median**/ 2nd quart.	0/**28**/57
	Peak 9	**Mean ± STD**	8 ± 22
		(Min→Max)	(0→ 80)
		1st quart./**Median**/ 2nd quart.	0/**0**/0
	Peak 10	**Mean ± STD**	8 ± 16
		Min→Max	(0→ 58)
		1st quart./**Median**/ 2nd quart.	0/**0**/0
	Peak 11	**Mean ± STD**	19 ± 30
		(Min→Max)	(0→ 104)
		1st quart./**Median**/ 2nd quart.	0/**0**/35
Monoglycerides	**Peak 12**	**Mean ± STD**	138 ± 50
		Min→Max	(0→ 242)
		1st quart./**Median**/ 2nd quart.	102/**132**/169
Cholesterol	**Peak 13**	**Mean ± STD**	8 ± 26
		Min→Max	(0→ 120)
		1st quart./**Median**/ 2nd quart.	0/**0**/0

Table 4.6. Tear lipid composition for asymptomatic and symptomatic populations. Descriptive statistics (Data reported as relative peak intensity).

Lipid classes	Peak	Parameters	Asymptomatic	Symptomatic	Exact p-value
Cholesterol esters	Peak 1	Mean ± STD	103 ± 31	137 ± 108	
		(Min→Max)	(65→ 170)	(22→ 408)	0.789
		Median	99	114	
	Peak 2	Mean ± STD	34 ± 47	35 ± 38	
		Min→Max	(0→ 134)	(0→ 100)	0.851
		Median	0	42	
Phospholipids/ triglycerides	Peak 4	Mean ± STD	77 ± 24	91 ± 57	
		Min→Max	(46→ 133)	(22→ 244)	0.782
		Median	68	80	
Fatty acids	Peak 6	Mean ± STD	19 ± 17	22 ± 28	
		Min→Max	(0→ 42)	(0→ 76)	0.820
		Median	24	0	
	Peak 7	Mean ± STD	7 ± 11	9 ± 23	
		Min→Max	(0→ 26)	(0→ 74)	0.377
		Median	0	0	
	Peak 8	Mean ± STD	24 ± 27	34 ± 32	
		Min→Max	(0→ 93)	(0→ 90)	0.328
		Median	22	34	
	Peak 9	Mean ± STD	17± 27	4 ± 17	
		Min→Max	(0→ 80)	(0→ 76)	**0.038**
		Median	0	0	
	Peak 10	Mean ± STD	5 ± 11	9 ± 18	
		Min→Max	(0→ 33)	(0→ 58)	0.503
		Median	0	0	
	Peak 11	Mean ± STD	12 ± 24	23 ± 34	
		Min→Max	(0→ 81)	(0→ 104)	0.498
		Median	0	0	
Monoglycerides	Peak 12	Mean ± STD	109 ± 19	155 ± 55	
		Min→Max	(75→ 147)	(0→ 242)	**0.002**
		Median	108	160	
Cholesterol	Peak 13	Mean ± STD	3 ± 9	11± 32	
		Min→Max	(0→ 31)	(0→ 120)	0.748
		Median	0	0	

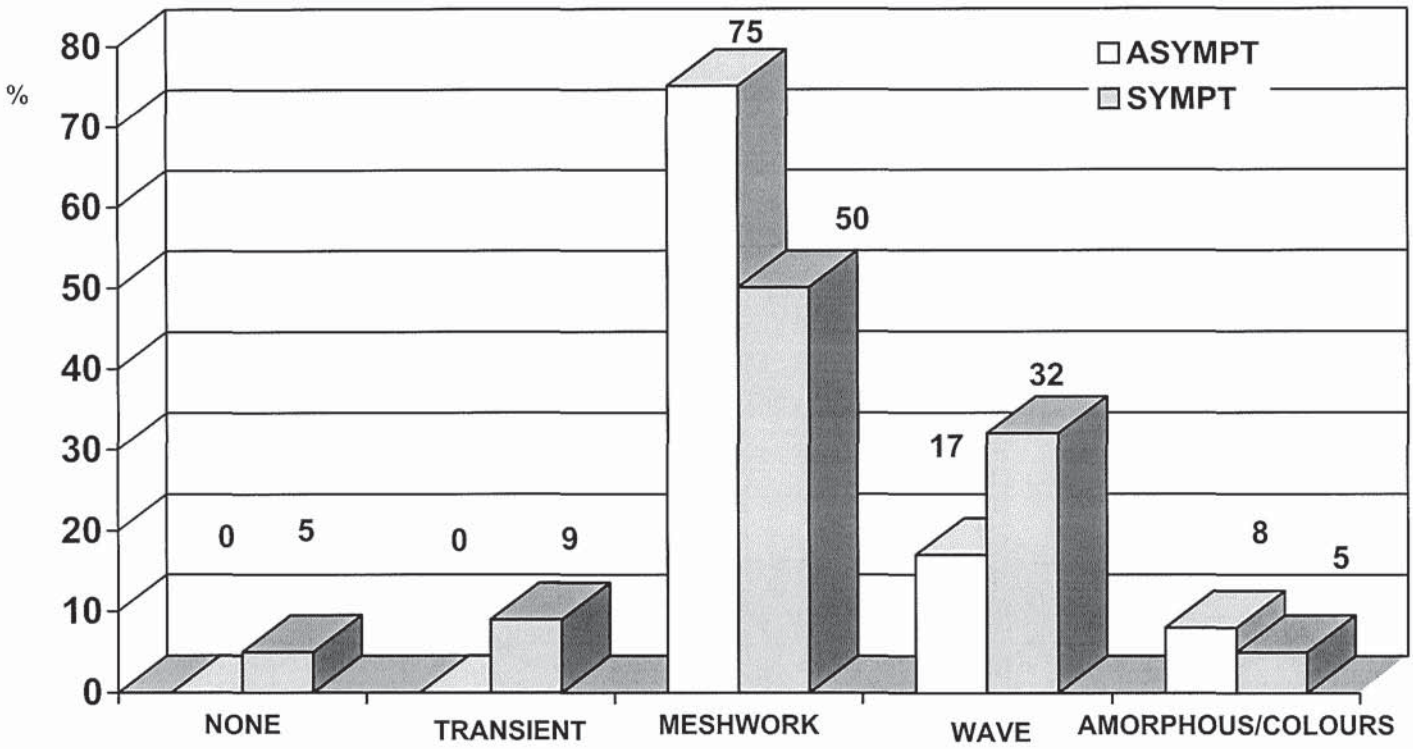


Figure 4.5. Pre lens tear film lipid layer mixing pattern - Distribution for asymptomatic and symptomatic populations.

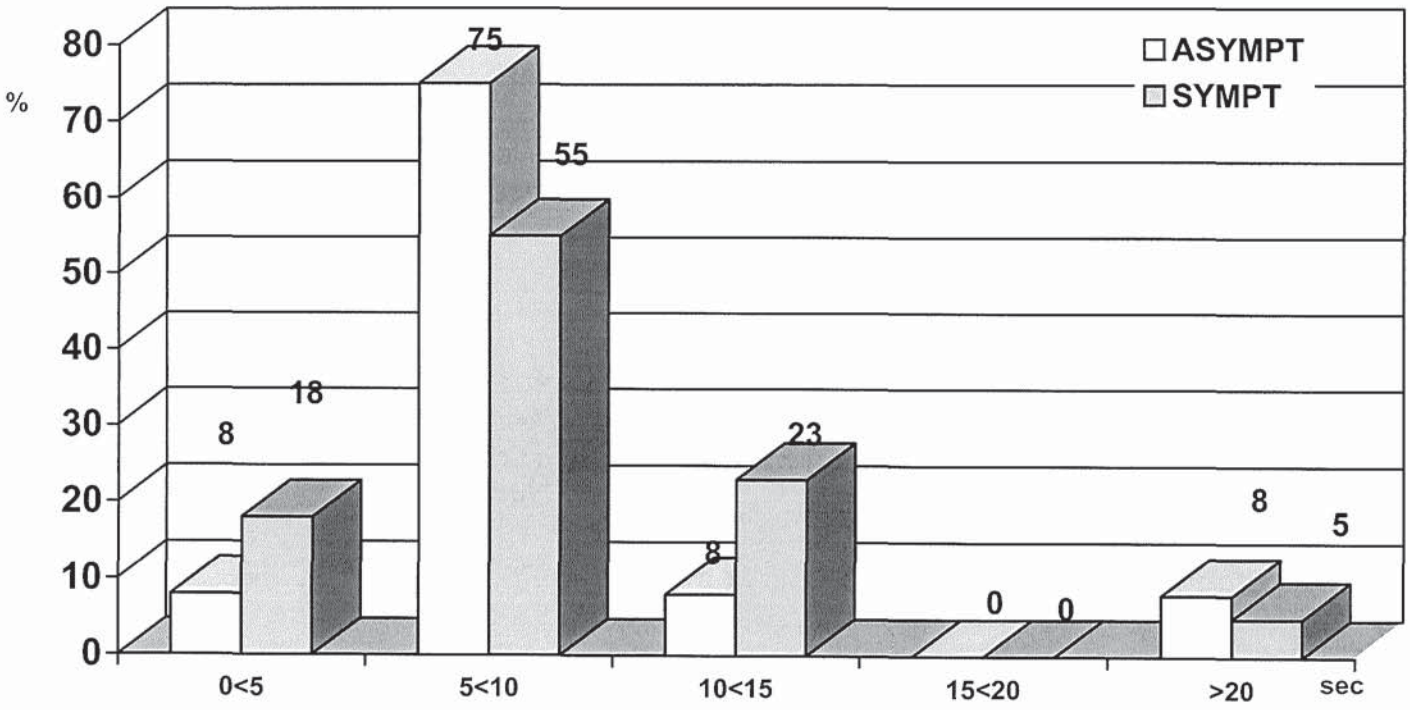


Figure 4.6. Pre lens tear film Non Invasive Break Up Time - Distribution for asymptomatic and symptomatic populations.

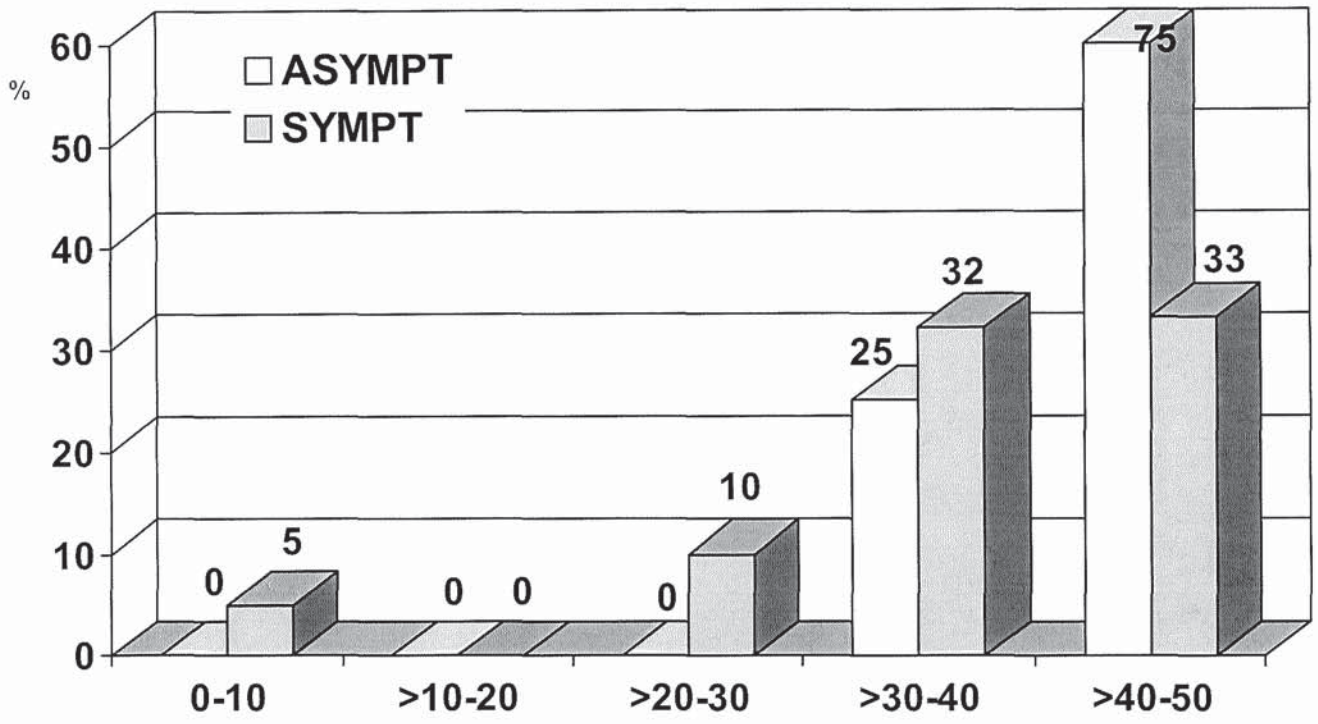


Figure 4.7. Comfort subjective ratings (0-50 point scale) – Distribution for asymptomatic and symptomatic populations.

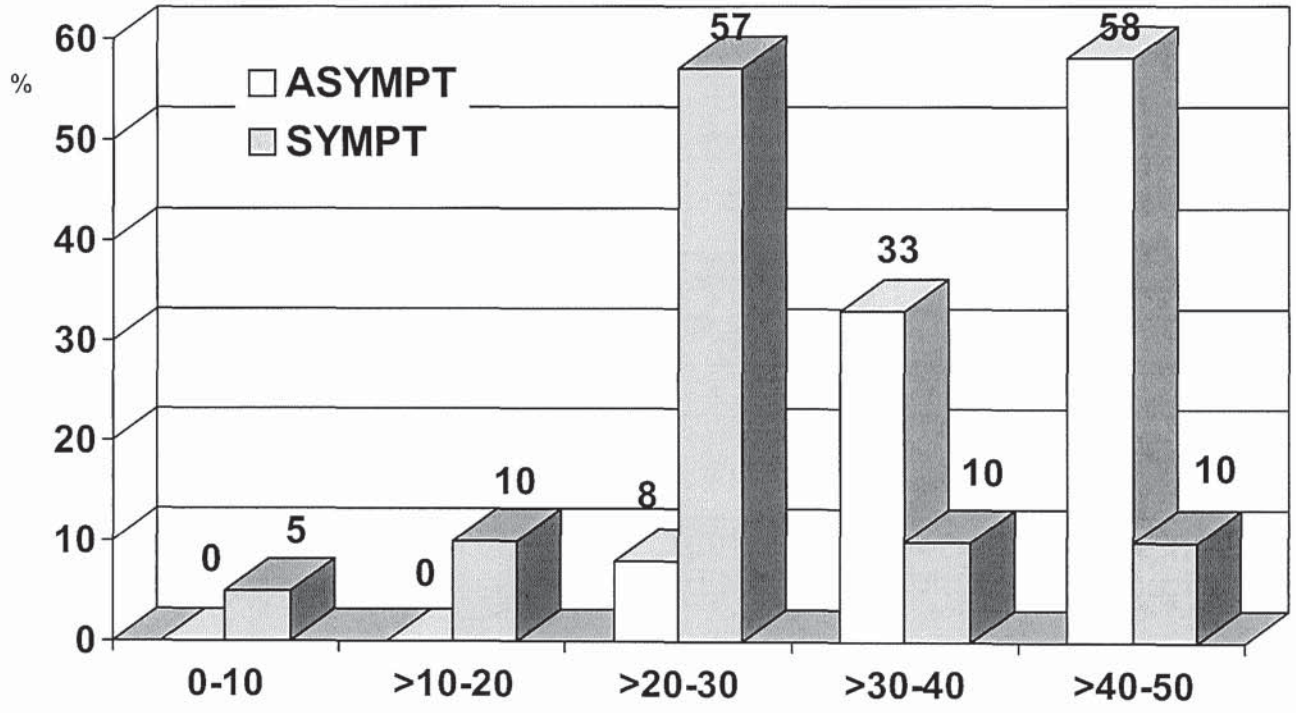


Figure 4.8. Dryness subjective ratings(0-50 point scale) – Distribution for asymptomatic and symptomatic populations.

Table 4.7. Comparison between concentration of cholesterol esters and tear film lipid layer structure by one way ANOVA and SNK test (*values joined by a continuous line are not significantly statistically different).

p<0.05	Amorphous Colours	Wave	Meshwork	None Not visible
intensity	65	97	133	251
SNK(5%)*				

Table 4.8. Comparison between ratio of phospholipids/cholesterol esters and tear film stability by one way ANOVA and SNK test (*values joined by a continuous line are not significantly statistically different).

p=0.015	Unstable Lowest 25%	Normal Mid 50%	Stable Highest 25%
RATIO	0.5	0.5	0.9
SNK(5%)*			

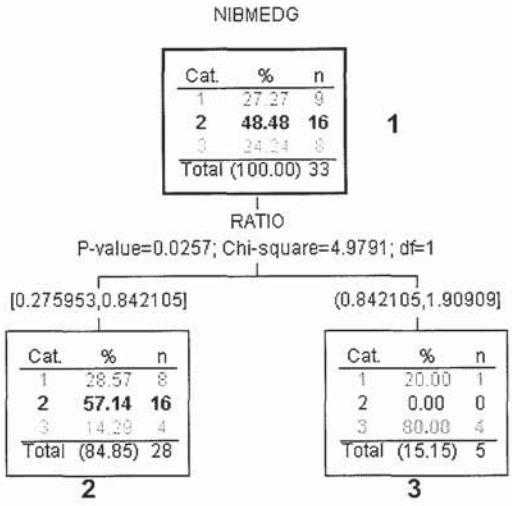


Table 4.9. NIBUT median vs. ratio phospholipids/cholesterol esters - Predictive analysis.

Main Predictor = Ratio of phospholipids/cholesterol esters

Box 1 = NIBUT values for overall population
Box 2 = NIBUT values for low ratio of phospholipids/cholesterol esters
Box 3 = NIBUT values for high ratio of phospholipids/cholesterol esters

Table 4.10. Comparison between lipid composition and comfort score by one way ANOVA and SNK test (*values joined by a continuous line are not significantly statistically different).

- fatty acids p=0.230	Bearable or less	Slight/good comfort most of the time	Good/excellent comfort all the time
	Lowest 25%	Mid 50%	Highest 25%
intensity	0.0	7.6	18.6
SNK(5%)*			

- monoglycerides p=0.006	Good/excellent comfort all the time	Slight/good comfort most of the time	Bearable or less
	Highest 25%	Mid 50%	Lowest 25%
intensity	109	144	176
SNK(5%)*			

Table 4.11. Comparison between lipid composition and dry eye symptomatology by one way ANOVA with SNK test (*values joined by a continuous line are not significantly statistically different).

- fatty acids p=0.026	Sometimes to rarely	Often to constantly	Very rarely to never
	Mid 50%	Mid 50%	Highest 25%
intensity	0.0	6.3	25.3
SNK(5%)*			

- monoglycerides p=0.006	Very rarely to never	Sometimes to rarely	Often to constantly
	Mid 50%	Mid 50%	Highest 25%
intensity	104	147	164
SNK(5%)*			

4.4.4. In vitro measurements

4.4.4.1. Phospholipids and cholesterol esters

The spreading behaviour of phospholipids and cholesterol esters onto an aqueous subphase was investigated using a mini-Langmuir trough. Phospholipids and cholesterol oleate were obtained from Sigma Chemicals Co. Lipid solutions, 0.1mg/ml in chloroform, with phospholipid to cholesterol oleate molar ratios ranging from 0.1 to 4.5, were prepared. The test consisted of depositing 5 μl of lipid solution to test at the air/aqueous interface on the mini Langmuir Trough. The aqueous phase was made up of 60 ml of HPLC grade water. The measurements were made by compressing and expanding the lipid layer for ten cycles. The pressure was recorded as a function of surface area, which ranged from 80 mm^2 to 20 mm^2 when fully compressed. The results obtained are reported in Table 4.12 and the typical compression cycles for cholesterol ester and phospholipids are shown on Figures 4.9 & 4.10. Figure 4.11 shows the change in spreading behaviour of the lipid layer with changes in relative concentration of phospholipids and cholesterol esters. A low pressure when fully compressed characterised lipid phases made of cholesterol ester exclusively or with low concentration of phospholipids. This is characteristic of components with low surface activity and a non-tendency to spread. On the other hand, the behaviour changed radically for phospholipids/cholesterol ester ratios over 0.9 where a high surface activity was recorded and became similar to that of a pure phospholipid layer.

4.4.4.2. Fatty acids

The spreading behaviour of fatty acids onto an aqueous subphase was investigated using a similar procedure to that described above. Linoleic and oleic acids were obtained from Sigma Chemicals Co. Solutions of 0.1 mg/ml of individual lipids in chloroform were prepared and analysed using the mini Langmuir Trough. Typical compression cycles of fatty acids are shown on Figures 4.12 & 4.13. They are characteristic of a medium surface activity and moderate to good spreading over the aqueous subphase.

Table 4.12. Pressure values at maximal compression for various ratios of phospholipids and cholesterol ester (ph/CE). Descriptive statistics.

Ratio (ph/CE)	Parameters	Pressure (dyn/cm^2)	Ratio (ph/CE)	Parameters	Pressure (dyn/cm^2)
0.1	Mean ± STD (Min →Max)	19.2 ± 2.2 (16.1→22.2)	**1.25**	Mean ± STD (Min →Max)	75.7 ± 1.3 (73.5→77.2)
0.25	Mean ± STD (Min →Max)	18.2 ± 2.3 (14.4→21.0)	**1.50**	Mean ± STD (Min →Max)	75.2 ± 1.5 (72.5→77.4)
0.50	Mean ± STD (Min →Max)	43.1 ± 1.2 (41.1→44.4)	**2.00**	Mean ± STD (Min →Max)	110.8 ± 3.7 (105.4→119.8)
0.75	Mean ± STD (Min →Max)	42.2 ± 1.0 (41.0→43.9)	**2.50**	Mean ± STD (Min →Max)	115.6 ± 4.9 (105.1→121.8)
0.9	Mean ± STD (Min →Max)	43.8 ± 1.4 (41.0→45.4)	**3.00**	Mean ± STD (Min →Max)	115.7 ± 4.9 (105.1→121.8)
1.00	Mean ± STD (Min →Max)	69.2 ± 0.9 (68.0→70.1)	**4.00**	Mean ± STD (Min →Max)	104.4 ± 3.3 (99.8→109.4)

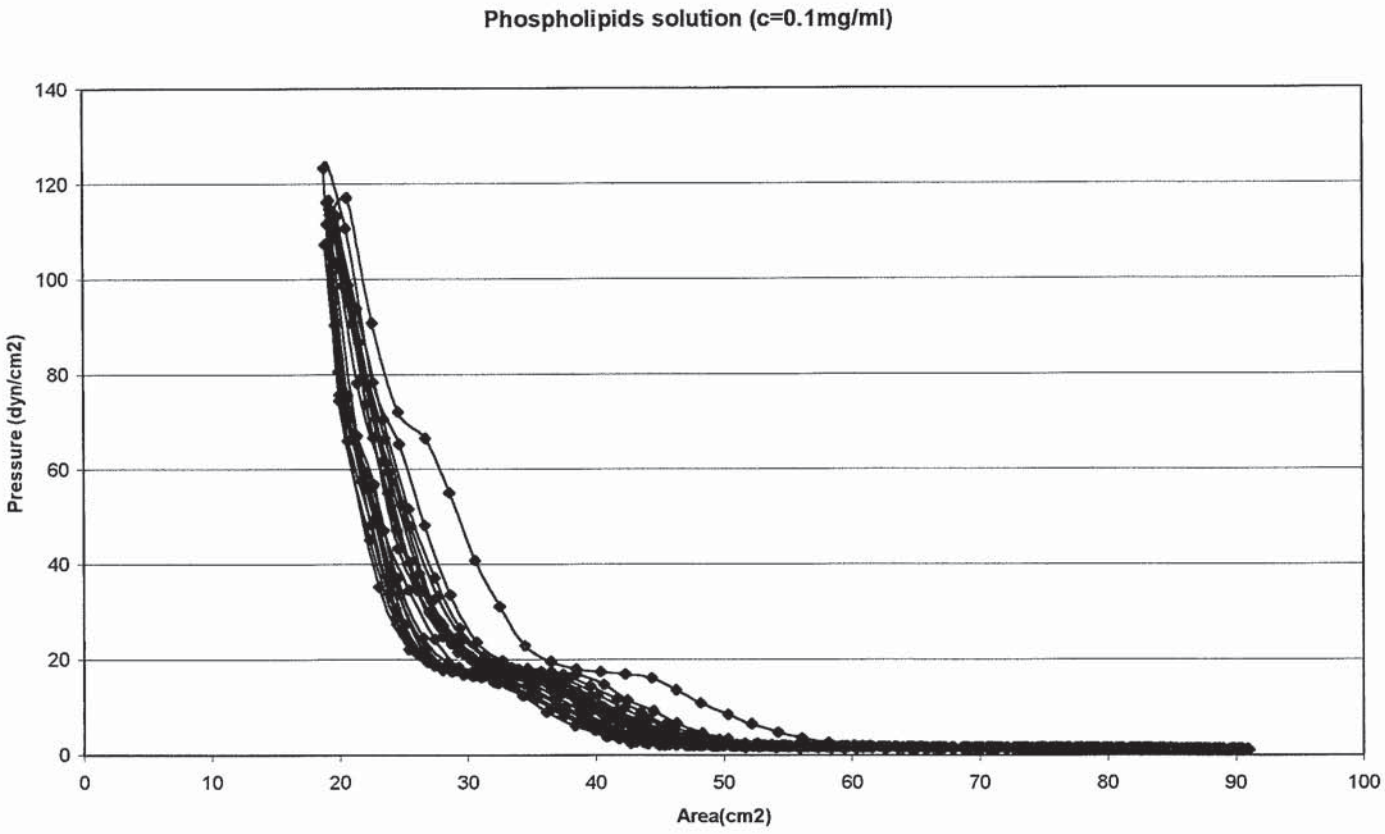


Figure 4.9. Compression and expansion cycles of phospholipids solution.

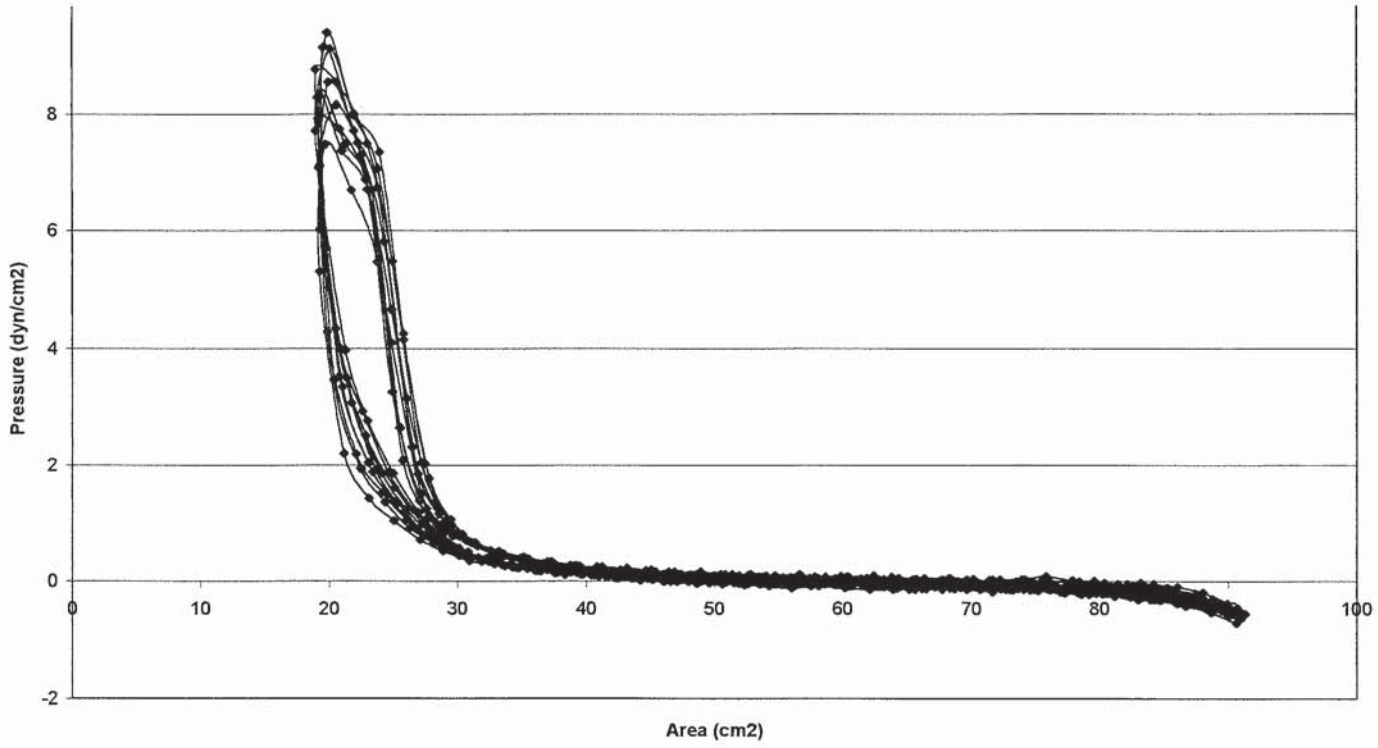


Figure 4.10. Compression and expansion cycles of cholesterol ester solution.

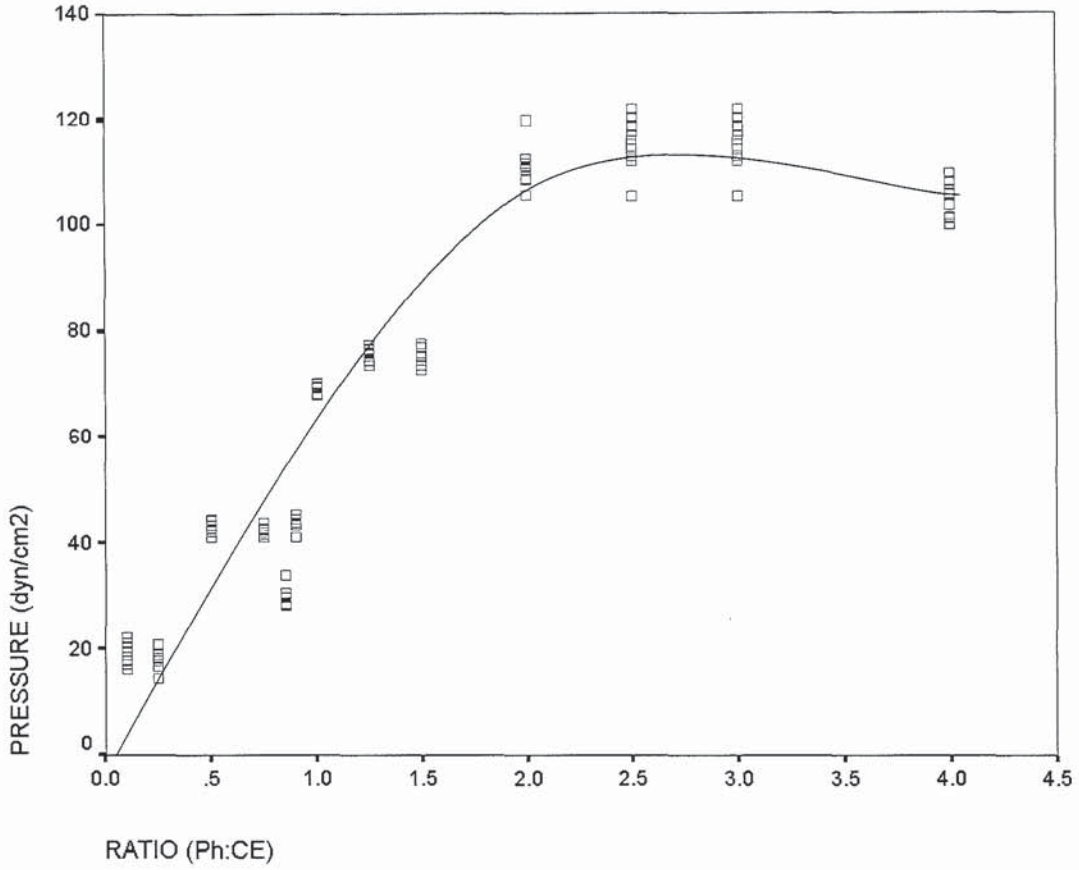


Figure 4.11. Pressure under maximal compression vs. ratio of phospholipids and cholesterol ester.

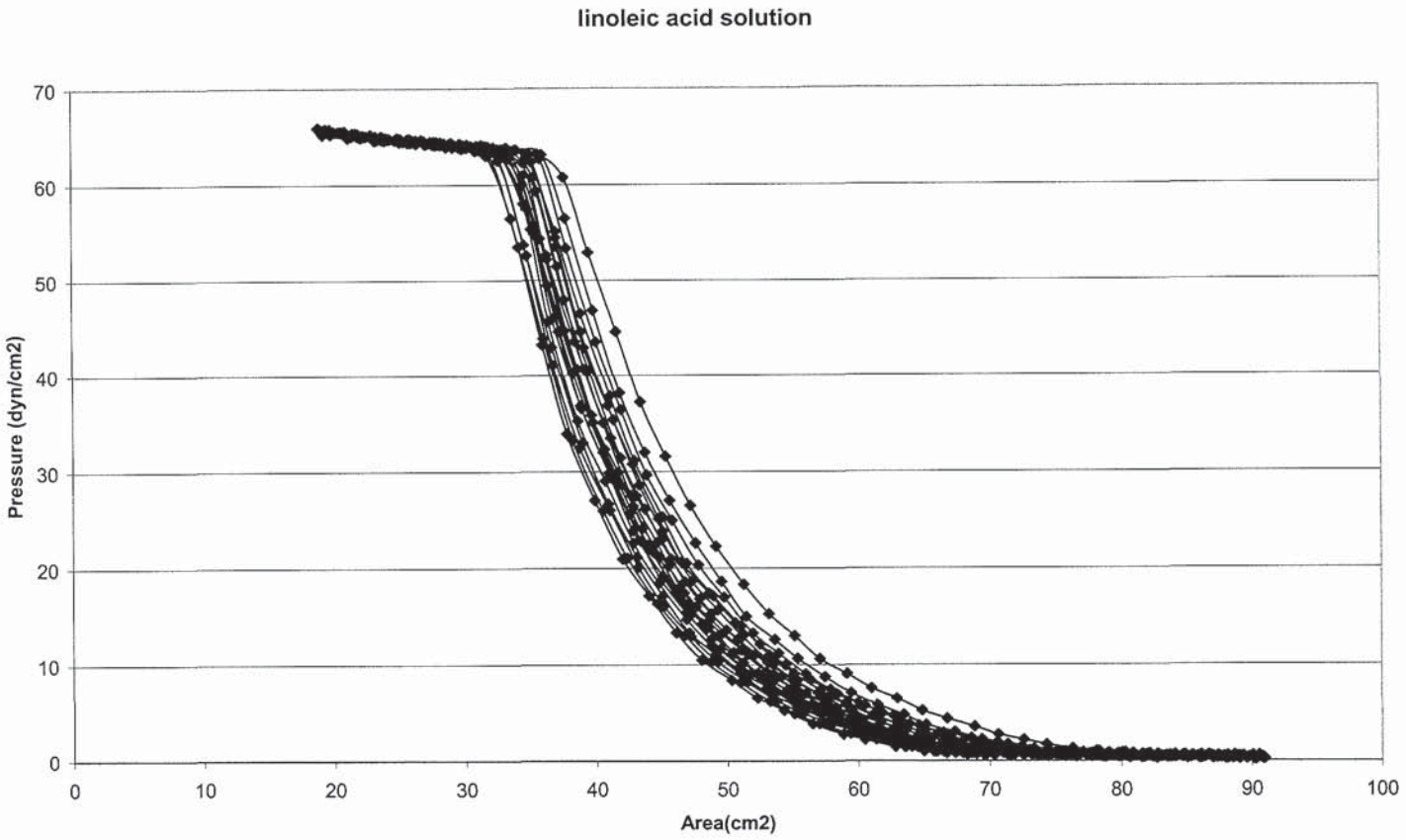


Figure 4.12. Compression and expansion cycles of linoleic acid solution.

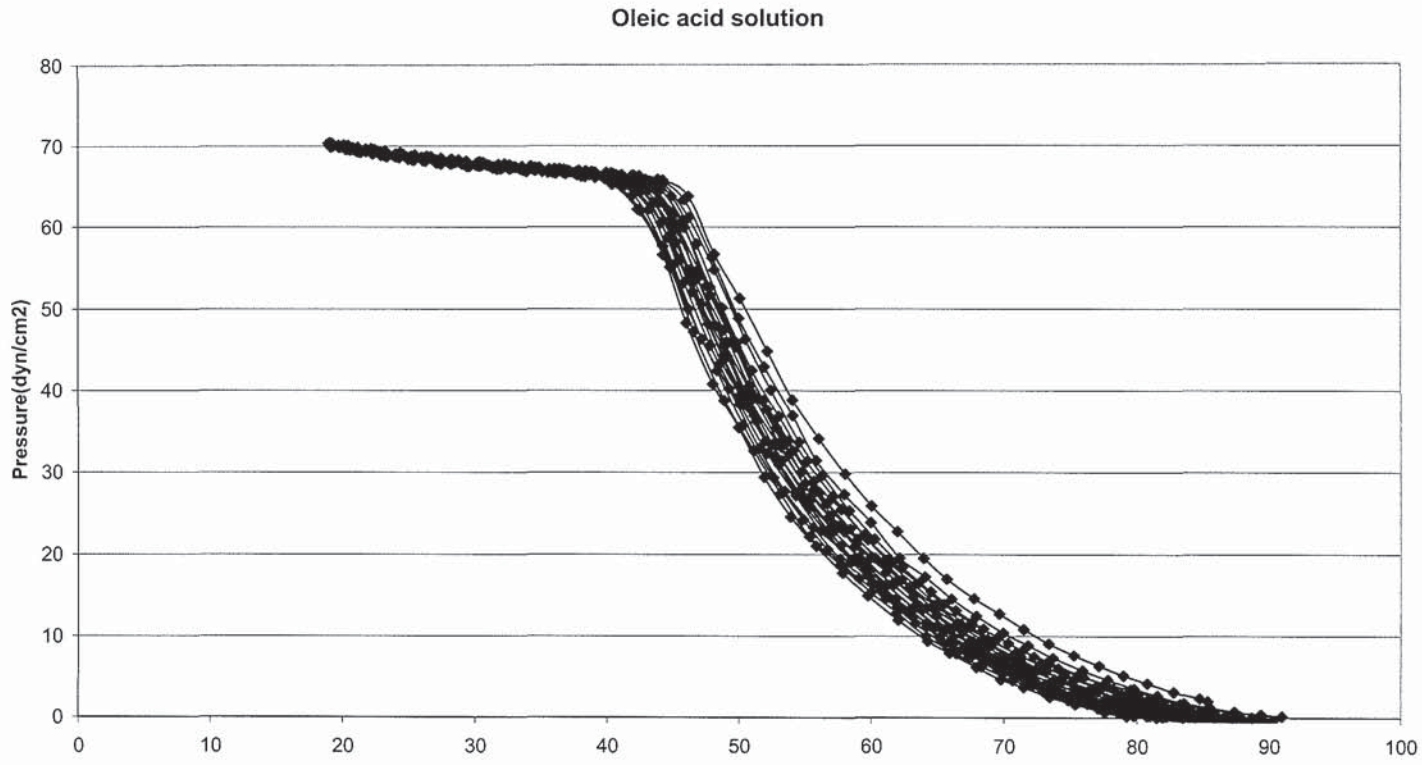


Figure 4.13. Compression and expansion cycles of oleic acid solution.

4.4.5. Discussion

The current technique using HPLC of individual non-reflex tear samples (2 μl) has been shown to be sensitive enough to detect the main lipid classes. The results obtained showed that the tear film lipid composition varied significantly between individuals.

For the first time an association has been identified between the PLTF clinical characteristics and tear film lipid composition. The observed lipid mixing pattern, indicative of the lipid thickness and spreading characteristics, the tear film stability have been shown to be influenced by the tear film lipid chemical composition. Specifically,

- a high concentration of hydrophobic cholesterol esters has been shown to play a deleterious role in the establishment of a thick and uniform lipid layer.
- the tear film stability was directly influenced by the ratio of polar vs. non-polar lipids; high ratios produced greater tear film stability.

The mechanism that underlies the influence of cholesterol esters and polar vs. non polar lipids was demonstrated in vitro by studying the effect of the components on the spreading of the lipid film on the aqueous layer. The findings made from the clinical sample were confirmed by in vitro measurements, which correlated to strictly different surface behaviours.

Biochemical differences in the composition of the lipid layer have been shown to influence the patient symptomatology. In that context biochemical analysis is superior to the clinical evaluation to identify risks of poor contact lens tolerance.

No clinical study to date has detected an association between tear film

characteristics and comfort. The two key lipid components were monoglycerides (low concentration = good comfort and low dryness) and fatty acids (high concentration = good comfort and low dryness).

4.4.6. Conclusion

The results obtained in this investigation led to the following conclusions:

(I) The analysis of individual tear samples (microlitre level) with HPLC enabled us to differentiate between five lipid classes, the ratios of which vary widely between subjects and to demonstrate significant associations between tear film structure and composition.

(ii) The subjects' symptomatology was associated with differences in the concentrations of fatty acids and monoglycerides present in the tear film. The symptomatic population was characterised by a higher concentration of monoglycerides and lower concentration of fatty acids compared to the asymptomatic population.

(iii) The study confirmed that the lipid composition of the tear film influenced its structure and stability:

- a high level of cholesterol esters was associated with a thinner lipid layer

- a high ratio of phospholipids vs. cholesterol esters was associated with a more stable tear film.

(iv) The study revealed statistical associations between patient comfort and dryness symptoms during contact lens wear and the level of monoglycerides and fatty acids present in the tear film.

CHAPTER 5

TEAR LIPID ANALYSIS AND CONTACT LENS EXTRACTS

5.1. Tear analysis of dry eye patients

5.1.1. Objective

Differences in lipid composition have been reported (Dry Eye Conference, 1996) between normal subjects and those suffering from dry eye symptoms[184]. The purpose of this investigation was:

i. to identify differences in the lipid composition that would be characteristic of a symptomatic contact lens population and,

ii. to detect markers for symptomatic subjects and validate their usefulness to detect these symptomatic contact lens wearers.

5.1.2. Materials and method

The test population consisted of myopic contact lens wearers with dry eye symptoms according to the modified McMonnies questionnaire. Subjects were considered symptomatic when they scored over 40 on dedicated McMonnies dry eye questionnaire for contact lens wearers. The population characteristics are summarised in Table 5.1. The investigation was a two weeks daily wear dispensing study. The subjects attended CLRC for an initial visit wearing their own soft contact lenses and were fitted with Vifilcon A, HEMA/NVP material (FOCUS, CIBAVision) contact lenses (Table 5.2). On the completion of the two weeks, the subjects found

to be symptomatic were enrolled in the second phase. Positive symptomatology was based on the following criteria:

- The subjects scored 50 or more on the modified McMonnies questionnaire and either rated comfort, dryness or grittiness less than 35 for on the 50 point scale (Appendix H) or their wearing time was less than 12 hours or they used more than 3 eye drops per day.
- The subjects scored 40 or more but less than 50 on the modified McMonnies questionnaire and, either rated comfort, dryness or grittiness less than 35 for on the 50 point scale (Appendix H) together with a wearing time of less than 12 hours or rated comfort, dryness or grittiness less than 25 on the 50 point scale or their wearing time was less than 8 hours.

Fifteen patients, out of the twenty-two enrolled in Phase I, were symptomatic and were therefore enrolled in the second phase of the study. During this second phase, they tested two soft contact lens materials: Etafilcon A, an HEMA-based material (ACUVUE, Johnson & Johnson) and a GMA/HEMA copolymer (BENZ55G, Benz) (Table 5.2). Each material was tested for two weeks of wear with follow up visits after 1 and 2 weeks of wear. One-week wash out period was implemented between each contact lens type.

Tears were collected using Visispear ophthalmic sponges at the following visits:

- at the Initial visit (Subjects wearing own contact lenses: n=22)
- at the Day 14 follow-up visit in Phase 1 (Subjects wearing Focus contact lenses: n=22)

- at the Dispensing visit for each contact lens type in Phase 2 (Subjects not wearing any contact lenses: n=15)
- at Day 7 & 14 follow-up visits for each contact lens type in phase 2 (Subjects wearing test contact lenses: n=15).

After collection, the tear samples were prepared and analysed by High Performance Liquid Chromatography (HPLC) as described in section 2.2.4.

Table 5.1. Population characteristics.

	N	Age (years)	Male	Female
Phase I	22	32.9 ± 6.9 (20 - 44)	8	14
Phase II	15	33.1 ± 6.4 (24 - 44)	6	9

Table 5.2. Contact lens material characteristics- Descriptive statistics

	FOCUS CIBAVision	ACUVUE Vistakon	BENZ55G Benz
Material	Vifilcon A (HEMA/NVP)	Etafilcon A (HEMA/MAA)	GMA/HEMA
Water content (%)	55	58	57
Back Optical Radius(mm)	8.60	8.40	8.60
Diameter (mm)	14.0	14.0	14.5

5.1.3. Results

5.1.3.1. Introductory remark

During the statistical analysis, a p-value of 0.05 or less was set as threshold of statistical significance and because of the small sample size in this investigation, a p-value of 0.2 or less was taken as threshold for a statistical trend.

5.1.3.2. Tear lipid composition during Phase I

The lipid composition of the Pre Lens Tear Film (PLTF) of the subjects either wearing their own soft contact lenses or after two weeks of wearing Focus is summarised in Tables 5.3 and 5.4, overall and for the symptomatic or asymptomatic groups separately.

Differences in the tear lipid profile were found while the subjects were wearing their own contact lenses and Focus contact lenses. The intensity of one of the fatty acids (Peak 8) was statistically significantly higher when the subjects wore their own lenses than when they wore Focus contact lenses (Table 5.5. p=0.048, median: 34 vs. 0). Similarly there was a trend towards a higher level for one of the fatty acid (Peak11) and for the monoglycerides peak (Peak 12) while the subjects wore their own contact lenses (Table 5.5. p=0.168 & 0.084). Here, biochemical differences are of limited clinical interest because in going from the subjects' own lenses to Focus lenses, we have simultaneously modified several parameters in particular the level of symptomatology and the age of the contact lens. Also the subjects' own lenses did not represent a homogeneous contact lens material baseline group.

For the subjects that became asymptomatic after two weeks of wearing Focus, no statistically significant differences in the tear profiles were found between the subjects' own lenses and Focus. However, there was a trend towards a lower intensity of the monoglycerides peak (Peak 12) for the samples taken after two weeks with Focus than with the subjects own lenses (Table 5.5. p=0.156, median: 168 vs. 144). This decrease in the level of monoglycerides associated with a decrease in symptomatology consolidated the findings described in Chapter 4

concerning the potential role of monoglycerides in the dry eye symptomatology of contact lens wearers.

For the subjects remaining symptomatic after two weeks of wear of Focus, some statistical differences and trends were found between the two PLTF tear profiles. The differences were limited to the fatty acid family; there was a statistically significantly higher intensity for Peak 8 (Table 5.5. p=0.004, median: 57 vs. 0) and a trend towards a higher level of Peak 11 (Table 5.5. p=0.186, median: 15 vs. 0) in the PLTF samples while subjects wore their own contact lenses than when they wore Focus contact lenses.

5.1.3.3. Tear lipid composition during Phase II

The tear lipid compositions of the Pre Ocular Tear Film (POTF) (baseline) and PLTF of the subjects, while wearing Acuvue and Benz, are summarised in Table 5.6.

No statistical differences were detected in the overall lipid composition of the pre lens tear film measured with Acuvue and with Benz contact lenses. Twelve out of thirteen peaks were not statistically different. The only significant difference recorded was for one of the fatty acids (Peak 11); a significantly higher level was recorded in the tears of the subjects while wearing Acuvue (Table 5.7. p=0.019, median: 31 vs. 15).

No overall effect of lens wear was detected. For the two contact lenses, the tear compositions were similar before and after two weeks of wear. Analysis of the tear lipid profile after two weeks of wearing either Acuvue or Benz revealed no significant

differences compared to the pre ocular tear lipid profile (Table 5.7ii). Some minor individual peak differences were recorded:

- For Benz contact lenses, a slight increase in the level of one of the fatty acids (Peak 7) was noticed after one week of wear compared to baseline (Table 5.7ii. p=0.109, median: 0 vs. 24). Further a trend towards a higher level of phospholipids/ triglycerides (Peak 4) was recorded for Benz after two weeks of wear than after one week (Table 5.7ii. p=0.127, median: 78 vs. 62).
- For Acuvue contact lenses, there was a statistically significant decrease in the level of one of the cholesterol esters (Peak 2) at two week follow up visit compared to one week (Table 5.7ii. p=0.031, median: 0 vs. 51) and a trend towards a decrease of the level of phospholipids/triglycerides (Peak 4) at one week follow up visit compared to baseline (Table 5.7ii. p=0.092, median: 61 vs. 78).

After two weeks of Acuvue and Benz contact lens wear, the subjects' symptomatology while wearing either type of contact lens was assessed using the criteria described in section 5.1.2. With Acuvue, 7 subjects became asymptomatic and 8 remained symptomatic over the two-week period of wear. With Benz, after two weeks of wear, 4 subjects out of 15 became asymptomatic. For each lens type, the tear lipid composition of each subgroup was compared and led to the following results:

- For Acuvue at the two week follow-up visit, there was a statistically significant difference in the tear level of monoglycerides (Peak 12) between asymptomatic

and symptomatic Acuvue contact lens wearers (Table 5.8. p=0.030). The level of monoglycerides was significantly higher in the tears of the symptomatic population, which is in agreement with earlier findings regarding the relationship between level of monoglycerides and symptomatology. A trend towards a higher intensity for one of the cholesterol esters (Peak 1) was also recorded for the asymptomatic group; no clinical significance however could be attributed to this difference.

- For Benz, there were no statistically significant differences between the tear lipid composition of asymptomatic and symptomatic contact lens wearers. The only possible trend recorded was towards a higher level of cholesterol (Peak 13) in the asymptomatic population. This finding has no relation with symptomatology and is probably due to the low and unequal sample sizes in each subgroup.

5.1.3.4. Tear composition after two weeks of wear of Focus, Acuvue & Benz

For the fifteen subjects enrolled in Phases I and II of this investigation, it was possible to compare the PLTF lipid composition while the subjects wore Acuvue and Benz vs. Focus. No statistically significant differences where found for either of the two contact lenses compared to Focus.

After two weeks of wear, the following statistical trends were recorded while comparing Focus and Acuvue:

- A lower level of cholesterol ester (Peak 2) was recorded with Acuvue (Table 5.9. p=0.063, median: 0 vs. 66);

- Higher levels of two fatty acids (Peak 8 and 11) were measured with Acuvue (Table 5.9. p=0.078 & 0.102, median: 30 vs. 0 and 31 vs. 0).

After two weeks of wear, the following statistical trends were found while comparing Focus and Benz:

- A lower level of cholesterol ester (Peak 1) was measured with Benz (Table 5.9. p=0.216, median: 102 vs. 122);
- A higher level of one of the fatty acids (Peak 7) was recorded with Benz (Table 5.9. p=0.094).

For both Acuvue and Benz, the tear lipid composition was characterized by a higher level of fatty acids and lower level of cholesterol ester compared to Focus.

Table 5.3. Overall tear lipid composition - Phase I (Own contact lenses and Focus contact lenses) - Descriptive statistics (n=22) (Relative peak intensity).

Lipid Identification		Parameters	Own CL	FOCUS
Cholesterol Ester	Peak 1	Mean±STD Median (Range)	137 ± 108 114 (22-408)	132 ± 62 122 (49-290)
	Peak 2	Mean±STD Median (Range)	35 ± 38 42 (0-100)	44 ± 47 52 (0-130)
Phospholipids/ Triglycerides	Peak 4	Mean±STD Median (Range)	91± 57 80 (22-244)	83 ± 44 66 (0-178)
Fatty acids	Peak 6	Mean±STD Median (Range)	22 ± 28 0 (0-76)	33 ± 45 34 (0-196)
	Peak 7	Mean±STD Median (Range)	9 ± 23 0 (0-74)	4 ± 13 0 (0-46)
	Peak 8	Mean±STD Median (Range)	34 ± 32 34 (0-90)	11 ± 19 0 (0-50)
	Peak 9	Mean±STD Median (Range)	4 ± 17 0 (0-76)	5 ± 17 0 (0-68)
	Peak 10	Mean±STD Median (Range)	9 ± 18 0 (0-58)	3 ± 9 0 (0-30)
	Peak 11	Mean±STD Median (Range)	23 ± 34 0 (0-104)	11 ± 22 0 (0-64)
Monoglycerides	Peak 12	Mean±STD Median (Range)	155 ± 55 160 (0-242)	133 ± 33 142 (57-198)
Cholesterol	Peak 13	Mean±STD Median (Range)	11 ± 32 0 (0-120)	3 ± 15 0 (0-65)

Table 5.4. Tear lipid composition for each subpopulation (Group 1 (n=7) subjects asymptomatic after 2 weeks of wear with Focus; Group 2 (n=15) subjects symptomatic after 2 weeks of wear with Focus) - Phase I (Own contact lenses and Focus contact lenses) - Descriptive statistics (Relative peak intensity).

Lipid Identification		Parameters	Own CL		FOCUS	
			Group 1 n=7	Group 2 n=15	Group 1 n=7	Group 2 n=15
Cholesterol Ester	Peak 1	Mean±STD Median (Range)	128 ± 95 136 (38-284)	141 ± 118 111 (22-408)	123 ± 43 124 (49-176)	136 ± 70 114 (52-290)
	Peak 2	Mean±STD Median (Range)	34 ± 36 42 (0-96)	36 ± 40 22 (0-100)	37 ± 44 26 (0-106)	48 ± 50 66 (0-130)
Phospholipids/ Triglycerides	Peak 4	Mean±STD Median (Range)	83± 62 82 (22-204)	94± 57 78 (32-244)	94 ± 47 88 (44-178)	77 ± 44 64 (0-152)
Fatty acids	Peak 6	Mean±STD Median (Range)	17 ± 24 0 (0-56)	25 ± 31 0 (0-76)	59 ± 70 38 (0-196)	21 ± 21 32 (0-56)
	Peak 7	Mean±STD Median (Range)	0 ± 0 0 (0-0)	14 ± 28 0 (0-74)	8 ± 19 0 (0-26)	2 ± 9 0 (0-32)
	Peak 8	Mean±STD Median (Range)	11 ± 19 0 (0-40)	46 ± 31 57 (0-90)	20 ± 23 16 (0-50)	7 ± 17 0 (0-48)
	Peak 9	Mean±STD Median (Range)	0 ± 0 0 (0-0)	5 ± 20 0 (0-76)	0 ± 0 0 (0-0)	7± 20 0 (0-68)
	Peak 10	Mean±STD Median (Range)	3 ± 8 0 (0-21)	12 ± 21 0 (0-58)	0 ± 0 0 (0-0)	4 ± 11 0 (0-30)
	Peak 11	Mean±STD Median (Range)	12 ± 32 0 (0-84)	29 ± 34 15 (0-104)	10 ± 24 0 (0-60)	11 ± 22 0 (0-64)
Monoglycerides	Peak 12	Mean±STD Median (Range)	171 ± 37 168 (122-232)	147 ± 61 160 (0-242)	138 ± 48 144 (57-198)	130 ± 25 132 (70-160)
Cholesterol	Peak 13	Mean±STD Median (Range)	4 ± 10 0 (0-27)	15 ± 38 0 (0-120)	11± 26 0 (0-65)	0 ± 0 0 (0-0)

Table 5.5. Tear lipid composition for each subpopulation (Group 1 (n=7) subjects asymptomatic after 2 weeks of wear with Focus; Group 2 (n=15) subjects symptomatic after 2 weeks of wear with Focus) - Phase I (Own contact lenses and Focus contact lenses) - Comparative statistics by Wilcoxon Matched Pairs Signed Ranks Exact Test.

	Comparison between contact lens type overall and for each group		
	Overall	Group 1 n=7	Group 2 n=15
Peak 1	0.840	0.438	0.791
Peak 2	0.622	1.000	0.547
Peak 4	0.799	0.844	0.569
Peak 6	0.699	0.375	0.676
Peak 7	0.875	1.000	0.500
Peak 8	**0.048**	0.375	**0.004**
Peak 9	0.500	1.000	0.500
Peak 10	0.625	1.000	0.625
Peak 11	**0.168**	1.000	**0.186**
Peak 12	**0.081**	**0.156**	0.266
Peak 13	0.500	1.000	0.500

Table 5.6. Overall tear lipid composition - Phase II (Acuvue and Benz contact lenses) - Descriptive statistics (n=15) (Relative peak intensity).

Lipid Identification		Parameter	ACUVUE			BENZ		
			DISP*	1/52	2/52	DISP*	1/52	2/52
Cholesterol Ester	Peak 1	Mean±STD Median (Range)	119 ± 80 98 (54-372)	114 ± 50 107 (61-204)	136 ± 42 142 (78-196)	115 ± 63 106 (58-312)	106 ± 54 109 (24-201)	106 ± 41 102 (45-188)
	Peak 2	Mean±STD Median (Range)	39 ± 52 0 (0-154)	54 ± 65 51 (0-209)	6 ± 21 0 (0-72)	23 ± 51 0 (0-172)	57 ± 100 30 (0-369)	33 ± 48 0 (0-140)
Phospholipids / Triglycerides	Peak 4	Mean±STD Median (Range)	100 ± 70 78 (31-276)	64± 29 61 (0-106)	70± 28 73 (0-106)	74 ± 19 77 (34-98)	59 ± 26 62 (0-90)	86 ± 60 78 (0-280)
Fatty acids	Peak 6	Mean±STD Median (Range)	28 ± 16 32 (0-52)	23 ± 21 29 (0-52)	31 ± 21 34 (0-56)	32 ± 25 32 (0-64)	32 ± 19 40 (0-50)	32 ± 20 32 (0-64)
	Peak 7	Mean±STD Median (Range)	12 ± 20 0 (0-62)	9 ± 14 0 (0-36)	11 ± 17 0 (0-46)	10 ± 17 0 (0-42)	15 ± 15 24 (0-34)	13 ± 20 0 (0-50)
	Peak 8	Mean±STD Median (Range)	23 ± 18 32 (0-50)	26 ± 22 25 (0-70)	23 ± 17 30 (0-44)	12 ± 18 0 (0-48)	17 ± 18 20 (0-52)	15 ± 17 0 (0-41)
	Peak 9	Mean±STD Median (Range)	8 ± 17 0 (0-50)	0 ± 0 0 (0-0)	6 ± 12 0 (0-32)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	4 ± 11 0 (0-32)
	Peak 10	Mean±STD Median (Range)	3 ± 9 0 (0-32)	3 ± 8 0 (0-26)	0 ± 0 0 (0-0)	7 ± 19 0 (0-58)	2 ± 9 0 (0-28)	0 ± 0 0 (0-0)
	Peak 11	Mean±STD Median (Range)	15 ± 18 0 (0-44)	20 ± 19 24 (0-48)	29 ± 15 31 (0-56)	17 ± 20 11 (0-52)	17 ± 17 22 (0-38)	15 ± 16 15 (0-44)
Monoglycerides	Peak 12	Mean±STD Median (Range)	150 ± 39 138 (90-232)	146 ± 25 138 (102-194)	132 ± 17 131 (104-164)	129 ± 20 134 (98-158)	129 ± 28 129 (68-172)	130 ± 23 136 (64-165)
Cholesterol	Peak 13	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	4 ± 9 0 (0-29)	0 ± 0 0 (0-0)	7 ± 9 0 (0-66)	5 ± 11 0 (0-31)

* Pre ocular tear film

Table 5.7. Overall tear lipid composition - Phase II (Acuvue and Benz contact lenses) - Comparative statistics (n=15).

i. Between contact lens types by Wilcoxon Matched-Pairs Signed-Ranks Exact Test

	Comparisons between Acuvue and Benz at each visit		
	DISP	1/52	2/52
Peak 1	0.733	0.625	0.301
Peak 2	0.563	0.258	0.219
Peak 4	0.519	0.846	0.339
Peak 6	0.465	0.301	1.000
Peak 7	1.000	0.563	1.000
Peak 8	0.232	0.301	0.426
Peak 9	0.250	1.000	0.910
Peak 10	0.500	1.000	1.000
Peak 11	0.578	0.570	**0.019**
Peak 12	0.910	0.770	1.000
Peak 13	1.000	0.500	0.625

ii. Between visits by Wilcoxon Matched-Pairs Signed-Ranks Exact Test

	Disp vs. 1/52		Disp vs. 2/52		1/52 vs. 2/52	
	ACUVUE	BENZ	ACUVUE	BENZ	ACUVUE	BENZ
Peak 1	1.000	0.622	0.320	0.463	1.000	0.946
Peak 2	0.625	0.734	0.219	0.813	**0.031**	0.557
Peak 4	**0.092**	0.233	0.465	0.670	0.734	**0.127**
Peak 6	0.831	0.813	0.508	0.961	0.820	0.635
Peak 7	1.000	**0.109**	0.688	0.578	1.000	0.652
Peak 8	0.898	0.762	0.770	0.773	0.287	0.770
Peak 9	0.250	1.000	0.625	0.500	0.500	1.000
Peak 10	0.875	1.000	0.500	0.500	0.500	1.000
Peak 11	0.426	0.695	0.240	0.734	0.322	0.920
Peak 12	0.791	0.733	0.206	0.761	0.322	0.893
Peak 13	1.000	1.000	0.500	0.500	0.500	0.750

Table 5.8. Tear lipid composition after two weeks of wear of Acuvue and Benz - Comparative statistics between symptomatic and asymptomatic groups by Mann Whitney Exact Test.

	ACUVUE	BENZ
Peak 1	**0.106**	0.343
Peak 2	1.000	0.640
Peak 4	0.287	0.733
Peak 6	0.636	0.292
Peak 7	0.558	0.429
Peak 8	0.610	0.558
Peak 9	0.841	0.790
Peak 10	1.000	1.000
Peak 11	0.876	0.462
Peak 12	**0.030**	0.753
Peak 13	1.000	**0.105**

Table 5.9. Overall tear lipid composition at Day 14 Follow up visit - Phases I & II (n=15) - Comparative statistics between contact lens types by Wilcoxon Matched Pairs Signed Ranks Exact Test.

	ACUVUE vs. FOCUS	BENZ vs. FOCUS
Peak 1	0.898	**0.216**
Peak 2	**0.063**	0.570
Peak 4	0.700	0.826
Peak 6	0.717	0.244
Peak 7	0.250	**0.094**
Peak 8	**0.078**	0.297
Peak 9	1.000	0.875
Peak 10	0.500	0.500
Peak 11	**0.102**	0.641
Peak 12	0.831	0.839
Peak 13	0.500	0.250

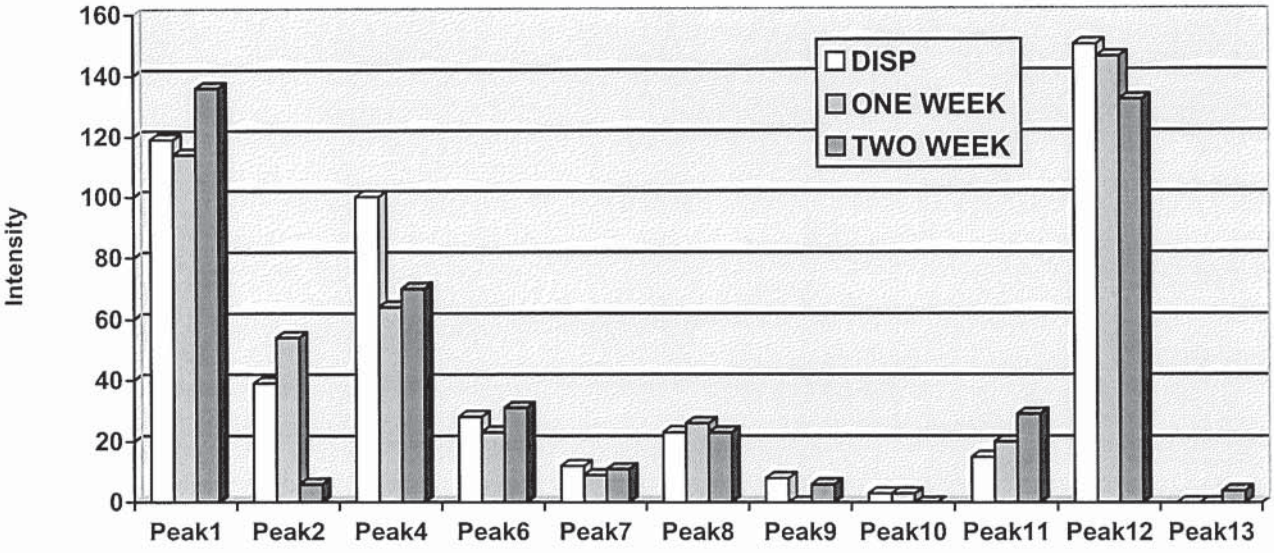


Figure 5.1. Tear lipid composition at each visit with Acuvue contact lenses - Median value for each lipid category.

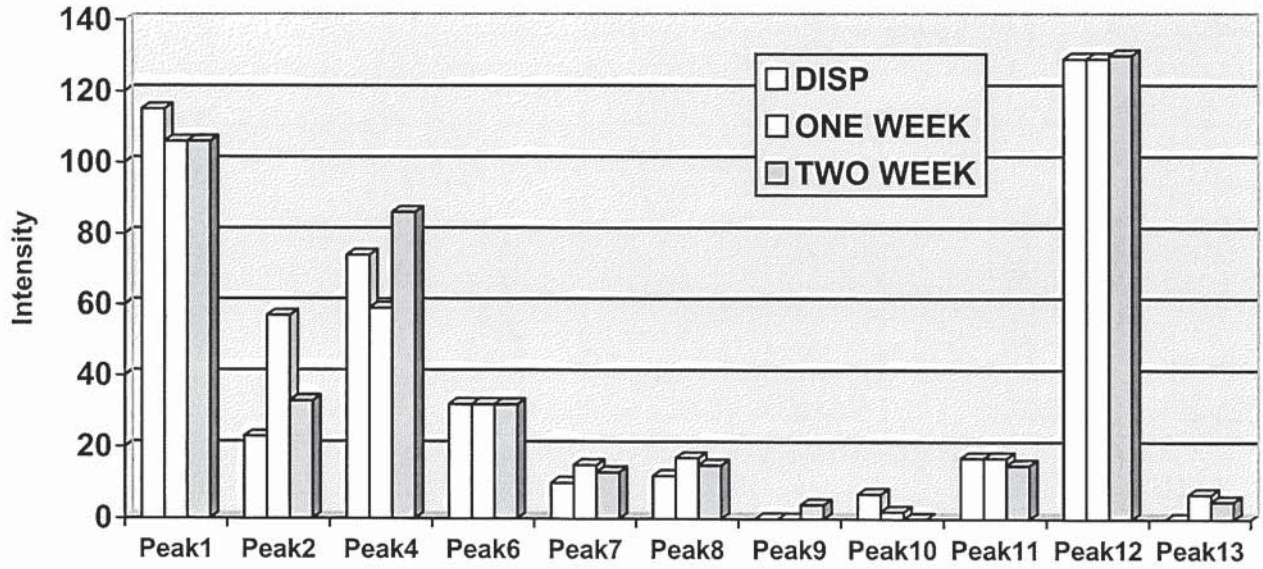


Figure 5.2. Tear lipid composition at each visit with Benz contact lenses - Median value for each lipid category.

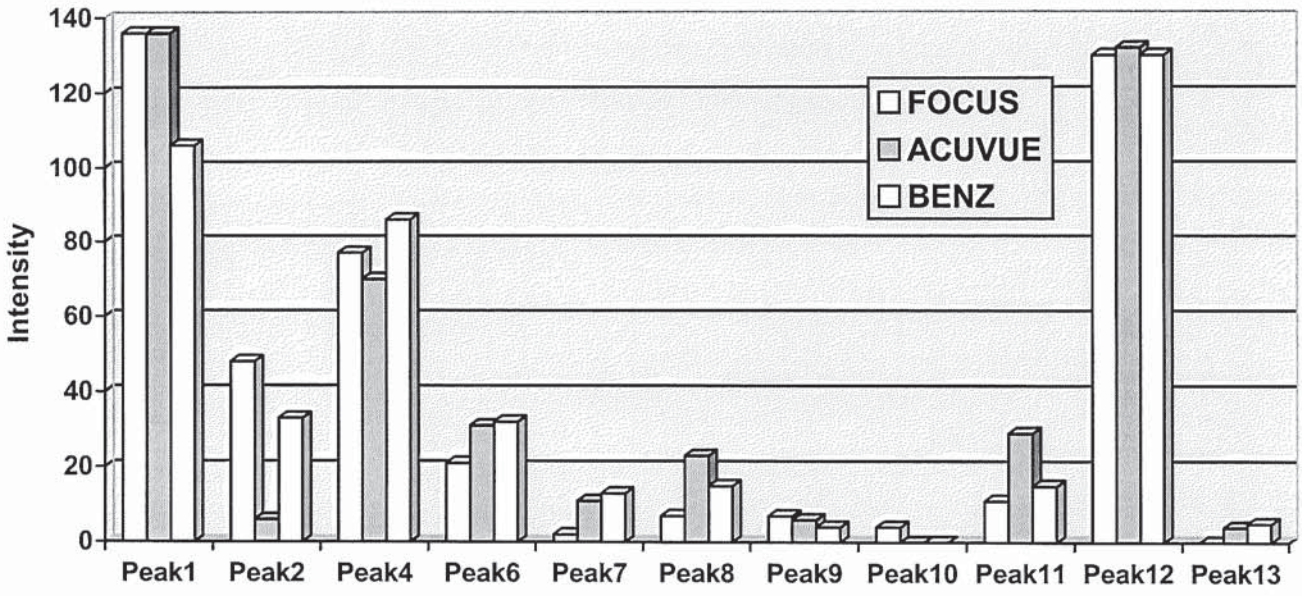


Figure 5.3. Tear lipid composition after two weeks of wear with each contact lens type - Median values.

5.2. Analysis of contact lens extracts

5.2.1. Objective

Whereas the gross lipid spoilation of various contact lens materials has been widely studied[121,123,124,125,126,181], little information is available on the nature of this deposition.

The purpose of this investigation was therefore to:

i) Identify any association between the nature of lipids deposited on the contact lenses and the contact lens materials composition or the tear characteristics of the contact lens wearer;

ii) Determine if the nature of lipids deposited affects the contact lens performance

iii) Detect any correlation between the tear lipid composition and the nature of lipid deposited.

5.2.2. Materials and methods

The test population described in section 5.1.2 consisted of symptomatic contact lens wearers according to the McMonnies questionnaire attending Contact Lens Research Consultants for soft contact lens follow up. The population characteristics are summarized in Table 5.1.

The three hydrogel materials tested were:

- FOCUS, FDA Group IV, ionic, NVP
- ACUVUE, FDA Group IV, ionic, non NVP
- BENZ55G, FDA Group II, non ionic, non NVP (Table 5.2).

The contact lenses were used under daily wear modality for a two-week period. The contact lenses were removed aseptically using hypoallergenic, powder free, sterile

disposable latex medical gloves (Shermond Surgical Supply Ltd). The contact lenses were stored at -20°C until analysis. The contact lenses were extracted for lipid analysis using MeOH and MeOH/Chloroform 1:3 as follows:

i). MeOH 2-3 hours

ii). MeOH overnight (12 hours)

iii). MeOH/Chloroform 2-3 hours

iv). MeOH/Chloroform overnight (12 hours).

The solvents were evaporated off by bubbling nitrogen over the surface. The lipid composition of the extracts was identified by High Performance Liquid Chromatography (HPLC).

5.2.3. Results

5.2.3.1. Introductory remarks

During the statistical analysis, a p-value of 0.05 or less was set as threshold of statistical significance and because of the small sample size in this investigation, a p-value of 0.2 or less was taken as threshold for a statistical trend.

5.2.3.2. Focus contact lens extracts at the end of Phase I

The results for each individual extraction are reported in Tables 5.10i & ii; the cumulative results of the consecutive extractions are reported in Tables 5.11i,ii,iii & iv.

No difference in the nature of the lipid deposition was found between symptomatic and asymptomatic contact lens wearers (Table 5.12).

5.2.3.3. Acuvue and Benz contact lens extracts at the end of Phase II

The results for each individual extraction are reported in Tables 5.13i & ii; the cumulative results of the consecutive extractions are reported in Tables 5.14i,ii,iii & iv.

After two weeks of wearing Acuvue and Benz, the symptomatology of the subjects with each contact lens type was assessed using the positive symptomatology criteria described in section 5.1.2. and based on the McMonnies questionnaire. For each contact lens type, it was therefore possible to divide the population into two subsets according to their symptomatology and compare the nature of the lipids deposition of each subpopulation.

For Acuvue contact lenses, the main difference between symptomatic and asymptomatic subjects was for the phospholipids/triglycerides peaks. Higher intensities were measured on contact lenses worn by the asymptomatic contact lens wearers. A trend was observed after the first extraction (Table 5.15. p=0.137, median: 31 vs. 0) and statistically significant differences for the cumulative results of extractions 1,2 and 1,2 & 3 (Table 5.15. p=0.028 & 0.028, median: 50 vs. 24 and 136 vs. 117). The cumulative results of the four extractions were not statistically different but the mean value measured for the asymptomatic group was still higher than that of symptomatic one (238 vs. 173). There were also some isolated differences for the cumulative results of extractions 1 & 2. For one of the fatty acids family (Peak c1) and for Peak a3, higher levels were recorded on contact lenses worn by asymptomatic contact lens wearers than by symptomatic ones. Also, the cumulative results of the first three extractions showed higher intensities for Peaks a2, c3 and d

(Table 5.15. p=0.214, 0.091 & 0.214) in the asymptomatic group compared to the symptomatic one but these differences had no clinical interest.

For Benz contact lenses, the differences in the tear lipid deposition between asymptomatic and symptomatic subpopulations were as follows:

- A statistically significantly higher mean level of phospholipids/triglycerides (Peak b1) was found in the contact lens samples from the asymptomatic population. This difference was noticeable after the first extraction (Table 5.15. p=0.154, median: 14 vs. 0) and became statistically significant for all cumulative results (Table 5.15. p=0.056, 0.024, 0.017 with respectively 26 vs. 0, 39 vs. 0, 45 vs. 0),
- A significantly higher intensity for one of the fatty acids (Peak c2) was measured in the contact lens extracts of the asymptomatic population. The difference was statistically significant from the first extraction (Table 5.15. p=0.189, 0.138 & 0.094, 28 vs. 0, 39 vs. 0, 45 vs. 0). After the fourth extraction, this difference was not statistically significant anymore but the median value observed was still higher for the asymptomatic population compared to the symptomatic one (Table 5.15. 51 vs. 18),
- A lower level of monoglycerides (Peak d) was found for the asymptomatic group (Table 5.15. p=0.110, 0.106 & 0.109, 124 vs. 136, 243 vs. 265, 337 vs. 380). After the fourth extraction, there was no statistical trend but the level remained higher for the symptomatic population (461 vs. 471).
- One of the cholesterol esters (Peak a2) and the cholesterol peak (Peak e) were also higher on contact lenses from asymptomatic contact lens wearers than

symptomatic. The influence of these differences on symptomatology is not known.

- The cumulative results of the first two extractions also revealed a higher level of Peak a3 (Table 5.15. p=0.077, 31 vs. 0) and of one fatty acid (Peak c1) (Table 5.15. p=0.150, 0 vs. 10). These seldom differences have little clinical interest.

After two weeks of wear, the comparison between the lipid composition of extracts from Acuvue and Benz contact lenses led to the following results:

- Slightly less cholesterol esters (Peak a2) were found with Benz (Table 5.16. p=0.219, 120 vs. 98 and p= 0.078, 35 vs. 24);
- A significantly lower intensity was recorded for Peak a3 with Benz (Table 5.16. p= 0.078, 35 vs. 24);
- A significantly lower level of one of the fatty acids detected (Peak c2) was found in Benz contact lens extracts (Table 5.16. p=0.232 (extractions 1,2) 24 vs. 0 and p=0.039 (extractions 1,2,3) 38 vs. 8);
- After the four extractions, the level of monoglycerides (Peak d) was marginally lower in Acuvue contact lens extracts than in Benz (Table 5.16. p=0.156, 442 vs.461).

5.2.3.4. Acuvue, Benz and Focus contact lens extracts after two weeks of wear

The comparisons of the lipid composition of extracts from Acuvue and Focus after two weeks of wear revealed the following findings:

- More cholesterol ester (Peak a2) was found in the extracts of Focus than those of Acuvue (Table 5.16. p=0.176 (extractions 1,2,3) 120 vs. 194)

- The intensity of Peak a3 was significantly higher in extracts from Acuvue than Focus contact lenses (Table 5.16. p=0.074 (extractions 1,2,3) 35 vs. 0 and, p=0.047 (extractions 1,2,3,4) 59 vs.19);
- The level of one fatty acid detected (Peak c2) was significantly lower in the Focus extracts than in Acuvue extracts (Table 5.16. p=0.064 (extractions 1,2) 24 vs. 0 and, p=0.109 (extractions 1,2,3,4) 42 vs.18);
- At the opposite, another fatty acid (Peak c3) was found in lower amount in Acuvue extracts than in Focus, but only after the third extraction only (Table 5.16. p=0.156 (extractions 1,2,3) 0 vs. 7);
- Finally, the level of monoglycerides (Peak d) was found to be higher (Table 5.16. p=0.204 (extractions 1,2,3) 361 vs. 370 and p=0.109 (extractions 1,2,3,4) 442 vs. 494) in Focus extracts than in Acuvue extracts.

The comparisons between the lipid composition of extracts from Benz and Focus contact lens materials produced the following findings:

- More cholesterol esters (Peaks a1 & a2) were found in Focus extracts than in Benz extracts (Table 5.16.). The difference was statistically significant for Peak a2 after third extraction (Table 5.16. p=0.042 (extractions 1,2,3) 98 vs. 194 and p=0.039 (extractions 1,2,3,4) 127 vs. 232);
- The intensity of Peak a3 was higher in extracts from Benz than from Focus (Table 5.16. p=0.109 (extractions 1,2,3) 24 vs. 0);

- The level of phospholipids/triglycerides (Peak b1) was lower in the Focus extracts than in the Benz extracts (Table 5.16. p=0.175 (extractions 1,2,3) 112 vs. 78 and p=0.098 (extractions 1,2,3,4) 142 vs. 110);
- At the opposite, the level of one of the fatty acids detected (Peak c3) was found to be statistically significantly lower in Benz contact lens extracts than in Focus after the third extraction (Table 5.16. p=0.031 (extractions 1,2,3) 0 vs. 16) and just marginally lower after the fourth extraction (Table 5.16. p=0.203 14 vs. 16);
- Finally, the level of monoglycerides (Peak d) was higher (Table 5.16. p=0.194 (extraction 1) and p=0.151 (extractions 1,2) in Focus than in Benz.

Table 5.10. Lipid composition of contact lens extracts - Phase I (Focus contact lenses) - Descriptive statistics for the overall population and for the asymptomatic (Group 1) and symptomatic (Group 2) groups (Relative peak intensity) – Individual results

i. after MeOH extraction

Lipid	Peak		Group1 (n=7)		Group2 (n=15)		Overall(n=22)	
		Extraction	1	2	1	2	1	2
Cholesterol Esters	a1	Mean±STD Median (Range)	135 ± 32 128 (88-186)	34 ± 20 32 (0-60)	130 ± 65 101 (54-258)	63 ± 62 39 (22-258)	132 ± 55 120 (54-258)	53 ± 53 37 (0-258)
	a2	Mean±STD Median (Range)	54 ± 40 62 (0-96)	58 ± 94 16 (0-259)	38 ± 34 44 (0-98)	120 ± 114 66 (0-358)	43 ± 36 46 (0-98)	100± 109 52 (0-358)
	a3	Mean±STD Median (Range)	0 ± 0 0 (0-0)	11 ± 30 0 (0-79)	0 ± 0 0 (0-0)	7 ± 21 0 (0-74)	0 ± 0 0 (0-0)	9 ± 24 0 (0-79)
Phospholi-pids/ trigly-cerides	b1	Mean±STD Median (Range)	18 ± 23 0 (0-48)	13 ± 19 0 (0-48)	20 ± 24 12 (0-70)	14 ± 20 0 (0-54)	20 ± 23 0 (0-70)	13± 19 0 (0-54)
	b2	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	4 ± 11 0 (0-28)	4 ± 11 0 (0-28)	10 ± 21 0 (0-58)	0 ± 0 0 (0-0)	8 ± 18 0 (0-58)	1± 6 0 (0-28)
	c2	Mean±STD Median (Range)	5 ± 14 0 (0-38)	18± 25 0 (0-56)	14 ± 30 0 (0-92)	3 ± 7 0 (0-21)	11 ± 26 0 (0-92)	8± 17 0 (0-56)
	c3	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	9 ± 23 0 (0-72)	2 ± 7 0 (0-24)	6 ± 19 0 (0-72)	1± 5 0 (0-24)
Monogly-cerides	d	Mean±STD Median (Range)	140 ± 32 144 (99-178)	121± 13 123 (100-136)	151 ± 43 138 (96-270)	119± 14 123 (82-135)	147 ±39 142 (96-270)	120 ±13 123 (82-136)
Cholesterol	e	Mean±STD Median (Range)	0 ± 0 0 (0-0)	4 ± 9 0 (0-25)	0 ± 0 0 (0-0)	4 ± 8 0 (0-24)	0 ± 0 0 (0-0)	3 ± 8 0 (0-25)

ii. after MeOH / chloroform extraction

Lipid	Peak		Group 1 (n=7)		Group2 (n=15)		Overall (n=22)	
		Extraction	3	4	3	4	3	4
Cholesterol Esters	a1	Mean±STD Median (Range)	55 ± 31 44 (22-104)	26 ± 8 26 (17-36)	52 ± 43 38 (0-139)	39 ± 53 22 (8-222)	53 ± 39 38 (0-139)	36 ± 45 23 (8-222)
	a2	Mean±STD Median (Range)	59 ± 69 36 (0-194)	21 ± 40 0 (0-101)	63 ± 44 68 (0-150)	20 ± 22 19 (0-75)	62 ± 51 53 (0-194)	20± 27 15 (0-101)
	a3	Mean±STD Median (Range)	2 ± 5 0 (0-14)	13 ± 14 9 (0-29)	4 ± 17 0 (0-64)	16 ± 17 19 (0-49)	4 ± 14 0 (0-64)	15 ± 16 18 (0-49)
Phospholi-pids / tri-glycerides	b1	Mean±STD Median (Range)	32 ± 19 34 (0-54)	42 ± 35 48 (0-80)	50 ± 36 44 (0-116)	52± 29 44 (0-115)	44 ± 32 39 (0-116)	49± 30 45 (0-115)
	b2	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	10 ± 11 8 (0-26)	12 ± 10 14 (0-21)	9 ± 10 6 (0-32)	11± 8 12 (0-22)	9 ± 10 7 (0-32)	11± 8 12 (0-22)
	c2	Mean±STD Median (Range)	11 ± 11 12 (0-28)	8 ± 12 0 (0-25)	11 ± 14 0 (0-42)	6 ± 8 0 (0-17)	11 ± 13 6 (0-42)	7± 9 0 (0-25)
	c3	Mean±STD Median (Range)	9 ± 11 0 (0-22)	2 ± 5 0 (0-13)	5 ± 10 0 (0-33)	2± 5 0 (0-16)	6 ± 10 0 (0-33)	2± 5 0 (0-16)
Monogly-cerides	d	Mean±STD Median (Range)	90 ± 27 98 (43-122)	113± 8 117 (102-120)	103 ± 30 112 (51-142)	104± 23 111 (38-128)	99 ±29 109 (43-142)	106 ±20 111 (38-128)
Cholesterol	e	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	2 ± 5 0 (0-19)	1 ± 3 0 (0-13)	1 ± 4 0 (0-19)	1 ±3 0 (0-13)

Table 5.11. Lipid composition of contact lens extracts - Phase I (Focus contact lenses) - Descriptive statistics for the overall population and for the asymptomatic (Group 1) and symptomatic (Group 2) groups (Relative peak intensity) – Cumulative results.

i. Results for first extraction

Lipid	Peak	Parameters	Group1 (n=7)	Group2 (n=15)	Overall(n=22)
Cholesterol Esters	a1	Mean±STD Median (Range)	135 ± 32 128 (88-186)	130 ± 65 101 (54-258)	132 ± 55 120 (54-258)
	a2	Mean±STD Median (Range)	54 ± 40 62 (0-96)	38 ± 34 44 (0-98)	43 ± 36 46 (0-98)
	a3	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Phospholi-pids / trigly-cerides	b1	Mean±STD Median (Range)	18 ± 23 0 (0-48)	20 ± 24 12 (0-70)	20 ± 23 0 (0-70)
	b2	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	4 ± 11 0 (0-28)	10 ± 21 0 (0-58)	8 ± 18 0 (0-58)
	c2	Mean±STD Median (Range)	5 ± 14 0 (0-38)	14 ± 30 0 (0-92)	11 ± 26 0 (0-92)
	c3	Mean±STD Median (Range)	0 ± 0 0 (0-0)	9 ± 23 0 (0-72)	6 ± 19 0 (0-72)
Monogly-cerides	d	Mean±STD Median (Range)	140 ± 32 144 (99-178)	151 ± 43 138 (96-270)	147 ±39 142 (96-270)
Cholesterol	e	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)

iii. Cumulative results for first & second extractions

Lipid	Peak	Parameters	Group1 (n=7)	Group2 (n=15)	Overall(n=22)
Cholesterol Esters	a1	Mean±STD Median (Range)	169 ± 31 164 (120-219)	193 ± 77 167 (82-336)	185 ± 65 164 (82-336)
	a2	Mean±STD Median (Range)	112 ± 107 96 (0-311)	159± 113 117 (0-358)	143 ± 110 106 (0-358)
	a3	Mean±STD Median (Range)	11 ± 30 0 (0-79)	7 ± 21 0 (0-74)	9 ± 23 0 (0-79)
Phospholipids / triglycerides	b1	Mean±STD Median (Range)	31 ± 34 26 (0-84)	34 ± 32 31 (0-95)	33 ± 32 30 (0-95)
	b2	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	8 ± 14 0 (0-28)	10 ± 21 0 (0-58)	10 ± 18 0 (0-58)
	c2	Mean±STD Median (Range)	24± 25 22 (0-56)	16 ± 29 0 (0-92)	19 ± 27 0 (0-92)
	c3	Mean±STD Median (Range)	0 ± 0 0 (0-0)	11± 23 0 (0-72)	7 ± 20 0 (0-72)
Monoglycerides	d	Mean±STD Median (Range)	261 ± 35 260 (199-303)	270 ± 46 257 (208-401)	267 ±42 259 (199-401)
Cholesterol	e	Mean±STD Median (Range)	4 ± 9 0 (0-25)	3 ± 8 0 (0-24)	3 ± 8 0 (0-25)

iv. Cumulative results for first, second and third extractions

Lipid	Peak	Parameters	Group1 (n=7)	Group2 (n=15)	Overall(n=22)
Cholesterol Esters	a1	Mean±STD Median (Range)	224 ± 47 208 (182-323)	246 ± 92 264 (95-382)	239 ± 79 234 (95-382)
	a2	Mean±STD Median (Range)	170± 121 122 (16-357)	215± 100 194 (72-373)	200 ± 107 181 (16-373)
	a3	Mean±STD Median (Range)	13± 29 0 (0-79)	12± 31 0 (0-94)	12± 30 0 (0-94)
Phospholipids / triglycerides	b1	Mean±STD Median (Range)	64 ± 38 54 (16-130)	85 ± 43 78 (38-164)	78 ± 42 56 (16-164)
	b2	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	18± 19 14 (0-54)	19 ± 25 9 (0-79)	18 ± 23 12 (0-79)
	c2	Mean±STD Median (Range)	35± 26 28 (0-66)	28 ± 37 16 (0-124)	30 ± 33 22 (0-124)
	c3	Mean±STD Median (Range)	9 ± 11 0 (0-22)	17± 23 7 (0-72)	14 ± 20 0 (0-72)
Monoglycerides	d	Mean±STD Median (Range)	351 ± 48 358 (275-425)	373 ± 65 370 (259-523)	366 ±59 363 (259-523)
Cholesterol	e	Mean±STD Median (Range)	4 ± 9 0 (0-25)	5 ± 9 0 (0-24)	5 ± 9 0 (0-25)

v. Cumulative results for first, second, third and four extractions

Lipid	Peak	Parameters	Group1 (n=7)	Group2 (n=15)	Overall(n=22)
Cholesterol Esters	a1	Mean±STD Median (Range)	249 ± 48 241 (202-346)	288 ± 106 310 (118-449)	275 ± 91 266 (118-449)
	a2	Mean±STD Median (Range)	191± 118 223 (16-357)	248± 95 232 (127-392)	228 ± 105 226 (16-392)
	a3	Mean±STD Median (Range)	24± 28 28 (0-79)	22± 30 19 (0-104)	23 ± 29 20 (0-104)
Phospholipids / triglycerides	b1	Mean±STD Median (Range)	105 ± 45 106 (48-176)	132± 49 110 (88-231)	123 ± 48 108 (48-231)
	b2	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	28± 15 23 (12-54)	31 ± 27 24 (0-98)	30 ± 23 23 (0-98)
	c2	Mean±STD Median (Range)	41± 35 28 (0-88)	37 ± 35 18 (0-124)	39 ± 34 25 (0-124)
	c3	Mean±STD Median (Range)	11±14 0 (0-31)	21± 24 16 (0-72)	17 ± 21 16 (0-72)
Monogly-cerides	d	Mean±STD Median (Range)	463 ± 50 461 (377-530)	489 ± 71 494 (377-634)	474 ±64 489 (377-634)
Cholesterol	e	Mean±STD Median (Range)	4 ± 9 0 (0-25)	6 ± 9 0 (0-24)	5 ± 9 0 (0-25)

Table 5.12. Lipid composition of contact lens extracts - Phase I (Focus contact lenses) - Comparative statistics between population groups (asymptomatic vs. symptomatic) by Mann-Whitney Exact Test.

	Extraction 1	Extractions 1&2	Extractions 1,2&3	Extractions 1,2,3&4
Peak a1	0.400	0.913	0.585	0.393
Peak a2	0.321	0.369	0.360	0.275
Peak a3	1.000	0.840	0.521	0.745
Peak b1	0.989	0.795	0.443	0.311
Peak b2	1.000	1.000	1.000	1.000
Peak c1	0.681	1.000	0.750	0.938
Peak c2	0.787	0.375	0.391	0.773
Peak c3	0.533	0.447	0.682	0.451
Peak d	0.730	0.971	0.443	0.588
Peak e	1.000	0.840	0.737	0.509

Table 5.13. Lipid composition of contact lens extracts – Phase II (Acuvue & Benz contact lenses) - Descriptive statistics (Relative peak intensity)- Individual results.

i. after MeOH extractions

Lipid	Peak	Parameters	ACUVUE		BENZ	
		Extraction	1	2	1	2
Cholesterol Esters	a1	Mean±STD Median (Range)	140 ± 72 121 (46-326)	44 ± 46 32 (18-185)	128 ± 95 92 (54-372)	40 ± 22 30 (22-84)
	a2	Mean±STD Median (Range)	23 ± 37 0 (0-100	74 ± 43 70 (42-204)	14 ± 29 0 (0-82)	60 ± 47 44 (16-186)
	a3	Mean±STD Median (Range)	2 ± 6 0 (0-20)	9 ± 14 0 (0-41)	0 ± 0 0 (0-0)	13 ± 28 0 (0-86)
Phospholi-pids / trigly-cerides	b1	Mean±STD Median (Range)	13 ± 17 0 (0-38)	19 ± 12 19 (0-42)	10 ± 22 0 (0-62)	23 ± 15 24 (0-62)
	b2	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	3 ± 9 0 (0-32)	8 ± 12 0 (0-30)	0 ± 0 0 (0-0)	7 ± 10 0 (0-24)
	c2	Mean±STD Median (Range)	14 ± 18 0 (0-40)	14 ± 13 18 (0-34)	14 ± 19 0 (0-52)	4 ± 9 0 (0-24)
	c3	Mean±STD Median (Range)	0 ± 0 0 (0-0)	1 ± 4 0 (0-14)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Monogly-cerides	d	Mean±STD Median (Range)	132 ± 14 129 (114-152)	121± 19 126 (84-142)	132 ± 11 132 (112-152)	126± 23 132 (66-154)
Cholesterol	e	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)	1 ± 4 0 (0-14)	2 ± 6 0 (0-20)

ii. after MeOH/chloroform extractions

Lipid	Peak		ACUVUE		BENZ	
		Extraction	3	4	3	4
Cholesterol Esters	a1	Mean±STD Median (Range)	51 ± 24 481 (17-99)	37 ± 44 20 (0-144)	41 ± 28 32 (19-131)	29 ± 21 25 (10-92)
	a2	Mean±STD Median (Range)	49 ± 33 50 (0-111)	43 ± 25 39 (0-78)	32 ± 16 32 (0-55)	25 ± 20 25 (0-65)
	a3	Mean±STD Median (Range)	59 ± 94 0 (0-302)	14 ± 39 0 (0-124)	29± 36 20 (0-119)	7 ± 22 0 (0-80)
Phospholi-pids / trigly-cerides	b1	Mean±STD Median (Range)	88 ± 34 89 (40-170)	82± 57 69 (15-223)	93 ± 15 87 (72-121)	66 ± 17 66 (32-89)
	b2	Mean±STD Median (Range)	0 ± 0 0 (0-0)	2 ± 7 0 (0-21)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	7 ± 8 0 (0-18)	18 ± 19 17 (0-68)	7 ± 6 9 (0-14)	12 ± 4 12 (0-15)
	c2	Mean±STD Median (Range)	5 ± 7 0 (0-18)	9 ± 8 11 (0-19)	5 ± 6 0 (0-16)	15 ± 6 15 (0-22)
	c3	Mean±STD Median (Range)	2 ± 5 0 (0-16)	8 ± 7 12 (0-16)	1 ± 4 0 (0-14)	12 ± 9 14 (0-29)
Monogly-cerides	d	Mean±STD Median (Range)	98 ± 18 99 (71-124)	96± 23 98 (50-130)	98 ± 19 106 (55-121)	100± 16 107 (68-120)
Cholesterol	e	Mean±STD Median (Range)	0 ± 0 0 (0-0)	4 ± 8 0 (0-22)	0 ± 0 0 (0-0)	1 ± 4 0 (0-15)

Table 5.14. Lipid composition of contact lens extracts - Phase II (Acuvue & Benz contact lenses) - Descriptive statistics (Relative peak intensity) – Cumulative results.

i. Results for first extraction

Lipid	Peak	Parameters	ACUVUE	BENZ
Cholesterol Esters	a1	Mean±STD Median (Range)	140 ± 72 121 (46-326)	128 ± 95 92 (54-372)
	a2	Mean±STD Median (Range)	23 ± 37 0 (0-100	14 ± 29 0 (0-82)
	a3	Mean±STD Median (Range)	2 ± 6 0 (0-20)	0 ± 0 0 (0-0)
Phospholi-pids / trigly-cerides	b1	Mean±STD Median (Range)	13 ± 17 0 (0-38)	10 ± 22 0 (0-62)
	b2	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	3 ± 9 0 (0-32)	0 ± 0 0 (0-0)
	c2	Mean±STD Median (Range)	14 ± 18 0 (0-40)	14 ± 19 0 (0-52)
	c3	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Monogly-cerides	d	Mean±STD Median (Range)	132 ± 14 129 (114-152)	132 ± 11 132 (112-152)
Cholesterol	e	Mean±STD Median (Range)	0 ± 0 0 (0-0)	1 ± 4 0 (0-14)

ii. Cumulative results for first & second extractions

Lipid	Peak	Parameters	ACUVUE	BENZ
Cholesterol Esters	a1	Mean±STD Median (Range)	185 ± 81 170 (80-370)	175 ± 105 138 (88-396)
	a2	Mean±STD Median (Range)	97 ± 58 71 (42-234)	76 ± 51 58 (16-186)
	a3	Mean±STD Median (Range)	10± 14 0 (0-41)	13± 28 0 (0-86)
Phospholi-pids / trigly-cerides	b1	Mean±STD Median (Range)	32 ± 15 29 (8-56)	34 ± 23 28 (0-80)
	b2	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	10± 17 0 (0-48)	7 ± 10 0 (0-24)
	c2	Mean±STD Median (Range)	28± 18 24 (0-59)	16 ± 20 0 (0-50)
	c3	Mean±STD Median (Range)	1 ± 4 0 (0-14)	0 ± 0 0 (0-0)
Monogly-cerides	d	Mean±STD Median (Range)	253 ± 26 256 (211-289)	255 ±28 260 (186-290)
Cholesterol	e	Mean±STD Median (Range)	0 ± 0 0 (0-0)	3 ± 7 0 (0-20)

iii. Cumulative results for first, second and third extractions

Lipid	Peak	Parameters	ACUVUE	BENZ
Cholesterol Esters	a1	Mean±STD Median (Range)	231 ± 83 201 (141-426)	226 ± 110 213 (115-426)
	a2	Mean±STD Median (Range)	144 ± 74 120 (58-284)	118 ± 59 98 (39-241)
	a3	Mean±STD Median (Range)	50 ± 57 35 (0-186)	46 ± 61 24 (0-205)
Phospholi-pids / trigly-cerides	b1	Mean±STD Median (Range)	120 ± 42 123 (60-217)	123 ± 27 112 (77-170)
	b2	Mean±STD Median (Range)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	17± 16 16 (0-48)	11 ± 10 10 (0-33)
	c2	Mean±STD Median (Range)	33 ± 18 38 (0-59)	19 ± 22 8 (0-56)
	c3	Mean±STD Median (Range)	3 ± 6 0 (0-16)	1 ± 4 0 (0-14)
Monogly-cerides	d	Mean±STD Median (Range)	353 ± 29 361 (295-396)	355 ± 31 359 (290-392)
Cholesterol	e	Mean±STD Median (Range)	0 ± 0 0 (0-0)	3 ± 7 0 (0-20)

iv. Cumulative results for first, second, third and four extractions

Lipid	Peak	Parameters	ACUVUE	BENZ
Cholesterol Esters	a1	Mean±STD Median (Range)	262 ± 101 209 (156-443)	261 ± 122 265 (127-460)
	a2	Mean±STD Median (Range)	186 ± 82 172 (98-354)	128 ± 43 127 (66-195)
	a3	Mean±STD Median (Range)	79 ± 57 59 (20-186)	40 ± 42 26 (0-117)
Phospholi-pids / trigly-cerides	b1	Mean±STD Median (Range)	200 ± 73 183 (81-338)	183 ± 25 142 (142-237)
	b2	Mean±STD Median (Range)	2 ± 7 0 (0-21)	0 ± 0 0 (0-0)
Fatty acids	c1	Mean±STD Median (Range)	35 ± 33 32 (0-116)	23 ± 10 21 (13-45)
	c2	Mean±STD Median (Range)	44 ± 19 42 (0-66)	31 ± 21 24 (9-74)
	c3	Mean±STD Median (Range)	10 ± 12 10 (0-32)	14 ± 9 14 (0-29)
Monogly-cerides	d	Mean±STD Median (Range)	441± 38 442 (367-492)	461 ± 27 461 (421-499)
Cholesterol	e	Mean±STD Median (Range)	4 ± 8 0 (0-22)	3 ± 6 0 (0-15)

Table 5.15. Lipid composition of contact lens extracts - Phase II (Acuvue & Benz contact lenses) - Comparative statistics between population groups (asymptomatic vs. symptomatic) by Mann-Whitney Exact Test.

i. ACUVUE

	Extraction 1	Extractions 1&2	Extractions 1,2&3	Extractions 1,2,3&4
Peak a1	0.840	0.933	0.933	1.000
Peak a2	0.265	0.368	**0.214**	0.548
Peak a3	1.000	**0.024**	0.685	0.548
Peak b1	**0.137**	**0.028**	**0.028**	0.262
Peak b2	1.000	1.000	1.000	1.000
Peak c1	0.333	**0.034**	**0.222**	0.714
Peak c2	0.519	0.976	0.808	0.805
Peak c3	1.000	0.333	**0.091**	0.786
Peak d	0.683	0.808	**0.214**	0.714
Peak e	1.000	1.000	1.000	1.000

ii. BENZ

	Extraction 1	Extractions 1&2	Extractions 1,2&3	Extractions 1,2,3&4
Peak a1	0.753	0.414	1.000	0.833
Peak a2	1.000	**0.199**	**0.164**	**0.117**
Peak a3	1.000	**0.077**	0.645	0.667
Peak b1	**0.154**	**0.056**	**0.024**	**0.017**
Peak b2	1.000	1.000	1.000	1.000
Peak c1	1.000	**0.150**	0.976	0.833
Peak c2	**0.189**	**0.138**	**0.094**	0.517
Peak c3	1.000	1.000	0.364	0.433
Peak d	**0.110**	**0.106**	**0.109**	0.383
Peak e	0.267	**0.077**	**0.109**	1.000

Table 5.16. Lipid composition of contact lens extracts - Comparative statistics between contact lens types by Wilcoxon Matched Pairs Signed Ranks Exact Test.

i. After first extraction

	ACUVUE vs. BENZ	ACUVUE vs. FOCUS	BENZ vs. FOCUS
Peak a1	0.339	0.791	0.903
Peak a2	0.844	0.250	**0.084**
Peak a3	1.000	1.000	1.000
Peak b1	0.945	0.641	0.359
Peak b2	1.000	1.000	1.000
Peak c1	1.000	0.500	0.250
Peak c2	0.773	0.625	0.742
Peak c3	1.000	1.000	0.500
Peak d	0.733	**0.151**	**0.194**
Peak e	1.000	1.000	1.000

ii. After first and second extractions

	ACUVUE vs. BENZ	ACUVUE vs. FOCUS	BENZ vs. FOCUS
Peak a1	0.465	0.733	**0.204**
Peak a2	0.638	0.519	**0.176**
Peak a3	0.931	0.563	0.875
Peak b1	0.577	1.000	0.970
Peak b2	1.000	1.000	1.000
Peak c1	0.719	0.844	0.578
Peak c2	**0.232**	**0.064**	0.742
Peak c3	1.000	0.500	0.250
Peak d	0.966	**0.233**	**0.151**
Peak e	0.500	0.500	1.000

iii. After first, second and third extractions

	ACUVUE vs. BENZ	ACUVUE vs. FOCUS	BENZ vs. FOCUS
Peak a1	0.625	0.733	**0.123**
Peak a2	0.492	**0.176**	**0.042**
Peak a3	0.844	**0.074**	**0.109**
Peak b1	0.922	0.266	**0.175**
Peak b2	1.000	1.000	1.000
Peak c1	0.426	0.765	0.814
Peak c2	**0.039**	0.301	0.910
Peak c3	0.500	**0.156**	**0.031**
Peak d	0.922	0.204	0.413
Peak e	0.500	0.500	0.625

iv. After first, second, third and fourth extractions

	ACUVUE vs. BENZ	ACUVUE vs. FOCUS	BENZ vs. FOCUS
Peak a1	0.297	0.461	0.250
Peak a2	**0.219**	0.313	**0.039**
Peak a3	**0.078**	**0.047**	0.578
Peak b1	0.813	0.313	**0.098**
Peak b2	1.000	1.000	1.000
Peak c1	0.688	0.945	0.910
Peak c2	0.813	**0.109**	1.000
Peak c3	0.469	0.688	**0.203**
Peak d	**0.156**	**0.109**	0.426
Peak e	1.000	0.875	0.625

5.2.3.5. Effect of nature of lipids deposited on the clinical performance

To test whether the nature of lipids deposited on the contact lens surface affects their performance, the data were analysed using Answer Tree^{TM}. The interactions between the nature of lipids and the clinical performance were as follow:

- The contact lens subjective acceptance was influenced by the level of phospholipids/ triglycerides (Peak b1) found at the contact lens surface after the first extraction (Table 5.17). Based upon the level of Peak b1 found on the contact lenses, the population was divided into two groups. When no detectable level of Peak b1 was measured, the mean comfort score decreased from 39.1 to 36.8. On the other hand, the group with a higher level of deposited phospholipid/triglycerides had significantly higher ratings with a mean score of 44.0. A deposition of loosely bound phospholipids/triglycerides seemed favourable to the contact lens biocompatibility.

- The comfort data was transformed and categorised into three groups according to the score recorded:

  i. Group 1 = The lowest quartile, subjects with lowest 25% comfort score representing the low comfort response,

  ii. Group 2 = The median quartile, subjects with the median 50% score characterising a normal population,

  iii. Group 3 = The highest quartile, subjects with highest 25% comfort score representing the good comfort response.

The distribution into the above groups was most influenced by the level of Peak a3 measured in contact lens extracts after the first two extractions (Table 5.18). The presence of Peak a3 was associated with a significant increase in the population comfort. The presence of Peak a3 resulted in an increase of Group 3 subjects to 73% (from the overall distribution of 25%) and a decrease of Group 1 subjects to 0% (from the overall population distribution of 25%).

- The pre lens tear film stability measured by the Non Invasive Break Up Time (NIBUT) seemed influenced by the level of cholesterol ester (Table 5.19). The population was divided into the three subgroups based upon the level of cholesterol esters. The NIBUT median value was shown to be influenced by the level of cholesterol ester for all three contact lenses together as listed below and for each contact lens type as in Table 5.20.

  i. A lower level of cholesterol ester was associated with a lower NIBUT (9.1 vs. 10.4 seconds for total population)

  ii. A median level of cholesterol ester which was associated with a longer break up time (17.4 vs. 10.4 seconds for total population)

  iii. A high level of cholesterol ester deposited on the contact lens was associated with a short NIBUT (8.5 vs. 10.4 seconds for total population).

Table 5.20. NIBUT median – Effect of cholesterol ester deposition

	NIBUT median		
	Acuvue	**Benz**	**Focus**
Low Cholesterol ester	8.5 ± 1.7 8.6 (5.6-10.2)	10.0 ± 6.5 7.6 (5.3-23.0)	8.7 ± 4.0 7.2 (6.2-20.2)
Median cholesterol ester	29.0 ± . 29.0 (29.0 -)	18.6 ± 16.3 18.6 (7.0-30.1)	16.3 ± 6.2 18.1 (7.6-21.2)
High Cholesterol ester	8.4 ± 1.6 8.4 (7.2-9.5)	10.4 ± 5.7 10.4 (6.3-14.4)	6.8 ± 1.0 6.8 (6.1-7.5)

- The lipid layer mixing pattern appeared to be most influenced by the total level of phospholipids/triglycerides (Peak b1) present on the contact lens (Fig. 5.21). An increase in the level of phospholipids / triglycerides (group 3) was associated with a thicker and more stable lipid layer with the majority of cases going from an open meshwork type of pattern to a wave type of pattern and with a significant increase in the number of cases showing an amorphous pattern, 13% vs. 6% in the starting the population. For the group with a low level of phospholipids/triglycerides (group 1), the lipid patterns observed were characteristics of a slightly thinner lipid layer compared to the overall population; the number of cases showing a wave type of pattern decreased from 28% to 6% and the percentage of amorphous patterns observed decreased from 11% to 6%.

- The type of break observed for the tear film was influenced by the level of deposited monoglycerides on the contact lenses measured after the fourth extraction (Table 5.22). The population was partitioned accordingly into two

groups, one with a low level of monoglycerides deposited and one with a high level of monoglycerides deposition. A high deposition of monoglycerides (Peak d) was associated with a higher incidence of slow destabilising breaks and a lower incidence of rapidly destabilising breaks: the percentage of cases in which the break was not applicable or as single spots increased from 14% to 50% and the incidence of cases in which the break was a surface or band type of breaks decreased from 69% to 17%.

- The level of hyperaemia in the limbal area[+] was also influenced by the type and level of lipids deposited on the contact lenses. The maximum limbal hyperaemia was most influenced by the level of one of the cholesterol ester (Peak a2) measured after the first extraction (Table 5.23) as follow:

  i. When no detectable level of Peak a2 were found on the contact lens, none to very slight hyperaemia was observed in 66% of cases;

  ii. When, after the first extraction, a detectable level of Peak a2 was measured the level of hyperaemia increased: The percentage of cases rated as showing slight to mild hyperaemia increased from 33% when no cholesterol ester (Peak a2) was deposited to 45% when some could be detected. For this category the level of limbal hyperaemia could be further discriminated based upon the level of fatty acids (Peak c2) measured in the contact lens extracts after the first extraction. A high level of Peak c2 was associated with a significant decrease in hyperaemia; all cases showing none to very slight

[+] Hyperaemia (Redness) is considered as a clinical indicator of the inflammatory status of the anterior eye.

hyperaemia. A low level of Peak c2 was associated with an increase in ocular hyperaemia, with most cases showing a slight to mild hyperaemia. The presence of cholesterol ester on the contact lens appeared deleterious to ocular integrity, probably generating ocular irritation, as shown by the increase in eye redness.

5.3.Discussion

The results obtained enabled us to further understand the effects of contact lens wear on the tear film lipid composition and the variation in the lipid composition with the subject symptomatology.

The analysis of the tear film lipid composition emphasised the importance of the concentration of monoglycerides on the symptomatology associated with contact lens wear. For both Focus and Acuvue, symptomatic contact lens wearers had a higher tear concentration of monoglycerides. And similarly during Phase I of the study, the group of subjects that went from symptomatic with their own contact lenses to asymptomatic after two weeks of wear of Focus were found simultaneously to have a decreased level of monoglycerides compared to their baseline level.

The effect of contact lens material on tear lipid profile was also assessed by comparing composition of tear film while the subjects wore Focus, Acuvue and Benz. No significant differences were found between Benz and Acuvue, but with Focus the tear profile of the subjects changed producing a higher level of cholesterol esters and lower level of fatty acids. Finally the lipid analysis of contact lens extracts enabled us to assess the effect of lipid deposition on symptomatology and its association between lipid level and contact lens materials. The level of phospholipids/triglycerides deposition was found to influence the symptomatology. A significantly higher level was measured in contact lens extracts of the asymptomatic group for both Acuvue and Benz. The effects of other lipid classes on symptomatology appeared to be more material dependent. A higher level of

deposition of fatty acids and Peak a3 on Acuvue contact lenses was associated with a decrease in symptomatology. For Benz contact lenses a lower monoglycerides deposition favoured a lower symptomatology. No symptomatology marker was found when analysing the nature of the lipid deposition on Focus contact lenses.

The nature of the lipid deposition was also shown to be material dependent. The difference between the three materials tested was particularly marked between Focus and the other two contact lens types. As discussed previously the special behaviour of Focus contact lenses in relation to lipid deposition is associated to its chemical composition, in particular the presence of vinyl pyrrolidone.

5.4. Conclusion

The results obtained in this investigation led to the following conclusions:

i. The contact lens wearers' symptomatology was associated with differences in the tear film concentration of monoglycerides. The symptomatic population was characterised by a higher concentration of monoglycerides compared to the asymptomatic population.

ii. The analysis of the nature of lipid deposition revealed significant associations between tear film characteristics, symptomatology and ocular integrity and the type of lipids deposited. The interactions found were as follow:

   - A high level of easily extracted phospholipids/triglycerides was associated with a higher comfort,

- Similarly the presence of Peak a3 after methanol extractions was also associated with an increase in comfort,
- The lipid layer mixing pattern was influenced by the total level of phospholipids/ triglycerides deposited; a high deposition was associated with a thicker and more stable lipid layer,
- The stability of the Pre Lens Tear Film was influenced by the level of cholesterol esters deposited. A median deposition level was associated with the longer break up times and a high deposition with the short break up times.
- A high deposition of monoglycerides was correlated with a higher incidence of slow destabilising breaks and a lower incidence of high destabilising breaks.
- The level of hyperaemia recorded increased in the presence of cholesterol ester deposited on the contact lens, and this increase could be counterbalanced by the level of fatty acids found on the contact lens.

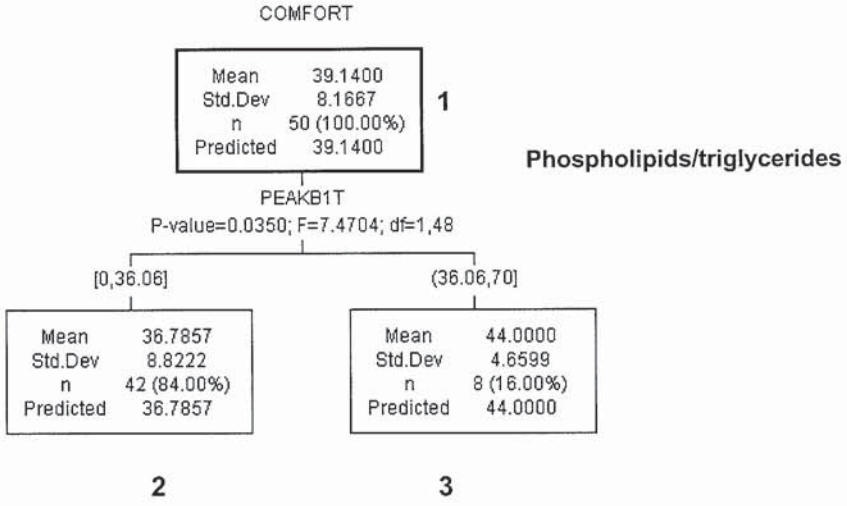


Table 5.17. Effect of lipids deposited on comfort score after first extraction - Predictive analysis by CHAID
Main predictor = PHOSPHOLIPIDS/TRIGLYCERIDES
Box 1 = Comfort scores for overall population
Box 2 = Comfort scores for low level of phospholipids/triglycerides (0 to 36)
Box 3 = Comfort scores for high level of phospholipids/triglycerides (> 36)

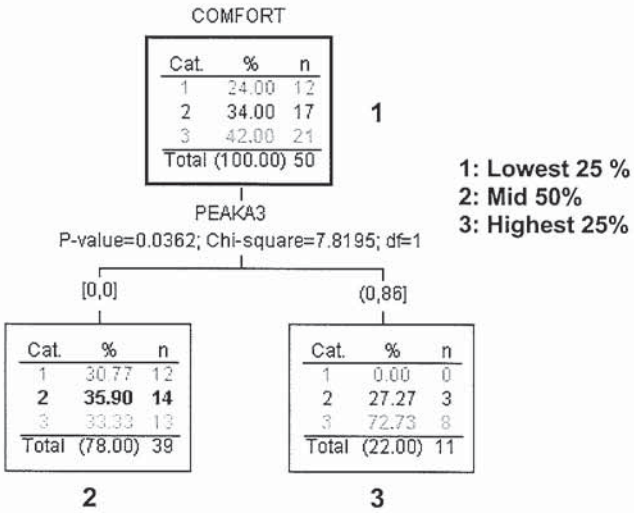


Table 5.18. Effect of lipids deposited on comfort distribution after first two extractions - Predictive analysis by CHAID

Main predictor = PEAK A3
Box 1 = Comfort scores for overall population
Box 2 = Comfort scores for low level of peak a3 (0)
Box 3 = Comfort scores for high level of peak a3 (>0)

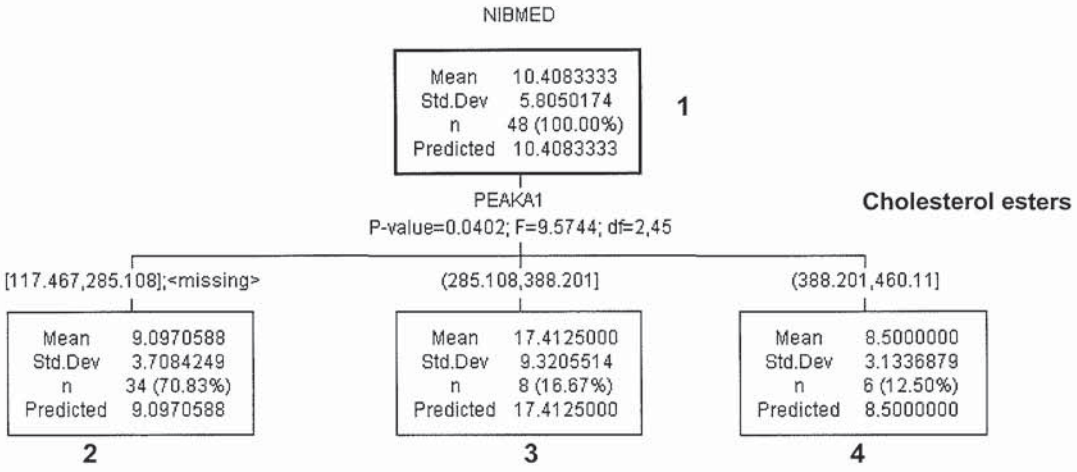


Table 5.19. Effect of lipids deposited on NIBUT median - Predictive analysis by CHAID

Main predictor = CHOLESTEROL ESTERS
Box 1 = NIBUT values for overall population
Box 2 = NIBUT values for low level of cholesterol esters
Box 3 = NIBUT values for medium level of cholesterol esters
Box 4 = NIBUT values for high level of cholesterol esters

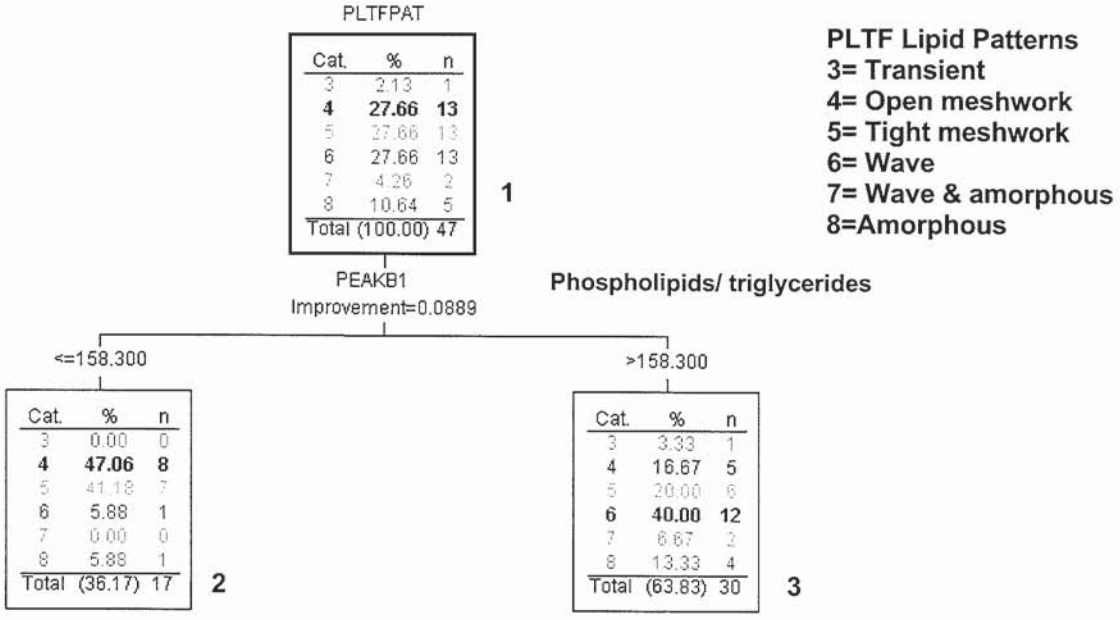
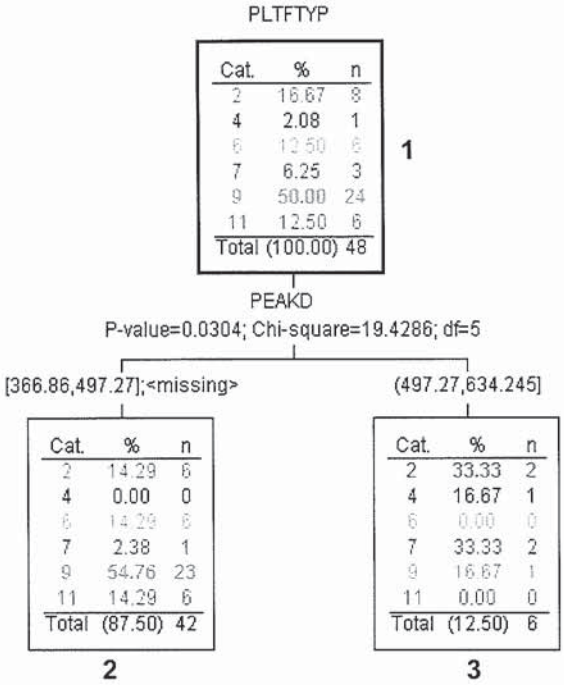


Table 5.21. Effect of lipids deposited on PLTF lipid mixing pattern - Predictive analysis by C&RT

Main predictor = PHOSPHOLIPIDS/TRIGLYCERIDES
Box 1 = PLTF lipid layer mixing pattern distribution for overall population
Box 2 = PLTF lipid layer mixing pattern distribution for low level of phospholipids/triglycerides (<158)
Box 3 = PLTF lipid layer mixing pattern distribution for high level of phospholipids/triglycerides (> 158)



PLTF Break Type
2= Not visible
4= Single spots
6= Horizontal Line
7= Vertical line
9= Surface
11=Band

Table 5.22. Effect of lipids deposited on PLTF break up type - Predictive analysis by CHAID

Main predictor = PEAK D
Box 1 = PLTF lipid break up type distribution for overall population
Box 2 = PLTF lipid break up type distribution for low level of Peak D (366 to 497)
Box 3 = PLTF lipid break up type distribution for high level of peak D (> 497)

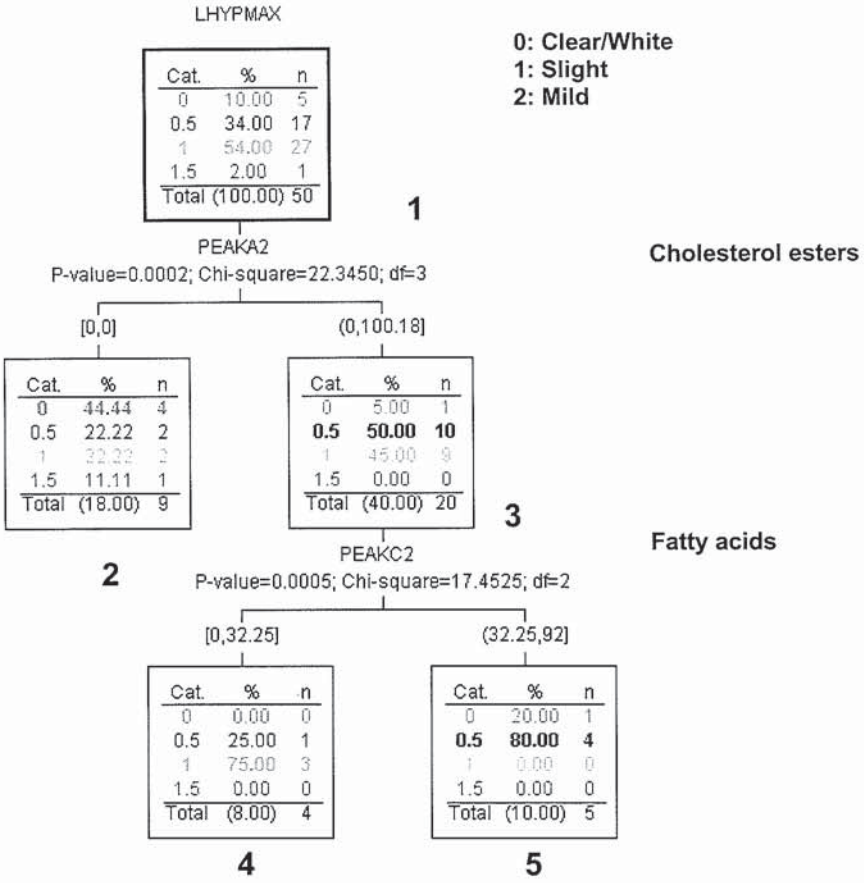


Table 5.23.Effect of lipids deposited on limbal hyperaemia - Predictive analysis by CHAID

Main predictors = PEAK A2 & PEAKC2

Box 1 = Limbal hyperaemia distribution for overall population
Box 2 = Limbal hyperaemia distribution for low level of Peak A2 (cholesterol ester)
Box 3 = Limbal hyperaemia distribution for high level of Peak A2 (cholesterol ester)
Box 4 = Limbal hyperaemia distribution for high level of Peak A2 (cholesterol ester) & low level of Peak C2 (fatty acids)
Box 5 = Limbal hyperaemia distribution for high level of Peak A2(cholesterol ester) & high level of Peak C2 (fatty acids)

CHAPTER 6

DAILY DISPOSABLE CONTACT LENSES AND SYMPTOMATOLOGY

6.1. Objective

The objective of the study was to assess the relative performance and acceptance of soft hydrophilic contact lenses used on a daily disposable regimen and to monitor the influence of patient tear characteristics on this performance.

6.2. Materials and methods

The study was a masked cross-over randomised study. The subjects attended CLRC for a dispensing visit not wearing contact lenses and were randomly dispensed with either Nelfilcon A, a PVA-based material (Dailies, CIBAVision) or Etafilcon A, a HEMA-based material (1-Day Acuvue, Johnson & Johnson) (Table 6.1). The patients were scheduled to attend the clinic for follow up visits at one and four weeks. After one-week wash out, the subjects were then dispensed with the other contact lens type.

The test population consisted of myopic soft contact lens wearers. The population was divided into 3 groups according to their symptomatology:

i. Poor performance with Dailies on daily disposable modality (Group1)
ii. Poor performance with 1-Day Acuvue on daily disposable modality (Group 2)

iii. Good performance with both contact lens types (Group 3).

The allocation to each of the groups was based upon the following criteria:

- To be classified in the ' Poor performance' category, the subject had to score less than 35 on the 50 point scale for either comfort or dryness or grittiness and this in conjunction with an habitual daily wearing time of less than 12 hours.
- To be classified in the ' Good performance' category, the subject had to score over 40 on the 50 point scale for comfort, dryness and grittiness and this in conjunction with a habitual daily wearing time of more than 12 hours.

The population characteristics are reported in Table 6.2.

Tear samples were collected at each visit (Dispensing, 1 week and 1 month) using Visispear™ ophthalmic sponges. After collection the samples were prepared and analysed using High performance Liquid Chromatography (HPLC) as described in Chapter 2. At the 1-month visit, the contact lenses were aseptically removed and kept dry at -20°C before analysis. The contact lenses were extracted for lipid analysis using methanol and the extracts analysed using HPLC.

Table 6.1. Contact lens material characteristics.

	CIBAVision Dailies	**Vistakon 1-Day Acuvue**
Material	Nelfilcon A	Etafilcon A
Water content	69%	58%
Base Curve(mm)	8.6	8.5
Diameter(mm)	13.8	14.0

Table 6.2. Population demographics (Age)-Descriptive statistics.

	Group 1 (years)	Group 2 (years)	Group 3 (years)
Male	31.8 ± 7.7 (23- 42) n=6	34.7 ± 8.4 (25- 40) n=3	34.3 ± 3.9 (31- 39) n=4
Female	38.8 ± 5.3 (33- 45) n=4	28.1 ± 6.2 (20- 40) n=8	35.0 ± 3.0 (31- 38) n=6
Overall	34.6 ± 7.4 (23- 45) n=10	29.9 ± 7.1 (20- 40) n=11	34.7 ± 3.2 (31- 39) n=10

6.3. Results

6.3.1. Tear analysis

6.3.1.1. Pre ocular tear film

The distribution of the different lipid classes, cholesterol esters, cholesterol, fatty acids and phospholipids/triglycerides are represented in Figures 6.1a,b,c&d. None of the individual lipid distributions were normally distributed. The distributions of most lipid classes were also leptokurtic with kurtosis values ranging from 2.250 to 28.157. Cholesterol esters and cholesterol were the lipids most closely approaching a normal distribution. The cholesterol ester distribution ranged from 0 to 280 and was slightly skewed towards the right (Mean: 74.3, Median: 54.0, Skewness: 1.499), with 60% of the cases with an intensity lower than 70. The cholesterol distribution ranged from 0 to 65 and was the most normally distributed with a slight trend for a Skewness to the left (Mean: 45.9, Median: 48.0, Skewness: -0.964) and a Kurtosis (Kurtosis: 1.172) closest to normality. Phospholipids/triglycerides (Mean: 19.7, Median: 13.0, Skewness:

5.051) and fatty acids (Mean: 7.7, Median: 0.0, Skewness: 3.810) were not normally distributed but both highly skewed to the right. In approximately 90% of cases, the intensity of the fatty acid peak was lower or equal to 10 and the intensity of phospholipids/triglycerides peaks inferior or equal to 20.

The distributions of the lipid concentrations at the first and second dispensing visits are plotted in Figures 6.2a,b,c&d. The plots revealed poor correlation for individual measurements, this was confirmed by the low correlation coefficients for each lipid class (r=0.005 to 0.242). However when looking at clinically significant ranges and not individual values, the variability between the two sets of measurements was significantly lower. For instance, the comparison of the cholesterol distributions by groups with peak intensities lower than 40 or between 40 and 50, 20 out of 30 subjects were classified in the same group for both visits. Four cases went from intensities lower than 40 at the first dispensing visit to intensities over 40 and, six cases went from a peak intensity between 40 and 50 to an intensity inferior to 40 from the first to the second visit. The distribution of the peak intensity of the phospholipids/triglycerides remained lower than 50 in most cases. Only four cases went from less than 50 at the first visit to an intensity ranging from 50 to 250 at the second. The fatty acid evaluation revealed that seventeen cases had and remained with an intensity of less than 10 and 22 out of 30 had a peak intensity inferior to 20 at both visits. There were only two outliners that went from a low value at one dispensing visit to high value at the second dispensing visit.

The lipid composition of the pre-ocular tear film for each population group and overall was measured twice and the results are reported in Tables 6.3a & 6.3b. The average values of these two measurements are reported in Table 6.4. Similar overall lipid profiles were measured for each population group before the two contact lens dispensing. Statistical differences were limited to few lipid classes. Overall comparisons between the three clinical groups showed a very significant statistical difference for two of the fatty acids (Table 6.5ai: Peak 6 $p=0.045$ & Peak 9 $p<0.001$). For Peak 9, the difference in mean intensity was greatest between the three clinical groups and the intensity of the Peak 9 was highest for Group 3, which was the best performing group. The individual comparisons (Table 6.5aii) revealed:

- a statistically significant difference between the group that achieves good performance with both contact lenses (Group 3) and the group characterised by a poor performance with Acuvue contact lenses (Group 2),
- a difference at the limit of clinical significance between the group that achieves good performance with both contact lenses (Group 3) and the group made up of subjects that performed poorly with Dailies contact lenses (Group 1), at the limit of statistical significance and,
- a similar performance for the two symptomatic groups (Groups 1 & 2).

Paired comparisons between the group that was asymptomatic with both contact lens types (Group 3) and either of the groups performing poorly with at least one of the contact lens type showed a significantly higher intensity for Peak 9 for the

well performing group (Group 3) than either of the two other (Groups 1 & 2) (Table 6.5b: p=0.009 & <0.001).

For one of the cholesterol esters (Peak 2), comparisons between the asymptomatic group with both contact lens types and either of groups performing poorly with one contact lens type revealed differences at the limit of statistical significance (Table 6.5b. p=0.067 (Group 1 vs. 3) and p=0.068 (Group 2 vs. 3)). In both cases the level of Peak 2 was slightly higher in the asymptomatic group (Group 3). The clinical significance of this difference is not known.

The distribution observed for the lipid pattern was as follows:

- The lipid layer mixing pattern was a wave (40.5%) or amorphous pattern (40.5%) in the majority of cases.
- Two other two types of patterns were also observed: a meshwork pattern in 5.6% of cases and a colour pattern in 13.5% of cases.

The interaction between pre ocular tear film (POTF) structure and lipid composition was investigated using the Answer Tree^{TM} statistical technique. The only significant interaction detected was between the POTF lipid composition and clinically observed lipid layer mixing pattern (Table 6.6).

The POTF lipid layer mixing pattern was most influenced by the level of phospholipids/triglycerides. A high level of phospholipids/triglycerides was associated with a thicker and more even pattern. In 90% of cases, against 80% in starting group, the lipid layer mixing pattern observed was a wave or amorphous pattern and the number of amorphous pattern observed increased

from 40.5% to 51.9%. Simultaneously there was a decrease in the percentage of cases where a colour pattern was observed, from 13.5 % to only 2% in the final group. Therefore a high level of phospholipids/triglycerides also associated with less contamination of lipid layer.

On the opposite, the group with a low level of phospholipids/triglycerides was characterised by a thinner and more contaminated lipid layer compared to the overall population. The percentage of cases in which an amorphous pattern was observed decreased from 40.5% to 24.3% and that when a colour pattern was observed increased to 29.7% of cases from only 13.5% in overall population.

Table 6.3a. Pre ocular tear lipid composition at first dispensing - Descriptive statistics

Lipid identification	Peak	Parameters	Overall	Group 1	Group 2	Group 3
Cholesterol ester	Peak 1	Mean ± STD Median (Range)	76 ± 61 55 (13 – 284)	80 ± 64 63 (15-232)	85 ± 74 56 (14-284)	60 ± 34 50 (13-138)
	Peak 2	Mean ± STD Median (Range)	13 ± 18 0 (0 - 81)	11 ± 16 0 (0-46)	6 ± 12 0 (0-45)	23 ± 23 20 (0-81)
	Peak 2b	Mean ± STD Median (Range)	4 ± 6 0 (0 - 17)	5 ± 7 0 (0 - 17)	3 ± 5 0 (0 - 14)	2 ± 5 0 (0 - 15)
Phospholipids/ triglycerides	Peak 3	Mean ± STD Median (Range)	13 ± 10 14 (0 - 41)	15 ± 11 13 (0 – 41)	13 ± 8 11 (0 – 27)	13 ± 11 11 (0 – 31)
Fatty acids	Peak 4	Mean ± STD Median (Range)	4 ± 13 0 (0 - 82)	2 ± 5 0 (0-15)	2 ± 6 0 (0-18)	10 ± 23 0 (0-82)
	Peak 5	Mean ± STD Median (Range)	1 ± 4 0 (0 - 24)	0	0	2 ± 7 0 (0 – 24)
	Peak 6	Mean ± STD Median (Range)	1 ± 3 0 (0 - 16)	0	1 ± 3 0 (0 - 9)	1 ± 5 0 (0 - 16)
	Peak 7	Mean ± STD Median (Range)	0	0	0	0
	Peak 8	Mean ± STD Median (Range)	6 ± 10 0 (0 - 51)	8 ± 13 4 (0-51)	4 ± 5 0 (0-13)	6 ± 8 0 (0-27)
	Peak 9	Mean ± STD Median (Range)	3 ± 6 0 (0 - 25)	1 ± 3 0 (0 - 9)	1 ± 2 0 (0 - 9)	9 ± 9 10 (0 - 25)
	Peak10	Mean ± STD Median (Range)	2 ± 5 0 (0 -14)	1 ± 4 0 (0 –12)	4 ± 6 0 (0 -14)	2 ± 4 0 (0 -12)
	Peak 11	Mean ± STD Median (Range)	11 ± 12 12 (0 -58)	8 ± 9 4 (0-24)	14 ± 10 15 (0-29)	12 ± 17 0 (0-58)
	Peak 12	Mean ± STD Median (Range)	8 ± 9 0 (0 -28)	6 ± 9 0 (0-27)	9 ± 10 9 (0-28)	7 ± 8 0 (0-22)
	Peak 13	Mean ± STD Median (Range)	5 ± 8 0 (0 -27)	3 ± 7 0 (0 – 24)	4 ± 8 0 (0 – 22)	8 ± 9 10 (0 – 27)
	Peak 14	Mean ± STD Median (Range)	0	0 ± 0 0 (0 – 0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Monoglycerides	Peak 15	Mean ± STD Median (Range)	0	0 ± 0 0 (0 – 0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Cholesterol	Peak 16	Mean ± STD Median (Range)	47 ± 13 49 (0-64)	50 ± 11 55 (22-62)	42 ± 15 45 (0-63)	49 ± 12 49 (27-64)

Table 6.3b. Pre ocular tear lipid composition at second dispensing - Descriptive statistics

Lipid identification	Peak	Parameters	Overall	Group 1	Group 2	Group 3
Cholesterol ester	Peak 1	Mean ± STD Median (Range)	72 ± 57 54 (10 – 273)	58 ± 50 44 (10-193)	73 ± 50 61 (17-187)	84 ± 70 65 (19-273)
	Peak 2	Mean ± STD Median (Range)	14 ± 19 9 (0 - 97)	9 ± 13 5 (0-40)	16 ± 25 9 (0-97)	16 ± 17 10 (0-44)
	Peak 2b	Mean ± STD Median (Range)	5 ± 7 0 (0 -19)	3 ± 5 0 (0 – 14)	5 ± 7 0 (0 - 18)	7 ± 7 5 (0 - 19)
Phospholipids/ triglycerides	Peak 3	Mean ± STD Median (Range)	26 ± 46 16 (0 – 227)	30 ± 62 15 (0 – 227)	16 ± 19 15 (0 – 80)	36 ± 54 23 (0 – 212)
Fatty acids	Peak 4	Mean ± STD Median (Range)	2 ± 6 0 (0 – 27)	1 ± 5 0 (0-17)	3 ± 7 0 (0-19)	2 ± 7 0 (0-27)
	Peak 5	Mean ± STD Median (Range)	2 ± 8 0 (0 -45)	1 ± 4 0 (0-14)	0	5 ± 13 0 (0 – 4524)
	Peak 6	Mean ± STD Median (Range)	1 ± 4 0 (0 - 20)	0	2 ± 6 0 (0 – 20)	0
	Peak 7	Mean ± STD Median (Range)	0	0	0	0
	Peak 8	Mean ± STD Median (Range)	10 ± 17 7 (0 – 93)	4 ± 6 0 (0-20)	15 ± 25 8 (0-93)	7 ± 7 8 (0-21)
	Peak 9	Mean ± STD Median (Range)	3 ± 11 0 (0 – 65)	2 ± 8 0 (0 – 26)	<1 ± 2 0 (0 – 8)	7 ± 17 0 (0 – 65)
	Peak10	Mean ± STD Median (Range)	3 ± 6 0 (0 – 24)	2 ± 5 0 (0 –15)	3 ± 7 0 (0 –24)	3 ± 5 0 (0 –12)
	Peak 11	Mean ± STD Median (Range)	9 ± 12 0 (0 – 47)	8 ± 13 0 (0-33)	8 ± 13 0 (0-47)	11 ± 9 10 (0-30)
	Peak 12	Mean ± STD Median (Range)	5 ± 8 0 (0 - 37)	6 ± 9 0 (0-22)	4 ± 10 0 (0-37)	5 ± 7 0 (0-21)
	Peak 13	Mean ± STD Median (Range)	5 ± 10 0 (0 → 40)	4 ± 9 0 (0 – 27)	6 ± 13 0 (0 – 40)	4 ± 7 0 (0 – 18)
	Peak 14	Mean ± STD Median (Range)	0	0 ± 0 0 (0 – 0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Monoglycerides	Peak 15	Mean ± STD Median (Range)	1 ± 3 0 (0 → 13)	0 ± 0 0 (0 – 0)	1 ± 4 0 (0-13)	1 ± 3 0 (0-13)
Cholesterol	Peak 16	Mean ± STD Median (Range)	44 ± 11 48 (17 – 61)	44 ± 12 48 (18-61)	45 ± 12 45 (17-60)	45 ± 11 48 (28-59)

Table 6.4. Pre ocular tear lipid composition - Mean values for first and second dispensing - Descriptive statistics

Lipid identification	Peak	Parameters	Overall	Group 1	Group 2	Group 3
Cholesterol ester	Peak 1	Mean ± STD Median (Range)	75 ± 58 61 (10 – 284)	72 ± 58 65 (10-232)	82 ± 61 61 (23-284)	70 ± 58 56 (13-273)
	Peak 2	Mean ± STD Median (Range)	8 ± 9 8 (0 – 41)	8 ± 9 6 (0-31)	5 ± 6 6 (0-23)	13 ± 11 10 (0-41)
	Peak 2b	Mean ± STD Median (Range)	4 ± 5 0 (0 –16)	4 ± 6 0 (0 – 16)	3 ± 4 0 (0 - 13)	5 ± 6 0 (0 – 16)
Phospholipids/ triglycerides	Peak 3	Mean ± STD Median (Range)	18 ± 22 14 (0 – 125)	19 ± 28 13 (0 – 125)	15 ± 11 14 (0 – 46)	21 ± 26 14 (0 – 116)
Fatty acids	Peak 4	Mean ± STD Median (Range)	3 ± 7 0 (0 – 41)	2 ± 3 0 (0-9)	3 ± 5 0 (0-18)	5 ± 12 0 (0-41)
	Peak 5	Mean ± STD Median (Range)	1 ± 4 0 (0 –23)	<1 ± 2 0 (0 - 7)	0	2 ± 6 0 (0 – 23)
	Peak 6	Mean ± STD Median (Range)	1 ± 2 0 (0 – 10)	0	2 ± 3 0 (0 – 10)	<1 ± 2 0 (0 - 8)
	Peak 7	Mean ± STD Median (Range)	0	0	0	0
	Peak 8	Mean ± STD Median (Range)	7 ± 10 5 (0 – 55)	6 ± 7 5 (0-30)	10 ± 15 5 (0-55)	6 ± 6 7 (0-18)
	Peak 9	Mean ± STD Median (Range)	3 ± 10 (0 – 65)	1 ± 3 0 (0 – 13)	<1 ± 1 0 (0 – 4)	9 ± 16 5 (0 – 65)
	Peak10	Mean ± STD Median (Range)	2 ± 3 0 (0 – 13)	1 ± 3 0 (0 –8)	4 ± 4 0 (0 –13)	2 ± 3 0 (0 –6)
	Peak 11	Mean ± STD Median (Range)	9 ± 9 10 (0 – 38)	7 ± 8 4 (0-24)	11 ± 7 12 (0-24)	10 ± 10 11 (0-38)
	Peak 12	Mean ± STD Median (Range)	7 ± 8 5 (0 – 37)	6 ± 7 5 (0-27)	8 ± 10 5 (0-37)	6 ± 7 4 (0-20)
	Peak 13	Mean ± STD Median (Range)	4 ± 6 0 (0 – 20)	3 ± 6 0 (0 – 20)	5 ± 7 0 (0 – 20)	5 ± 6 3 (0 – 16)
	Peak 14	Mean ± STD Median (Range)	0	0 ± 0 0 (0 – 0)	0 ± 0 0 (0-0)	0 ± 0 0 (0-0)
Monoglycerides	Peak 15	Mean ± STD Median (Range)	<1 ± 2 0 (0 - 13)	0 ± 0 0 (0 – 0)	1 ± 2 0 (0-17)	1 ± 3 0 (0-13)
Cholesterol	Peak 16	Mean ± STD Median (Range)	45 ± 11 47 (20 – 62)	47 ± 11 51 (22-62)	43 ± 9 46 (20-60)	46 ± 11 45 (27-61)

Table 6.5a. Pre ocular tear lipid composition – Mean values for first and second dispensing visits - Comparative statistics

i. Overall by Kruskall-Wallis Test

Peak	P-value
1	0.641
2	0.105
2b	0.805
3	0.731
4	0.851
5	0.116
6	**0.045**
7	1.000
8	0.897
9	**<0.001**
10	0.174
11	0.326
12	0.941
13	0.226
14	1.000
15	0.431
16	0.398

ii. Individual comparisons (* values joined by a continuous line are not significantly statistically different)

- Peak 6

	1	3	2
Mean rank	26.00	27.67	32.71
NP Tukey (*)	———	———	

- Peak 9

	2	1	3
Mean rank	23.71	25.94	38.22
NP Tukey (*)	———	———	

Table 6.5b. Pre ocular tear lipid composition – Mean values for first and second dispensing visits – Comparison between good and poor performance groups by Mann - Whitney Exact Test

Peak	1 vs. 3	2 vs. 3
1	1.000	0.443
2	**0.067**	**0.068**
2b	0.713	0.521
3	0.505	0.498
4	0.646	0.779
5	0.229	0.089
6	1.000	0.171
7	1.000	1.000
8	0.739	0.778
9	**0.009**	**<0.001**
10	0.598	0.217
11	0.336	0.638
12	0.956	0.752
13	0.078	0.619
14	1.000	1.000
15	1.000	1.000
16	0.981	0.512

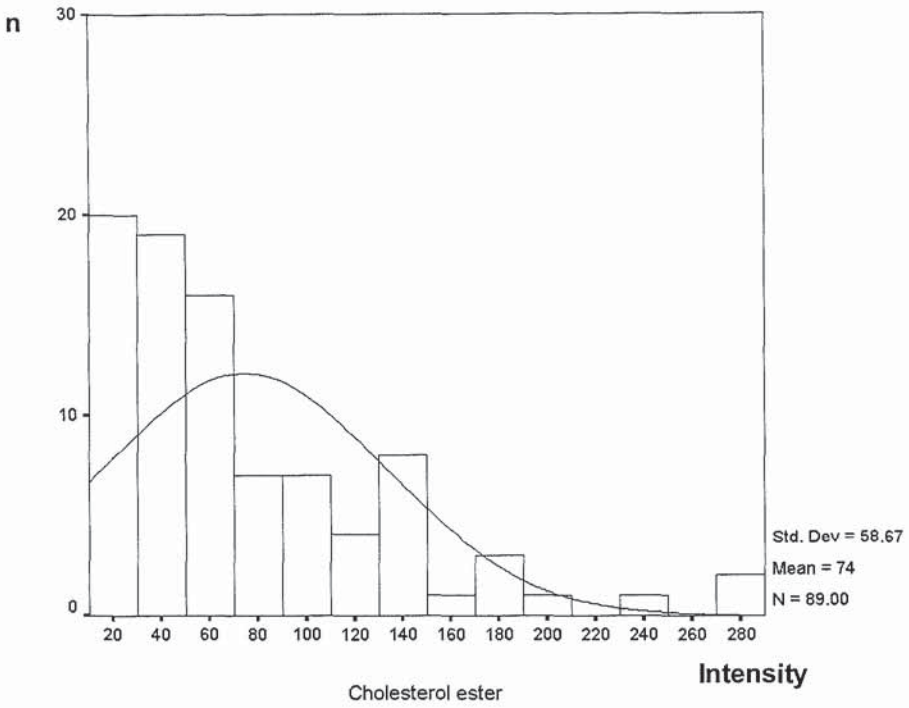


Figure 6.1a. Cholesterol esters distribution in the pre ocular tear film. The continuous line surimposed over the histogram is the normal curve with same mean and variance as data.

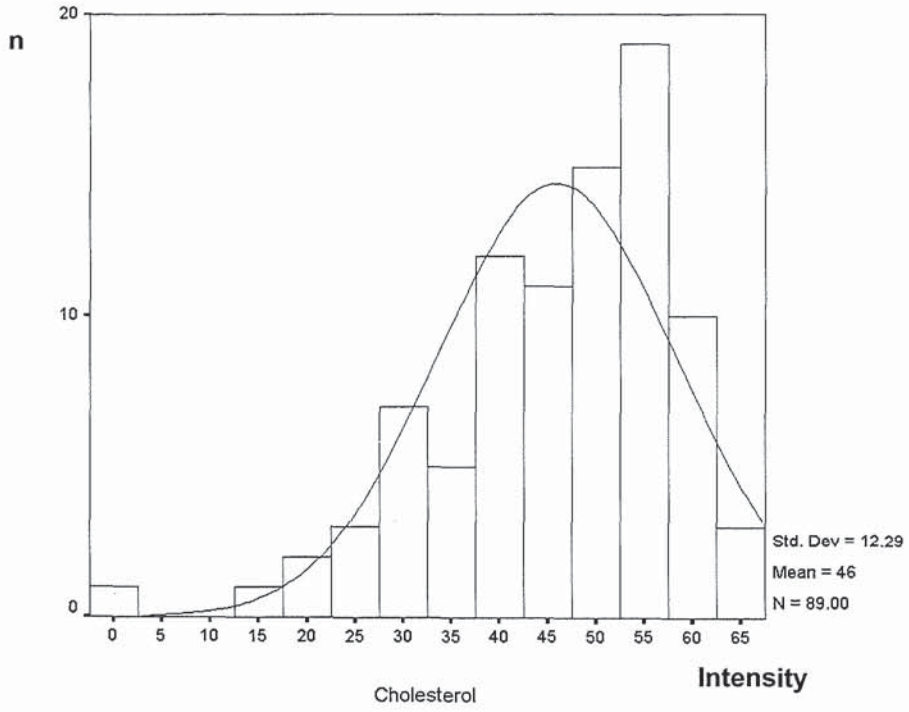


Figure 6.1b. Cholesterol distribution in the pre ocular tear film. The continuous line surimposed over the histogram is the normal curve with same mean and variance as data.

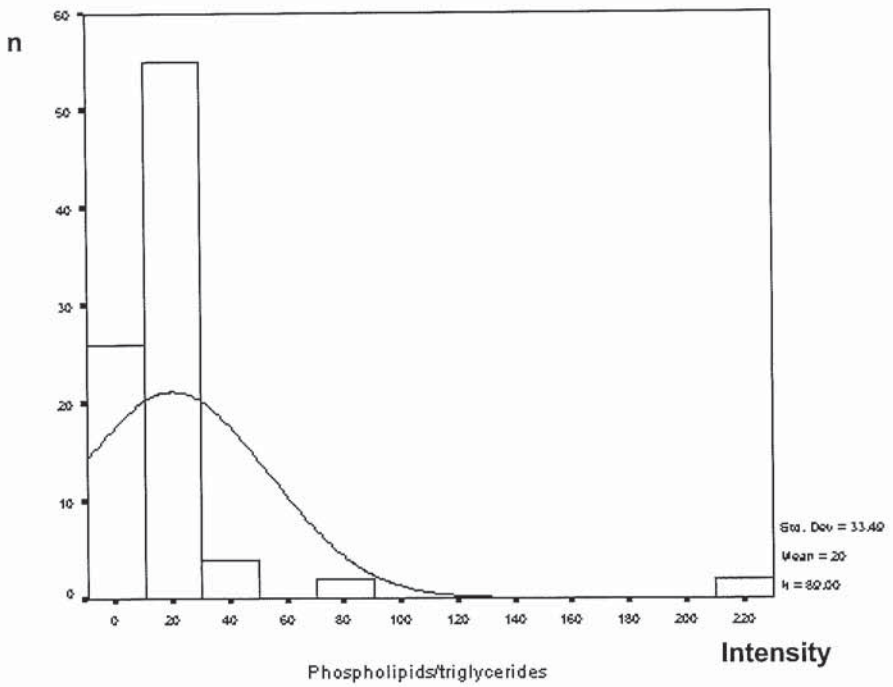


Figure 6.1c. Phospholipids/triglycerides distribution in the pre ocular tear film. The continuous line surimposed over the histogram is the normal curve with same mean and variance as data.

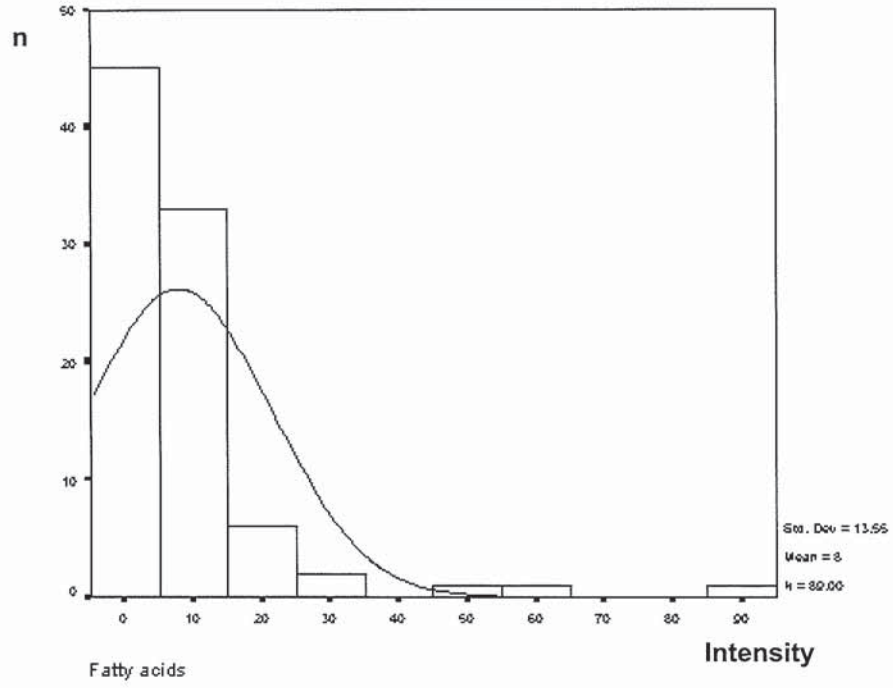


Figure 6.1d. Fatty acids distribution in the pre ocular tear film. The continuous line surimposed over the histogram is the normal curve with same mean and variance as data.

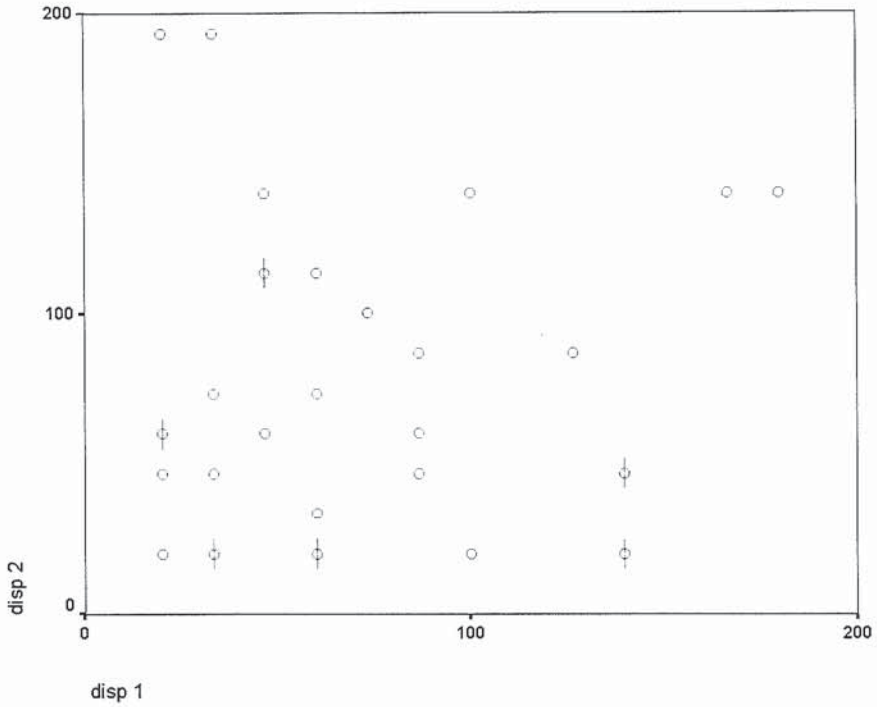


Figure 6.2a. Scatterplot of cholesterol ester peak intensities at first and second dispensing visits.[+]

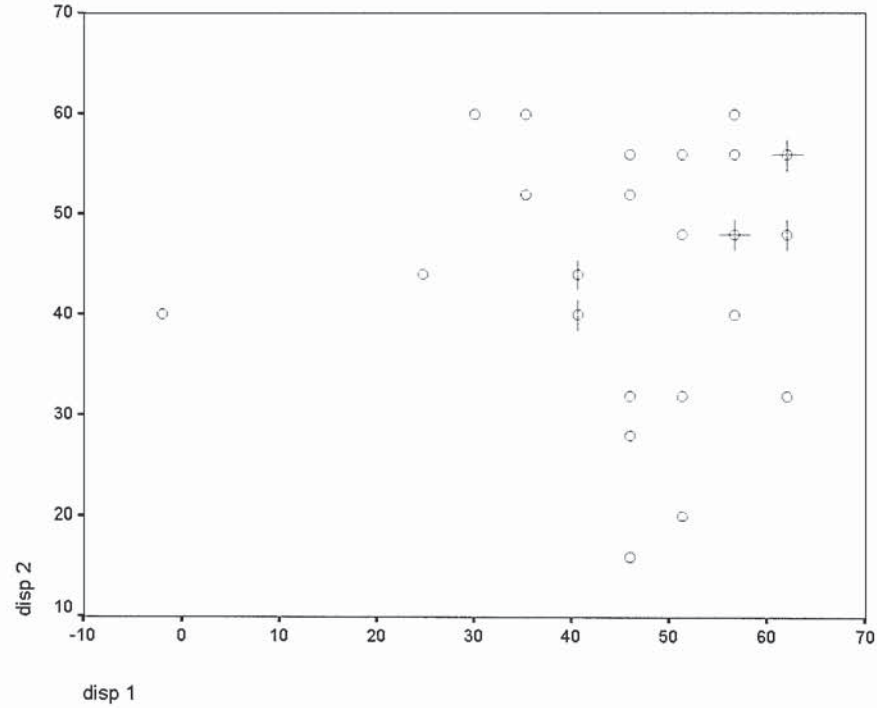


Figure 6.2b. Scatterplot of cholesterol peak intensities at first and second dispensing visits.[+]

[+] Overlapping points are represented by 'sunflowers'. If a cell contains only one point, it is represented by a small circle; if a cell has more than one point each point is represented by a short line (a 'petal') originating from the circle.

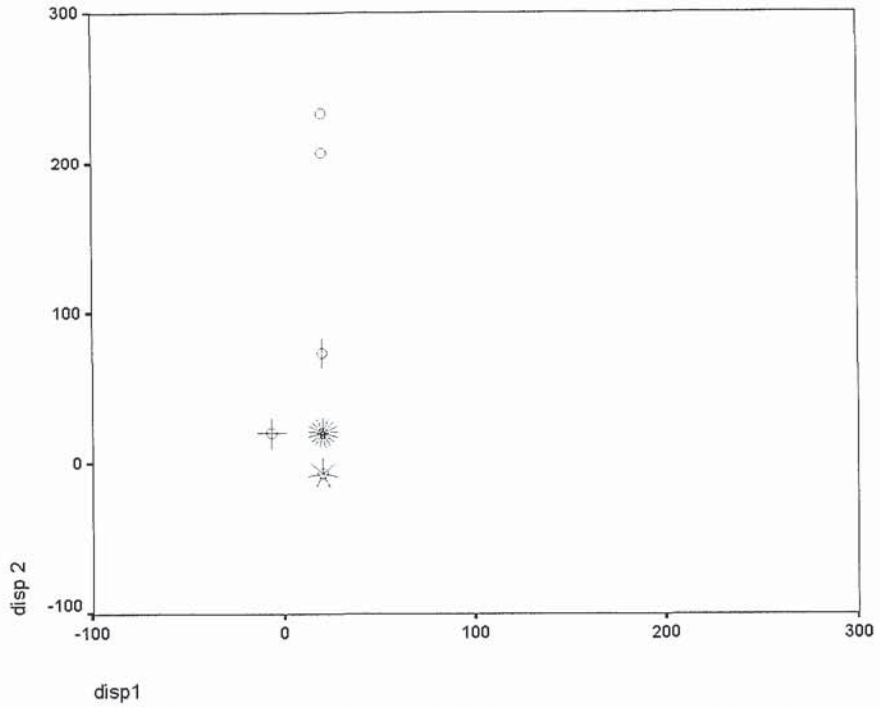


Figure 6.2c. Scatterplot of phospholipids/triglycerides peak intensities at first and second dispensing visits[+]

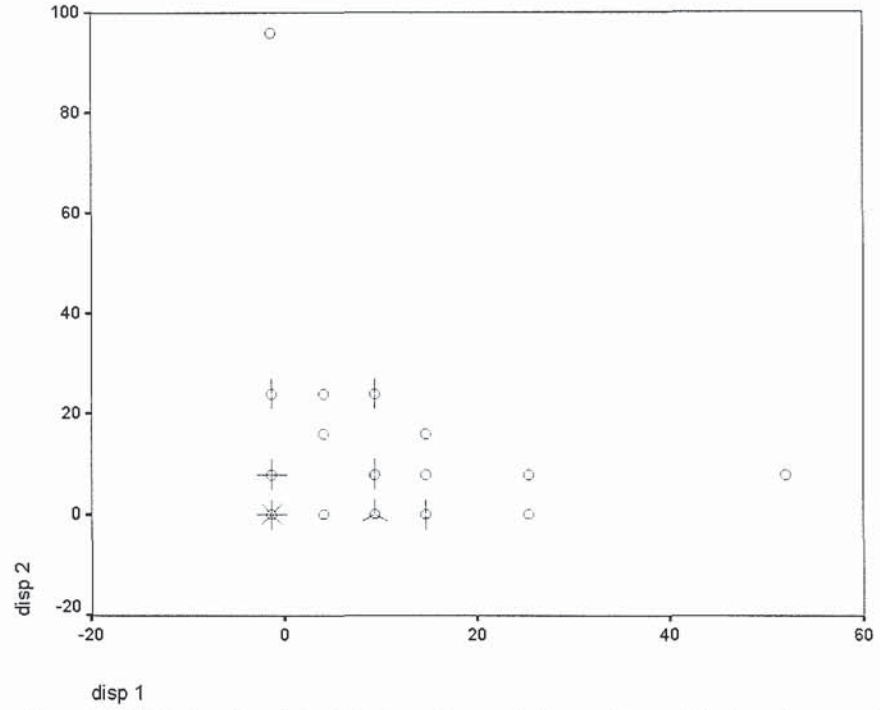
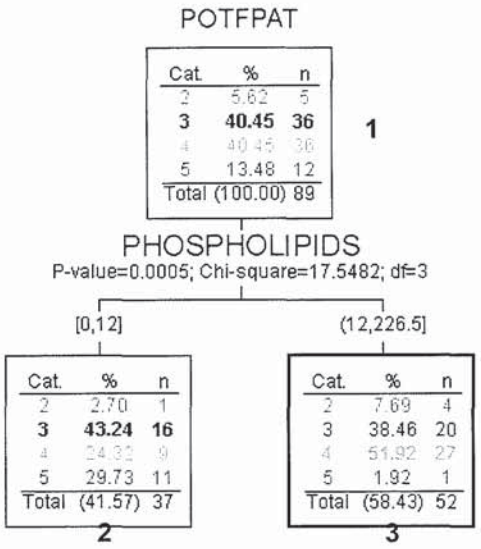


Figure 6.2d. Scatterplot of fatty acids peak intensities at first and second dispensing visits [+]

[+] Overlapping points are represented by 'sunflowers'. If a cell contains only one point, it is represented by a small circle; if a cell has more than one point each point is represented by a short line (a 'petal') originating from the circle.



2= meshwork
3= wave
4= amorphous
5= colors

Table 6.6. Pre ocular lipid layer mixing pattern - Predictive analysis with CHAID.

Main predictor selected = Phospholipids
Box 1= POTF lipid layer mixing pattern distribution for the overall population
Box 2= POTF lipid layer mixing pattern distribution for low levels of phospholipids (0 to 12)
Box 3= POTF lipid layer mixing pattern distribution for high levels of phospholipids (>12)

6.3.1.2. Pre Lens tear film

The distributions of the different lipid classes in the pre lens tear film (PLTF), reported in Figures 6.3a,b,c&d, were similar to those observed for the pre ocular tear film with however some differences. Higher cholesterol ester peak intensities were measured in the pre lens tear film than in the pre-ocular tear film. The highest individual peak intensity increased from 250 for the POTF to 850 for PLTF. In addition, the distributions of individual cholesterol esters peak intensities in the PLTF were also more skewed to right and kurtosed than in the POTF (Mean: 84, Median: 59, Skewness: 4.860 and Kurtosis: 33.593). The mean of cholesterol peak intensities measured in the tear samples for the PLTF was very similar to that obtained for the POTF, the majority of peak intensities were around 50. The main difference in the distribution of the individual peak intensities for the POTF and PLTF was a normal distribution for the latter (Mean: 42, Median: 43, Skewness: 0.696 and Kurtosis: 2.320).

The distribution of the PLTF phospholipids/triglycerides was similar to that of the POTF. The distribution was skewed to the right and highly kurtosed (Mean: 19, Median: 14, Skewness: 4.518 and Kurtosis: 22.645), most of the peak intensities were again inferior to 20. The peak intensities of the PLTF fatty acids measured in the PLTF were similar to those of the POTF. The highest peak intensity measured was however lower for the PLTF (maximum intensity= 50) than for the POTF (maximum intensity= 90). The distribution was kurtosed with a peak intensity of zero for 55% of cases (Mean: 6, Median: 0).

The measurements obtained for individual eyes at the one-week and one month visits were statistically correlated for most lipid classes (Fig. 6.4a.b.c&d). The statistical significance was highest for the cholesterol esters and phospholipids/triglycerides distributions (p=0.008 for cholesterol esters and p=0.018 for phospholipids/triglycerides). The level of cholesterol showed the greatest variability with length of wear.

The peak intensities recorded overall and for the three clinical groups (Tables 6.7 & 6.8) revealed similar average peak intensities. Statistical comparisons between the individual clinical groups (Tables 6.9 & 6.10) did not reveal any statistically significant differences.

The interactions between the PLTF lipid composition and tear film clinical characteristics (lipid layer mixing pattern, tear break up time, the position and type of break) and the contact lens subjective acceptance (comfort, dryness) were tested with a view to produce predictive factors. Predictive data analysis was carried using the Answer Tree^{TM} CHAID test. The interactions were as follow:

- A significant interaction was detected between the pre lens tear film stability, characterised clinically by the NIBUT and the level of cholesterol in the pre lens tear film (Table 6.11). The population was automatically divided into two subgroups. For the first subgroup, for which a non-detectable or low level of cholesterol was measured, the average minimum NIBUT value increased

significantly from 7.1 to 9.3 seconds. The second group, characterised by a higher level of cholesterol had a lower average minimum NIBUT with a value of 6.5 seconds.

- The lipid layer thickness, characterised by the observed lipid mixing pattern was influenced by the level of cholesterol ester (Table 6.12). The population was automatically partitioned into three categories:

  i. A low level of cholesterol ester was influential in producing a thin lipid layer. A meshwork pattern was observed in 63% of cases. The tendency towards a thin lipid layer was confirmed by the increase in the incidence of transient patterns (Table 6.12. 4% vs. 3%) and the decrease in the incidence of amorphous and wave patterns (Table 6.12. 8% vs. 15% and 25% vs. 31% respectively).

  ii. A median level of cholesterol ester in the pre lens tear film was associated with a thicker lipid layer compared to the overall population and to the first group described above. The mixing patterns observed were mostly of three types: meshwork, wave and amorphous in respectively 21%, 47% and 24% of cases (Table 6.12). No transient patterns were observed and only a small percentage of colour patterns were visible.

  iii. Finally, the third group for which high cholesterol ester peak intensities were recorded for the pre lens tear film, was characterised by an increased contamination of the lipid layer, associated with an increase in the incidence of colour pattern (Table 6.12. 9% vs. 4%). The

thickness of the lipid layer for this population subgroup was similar to that of the overall population but thinner to that of group 2 above.

- The type of break observed appeared to be most influenced by the level of fatty acids in the tear film (Table 6.13). The population was automatically divided into two groups according to their fatty acids peak intensities. The comparison between these two groups revealed that the group with the high fatty acids peak intensity was associated with a higher incidence of slow destabilising breaks than the group with low fatty acids peak intensities (77% in high fatty acids group vs. 54% in the low fatty acids groups), and a lower incidence of highly destabilising breaks such as surface or band breaks (15% vs. 33%). The fatty acids appeared to help in the attachment of the aqueous tear film to the contact lens surface once the first break appears within the tear film.
- The dryness scores recorded in the clinic were used to partition the population into three groups according to their dry eye symptomatology. The lowest quartile group was characteristic of a population with high dry eye symptoms and the highest quartile of a population with low symptoms. The group defined by the median 50% of the population was representative of normal contact lens wearers with an average symptomatology. The dryness symptomatology scores recorded for each subject while they wore the test lenses was influenced by the ratio of polar vs. non-polar lipids (Table 6.14). The CHAID algorithm divided the population into four groups. The first three

groups showed no clinically significant differences but the population of the fourth group characterised by the highest measured level of fatty acids in the tear film showed a significant decrease in dryness symptomatology. 48% of the subjects in this group were classified as experiencing low dryness symptoms vs. only. 28% in the initial population. Further the percentage of subjects showing high dryness symptoms decrease significantly from 24% in the overall population to 14% in the population with high level of fatty acids.

Similarly the level of phospholipids/triglycerides (Peak 3) was statistically significantly higher (Table 6.15. p=0.020) between the low dryness symptoms group (Highest 25%) compared to other two groups.

The level of cholesterol ester in tear film was also found to be related to comfort score and wearer symptomatology level. A high level of cholesterol ester was associated with a lower comfort score. The population was divided into 3 groups according to their comfort score as follows: i) Lowest quartile characteristic of poor comfort (1), ii) Median quartile characteristic of a normal comfort (2), iii) Highest quartile characteristic of good comfort (3).

There was a statistically significant difference between the high and low comfort groups with a significantly higher level of cholesterol ester associated with the lowest comfort score and higher symptoms groups (Table 6.16).

Table 6.7. Overall pre lens tear film lipid composition - Descriptive statistics.

Lipid Identification	PEAK	Parameters	1/52		1/12	
			1-Day ACUVUE	DAILIES	1-Day ACUVUE	DAILIES
Cholesterol ester	1	Mean ± STD Median (Range)	53 ± 27 46 (21 → 126)	59 ± 62 46 (15 → 408)	108 ± 133 82 (0 → 874)	110 ± 85 85 (17 → 402)
	2	Mean ± STD Median (Range)	15 ± 16 16 (0 → 60)	12 ± 15 0 (0 → 51)	14 ± 23 0 (0 → 128)	14± 35 0 (0 → 242)
	2b	Mean ± STD Median (Range)	2 ± 5 0 (0 → 22)	1 ± 6 0 (0 → 38)	<1 ± 2 0 (0 → 16)	2 ± 4 0 (0 → 16)
Phospholipids/ triglycerides	3	Mean ± STD Median (Range)	19 ± 27 14 (0 → 177)	17 ± 16 15 (0 → 89)	17 ± 24 14 (0 → 129)	22 ± 39 15 (0 → 196)
Fatty acids	4	Mean ± STD Median (Range)	1 ± 6 0 (0 → 34)	1 ± 5 0 (0 → 24)	3 ± 10 0 (0 → 52)	4 ± 10 0 (0 → 44)
	5	Mean ± STD Median (Range)	0	< 1± 2 0 (0 → 12)	<1 ± 2 0 (0 → 13)	<1 ± 1 0 (0 → 9)
	6	Mean ± STD Median (Range)	2 ± 6 0 (0 → 36)	1 ± 3 0 (0 → 12)	2 ± 5 0 (0 → 21)	<1 ± 2 0 (0 → 11)
	7	Mean ± STD Median (Range)	0	0	<1 ± 2 0 (0 → 12)	<1 ± 2 0 (0 → 12)
	8	Mean ± STD Median (Range)	3 ± 8 0 (0 → 49)	3 ± 6 0 (0 → 26)	9 ± 8 8 (0 → 26)	10 ± 10 9 (0 → 41)
	9	Mean ± STD Median (Range)	<1 ± 2 0 (0 → 12)	<1 ± 1 0 (0 → 9)	2 ± 4 0 (0 → 16)	2 ± 4 0 (0 → 15)
	10	Mean ± STD Median (Range)	1 ± 3 0 (0 → 16)	1 ± 3 0 (0 → 13)	4 ± 6 0 (0 → 19)	4 ± 6 0 (0 → 20)
	11	Mean ± STD Median (Range)	6 ± 8 0 (0 → 29)	5 ± 7 0 (0 → 23)	11 ± 10 12 (0 → 32)	15 ± 16 14 (0 → 104)
	12	Mean ± STD Median (Range)	0	<1 ± 2 0 (0 → 12)	1 ± 4 0 (0 → 19)	0
	13	Mean ± STD Median (Range)	3 ± 6 0 (0 → 20)	3 ± 8 0 (0 → 41)	6 ± 8 0 (0 → 26)	11 ± 14 11 (0 → 71)
	14	Mean ± STD Median (Range)	0	1 ± 4 0 (0 → 28)	<1 ± 3 0 (0 → 24)	<1 ± 1 0 (0 → 7)
Monoglycerides	15	Mean ± STD Median (Range)	1 ± 4 0 (0 → 16)	2 ± 5 0 (0 → 15)	0	3 ± 12 0 (0 → 79)
Cholesterol	16	Mean ± STD Median (Range)	44 ± 7 44 (24 → 58)	44 ± 8 45 (21 → 63)	40 ± 10 41 (0 → 64)	41 ± 9 42 (21 → 58)

Table 6.8. Pre lens tear film lipid composition by group - Descriptive statistics.

- **Group 1: Poor performance with Dailies**

Lipid Identification	PEAK		1/52		1/12	
			1-Day ACUVUE	DAILIES	1-Day ACUVUE	DAILIES
Cholesterol ester	1	Mean ± STD Median (Range)	55 ± 31 50 (22 → 104)	70 ± 89 47 (20 → 408)	121 ± 110 90 (53→ 512)	110 ± 58 108 (36 → 208)
	2	Mean ± STD Median (Range)	12 ± 16 0 (0 → 42)	6 ± 11 0 (0 → 29)	15 ± 33 0 (0 → 128)	22 ± 60 0 (0 → 242)
	2b	Mean ± STD Median (Range)	0	<1 ± 1 0 (0 → 3)	0	2 ± 5 0 (0 → 16)
Phospholipids/ triglycerides	3	Mean ± STD Median (Range)	15 ± 7 13 (8 → 33)	17 ± 20 13 (0 → 89)	20 ± 28 15 (0 → 120)	23 ± 46 14 (0 → 196)
Fatty acids	4	Mean ± STD Median (Range)	0	1 ± 6 0 (0 → 24)	2 ± 8 0 (0 → 31)	6 ± 12 0 (0 → 44)
	5	Mean ± STD Median (Range)	0	0	0	1 ± 2 0 (0 → 9)
	6	Mean ± STD Median (Range)	1 ± 3 0 (0 → 9)	0	1 ± 4 0 (0 → 14)	1 ± 3 0 (0 → 11)
	7	Mean ± STD Median (Range)	0	0	0	1 ± 3 0 (0 → 12)
	8	Mean ± STD Median (Range)	3 ± 6 0 (0 → 19)	3 ± 6 0 (0 → 24)	8 ± 7 8 (0 → 21)	8 ± 10 6 (0 → 33)
	9	Mean ± STD Median (Range)	0	0	1 ± 3 0 (0 → 10)	1 ± 4 0 (0 → 13)
	10	Mean ± STD Median (Range)	0	0	2 ± 5 0 (0 → 13)	3 ± 6 0 (0 → 18)
	11	Mean ± STD Median (Range)	5 ± 7 0 (0 → 20)	2 ± 5 0 (0 → 18)	10 ± 8 13 (0 → 21)	19 ± 24 13 (0 → 104)
	12	Mean ± STD Median (Range)	0	0	2 ± 6 0 (0 → 19)	0
	13	Mean ± STD Median (Range)	3 ± 6 0 (0 → 17)	1 ± 3 0 (0 → 11)	2 ± 5 0 (0 → 13)	9 ± 9 11 (0 → 27)
	14	Mean ± STD Median (Range)	0	0	0	<1 ± 2 0 (0 → 7)
Monoglycerides	15	Mean ± STD Median (Range)	2 ± 4 0 (0 → 14)	3 ± 6 0 (0 → 15)	0	2 ± 6 0 (0 → 21)
Cholesterol	16	Mean ± STD Median (Range)	44 ± 6 44 (33 → 53)	43 ± 7 45 (24 → 52)	40 ± 9 40 (28 → 60)	39 ± 11 41 (22 → 58)

- **Group 2: Poor performance with 1-Day Acuvue**

Lipid Identification	PEAK	Parameters	1/52		1/12	
			1-Day ACUVUE	DAILIES	1-Day ACUVUE	DAILIES
Cholesterol ester	1	Mean ± STD Median (Range)	49 ± 19 46 (21 → 89)	58 ± 39 45 (15 → 123)	120 ± 205 60 (16→ 874)	132 ± 110 111 (17 → 402)
	2	Mean ± STD Median (Range)	18 ± 19 17 (0 → 60)	19 ± 17 20 (0 → 51)	12 ± 15 0 (0 → 35)	9 ± 14 0 (0 → 42)
	2b	Mean ± STD Median (Range)	1 ± 4 0 (0 → 15)	3 ± 11 0 (0 → 38)	0	1 ± 3 0 (0 → 13)
Phospholipids/ triglycerides	3	Mean ± STD Median (Range)	19 ± 20 14 (0 → 92)	17 ± 15 15 (0 → 56)	14 ± 13 12 (0 → 49)	14 ± 8 16 (0 → 26)
Fatty acids	4	Mean ± STD Median (Range)	1 ± 5 0 (0 → 20)	0	4 ± 13 0 (0 → 52)	3 ± 9 0 (0 → 32)
	5	Mean ± STD Median (Range)	0	0	1 ± 3 0 (0 → 13)	0
	6	Mean ± STD Median (Range)	4 ± 9 0 (0 → 36)	0	3 ± 5 0 (0 → 16)	0
	7	Mean ± STD Median (Range)	0	0	0	<1 ± 2 0 (0 → 9)
	8	Mean ± STD Median (Range)	4 ±12 0 (0 → 49)	3 ± 5 0 (0 → 12)	9 ± 8 11 (0 → 22)	14 ± 12 13 (0 → 41)
	9	Mean ± STD Median (Range)	0	0	2 ± 5 0 (0 → 14)	0
	10	Mean ± STD Median (Range)	1 ± 4 0 (0 → 16)	1 ± 3 0 (0 → 11)	5 ± 7 0 (0 → 19)	4 ± 7 0 (0 → 20)
	11	Mean ± STD Median (Range)	6 ± 8 0 (0 → 23)	6 ± 8 0 (0 → 23)	11 ± 11 10 (0 → 32)	13 ± 11 14 (0 → 43)
	12	Mean ± STD Median (Range)	0	1 ± 4 0 (0 → 12)	0	0
	13	Mean ± STD Median (Range)	2 ± 5 0 (0 → 20)	5 ± 12 0 (0 → 41)	8 ± 11 0 (0 → 26)	13 ± 20 0 (0 → 71)
	14	Mean ± STD Median (Range)	0	0	0	0
Monoglycerides	15	Mean ± STD Median (Range)	<1± 2 0 (0 → 7)	2 ± 4 0 (0 → 11)	0	6 ± 19 0 (0 → 79)
Cholesterol	16	Mean ± STD Median (Range)	45 ± 7 45 (32 → 58)	45 ± 10 48 (21 → 61)	42 ± 9 42 (31 → 64)	41 ± 8 42 (20 → 52)

- **Group 3: good performance with both contact lenses**

Lipid identification	PEAK	parameters	1/52		1/12	
			1-Day ACUVUE	DAILIES	1-Day ACUVUE	DAILIES
Cholesterol ester	1	Mean ± STD Median (Range)	56 ± 32 47 (23 → 126)	45 ± 23 44 (16 → 98)	85 ± 49 78 (26→ 171)	84 ± 70 53 (27 → 310)
	2	Mean ± STD Median (Range)	15 ± 14 18 (0 → 41)	14 ± 15 16 (0 → 41)	14 ± 19 0 (0 → 54)	11± 15 0 (0 → 43)
	2b	Mean ± STD Median (Range)	5 ± 8 0 (0 → 22)	1 ± 4 0 (0 → 10)	1 ± 4 0 (0 → 16)	2 ± 5 0 (0 → 16)
Phospholipids/ triglycerides	3	Mean ± STD Median (Range)	24 ± 42 14 (0 → 177)	17 ± 11 17 (0 → 41)	19 ± 29 13 (0 → 129)	31 ± 52 16 (0 → 196)
Fatty acids	4	Mean ± STD Median (Range)	2 ± 9 0 (0 → 34)	1 ± 5 0 (0 → 19)	3 ± 9 0 (0 → 34)	4 ± 9 0 (0 → 32)
	5	Mean ± STD Median (Range)	0	1 ± 4 0 (0 → 12)	1 ± 3 0 (0 → 11)	0
	6	Mean ± STD Median (Range)	2 ± 5 0 (0 → 19)	3 ± 4 0 (0 → 12)	3 ± 6 0 (0 → 21)	1 ± 2 0 (0 → 8)
	7	Mean ± STD Median (Range)	0	0	1 ± 3 0 (0 → 12)	0
	8	Mean ± STD Median (Range)	2 ± 4 0 (0 → 11)	3 ± 7 0 (0 → 26)	9 ± 9 8 (0 → 26)	7 ± 7 8 (0 → 20)
	9	Mean ± STD Median (Range)	1 ± 3 0 (0 → 12)	1 ± 2 0 (0 → 9)	1 ± 4 0 (0 → 16)	4 ± 6 0 (0 → 15)
	10	Mean ± STD Median (Range)	1 ± 4 0 (0 → 14)	2 ± 4 0 (0 → 13)	4 ± 7 0 (0 → 19)	3 ± 5 0 (0 → 14)
	11	Mean ± STD Median (Range)	6 ± 10 0 (0 → 29)	6 ± 8 0 (0 → 22)	11 ± 10 14 (0 → 31)	13 ± 10 15 (0 → 33)
	12	Mean ± STD Median (Range)	0	0	0	0
	13	Mean ± STD Median (Range)	4 ± 7 0 (0 → 20)	4 ± 8 0 (0 → 21)	7 ± 8 5 (0 → 21)	9 ± 10 6 (0 → 24)
	14	Mean ± STD Median (Range)	0	2 ± 8 0 (0 → 28)	1 ± 6 0 (0 → 24)	0
Monoglycerides	15	Mean ± STD Median (Range)	2 ± 4 0 (0 → 16)	1 ± 3 0 (0 → 11)	0	0
Cholesterol	16	Mean ± STD Median (Range)	43 ± 7 42 (24 → 57)	45 ± 9 45 (32 → 63)	38 ± 12 40 (0 → 53)	43 ± 9 45 (29 → 55)

Table 6.9. Pre lens tear lipid composition- Comparison for each contact lens type between good and poor performance by Mann-Whitney Exact Test.

	1-Day Acuvue Group 2 vs. 3		Dailies Group 1 vs. 3	
Peak	1/52	1/12	1/52	1/12
1	0.805	0.702	0.408	0.085
2	0.730	0.858	0.120	0.908
2b	0.126	1.000	0.347	0.867
3	0.831	0.778	0.305	0.495
4	0.743	0.868	1.000	0.552
5	1.000	0.743	0.179	1.000
6	0.351	0.949	0.026	1.000
7	1.000	0.487	1.000	1.000
8	0.877	0.937	0.545	0.868
9	0.471	0.587	0.433	0.187
10	1.000	0.746	0.179	0.864
11	0.911	0.965	0.139	1.000
12	1.000	1.000	1.000	1.000
13	0.149	0.947	0.202	0.992
14	1.000	1.000	0.433	1.000
15	0.350	1.000	0.225	0.484
16	0.448	0.580	0.556	0.266

Table 6.10. Pre lens tear lipid composition - Comparison for each group between contact lens types by Mann-Whitney Exact Test.

	Group1		Group2		Group3	
Peak	1/52	1/12	1/52	1/12	1/52	1/12
1	0.191	0.761	0.938	0.151	0.820	0.903
2	0.578	1.000	0.469	0.734	0.844	0.496
2b	1.000	0.500	0.500	1.000	1.000	1.000
3	0.542	0.960	0.438	0.639	0.129	0.426
4	1.000	0.625	1.000	1.000	1.000	0.875
5	1.000	1.000	1.000	1.000	1.000	1.000
6	0.500	1.000	0.500	0.250	0.500	0.500
7	1.000	1.000	1.000	1.000	1.000	1.000
8	0.813	0.455	0.750	0.110	1.000	0.497
9	1.000	0.750	1.000	0.250	1.000	0.578
10	1.000	0.844	1.000	0.807	1.000	0.578
11	0.383	0.850	0.438	0.903	0.688	0.787
12	1.000	0.500	1.000	1.000	1.000	1.000
13	0.250	0.047	1.000	0.677	0.500	0.770
14	1.000	1.000	1.000	1.000	1.000	1.000
15	0.875	0.500	1.000	0.500	1.000	1.000
16	0.946	0.670	0.375	1.000	0.652	0.463

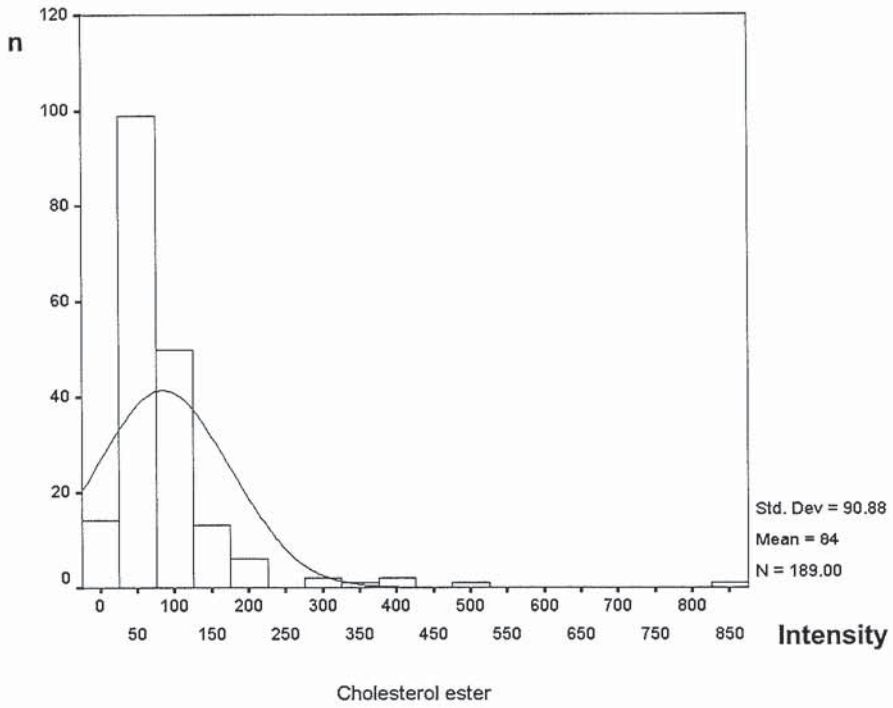


Figure 6.3a. Cholesterol ester distribution in the pre lens tear film. The continuous line surimposed over the histogram is the normal curve with same mean and variance as data.

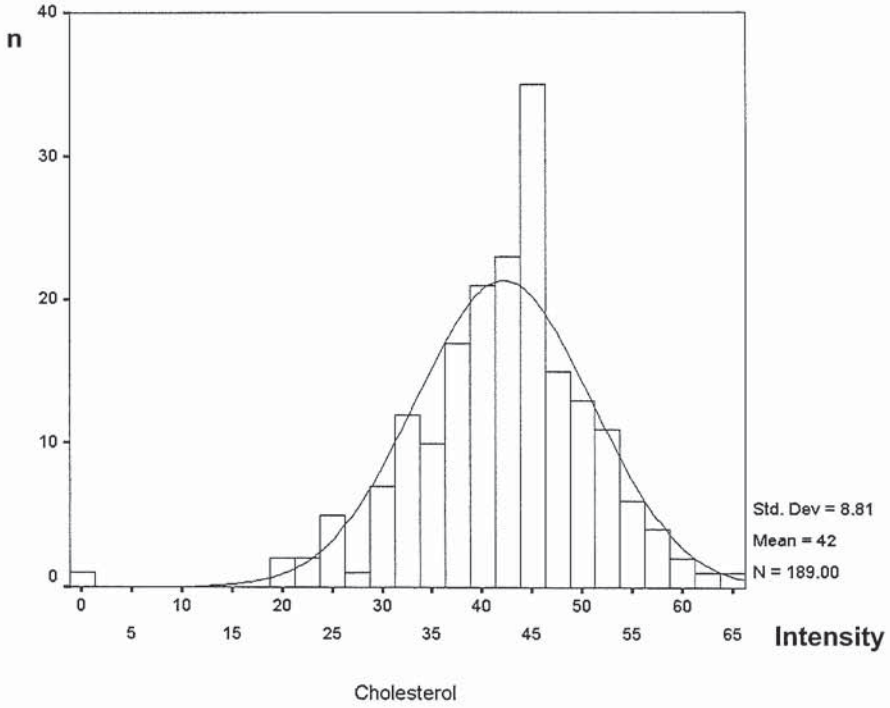


Figure 6.3b. Cholesterol distribution in the pre lens tear film. The continuous line surimposed over the histogram is the normal curve with same mean and variance as data.

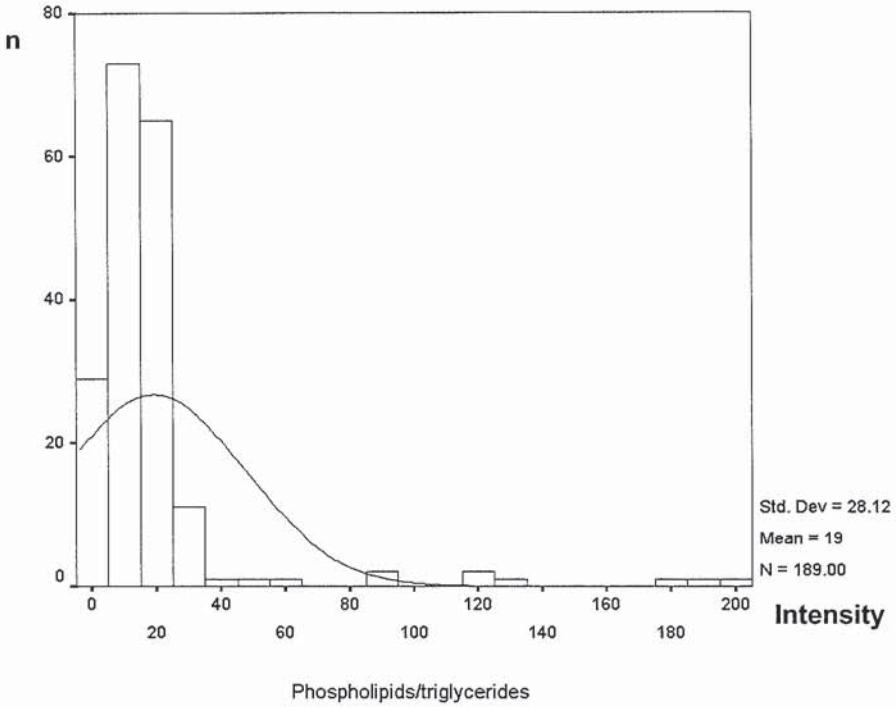


Figure 6.3c. Phospholipids/triglycerides distribution in the pre lens tear film. The continuous line surimposed over the histogram is the normal curve with same mean and variance as data.

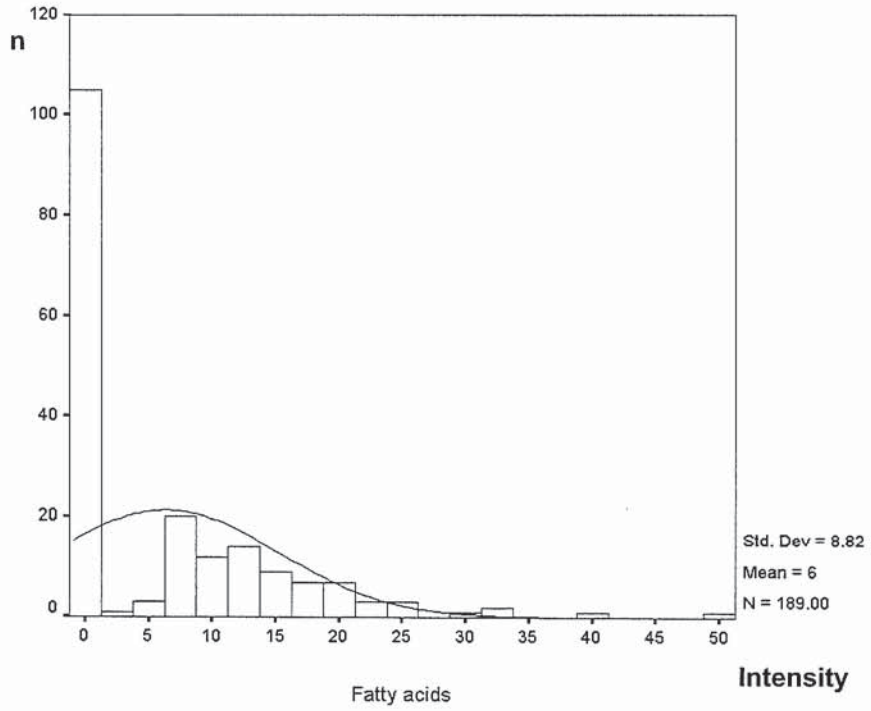


Figure 6.3d. Fatty acids distribution in the pre lens tear film. The continuous line surimposed over the histogram is the normal curve with same mean and variance as data.

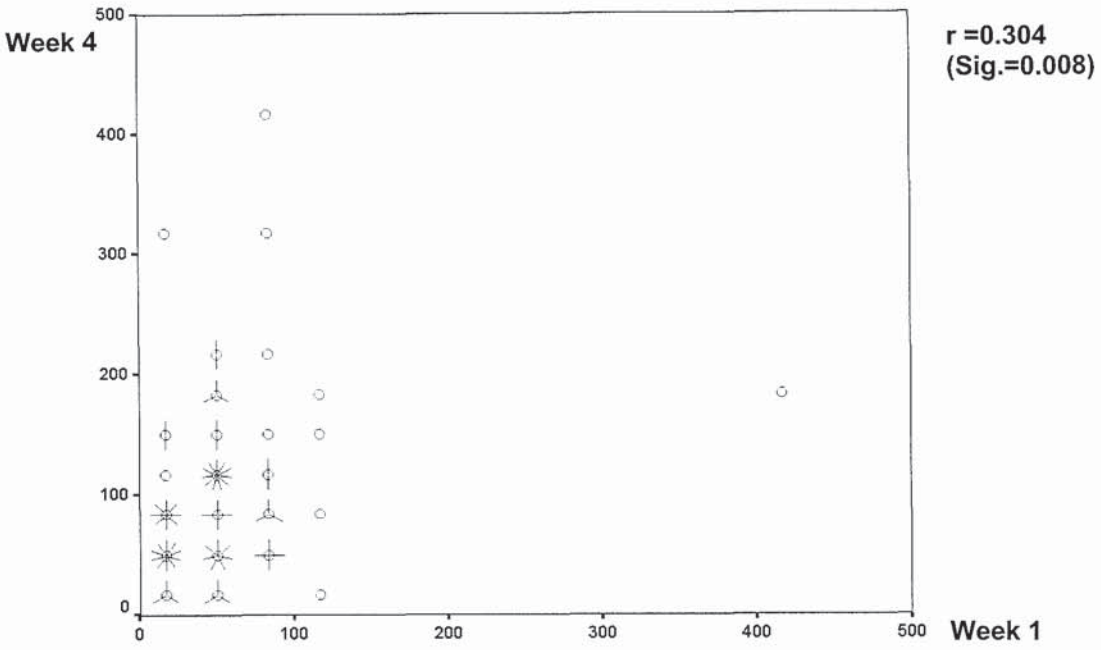


Figure 6.4a. Scatterplot of cholesterol ester levels after 1 week and 1 month of wear [+]

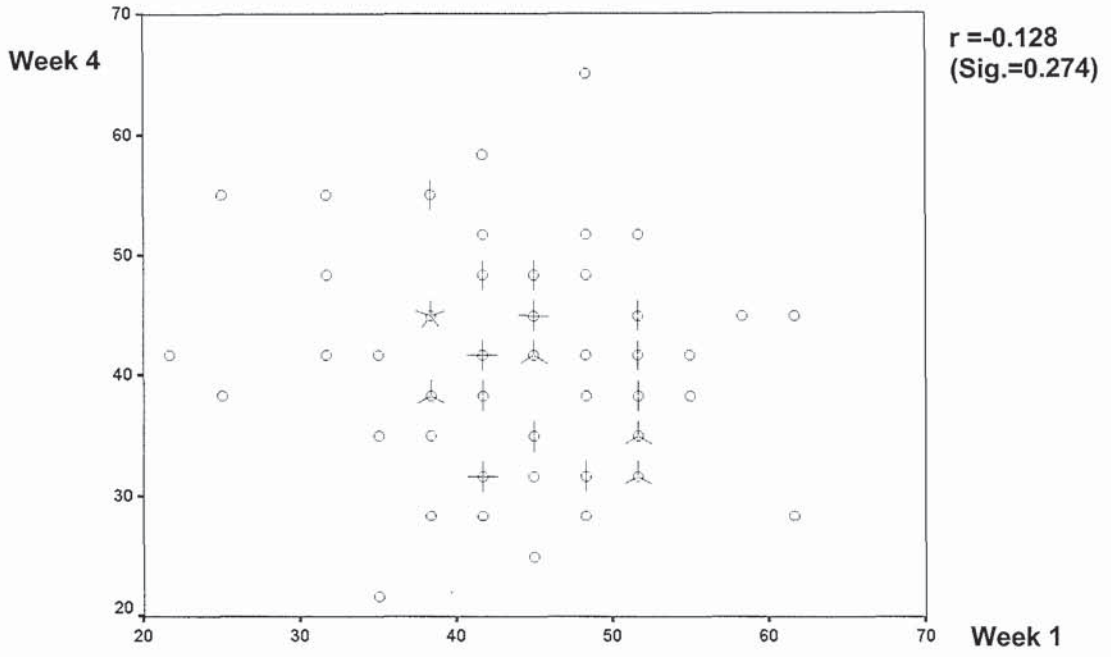


Figure 6.4b. Scatterplot of cholesterol levels after 1 week and 1 month of wear [+]

[+] Overlapping points are represented by 'sunflowers'. If a cell contains only one point, it is represented by a small circle; if a cell has more than one point each point is represented by a short line (a 'petal') originating from the circle.

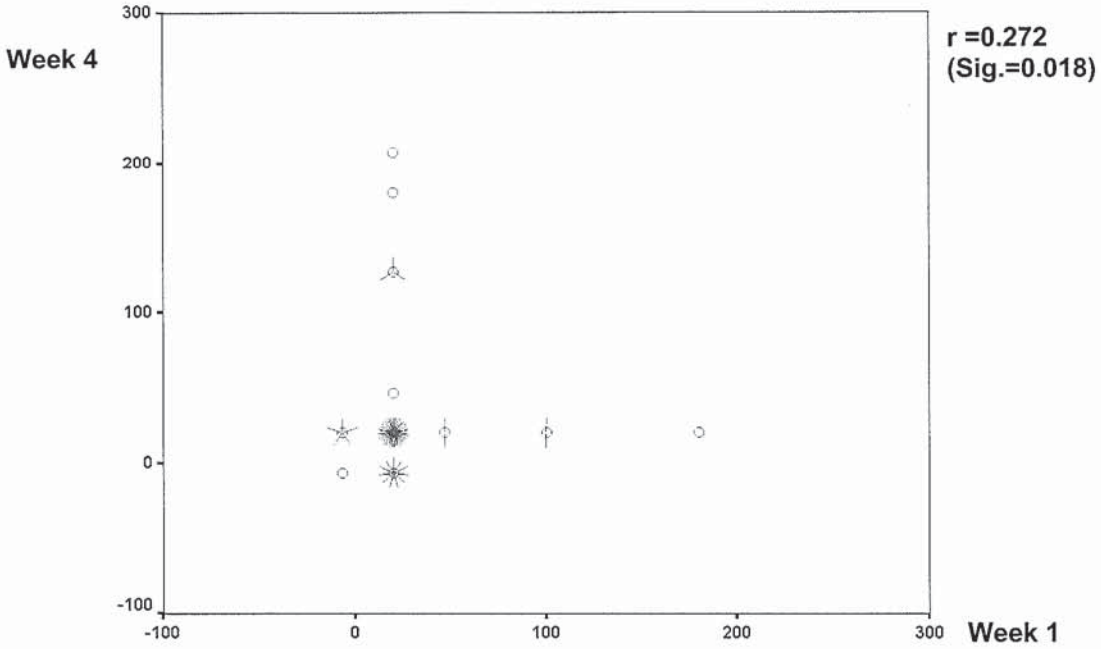


Figure 6.4c. Scatterplot of Phospholipids/triglycerides levels after 1 week and 1 month of wear[+]

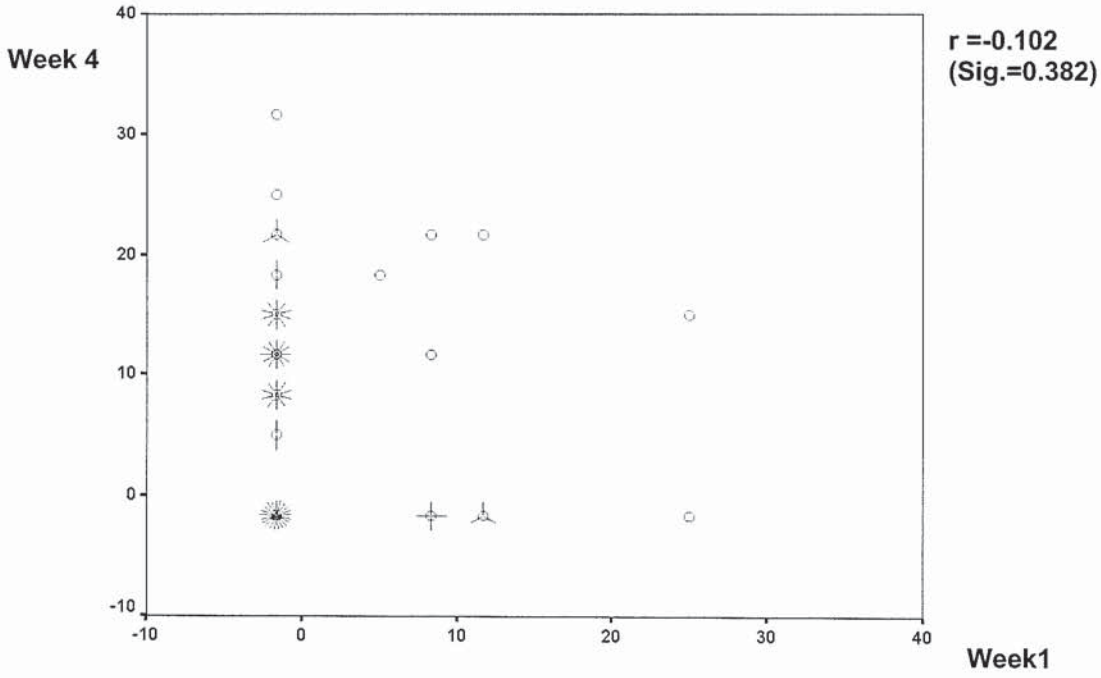


Figure 6.4d. Scatterplot of fatty acids levels after 1 week and 1 month of wear[+]

[+] Overlapping points are represented by 'sunflowers'. If a cell contains only one point, it is represented by a small circle; if a cell has more than one point each point is represented by a short line (a 'petal') originating from the circle.

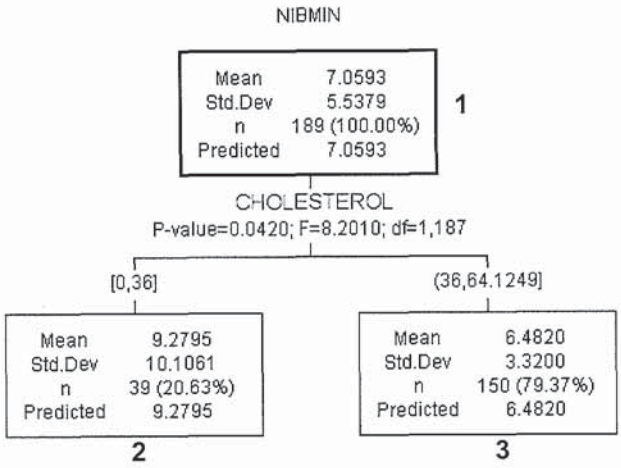


Table 6.11. Pre Lens Tear Film Non Invasive Break Up Time - Predictive analysis

Main predictor selected = CHOLESTEROL
Box 1= NIBUT values for the overall population
Box 2= NIBUT values for low levels of cholesterol (0 to 36)
Box 3= NIBUT values for high levels of cholesterol (>36)

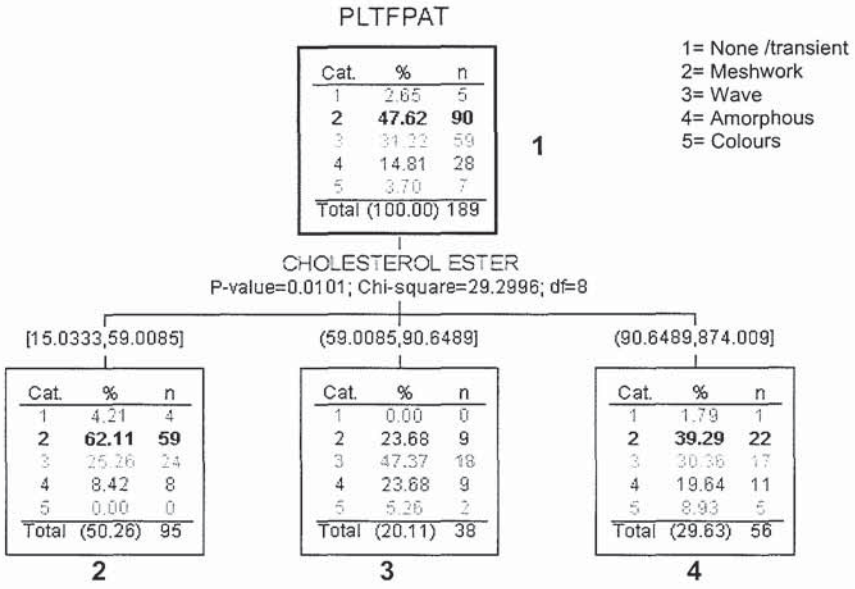


Table 6.12. Pre Lens Tear Film lipid layer mixing pattern - Predictive analysis

Main predictor selected = CHOLESTEROL ESTER
Box 1= PLTF lipid layer mixing pattern distribution for the overall population
Box 2= PLTF lipid layer mixing pattern distribution for low levels of cholesterol esters (15 to 59)
Box 3= PLTF lipid layer mixing pattern distribution for medium levels of cholesterol esters (>59 to 91)
Box 3= PLTF lipid layer mixing pattern distribution for high levels of cholesterol esters (>91 to 874)

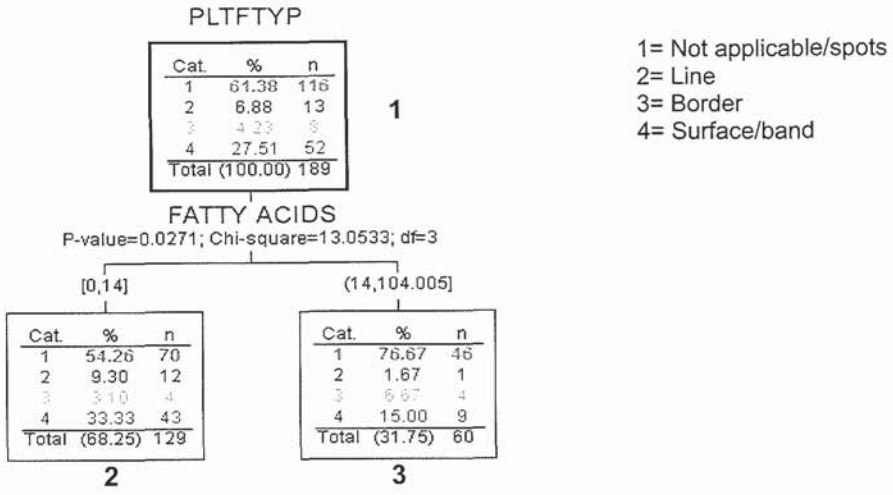


Table 6.13. Pre Lens Tear Break Up type - Predictive analysis

Main predictor selected = FATTY ACIDS
Box 1= PLTF break up type distribution for the overall population
Box 2= PLTF break up type distribution for low levels of fatty acids (0 to 14)
Box 3= PLTF break up type distribution for high levels of fatty acids (>14)

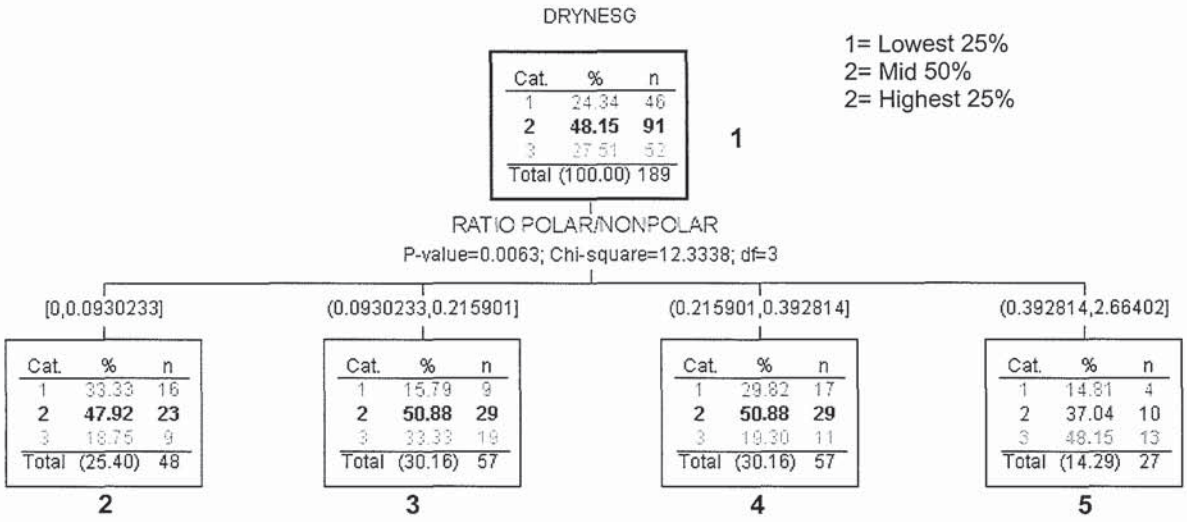


Table 6.14. Dryness symptomatology while wearing contact lenses - Predictive analysis

Main predictor selected = RATIO POLAR/NONPOLAR
Box 1= Dryness distribution for the overall population
Box 2,3&4= Dryness distribution for low ratio (0 to 0.39)
Box 5= Dryness distribution for high ratio (>0.39)

Table 6.15. Comparison between phospholipid/triglycerides level and dryness groups by One way ANOVA with SNK Test (* values joined by a continuous line are not statistically significant).

	1	2	3
Intensity	14	17	28
SNK (5%)*	_____		

1= Lowest 25%(Highest dryness symptoms)
2= Mid 50%
3=Highest 25% (Lowest dryness symptoms)

Table 6.16. Comparison between cholesterol ester level and comfort groups by One way ANOVA with SNK Test (* values joined by a continuous line are not statistically significant).

	3	2	1
Intensity	9	13	20
SNK (5%)*	_____		
		_____	

1= Lowest 25%
2= Mid 50%
3=Highest 25%

6.3.2. Lipid analysis of contact lens extracts

For each contact lens type, the lipid composition of contact lens extracts was analysed. The results obtained are reported overall and for each clinical group in Tables 6.17 & 6.19. The mean values of main lipid peaks were plotted on Fig.6.5a&b respectively for Dailies and Acuvue. A trend towards lower peak intensity values for most lipid classes was apparent for the group performing poorly with 1-Day compared to the group achieving good clinical performance. For Dailies, the lipid profile of both poor and well performing groups was similar. The main differences recorded were for one cholesterol peak (Peak 1) and one fatty acid (Peak 12). The intensity of Peak 1 seemed significantly higher in the poor performing group. On the opposite, for Peak 12, the intensity recorded was significantly lower for the poor performing group.

For each contact lens types, the nature of lipid deposited was compared for the well and poor performing groups was compared. For 1-Day Acuvue, there was no statistically significant differences between the two groups (Table 6.18. p=NS). A possible trend towards a difference was identified for only one peak. However, the recorded intensity of Peak 15 was slightly higher for the group achieving good performance compared to the group performing poorly with 1-Day Acuvue (Table 6.18. p=0.118, Median: 0 vs. 0, Mean: 3 vs. 8). For Dailies, there was a statistical trend towards a higher level of fatty acids for the well performing group. The difference was statistically significant for Peak 12 (Table 6.20. p=0.034, Median: 13 vs. 21). For three other fatty acids (Peaks 8, 11 and 12b)

the difference was not statistically significant, but a statistical trend was identified (Table 6.20. p=0.188,0.192 & 0.188).

The nature of lipid deposition with each contact lens type was also compared within each group. As expected, the group performing well with both contact lens types (Group 3) did not produce any difference in the nature of lipid deposition for the lipids extracted from both contact lens types.

In Group 1, which subjects performed poorly with Dailies, produced a statistically significant difference in the peak intensity of one of the fatty acids (Peak 12) measured in contact lens extracts (Table 6.21. p=0.051). A significantly lower intensity was found for Peak 12 with Dailies compared to 1-Day Acuvue.

In Group 2, which subjects performed poorly with 1-Day Acuvue, the only significant difference recorded was for another fatty acid (Peak 15) (Table 6.21. p=0.042) with significantly lower level with 1-Day Acuvue than with Dailies.

To test whether the nature of lipids deposited on the contact lens surface affected their clinical performance, the data was analysed using Answer Tree™ which test for interactions in small sample size groups. The interactions identified between the nature of lipids and the clinical performance were as follow:

- The type of tear film break observed was influenced by the level of cholesterol deposited on the contact lens (Peak 16) as follows (Table 6.22):

- The high cholesterol deposition group produced breaks classified as "None, spots or line" in 66.7% of cases and as "surface or bands" in 30.1%.

- The low cholesterol deposition group produced breaks which were "None, spots or line" breaks in 80.0% of cases and "surface or bands" breaks in 13.3%.

Hence, an increase in cholesterol deposition was associated with a lower incidence of slow destabilising breaks (80.0% vs. 66.7%) and a higher incidence of highly destabilising breaks (13.3% vs. 30.1%).

The group characterised by a low level of cholesterol deposition could be further discriminated by the deposition level of one of the fatty acids (Peak 12). The subgroup with a low deposition (low peak intensity) of Peak 12 was associated with a increase in the percentage of destabilising breaks (13.3% vs. 22.2%) and a decrease in slow destabilising breaks (80.0% vs. 66.7%) whereas the subgroup showing a higher deposition of Peak 12 was characterised by slow destabilising breaks such as "none or spots" type of breaks, in 100% of cases.

Symptomatology was also influenced by the nature of lipid deposition. The ratio of fatty acids and cholesterol esters deposited on contact lenses appeared to be the key influential factor for symptomatology (Table 6.23). An increase of this ratio was associated with a decrease in symptomatology; the incidence of asymptomatic contact lens wearers went

from 25.0% to 62.8% and that of symptomatic wearers from 75.0% to 37.3%.

The symptomatology for the high ratio group was also influenced by the level of another fatty acid (Peak 15). A higher level was characterised by a further decrease in symptomatology: in this subgroup, all subjects were asymptomatic. The group with a low ratio was further partitioned according to the intensity of phospholipids/triglycerides peak (Peak 4). The group with highest peak intensity was significantly less symptomatic and the population of the group with low peak intensity was distributed evenly between asymptomatic and symptomatic contact lens wearers, 52.5% asymptomatic and 47.5 % symptomatic.

Table 6.17. Lipid composition of 1-Day Acuvue contact lens extracts - Descriptive statistics

Lipid identification	Peak	Parameters	Overall	Group 1	Group 2	Group 3
Cholesterol ester	Peak 1	Mean ± STD Median (Range)	72 ± 54 57 (14-290)	89 ± 73 69 (14-290)	59 ± 38 46 (17-158)	69 ± 42 55 (18-148)
	Peak 2	Mean ± STD Median (Range)	23 ± 27 14 (0-100)	30 ± 31 24 (0-100)	15 ± 18 11 (0-50)	24 ± 29 7 (0-84)
Phospholipids/ Triglycerides	Peak 4	Mean ± STD Median (Range)	2 ± 6 0 (0-27)	4 ± 9 0 (0-27)	0.0 ± 0.0 0 (0-0)	1 ± 4 0 (0-18)
Fatty acids	Peak 8	Mean ± STD Median (Range)	2 ± 6 0 (0-23)	4 ± 7 0 (0-19)	2 ± 6 0 (0-23)	1 ± 4 0 (0-18)
	Peak 11	Mean ± STD Median (Range)	4 ± 9 0.0 (0-48)	4 ± 7 0 (0-21)	2 ± 6 0 (0-25)	6 ± 13 0 (0-48)
	Peak 12	Mean ± STD Median (Range)	17 ± 12 17 (0-55)	21 ± 10 21 (0-40)	14 ± 11 16 (0-32)	18 ± 16 13.4 (0-55)
	Peak 15	Mean ± STD Median (Range)	6 ± 10 0 (0-47)	7 ± 13 0 (0-47)	3 ± 6 0 (0-17)	8± 11 0 (0-35)
Monoglycerides	Peak 16o	Mean ± STD Median (Range)	11 ± 10 12 (0-31)	11 ± 11 13 (0-31)	9 ± 10 11 (0-29)	12 ± 8 13 (0-27)
Cholesterol	Peak 16	Mean ± STD Median (Range)	78 ± 33 70 (20-140)	84 ± 37 89 (20-129)	74 ± 32 62 (39-138)	77 ± 29 68 (40-140)

Table 6.18. Comparisons between good (group 3) and poor (group 2) performance with 1-Day Acuvue by Mann-Whitney Test

	P value
Peak 1	0.486
Peak 1b	1.000
Peak 2	0.570
Peak 2b	0.968
Peak 4	0.317
Peak 8	0.553
Peak 10	1.000
Peak 11	0.354
Peak 12o	0.317
Peak 12	0.762
Peak 12b	0.598
Peak 15	0.113
Peak 15b	0.509
Peak 16o	0.430
Peak 16	0.613

Table 6.19. Lipid composition of Dailies contact lens extracts - Descriptive statistics

Lipid identification	Peak	Parameters	Overall	Group 1	Group 2	Group 3
Cholesterol ester	Peak 1	Mean ± STD Median (Range)	93 ± 84 49 (11-315)	123 ± 99 86 (17-315)	68 ± 62 43 (11-257)	91± 85 45 (15-288)
	Peak 2	Mean ± STD Median (Range)	23 ± 29. 14 (0-132)	24 ± 38 5.6 (0-132)	26 ± 27 24 (0-88)	19 ± 24 14 (0-73)
Phospholipids/ Triglycerides	Peak 4	Mean ± STD Median (Range)	4 ± 8 0 (0-33)	3 ± 9 0 (0-33)	4 ± 8 0 (0-25)	4 ± 7 0 (0-19)
Fatty acids	Peak 8	Mean ± STD Median (Range)	1 ± 3 0 (0-15)	0 ± 0 0 (0-0)	1 ± 3 0 (0-11)	2± 5 0 (0-15)
	Peak 11	Mean ± STD Median (Range)	3 ± 8 0 (0-32)	1 ± 3 0 (0-14)	4 ± 10 0 (0-32)	4 ± 7 0 (0-23)
	Peak 12	Mean ± STD Median (Range)	15 ± 12 16 (0-48)	11 ± 11 13 (0-28)	13 ± 9 18 (0-26)	21 ± 13 21 (0-48)
	Peak 15	Mean ± STD Median (Range)	9 ± 10 5 (0-40)	10 ± 10 11 (0-29)	9 ± 9 12 (0-21)	8± 11 0 (0-40)
Monoglycerides	Peak 16o	Mean ± STD Median (Range)	9 ± 10 8 (0-31)	8 ± 10 0 (0-27)	11 ± 9 14 (0-31)	8 ± 10 0 (0-29)
Cholesterol	Peak 16	Mean ± STD Median (Range)	76 ± 35 69 (0-135)	79 ± 33 86 (20-123)	70 ± 35 61 (0-124)	79 ± 38 82 (17-135)

Table 6.20. Comparisons between good (group 3) and poor (group 1) performance with Dailies by Mann-Whitney Test

	P value
Peak 1	0.337
Peak 1b	0.869
Peak 2	0.944
Peak 2b	1.000
Peak 4	0.448
Peak 8	0.188
Peak 10	0.934
Peak 11	0.192
Peak 12o	0.449
Peak 12	0.034
Peak 12b	0.188
Peak 15	0.345
Peak 15b	0.934
Peak 16o	0.971
Peak 16	0.947

Table 6.21. Lipid composition of contact lens extracts - Comparison between two lens types for each group by Wilcoxon Matched-Pairs Signed Ranks Test.

	Group 1	Group 2	Group 3
Peak 1	0.528	0.791	0.431
Peak 1b	1.000	1.000	1.000
Peak 2	0.762	0.348	0.519
Peak 2b	1.000	1.000	1.000
Peak 4	1.000	0.250	0.219
Peak 8	0.250	1.000	1.000
Peak 10	1.000	1.000	1.000
Peak 11	0.375	0.625	0.813
Peak 12o	1.000	1.000	0.250
Peak 12	0.051	0.934	0.548
Peak 12b	1.000	0.500	1.000
Peak 15	0.301	0.042	0.685
Peak 15b	1.000	0.250	1.000
Peak 16o	0.635	0.708	0.193
Peak 16	0.860	0.821	0.782

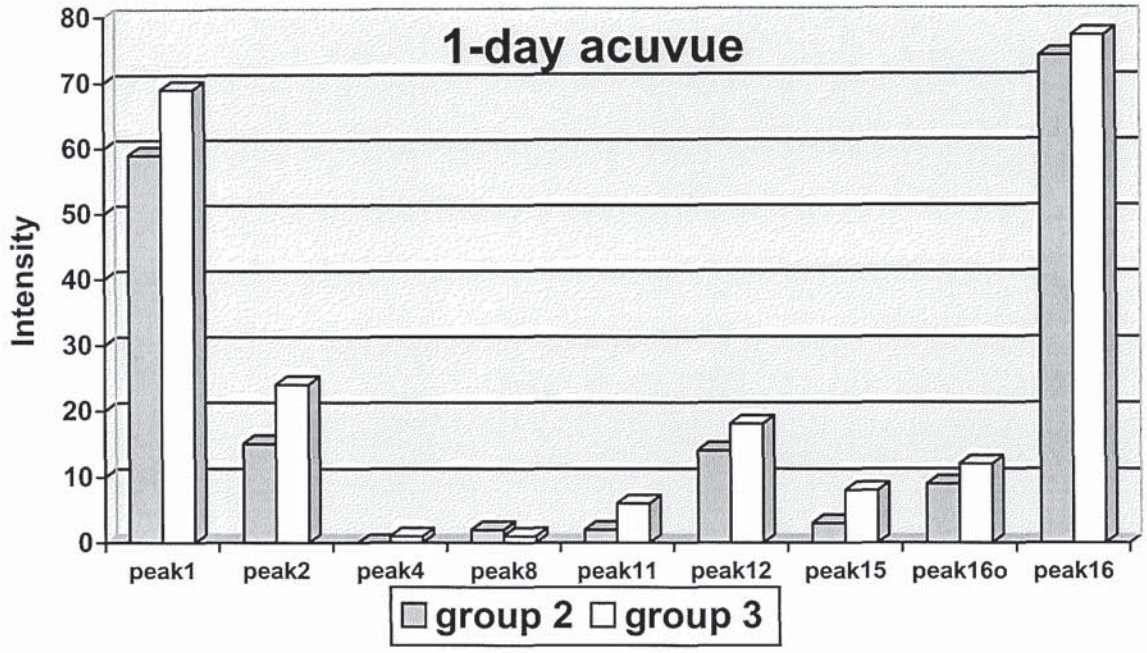


Figure 6.5a. Lipid composition of 1-Day Acuvue contact lens extracts for good (group 3) and poor (group2) performance groups

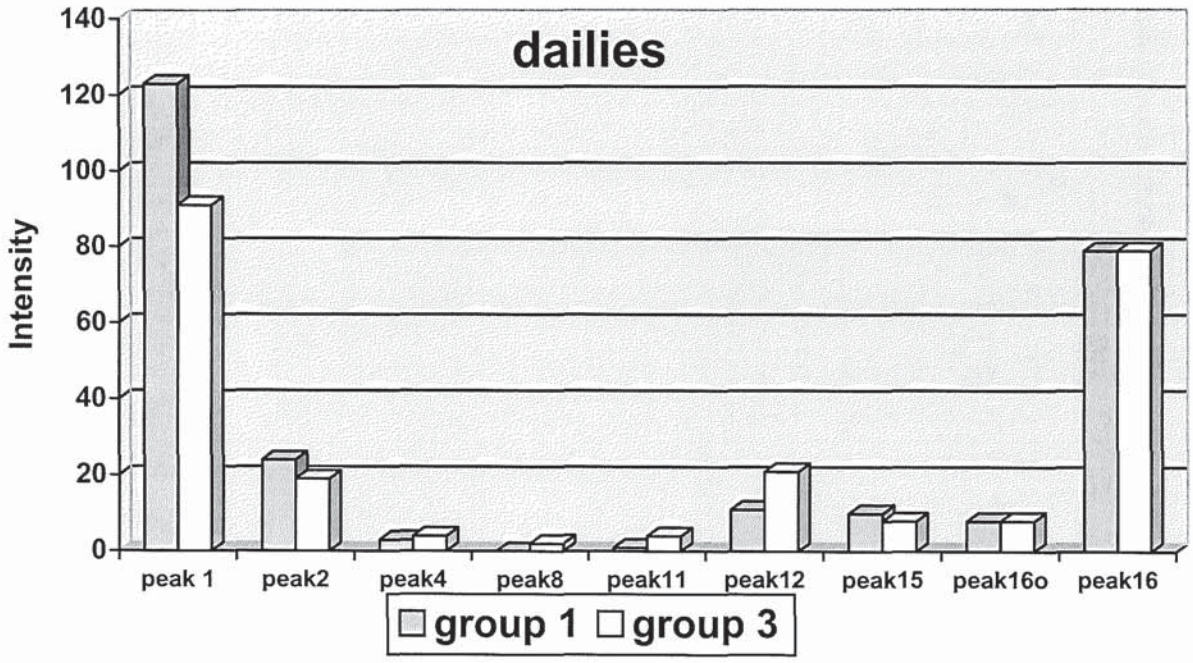


Figure 6.5b. Lipid composition of Dailies contact lens extracts for good (group 3) and poor (group1) performance groups

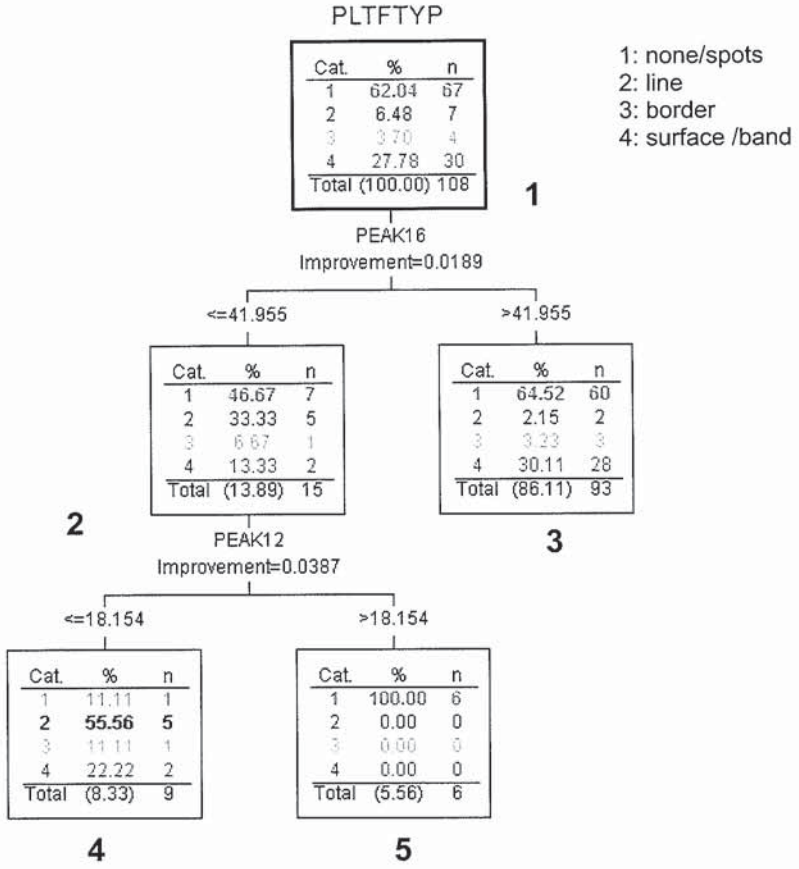


Table 6.22. PLTF break up type - Predictive analysis using C&RT

Main predictors = Peak 16 & Peak12

Box 1 = PLTF break up type for overall population
Box 2 = PLTF break up type for low level of peak 16
Box 3 = PLTF break up type for high level of peak 16
Box 4 = PLTF break up type for low level of peak 16 & low level of peak 12
Box 5 = PLTF break up type for low level of peak 16 & high level of peak 12

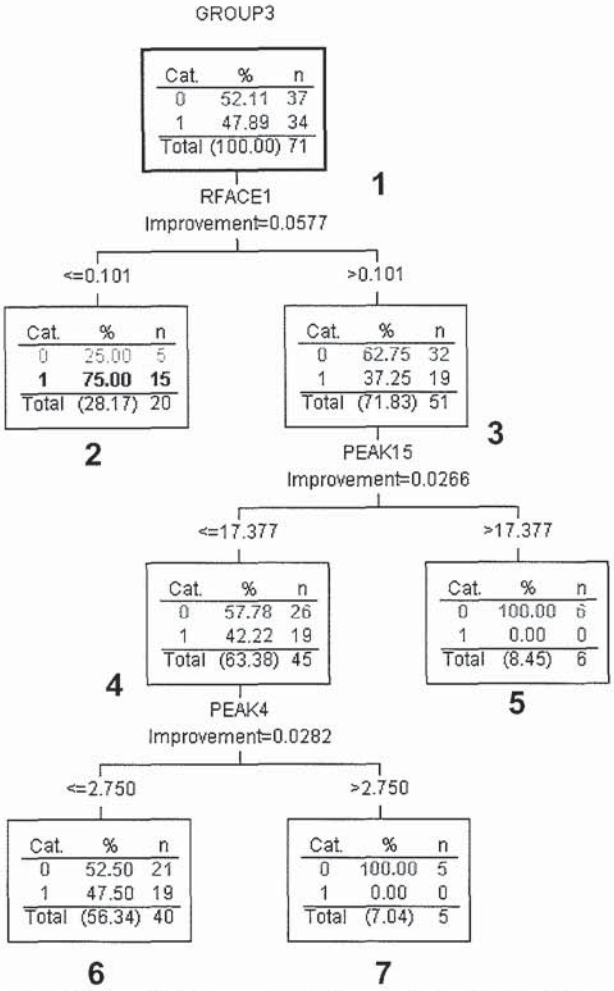


Table 6.23. Symptomatology prediction using nature of lipid deposition - Predictive analysis using C& RT

Main predictors = Ratio fatty acids/cholesterol esters, Peak 15 & Peak4

Box 1 = Symptomatology for overall population
Box 2 = Symptomatology for low ratio fatty acids/cholesterol esters
Box 3 = Symptomatology for high ratio fatty acids/cholesterol esters
Box 4 = Symptomatology for high ratio fatty acids/cholesterol esters & low level of peak 15
Box 5 = Symptomatology for high ratio fatty acids/cholesterol esters & high level of peak 15
Box 6 = Symptomatology for high ratio fatty acids/cholesterol esters & low level of peak 15 & low level of peak 4
Box 7 = Symptomatology for high ratio fatty acids/cholesterol esters & low level of peak 12 & high level of peak 4

6.3. Discussion

The analysis by HPLC of different types of lipids present in the pre ocular and pre lens tear film or deposited onto the contact lens for population groups with various symptomatology levels enabled us to reveal the influence of lipids on contact lens wearers symptomatology. When looking at the symptomatology the key lipids seemed to be the fatty acids and cholesterol esters. High levels of fatty acids in the pre ocular tear and in the contact lens extracts were both associated with a decrease in symptomatology. In addition the symptomatology of contact lens wearers was influenced by the nature of lipids found on their contact lenses: a high ratio of fatty acids vs. cholesterol esters favoured a decrease in symptoms.

On the opposite, high levels of cholesterol esters seemed to have a deleterious effect on symptomatology and were associated with a decrease in comfort during contact lens wear.

The levels of dryness symptoms reported by the subjects were affected by the ratio of polar vs. non-polar lipids in tears: a high ratio was correlated with significantly lower dryness symptoms.

As the sample size was larger in this investigation, it was possible using Answer Tree^{TM} to find multiple interactions between the tear film clinical characteristics, and the type of lipids present in the tear film or on the contact lens. These interactions could be summarised as follow:

- The lipid layer mixing patterns observed in the pre ocular tear film were most influenced by the level of phospholipids/triglycerides measured in tears. A high level was associated with a thicker and more even lipid layer and therefore probably a more stable tear film.
- When observing the lipid layer mixing patterns of pre lens tear film, the most influential factor was the level of cholesterol esters. The interaction between the two factors was complex: a median level of cholesterol ester was associated with the thickest lipid layer. A higher level generated an increase in contamination of this now uneven lipid layer characterised by an alternate of thicker and thinner zones. A lower level of cholesterol produced a generally thinner and even lipid layer.
- The tear film break up type was affected by the level of fatty acids in the tear film. The presence of fatty acids facilitated in the attachment of the aqueous part of the tear film over the contact lens area once the first break had happened. The tear film break up type was also affected by the type and level of lipids found on the contact lens. At the opposite of fatty acids, a high deposition of cholesterol on the contact lens surface was associated with a more rapid destabilisation of the tear film once the first break has appeared.
- The tear film stability, measured by the NIBUT, was most influenced by the level of cholesterol in the tear film: a high level was associated with a significantly shorter NIBUT.

6.4. Conclusion

The results obtained from this investigation led to the following conclusions:

i. The nature of lipids present on the contact lenses influenced the subjects symptomatology during wear as follows:
   - A high amount of fatty acids was associated with a decrease in symptomatology;
   - A high level of cholesterol esters deposited was associated with an increase in symptoms and a decrease in overall comfort;
   - Finally, a high ratio of fatty acids vs. cholesterol esters on the contact lenses was associated with a decrease in symptoms.

ii. Additionally, the influence of the lipid composition of the pre ocular and pre lens tear film on subjects' symptomatology could be summarised as follows:
   - A high level of fatty acids in the POTF was correlated with a decrease in symptoms;
   - And a high ratio of polar vs. non-polar lipids in the PLTF was associated with a decrease in the incidence of dryness symptoms.

iii. The key findings in the interaction between tear lipid composition, lipid deposition and the clinical characteristics of the tear film are reported below:
   - A high level of phospholipids/triglycerides in the POTF was in favour of a thicker and more even lipid layer and therefore a more stable tear film;

- In the PLTF, the thickness of the lipid layer was most influenced by the level of cholesterol esters;
- The stability of the PLTF was correlated to the concentration of cholesterol in the tears;
- The presence of fatty acids in the PLTF maintained the attachment of the aqueous part of the tear film over the contact lens after the first dry spot had appeared;
- The presence of cholesterol ester deposited on contact lenses was associated with an easier and more rapid destabilisation of the tear film over the contact lens surface.

CHAPTER 7

CONCLUSION AND FUTURE WORK

The aim of this work was to identify biochemical markers present in the tear film and/or at the surface of the worn contact lenses that would enable to classify contact lens wearers according to their performance. The rationale for the work was that in the long term biochemical kits could be develop to be used by clinicians to i) identify candidate contact lens wearers that would be likely to experience problems; ii) select contact lens material best suited for individual contact lens wearers.

The first part of this work studied the influence of proteins and lipids deposition on the contact lens performance and on contact lens wearer's subjective response. The results confirmed the material and time dependence of protein and lipid deposition on contact lenses and unveiled some characteristics of lipid deposition that could be basis for future work:

- FDA group IV materials deposit mainly protein due to their ionic charges. The level of deposition is strictly correlated with the percentage of ionic groups present.
- In the ionic group IV materials, the proteins move progressively into the contact lens matrix, depending on its degree of ionicity, and the deposition increases with time until a plateau occurs.
- FDA Group IV materials such as Focus deposit also high levels of lipids, due to the presence of N-vinyl pyrrolidone in its composition.
- At the opposite of proteins, which keep their lability once in contact with the contact lens, the lipid species are immobilised to a large extent when

deposited on a contact lens. This immobilisation is responsible for the significant difference in deposition between the front and back surface of a contact lens. In most published studies dealing with lipid deposition, no reference has been made as to whether the deposition measured was front or back surface due to the limitation of techniques available. The advantage of Fluorescence spectrophotometry, which was used, had the advantage to allow studying separately the deposition at the front and back surfaces of a contact lens. This investigation confirmed that most of lipid deposition happens at the front of the contact lens. However the deposition on the back surface even in lower amounts may also have significant clinical implications as the posterior surface is in contact with an area where the tear flow is reduced. The presence of high levels of lipids may decrease lubricity, hence decreasing tear exchange and/or increasing the attachment of hydrophobic components to the lens surface.

- Total protein and lipid depositions were poorly correlated with the clinical performance ratings or the subjects' symptomatology.

The second and main part of this work hence focused on studying the nature of the lipid species present in the tear film or found on contact lens surfaces. A similar analysis of the proteins could not be undertaken due to time restriction but is currently under investigation as part of a post graduate research project at the University of Aston.

Different lipid families were found to influence the subject's symptomatology and other aspects of contact lens clinical performance. The investigations that studied these possible markers led to the following general conclusions, regarding symptomatology, tear film structure and ocular integrity.

Symptomatology

1. The symptomatology was influenced by the tear lipid composition as follows:
    - Symptomatology was significantly higher when monoglycerides were present in high concentration in the tear film;
    - The presence of fatty acids in higher concentration either in the pre ocular tear film or in the pre lens tear film in higher concentration was on the opposite associated with a decrease symptomatology characterised by higher comfort scores and lower dryness symptoms;
    - Additionally, a high ratio of polar vs. non-polar lipids was associated with lower dryness symptoms.
2. The symptomatology was influenced by the nature of lipids deposited on contact lens:
    - Deposition of fatty acids was associated with a decrease in symptomatology;
    - Deposition of cholesterol esters generated an increase in symptoms and a decrease in comfort;
    - Consequently symptomatology was highly influenced by the ratio of fatty acids vs. cholesterol esters: a higher ratio was associated with lower symptoms;
    - Finally, deposition of phospholipids/triglycerides was correlated with higher comfort.

Tear film structure

1. The tear film structure was influenced by the tear lipid composition:

- The tear film stability was related to the ratio of phospholipids vs. cholesterol esters: an increase in ratio was associated with a more stable tear film. Additionally, the Pre Lens Tear Film (PLTF) stability was the lowest when a high level of cholesterol was present in the tear film;
- The thickness of the lipid layer was directly correlated to the level of phospholipids/triglycerides found in the Pre Ocular Tear Film (POTF) and inversely correlated to the concentration of cholesterol esters in PLTF during contact lens wear;
- The fatty acids were shown to facilitate attachment of the aqueous part of the tear film to the contact lens surface, increasing the incidence of slow destabilising breaks.

2. The tear film structure was influenced by the nature of lipids deposited:
   - The pre lens tear film stability recorded was lowest when the amount of cholesterol ester deposited was the highest;
   - Deposition of cholesterol had a tendency to facilitate the destabilisation of the PLTF once the first dry spot has appeared, whereas the deposition of monoglycerides increased the incidence of slow destabilising breaks.

Whereas the symptomatology was affected by both the tear film lipid composition and the nature of the lipid deposition, the structure of the tear film and its stability were mainly influenced by the tear film lipid composition.

Ocular integrity

The ocular integrity also appeared to be influenced by the nature of the lipid deposition. The presence of cholesterol esters on the contact lens was associated with an increase in hyperaemia, but this deleterious effect could be limited by the presence of fatty acids.

Study implication

This investigation enabled us to identify some possible markers within the lipid species. Particular interests should be paid to fatty acids, cholesterol esters or cholesterol itself and phospholipids. The technique chosen for this study only enabled a general separation of the main lipid families. By using a different separative HPLC column, it would be possible to separate further the different lipids and for instance to separate and identify the various fatty acids present within the fatty acid family.

Hence further applications for clinicians could be as follows:

- When required in order to identify a problematic wearer or to match the contact lens material to the contact lens wearer, tear samples collected by the clinician could be dispatched to an analytical laboratory where lipid analysis could be carried out by HPLC.
- A colorimetric kit based on the lipid markers could also be developed and used by clinician directly in the practice; such a kit would involve tear sampling and classification according to the colour into " Problem", " Border line " and "Good" contact lens wearers groups.

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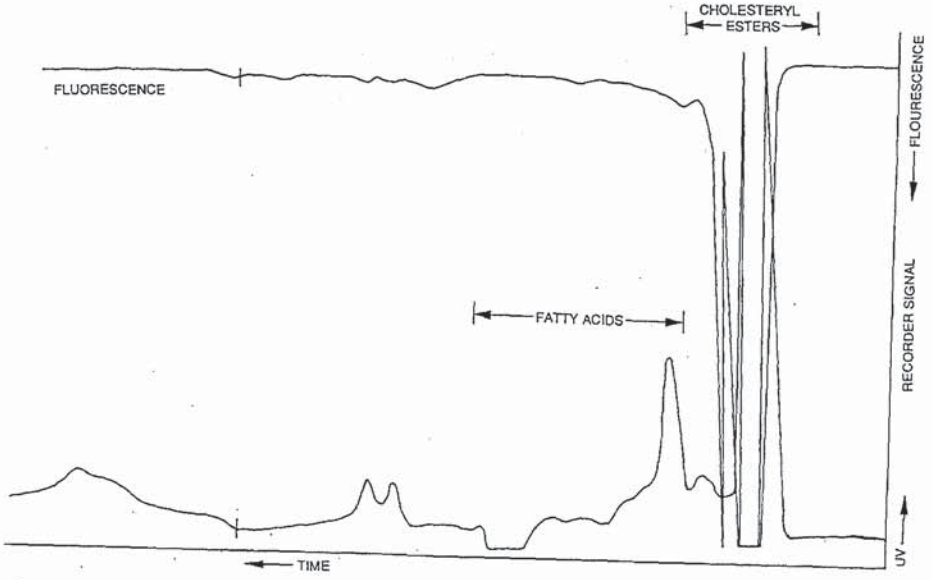
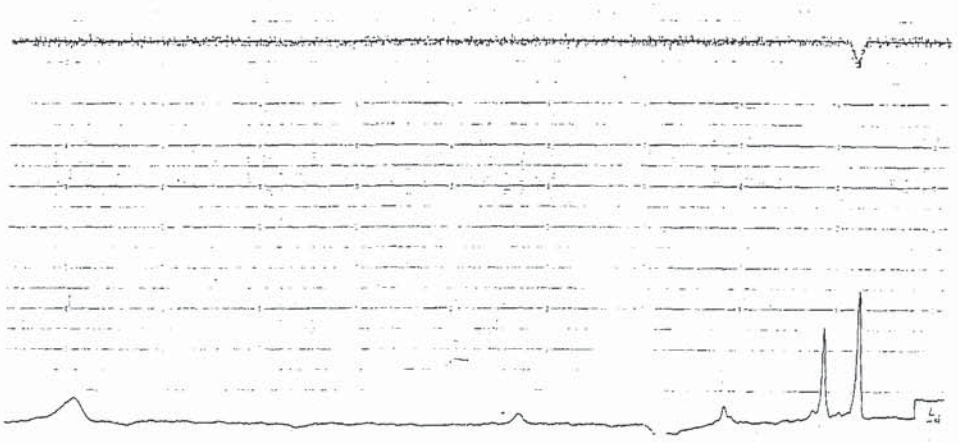
Appendix A



Lipid profile



Recorded trace

Appendix B

Mean, standard deviation, range and repeatability of UV measurements for unworn contact lenses.

- Acuvue

	Acuvue	Acuvue	Acuvue	Acuvue	Acuvue	Acuvue
	+3.75	-0.5	-2	-5.25	-5.25	-6
Mean	0.0266	0.0175	0.0222	0.0256	0.0264	0.0271
Minimum	0.024	0.015	0.020	0.023	0.023	0.023
Maximum	0.03	0.023	0.024	0.031	0.03	0.032
StdDev	0.0019	0.00227	0.00148	0.00263	0.00196	0.00302
R%	16.1	29.3	15.1	23.2	16.8	25.2
Rmean(%)	21.6					
n10%	5					
n20%	2					

	Acuvue(tinted)	Acuvue(tinted)
	-2.5	-2.5
Mean	0.0444	0.0578
Minimum	0.04	0.052
Maximum	0.048	0.065
StdDev	0.00250	0.00382
R(%)	12.7	14.9
Rmean(%)	13.9	
n10%	2	
n20%	1	

- NewVues

	NewVues	NewVues	NewVues	NewVues	NewVues	NewVues
	-1.25	-2	-2.75	-3	-4	-4.75
Mean	0.1035	0.1258	0.0915	0.0877	0.1109	0.1015
Minimum	0.096	0.114	0.068	0.075	0.095	0.086
Maximum	0.112	0.139	0.108	0.1	0.126	0.115
StdDev	0.00519	0.00764	0.01101	0.01048	0.00956	0.00923
R(%)	11.3	13.7	27.2	27	19.5	20.6
Rmean(%)	20.8					
n10%	5					
n20%	2					

- Excelens

	Excelens	Excelens	Excelens	Excelens	Excelens	Excelens
	-0.5	-0.75	-1	-2	-2	-3
Mean	0.2057	0.1952	0.206	0.2108	0.2556	0.1293
Minimum	0.198	0.18	0.192	0.192	0.245	0.119
Maximum	0.211	0.216	0.22	0.233	0.267	0.139
StdDev	0.00481	0.01144	0.00925	0.01268	0.00688	0.00646
R	0.01087	0.02585	0.020905	0.0286568	0.0155488	0.0145996
R(%)	5.3	13.2	10.1	13.6	6.1	11.3
Rmean(%)	10.5					
n10%	2					
n20%	1					

- SeeQuence2

	SeeQ2	SeeQ2	SeeQ2	SeeQ2	SeeQ2	SeeQ2
	-0.75	-1.75	-2	-2.75	-3.25	-3.5
Mean	0.0457	0.0454	0.0454	0.0487	0.0575	0.0599
Minimum	0.042	0.04	0.043	0.044	0.051	0.055
Maximum	0.05	0.063	0.053	0.057	0.062	0.069
StdDev	0.00241	0.0067	0.00317	0.00437	0.00363	0.00396
R(%)	11.9	33.4	15.8	20.3	14.3	14.9
Rmean(%)	19.8					
n10%	4					
n20%	1					

- SeeQuence

	SeeQ	SeeQ	SeeQ
	-2.75	-3	-4
Mean	0.0429	0.0442	0.0487
Minimum	0.039	0.037	0.038
Maximum	0.056	0.056	0.060
StdDev	0.00526	0.00621	0.00726
R	0.0118876	0.0140346	0.0164076
R(%)	27.710023	31.752489	33.69117
Rmean(%)	31.15103		
n10%	10		
n20%	3		

- Focus

	Focus	Focus	Focus
	-1.25	-2.75	-3.25
Mean	0.1729	0.1575	0.1986
Minimum	0.144	0.142	0.177
Maximum	0.197	0.185	0.22
StdDev	0.01456	0.01428	0.0126
R(%)	19	20.5	14.3
Rmean(%)	18.1		
n10%	4		
n20%	1		

Appendix C

Mean, standard deviation, range and repeatabillity of UV measurements for worn contact lenses.

- Acuvue

	Acuvue	Acuvue
	-6	-2
Mean	0.3744	0.123
Minimum	0.366	0.118
Maximum	0.383	0.128
StdDev	0.00502	0.00327
R(%)	3	6
Rmean(%)	4.5	
n10%	1	
n20%	1	

	Acuvue(tinted)
	-2.5
Mean	0.3027
Minimum	0.282
Maximum	0.346
StdDev	0.01754
R(%)	13.1
Rmean(%)	13.1
n10%	2
n20%	1

- NewVues

	NewVues	NewVues	NewVues
	-2	-1.25	-4
Mean	0.2751	0.206	0.1732
Minimum	0.263	0.192	0.145
Maximum	0.285	0.231	0.198
StdDev	0.00792	0.01074	0.01468
R(%)	6.5	11.8	19.2
Rmean(%)	13.5		
n10%	2		
n20%	1		

-Excelens

	Excelens	Excelens	Excelens
	-0.5	-3	-2
Mean	0.1293	0.154	0.1279
Minimum	0.115	0.146	0.121
Maximum	0.136	0.166	0.137
StdDev	0.0063	0.00521	0.00448
R(%)	11.5	7.6	7.9
Rmean(%)	9.2		
n10%	1		
n20%	1		

- SeeQuence2

	SeeQ2	SeeQ2	SeeQ2
	-2	-2.75	-3.5
Mean	0.0457	0.0447	0.0561
Minimum	0.042	0.04	0.051
Maximum	0.049	0.05	0.061
StdDev	0.00258	0.00295	0.003
R	0.0058308	0.006667	0.00678
R(%)	12.7	14.9	12.1
Rmean(%)	13.3		
n10%	2		
n20%	1		

Appendix D

- **Acuvue unworn contact lenses- Repeatability of fluorescence measurements-Individual results**

lens1	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	342.8	426.0	42.51	10.67
	337.8	428.0	46.09	8.05
	335.8	427.8	26.18	8.73
	340.8	433.0	29.91	8.98
	336.2	432.0	41.94	17.03
Mean	338.7	429.4	37.33	10.69
STDev	2.7	2.7	7.80	3.28
R	7.5	7.4	21.65	9.12
R%	2.2	1.7	58.01	85.27
lens2	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	334.6	436.0	29.51	11.00
	336.2	429.2	34.35	9.54
	341.6	438.0	29.60	12.20
	342.2	425.2	17.38	6.56
	342.4	439.6	25.87	13.23
Mean	339.4	433.6	27.34	10.51
STDev	3.3	5.5	5.66	2.33
R	9.2	15.3	15.72	6.46
R%	2.7	3.5	57.49	61.44
lens3	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	349.4	429.6	32.39	11.69
	342.0	430.0	37.32	10.65
	347.0	425.8	37.24	8.21
	341.8	425.6	25.56	7.78
	337.6	428.0	22.86	8.91
Mean	343.6	427.8	31.07	9.45
STDev	4.2	1.8	5.94	1.49
R	11.6	5.1	16.50	4.13
R%	3.4	1.2	53.10	43.73

- **Acuvue worn contact lenses- Repeatability of fluorescence measurements- Individual measurements**

lens1	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	339.0	433.0	336.14	17.97
	340.0	425.4	322.61	15.23
	340.0	427.0	322.61	9.04
	341.6	425.2	362.00	16.00
	341.6	440.4	362.00	19.38
Mean	340.4	430.2	341.07	15.52
STDev	1.0	5.8	17.79	3.56
R	2.8	16.2	49.38	9.87
R%	0.8	3.8	14.48	63.58
lens2	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	343.4	440.0	210.02	11.30
	340.0	440.0	192.58	11.30
	340.0	444.0	192.58	27.47
	336.2	442.2	208.78	12.42
	341.8	425.8	203.19	15.35
Mean	340.3	438.4	201.43	15.57
STDev	2.4	6.5	7.58	6.13
R	6.7	18.0	21.05	17.02
R%	2.0	4.1	10.45	109.35
lens3	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	339.0	454.4	204.79	37.78
	342.6	452.6	225.56	38.06
	342.6	443.6	225.56	9.95
	340.2	438.2	204.47	12.16
	335.8	440.0	215.26	9.80
Mean	340.0	445.8	215.13	21.55
STDev	2.5	6.6	9.36	13.39
R	7.0	18.3	25.99	37.18
R%	2.1	4.1	12.08	172.52

- **NewVue unworn contact lenses- Repeatability of fluorescence measurements- Individual measurements**

lens1	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	320.6	436.8	244.10	31.75
	320.6	436.8	244.10	31.75
	320.6	436.8	244.10	31.75
	321.8	436.2	237.40	27.50
	321.2	432.2	271.15	28.13
Mean	321.0	435.8	248.17	30.18
STDev	0.5	1.8	11.78	1.94
R	1.3	5.0	32.70	5.38
R%	0.4	1.1	13.18	17.83
lens2	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	320.4	426.6	308.40	31.54
	320.6	438.2	272.88	32.29
	320.2	441.0	251.33	35.23
	321.0	437.2	306.88	42.63
	321.8	439.6	220.57	37.06
Mean	320.8	436.5	272.01	35.75
STDev	0.6	5.1	33.51	3.98
R	1.6	14.2	93.02	11.04
R%	0.5	3.3	34.20	30.87
lens3	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	324.2	436.2	332.24	31.99
	321.6	441.8	359.61	38.81
	321.0	441.0	362.62	55.94
	320.8	435.4	356.25	47.43
	321.1	432.8	331.03	48.64
Mean	321.7	437.4	348.35	44.56
STDev	1.3	3.4	13.80	8.31
R	3.5	9.5	38.31	23.07
R%	1.1	2.2	11.00	51.78

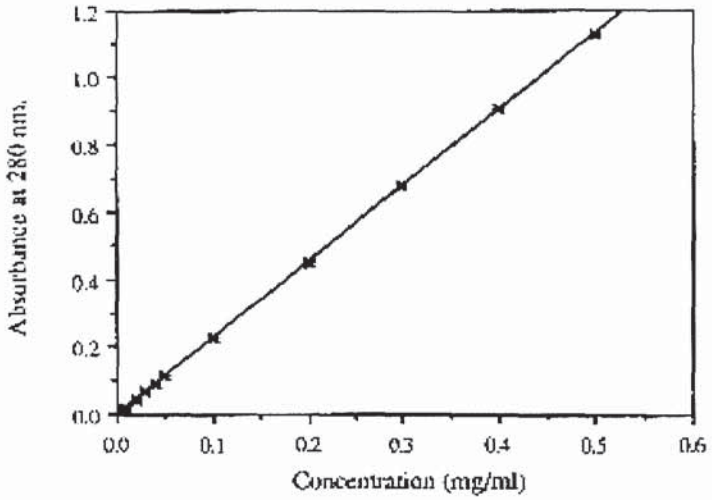
- **NewVue worn contact lenses- Repeatability of fluorescence measurements- Individual measurements**

lens1	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	335.2	433.6	188.58	52.61
	332.6	442.6	181.61	40.74
	335.4	439.6	157.84	44.52
	335.2	440.8	188.58	42.68
	335.2	432.2	188.58	
Mean	334.7	437.8	181.04	45.14
STDev	1.1	4.1	11.91	4.52
R	3.0	11.4	33.06	12.54
R%	0.9	2.6	18.26	27.78
lens2	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	337.2	440.8	137.81	43.43
	334.6	433.0	158.48	41.79
	336.2	434.8	157.98	42.65
	334.4	431.0	185.71	37.26
	336.6	436.6	235.00	43.00
Mean	335.8	435.2	175.00	41.63
STDev	1.1	3.3	33.65	2.25
R	3.1	9.3	93.40	6.24
R%	0.9	2.1	53.37	15.00
lens3	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	323.8	437.2	168.85	34.02
	325.6	426.8	162.80	31.67
	327.4	433.0	197.22	44.69
	336.4	431.0	202.50	51.13
	334.2	435.8	191.60	41.66
Mean	329.5	432.8	184.59	40.63
STDev	4.9	3.7	15.82	7.10
R	13.7	10.2	43.93	19.70
R%	4.2	2.4	23.80	48.48

- **SeeQuence2 unworn contact lenses- Repeatability of fluorescence measurements- Individual measurements**

lens1	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	324.4	426.2	58.54	14.87
	325.6	425.2	69.72	13.21
	323.2	425.2	84.01	19.11
	321.8	435.6	84.88	18.75
	324.2	435.6	98.08	44.36
Mean	323.8	429.6	79.05	22.06
STDev	1.3	4.9	13.63	11.38
R	3.5	13.7	37.83	31.58
R%	1.1	3.2	47.86	143.14
lens2	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	320.6	425.6	68.18	16.19
	328.8	425.2	75.82	12.43
	321.2	425.9	66.27	13.55
	323.2	425.2	72.17	13.76
	320.6	426.6	74.83	13.54
Mean	322.9	425.7	71.45	13.89
STDev	3.1	0.5	3.70	1.24
R	8.6	1.4	10.28	3.44
R%	2.7	0.3	14.39	24.76
lens3	Peak		Intensity of peak	
	Protein	Lipid	Protein	Lipid
	320.6	432.8	164.01	18.65
	325.0	438.2	63.92	7.27
	324.6	430.2	75.47	13.09
	320.6	435.2	158.01	12.37
	322.0	431.2	73.87	12.38
Mean	322.6	433.5	107.06	12.75
STDev	1.9	2.9	44.27	3.61
R	5.3	8.0	122.90	10.03
R%	1.6	1.8	114.80	78.63

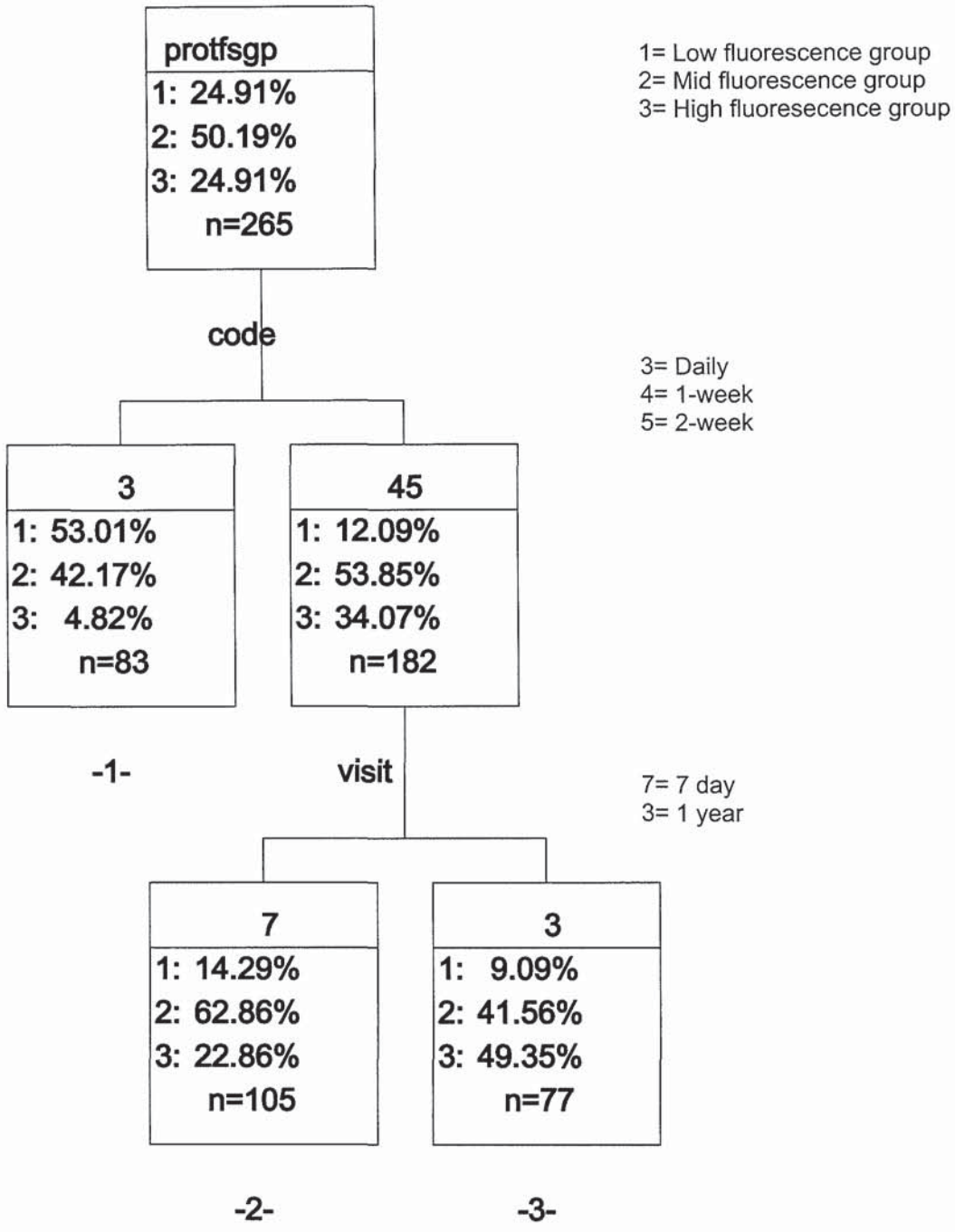
Figure 2.25 The Hitachi U.V. spectrophotometer.



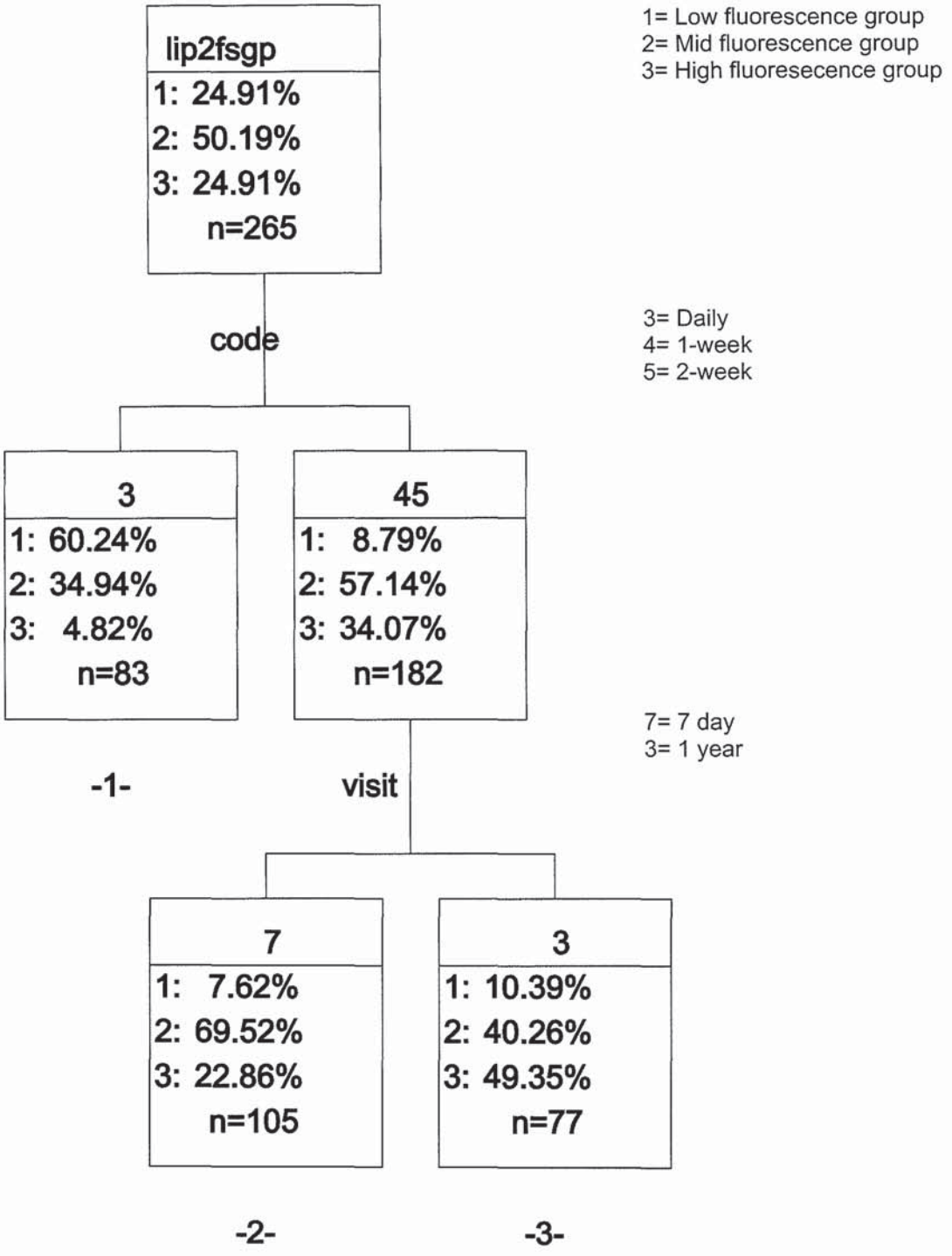
Calibration curve for the U.V. absorption of standard lysozyme solutions.

Appendix F

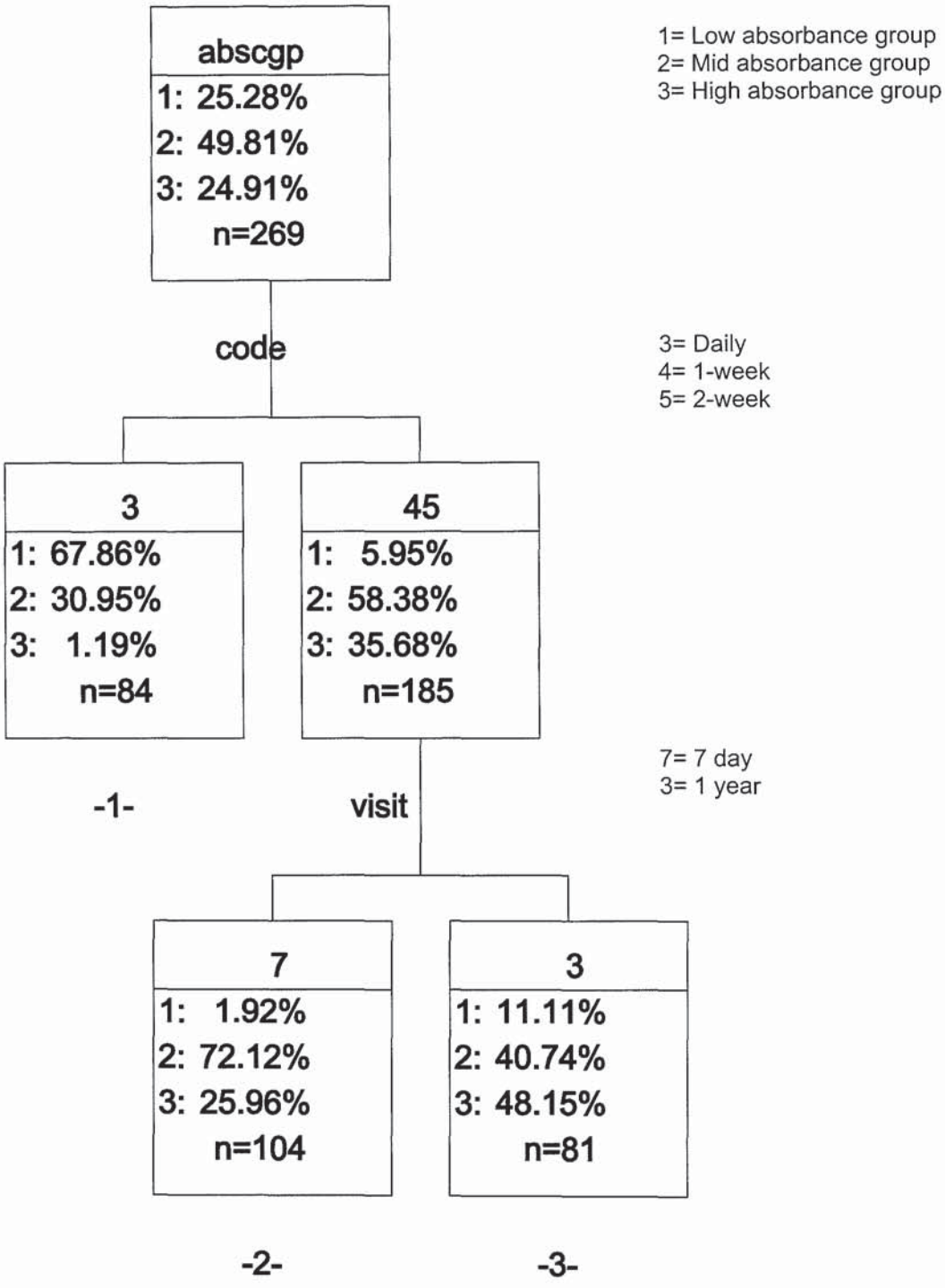
F.1. Protein fluorescence emission (front surface)



F.2. Lipid fluorescence emission (front surface)

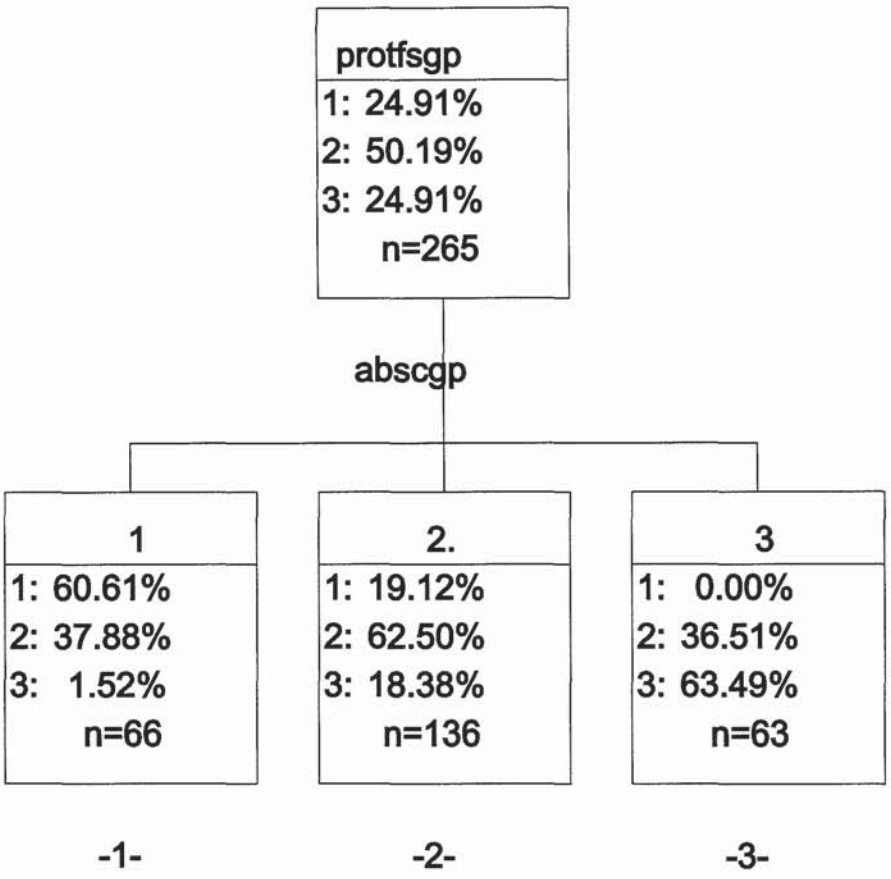


F.3. UV absorbance at 280 nm (total protein adsorbed and absorbed by the contact lens)

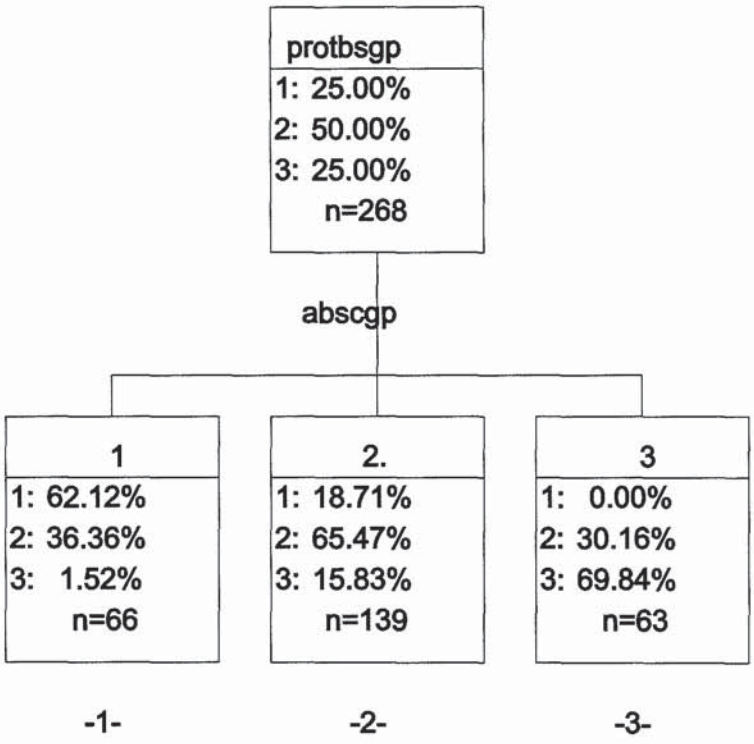


Appendix G

G.1. Front surface protein fluorescence emission



G.2. Back surface protein fluorescence emission



Appendix H

Contact lens subjective acceptance rating scale

The subjective rating scales were 50 point continuous scales with subjective descriptors to help the subjects describe their symptomatology.

COMFORT

0 = Impossible to wear
8 = Can wear very rarely without discomfort
17 = Can wear for short periods only
25 = Bearable
33 = Slight discomfort at times
42 = Good comfort most of the time
50 = Excellent comfort all the time

DRYNESS

0 = Constantly
8 = Very often
17 = Often
25 = Sometimes
33 = Rarely
42 = Very rarely
50 = Never

PAGE NUMBERING AS IN THE ORIGINAL THESIS