PLASTICISED POLY (VINYL CHLORIDE): SIGNIFICANCE OF PLASTICISER AND SURFACE MODIFICATION FOR PROTEIN ADSORPTION

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A thesis submitted in accordance with the regulations governing the award of the degree of Doctor of Philosophy in Bioengineering.

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I declare that this study was entirely my own work and has not previously been submitted to this or any other university.

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ABSTRACT

Plasticised poly(vinyl chloride)(PVC-P) remains the most widely used blood-contacting biomaterial. With respect to the blood compatibility of PVC-P, the plasticiser plays a more important role than the PVC polymer itself, since the blood contacting surface is highly distributed with plasticiser. Therefore, the objective of this project was to study the significance of the plasticiser on protein adsorption onto PVC-P, where the features of plasticiser considered were plasticiser selection (nature of plasticiser), the plasticiser surface level and plasticiser surface distribution. To evaluate this, three types of medical grade PVC-P, plasticised with 2-diethylhexyl phthalate (DEHP), tri-(2-ethylhexyl) trimellitate (TEHTM) and n-butyryl-tri-n-hexyl citrate (BTHC) respectively in sheet form with the same softness, were selected. Protein adsorption was carried out using ^{125}I radiolabelled human fibrinogen and bovine serum albumin. The *in vitro* protein /material contact was achieved with a modified 24-well incubation test cell. A reduced plasticiser surface level was obtained with methanol surface treatment. Surface characterisation was carried out using ATR-FTIR and UV-spectrophotometer. Results obtained indicate that fibrinogen adsorption on PVC-P strongly correlates with the plasticiser surface level. A reduced plasticiser level can reduce fibrinogen adsorption and increase albumin adsorption. However, excess surface washing might lead to a surface topographical change, initiating an increased fibrinogen adsorption. Protein adsorption is also dependent on the plasticiser nature and plasticiser surface distribution. A high level of plasticiser does not necessarily mean a high reactivity towards fibrinogen adsorption. Based on this study, surface modification of PVC-P was carried out using cyclodextrins (CDs) by blending. The combination of CDs with polyethylene oxide (PEO) or polyethylene (PEO)-poly(propylene oxide) (PPO) triblock copolymer (Pluronic surfactant) was also studied. Protein adsorption results indicate that the surface enriched CDs, CD/PEO, CD/Pluronic physical mixture or CD inclusion complex (CIC) can achieve a reduced protein adsorption. Finally, a possible mechanism was hypothesised and a proposal was made for a novel form of surface modification.

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CHAPTER 8 FINAL DISCUSSION AND FURTHER WORK

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TO MY WIFE HONG AND DAUGHTER MINGSU

LIST OF ABBREVIATION

ACD	acid citrate dextrose
α-CD	α-cyclodextrin
ATBC	acetyltributyl citrate
ATEC	acetyl tri-2-ethylhexyl citrate
ATHC	acetyltri-n-hexyl citrate
ATR-FTIR	attenuated total reflectance fourier transform infrared
β-CD (B-CD)	β-cyclodextrin
BTHC	n-butyryltri-n-hexyl citrate
γ-CD	γ-cyclodextrin
CDs	cyclodextrins
CICs	cyclodextrin inclusion complexes
DB-*n*	B-CD modified PVC-DEHP with n%B-CD in blend
DBP	dibutyl phthalate
DBS	dibutyl sebate
DBP- n_1n_2	B-CD/PEO mixture modified PVC-DEHP with n_1%B-CD and n_2% PEO
DBPP- n_1n_2	B-CD/PEO-PPO-PEO(F68) modified PVC-DEHP with n_1%B-CD and n_2% PEO-PPO-PEO
D-CIC (DCIC)	cyclodextrin inclusion complex modified PVC-DEHP
DEHP	2-di(ethylhexyl) phthalate
DEHA	2-di(ethylhexyl)apitate
DEHS	2-di(ethylhexyl)sebate
DH-*n*	HP-B-CD modified PVC-DEHP with n% of HP-B-CD
DHP-n_1n_2	HP-B-CD/PEO modified PVC-DEHP with n_1%HP-B-CD and n_2%PEO in blend
DHPP-n_1n_2	HP-B-CD/PEO-PPO-PEO(F68) modified PVC-DEHP with n_1%HP-B-CD and n_2%F68 in blend
DIBP	di-isobutyl phthalate

DIDP	di-isodecyl phthalate
DINP	di-isononyl phthalate
DIOP	di-isooctyl phthalate
DM-1, 3-6	B-CD/L81mixture modified PVC-DEHP with different proportion
DM-2,7	B-CD/PPG-PEG-PPG mixture modified PVC-DEHP with different proportion
DUP	diundecyl phthalate
ESBO	epoxidised soybean oil
EB	B-CD modified polyethylene
EBP	B-CD/PEO modified polyethylene
EBPP	B-CD/PEO-PPO-PEO (F68) modified polyethylene
HP-B- CD	hydroxypropyl-β-cyclodextrin
HLB	Hydrophile-lipophile balance
IPN	interpenetrating network
MEHP	mono(2-ethylhexyl)phthalate
PA	polymeric adipate
PHR	per hundred ratio
PVC	poly(vinyl chloride)
PVC-BTHC (or PB)	BTHC plasticised PVC
PVC-DEHP (PD, D)	DEHP plasticised PVC
PVC-P	plasticised poly(vinyl chloride)
PVC-TEHTM (PT or T)	TEHTM plasticised PVC
PVC-U	un-plasticised poly(vinyl chloride)
TEHTM	tri-(2-ethylhexyl) trimellitate
TB-*n*	B-CD modified PVC-TEHTM
TBP- n_1n_2	B-CD/PEO modified PVC-TEHTM with n_1%B-CD and n_2% PEO
TBPP- n_1n_2	B-CD/PEO-PPO-PEO modifed PVC-TEHTM with n_1%B-CD and n_2%PEO-PPO-PEO
VCM	vinyl chloride monomer

CHAPTER 1

INTRODUCTION

1.1 Plasticised poly(vinyl chloride)

Poly(vinyl chloride), abbreviated as PVC, is the most versatile of all the commodity polymers. It can satisfy a wide range of product function, safety, performance and cost criteria. PVC can be divided into plasticised PVC and un-plasticised PVC. The standard designations PVC-U (un-plasticised) and PVC-P (plasticised) have now been adopted by IUPAC for the two forms of PVC (Wilson, 1995). In this thesis, P will represent different type of plasticiser. For example, PVC-DEHP is the PVC plasticised with 2-di(ethylhexyl) phthalate (DEHP).

PVC-U is a rigid material. The use of PVC-U did not become significant until the 1960s when the processing technology was available. Nowadays, PVC-U is used extensively for the construction market because of its low cost and fire resistance.

PVC alone is of little value and must be compounded with various additives to make a useful plastic to achieve a broad range of properties. One of the most important additives for PVC is the plasticiser. It is used to increase the flexibility, softness, and workability of PVC. The process to achieve this transformation of PVC and plasticiser into a homogeneous plasticised compound is called plasticisation and the final product is plasticised PVC (PVC-P).

When a plasticiser is blended with PVC, a portion of it forms an intimate bond with the PVC, while the remainder is held in the polymer matrix. There is no covalent bond between PVC and plasticiser but they are very compatible and become an integral part of the matrix (Fig 1.1). In the case of extra soft PVC-P, the plasticiser content can be up to near 50% (Bowry, 1981).

In terms of volume, PVC resin is the most widely used polymeric biomaterial for single use, pre-sterilised medical devices (Blass, 1992). Plasticised PVC based film, sheet and tubing are used in numerous medical products. Most of them are relevant to blood-contacting applications, which are summarised in Fig 1.2. The research focus of

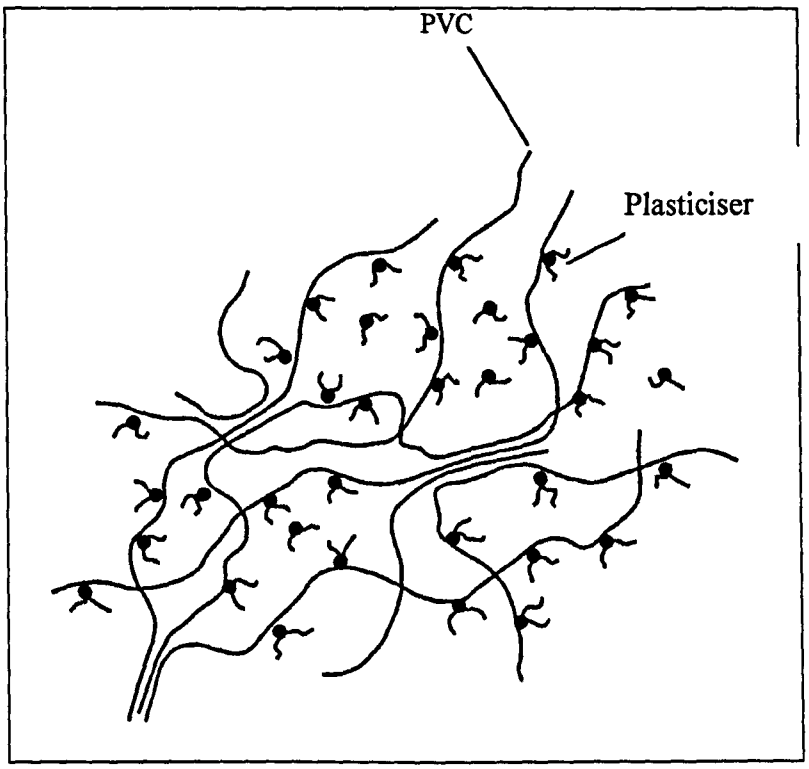


Fig 1.1 Illustration of a plasticiser/PVC compounding matrix
(modified from Wilson, 1995)

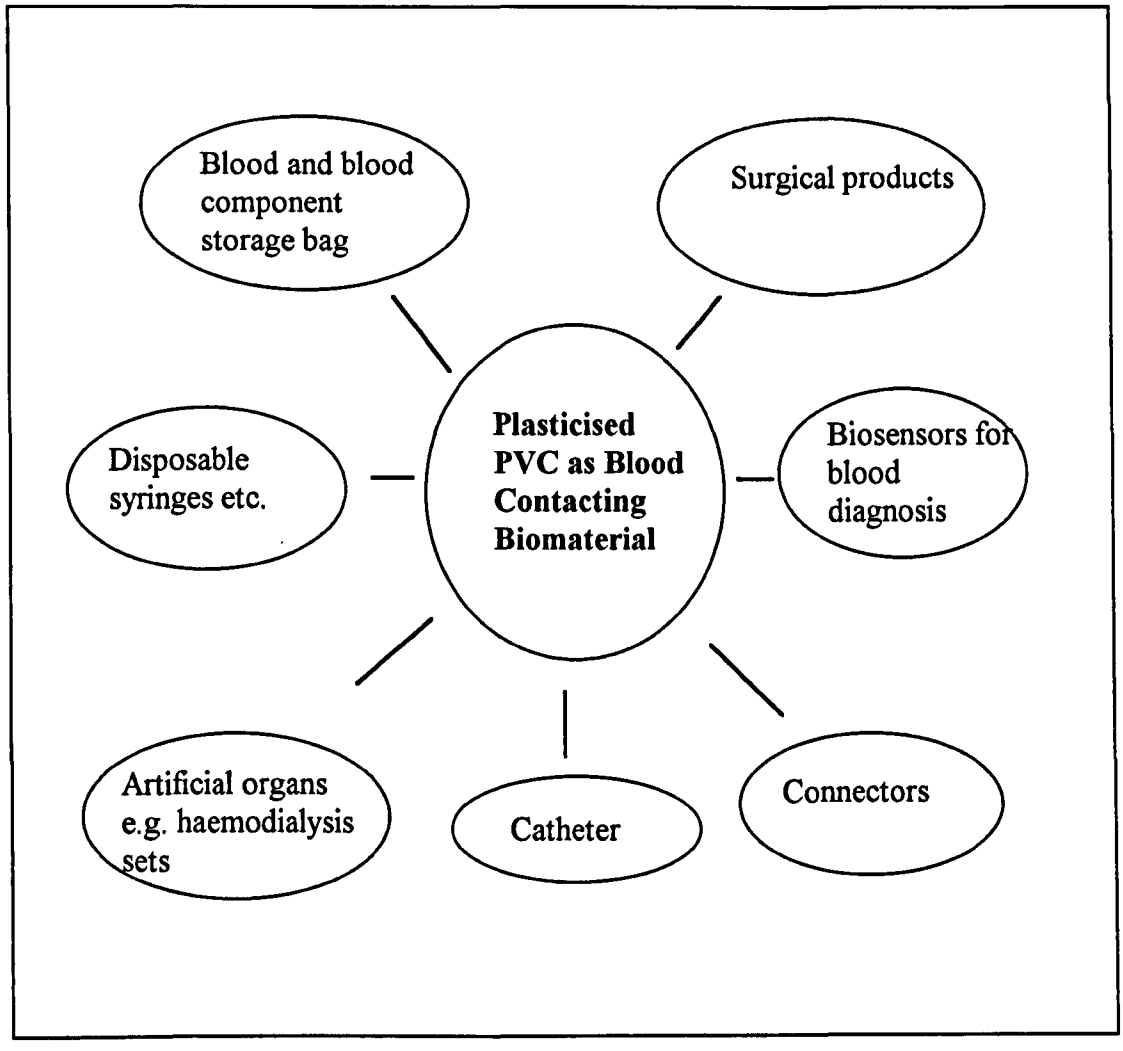


Fig1.2 Application of plasticised PVC as blood-contacting biomaterials

this thesis is on flexible PVC-P and particularly on PVC-P as a blood-contacting biomaterial.

From the material point of view, the blood compatibility of plasticised PVC is influenced by the PVC formulation (plasticiser selection and utilisation of other additives or modifiers) and PVC surface modification (alteration of plasticiser surface distribution, plasticiser surface level and other surface properties). PVC formulation determines the properties of both bulk and surface, while surface modification only influences the surface properties. The relationship between PVC formulation, PVC surface modification and blood compatibility are highlighted in Fig 1.4 (page 7).

1.2 Plasticiser selection

PVC is a very hard and rigid substance, which is also very sensitive to heat. It needs the addition of plasticiser to provide flexibility and a stabiliser to prevent degradation at high temperature. The composition of plasticised PVC formulation used in devices for blood collection, storage and delivery is shown in Fig 1.3 (Blass, 1992).

Fig 1.3 PVC-P formulation for medical device

VI 1.2.1.1 Materials based on plasticised PVC for containers for human blood, blood components, and aqueous solutions for intravenous perfusion

>=	55%	PVC polymer
<=	40%	DEHP plasticiser
<=	1%	Zinc octanoate
<=	1%	Calcium or Zinc stearate of mixture
<=	1%	NN'-Di-acylethylene diamines
<=	10%	Epoxy soya or linseed oil
		No colouring matter

In the formulation, plasticiser selection is critical in the medical application of PVC-P. Di-2-ethylhexyl phthalate (DEHP) is the most commonly utilised plasticiser and comprises 30-40% of the final polymer weight (Ljunggren, 1984). Also, DEHP is the

only plasticiser mentioned in any European Pharmacopoeia Monograph. It remains by far the largest tonnage plasticiser used in medical products (Wilson, 1995). It is probably one of the best-studied substances in the world and it is estimated that over 3,000 scientific papers on its biological activity have appeared (Van Dooren, 1991).

Since DEHP is not covalently bound within the PVC-DEHP matrix, it might leach from the material into the contacting physiological medium (Rubin & Ness, 1989). The migration problem of DEHP encouraged both the research and development of new generation plasticisers as alternatives to DEHP and also PVC-P alternatives.

The new generation PVC-P includes PVC plasticised with triethylhexyl trimellitate (TEHTM) (Simm et al, 1983) and butyryl trihexylcitrate (BTHC) (Kevy et al, 1985). Both of them have been shown to leach from PVC-P into blood components at a lesser extent than DEHP (Flaminio et al, 1988 ; Seidl et al , 1991).

The blood compatibility of PVC-P is strongly dependent on plasticiser selection. PVC-TEHTM was found to be unsuitable for red cell storage because it had no stabilising effect on red cell membranes (Estep et al, 1984; Rock, 1984) and reduced *in vivo* survival time, while PVC-DEHP was shown to confer stability on red cell membranes, reducing haemolysis and increasing *in vivo* survival (Estep et al, 1984; Rock et al, 1984; AuBuchon et al, 1988). PVC-BTHC has been shown to have a stabilising effect on red cell membranes similar to that of DEHP (Buchholz etal, 1989) and has proved to be an excellent platelet storage polymer for high concentrations of machine-derived platelets (Simon et al, 1991).

The content of plasticiser in PVC-P formulation also influences the blood compatibility. Bowry (1981) compared extra soft (48% DEHP) and standard PVC (39% DEHP) and found an enhanced platelet adhesion and aggregation with extra soft PVC. Protein adsorption has been found to be dependent on the DEHP concentration either at the PVC surface (Kim et al, 1976) or the total formulation (Kicheva et al, 1995).

It has also been found that plasticiser surface distribution has pronounced effects on blood compatibility (Yin et al, 1999a).

In summary, the blood compatibility of PVC-P is influenced by the PVC formulation mainly by plasticiser selection and plasticiser-incorporated level. The research and development of plasticised PVC as a biomaterial will be focused on understanding the relationship between surface nature of PVC-P and blood. PVC formulation (Chapter 2) and surface modification (Chapter 3) are two main approaches to achieve improved performances such as plasticiser migration resistance and blood compatibility for PVC-P.

1.3 Surface modification of PVC

Options for altering the influence of blood on a polymeric biomaterial are polymer synthesis, polymer formulation and polymer modification (Courtney et al, 1994). Most of the polymer modifications focus on the surface modification. Techniques include physical, chemical, biological and pharmaceutical modification (Chapter 3). Generally, surface modification of PVC biomaterials can be either by removal of material, addition of material or changing the material already present at the surface.

Kim et al (1976) reported that methanol extracted PVC-DEHP surface exhibited lower platelet adhesion and aggregation compared with non-cleansed samples. Agents such as prostaglandin have been incorporated into PVC-P systems in order to enhance the blood compatibility (Kim, 1980). Lakshmi et al (1998) grafted polyethylene glycol (PEG) onto PVC-DEHP to obtain an increased hydrophilicity at the surface, with little platelet adhesion to the modified surface, whereas the un-modified PVC-DEHP surface promoted extensive adhesion of platelets. Polymer blending modification of PVC-P with poly(ethylene oxide) (PEO) has been shown to be able to achieve a PEO-enriched surface, which resulted in a protein-resistant surface (Ding et al, 1996). Attachment of heparin onto a PVC surface is one of the most widely accepted

techniques for improving the blood compatibility of PVC. Heparinised PVC-DEHP tubing prepared according to the Carmeda end-point attachment method have been shown to have an improved blood compatibility (Yin, 1996).

For retarding plasticiser migration, surface coating with urethane material, surface crosslinking and surface grafting with hydrophilic polymers have been reported. Beta-cyclodextrin has been added to a PVC-DEHP solvent casting system to retard DEHP migration (Sreenivasan, 1996). Cyclodextrins (CDs) are a series of cyclic polysaccharides consisting of 6, 7, or 8 glucose units, which have a hydrophobic cavity surrounded by a hydrophilic shell. They have been utilised for modification of biomedical adsorbents to achieve biospecific removal of some endogenous or exogenous toxins (Zhao & He, 1994a,b). Modified PVC-P with cylodextrins and their combination with PEO and PEO-PPO-PEO surfactant represent a novel biomaterial.

1.4 Material selection, characterisation and blood component alteration

The focus of this thesis is on the evaluation of blood compatibility of PVC-P in sheet or film form. Assessment was restricted to the alteration to protein adsorption. For investigation of the influence of plasticiser selection on blood components, medical grade PVC plasticised respectively with DEHP, TEHTM and BTHC with similar softness were selected for blood compatibility evaluation. PVC-U and Cuprophan PM-150 were selected as controls.

It has been shown that surface contamination or cleanliness of a biomaterial has great influence on the blood compatibility (Kaswmo & Lausmao, 1988). Also, Kim et al (1976) discovered that protein adsorption on PVC-DEHP was affected by the surface methanol extraction. With the cleansed PVC-DEHP surface, there was a reduced fibrinogen adsorption and increased albumin adsorption compared to non-cleansed PVC-DEHP. For the correlation of protein adsorption with plasticiser surface level, surface modification of PVC-P was achieved using surface methanol extraction to obtain a reduced plasticiser level at the surface (Chapter 5). For improving blood

compatibility, a novel modification of PVC by utilisation of cyclodextrins and their combination with PEO and PEO-PPO-PEO surfactants was studied (Chapter 6 and 7). The modifications were achieved by polymer solution casting and polymer blending.

Since the interaction between blood and material takes place on the outermost surface within only a few molecular layers, information on the surface, such as physico-chemical properties, and chemical composition, is very crucial to an understanding of the blood response. Many surface characterisation techniques have been developed, among which, X-ray Photoelectron Spectroscopy (XPS) or Electron Spectroscopy for Chemical Analysis (ESCA) and Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) are the two most commonly applied. In this thesis, the surfaces of PVC-P and modified materials were characterised by UV-spectrophotometry and ATR-FTIR. The surface DEHP and TEHTM plasticiser levels were evaluated using UV-spectrophotometer (Pharmacopoeia, 1997) and ATR-FTIR. The surface characterisation of cyclodextrins modified PVC-P was achieved using ATR-FTIR.

The first event when a foreign surface comes into contact with blood is protein adsorption and the protein layer adsorbed onto the surface determines the subsequent coagulation reaction and cellular responses (Forbes & Courtney, 1994). In this thesis, *in vitro* protein adsorption using radiolabelled fibrinogen and albumin was employed for the evaluation of the blood compatibility of PVC-P biomaterials and the investigation of the interaction of plasma components such as fibrinogen, albumin with plasticised PVC or modified plasticised PVC-P is outlined in Fig 1.4.

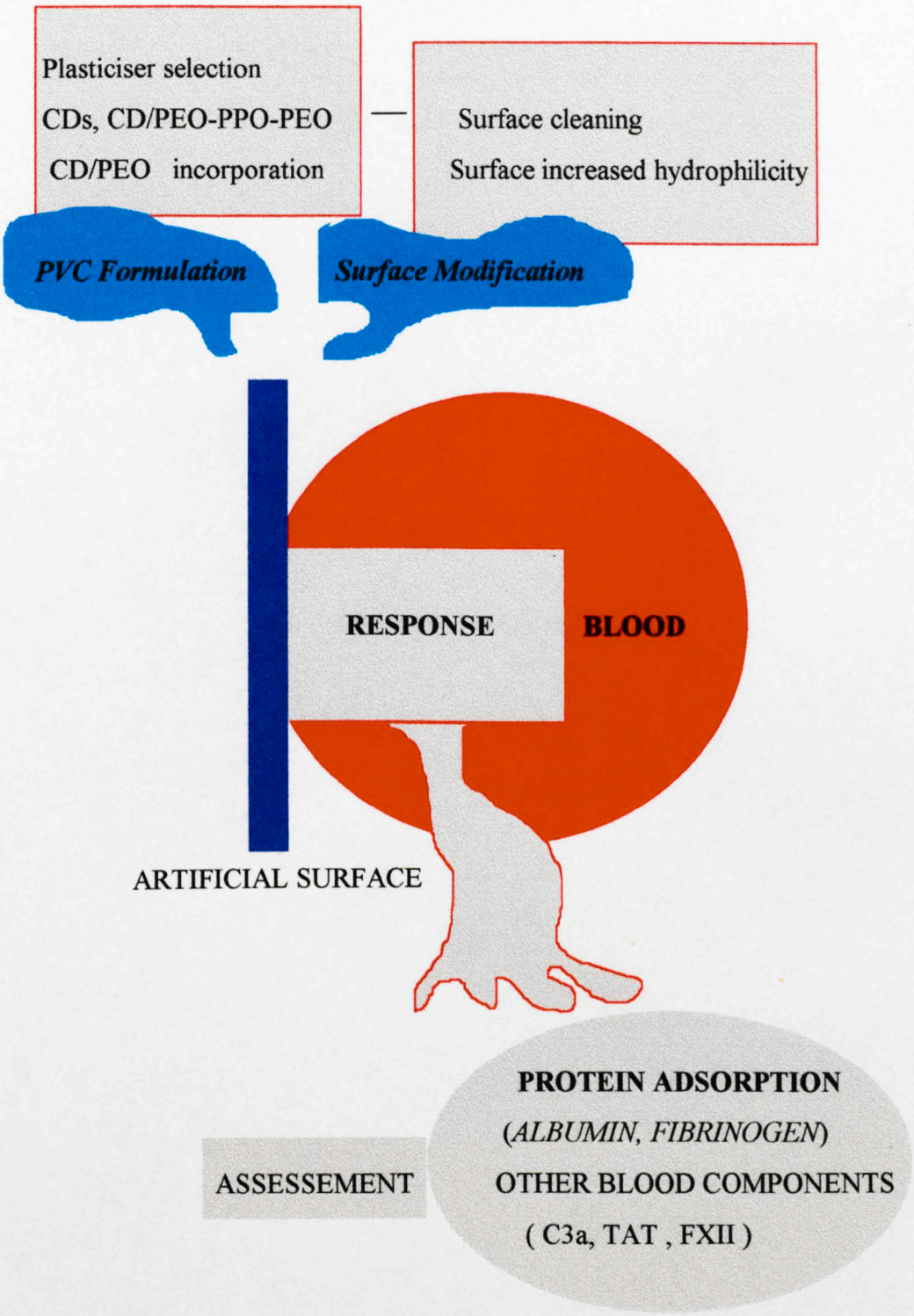


Fig 1.4 Schematic representation of research project on blood components interaction with plasticised poly(vinyl chloride): Correlation of surface with blood compatibility

1.5 Thesis objectives

An objective of this thesis was to evaluate the blood compatibility of plasticised PVC in terms of protein adsorption and correlate this with plasticiser selection and plasticiser surface level. Another important objective was to develop a novel approach for modification of PVC-P by utilisation of cyclodextrins and their combination with PEO and PEO-PPO-PEO surfactant. Following literature reviews on PVC-P as a biomaterial (Chapter 2) and surface modification of PVC-P for improved blood compatibility (Chapter 3), materials and methods are presented in Chapter 4, Chapters 5,6 and 7 deal with the detailed objectives as follows:

Chapter 5

1. To study the influence of plasticiser selection on protein adsorption
2. To correlate protein adsorption with plasticiser surface level
3. To investigate the influence of adsorption conditions on protein adsorption
4. To investigate the protein adsorption mechanism

Chapter 6

1. To study the influence of incorporation of cyclodextrins into PVC-DEHP on fibrinogen adsorption by polymer solution casting and melting
2. To study the influence of incorporation of cyclodextrins, and their combination with PEO and PEO-PPO-PEO into PVC-DEHP and PVC-TEHTM on protein adsorption.
3. To study the migration properties of cyclodextrin modified PVC-P

Chapter 7

1. To investigate the protein adsorption on cyclodextrin/PEO-PPO-PEO inclusion complex (CIC) as a supramolecule modified PVC-P
2. To present a hypothesis to correlate cyclodextrin modified surface with blood compatibility

CHAPTER 2

PLASTICISED POLY(VINYL CHLORIDE) AS A BIOMATERIAL

2.1 Plasticised poly(vinyl chloride) (PVC-P)

2.1.1 History of PVC

Poly(vinyl chloride)(PVC) is produced by polymerisation of vinyl chloride monomer. The first recorded use of the name vinyl chloride appeared in 1854, in Kolbe's *Lehrbuch der Organischen Chemie.* It was Baumann (1872) who first reported that on exposing vinyl chloride to sunlight, a white solid with a specific gravity of 1.406, which could be heated at 130°C without decomposition, was obtained.

In 1912, Ostromislensky (1912) patented the polymerisation of vinyl chloride and related substances, but the high decomposition rate at processing temperatures proved an insurmountable problem for over 15 years (Brydson, 1995). After 1930, when it was discovered how to process PVC using heat stabilisers, commercial interest shifted to this synthetic polymer, and today, PVC is one of the two largest tonnage plastics materials, second only to polyethylene (PE). In 1996, the PVC production by West European manufacturers reached 5,209,000 tonnes and the total PVC sales were about 5,222,000 tonnes (ECVM, 1996).

The commercial success of PVC is strongly linked to the discovery and development of suitable additives, including plasticisers. The first use of a plasticiser was in the 1860, when Parks and Hyatt used camphor to plasticise cellulose nitrate. Later, cellulose nitrate was plasticised to make motion picture film in 1882 (Simonds & Church, 1967). As early as 1928, two approaches had been tried by external (using tritolyl phosphate) and internal (using vinyl acetate as the co-monomer with vinyl chloride) plasticisation to reduce the processing temperature in order to mitigate the instability problem. These initiatives led to a rapid expansion in the production and application of PVC-P as a rubber substitute in the early 1930s. The existing rubber processing machinery was modified to compound and fabricate PVC-P and routine plasticisers for nitrocellulose such as tricresyl phosphate and dibutyl phthalate, were selected for PVC plasticisation (Wilson, 1995).

In 1933, Kyrides (1933) patented the use of di-beta ethylhexyl phthalate for platicisation of nitrocellulose, acetylcellulose and other plastics. In this patent, di-2-ethylhexyl phthalate (DEHP) was also covered. Two months later, Semon's patent on plasticisation of PVC with DEHP was issued (Semon, 1933). Since then, DEHP continued its growth and became the largest volume plasticiser in PVC industry. In 1934, some "non-toxic" plasticisers appeared and achieved FDA regulation in food packaging, adhesives, coatings and tubing used in food processing (Sears & Derby, 1983).

The use of plastic blood processing equipment was pioneered by Carl Walter as early as 1949(Walter, 1949). In 1952, Walter and Murphy introduced the use of a plastic blood bag to store blood in the presence of acid citrate dextrose (ACD) (Walter & Murphy 1952). In 1955, Strumia et al (1955) identified blood bag plastics as poly (vinyl chloride) plasticised with a plasticiser and they were the first to report differences among different plastic formulations based on the *in vitro* and *in vivo* testing of stored blood. Since then, PVC-P as a blood-contacting biomaterial in blood storage, blood transfusion and other medical uses has been widely applied and extensively investigated.

2.1.2 PVC-P formulation

2.1.2.1 PVC raw material

Poly (vinyl chloride) (PVC) is a thermoplastic formed from the addition polymerisation of vinyl chloride monomer (VCM), which is produced from the reaction of ethylene with chlorine and followed by a pyrolysis. (Fig2.1a)

Fig 2.1a Polymerisation of VCM to PVC

There are three major ways to manufacture PVC raw material:

1. Suspension polymerisation
2. Emulsion polymerisation
3. Mass or bulk polymerisation

Suspension polymerisation (Whelan & Craft, 1977; Matthews, 1996)

The process is shown in Fig 2.1.

A mixture of water, VCM, a free radical initiator and a protective colloid or suspension agent (usually a water soluble polymer such as hydrolysed polyvinyl acetate, gelatin or dextran) is agitated in a jacketed pressure vessel capable of withstanding the pressure generated by liquid VCM at the polymerisation temperature. Hot water or cold water in the jacket can control the temperature. After 70~90% conversion of VCM to PVC in a given time, most of the residual VCM (10~20% of the original charge) is recovered by gasification and liquefaction, but because of its carcinogenic nature (Whelan & Craft, 1977) it is necessary to reduce the monomer content still further. The most satisfactory and common procedure for achieving this is using steam to heat the slurry of PVC particles in water to between 80 and 110°C. The steam acts as a carrier for VCM residue, which later is separated from the water in a suitable condenser (Burgess, 1982). In this way, the VCM residue level can be reduced to $\leq$ 1 PPM.

Emulsion polymerisation

The process is similar to that shown in Fig 2.1 for suspension polymerisation except that the polymerisation autoclave is linked to either a homogenising mill or emulsifier/initiator injection equipment. The water is removed by evaporation in a spray dryer instead of using a centrifuge and hot air drying system. In normal emulsion polymerisation, a water-soluble initiator, such as ammonium or potassium

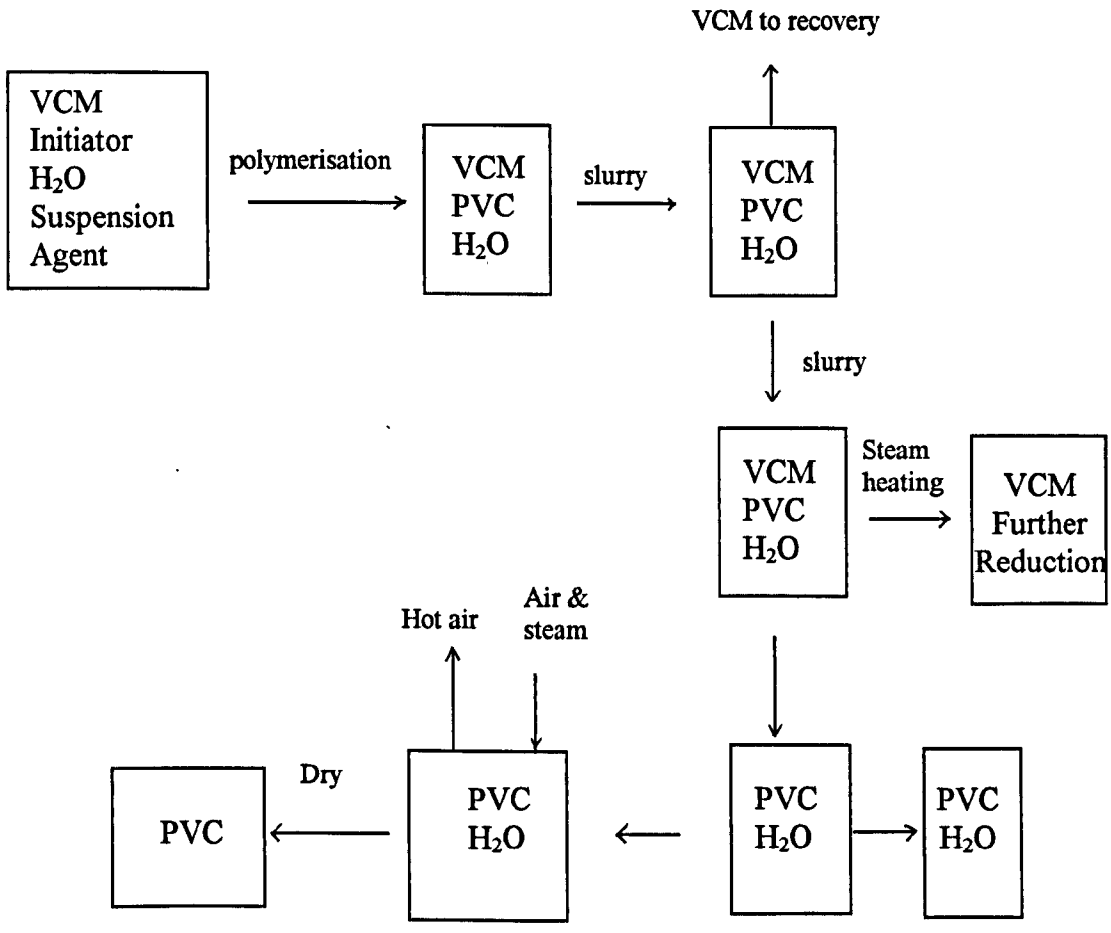


Fig 2.1 Diagram of suspension polymerisation process (from Whelan & Craft, 1977)

persulphate, is employed and the desired latex particle size is obtained by controlling the rate of initiation, the type and amount of emulsifier present and the agitation rate. Emulsion polymerisation is used to make general purpose polymers for speciality applications, such as calendered film and thin profile extrusion, where particularly easy processing is required. It is also used for the production of PVC paste, i.e. PVC suspended in plasticiser, which can be used in the fabrication of gloves and fabric coating etc.

Mass or bulk polymerisation

In mass polymerisation, VCM is polymerised to PVC in the absence of water. The process is divided into pre-polymerisation and post-polymerisation. Pre-polymerisation is to produce PVC seeds with an adjustable particle size using high-speed agitation. The final PVC particle type is substantially determined by the nature of the seed. The advantages of mass polymers are their high purity and enhanced clarity and they are intended particularly for the bottle market (Penn, 1971).

The features, which distinguish PVC raw materials one from another and account for the differences in the processibility and physical properties of their compounds, are the following:

1. Molecular weight
2. Particle size and morphology
3. Impurities
4. Polymeric composition (homopolymer or copolymer)

The molecular weight (MW) of PVC raw material affects both the processibility and the physical properties of the compound. In general, the higher the MW, the greater the difficulty in processing and the higher the physical properties such as mechanical strength. The MW of most commercial PVC resins lies within the range 30,000~75,000 (number-average molecular weight). With respect to the surface morphology, the granular PVC resin with a lower surface area, presents slow processing characteristics while the porous emulsion PVC latex, made up of a large

number of very small particles, is of a great processibility and particularly in plasticisation.

The interaction between PVC and the stabiliser system may be affected by the impurities left during the manufacture. For example, in the case of colour, emulsion PVC usually gives compounds which initially are more yellow than those from the granular PVC. In some cases, PVC resin is produced by copolymerisation with another vinyl monomer, such as vinyl acetate or vinylidene chloride. The copolymer component actually acts as an internal plasticiser. The more of this component added, the lower the processing temperatures.

2.1.2.2 Additives

A great variety of additives are used in the PVC formulation to give PVC useful properties such as colour, resistance to fire, strength and flexibility. Those majoring in importance and/or proportion incorporated are plasticiser, heat stabiliser and fillers.

Plasticiser

Plasticisers are organic compounds added to polymers (especially PVC) to facilitate processing and to increase flexibility of the final plasticised product by an external modification of the polymer molecule. PVC can be modified chemically such as copolymerisation with vinyl acetate to make the product more flexible or show better low temperature properties. This plasticisation process is through the polymer itself and the copolymer component is called the internal plasticiser.

Plasticisers may be divided into two main groups: primary plasticisers and secondary plasticisers. The former is highly compatible with the resin. As a guide, about 150 PHR should be freely compatible in this division (Penn, 1971). The primary plasticisers can be readily used alone. The secondary plasticisers are less compatible and are usually employed together with the primary plasticisers to confer some special properties.

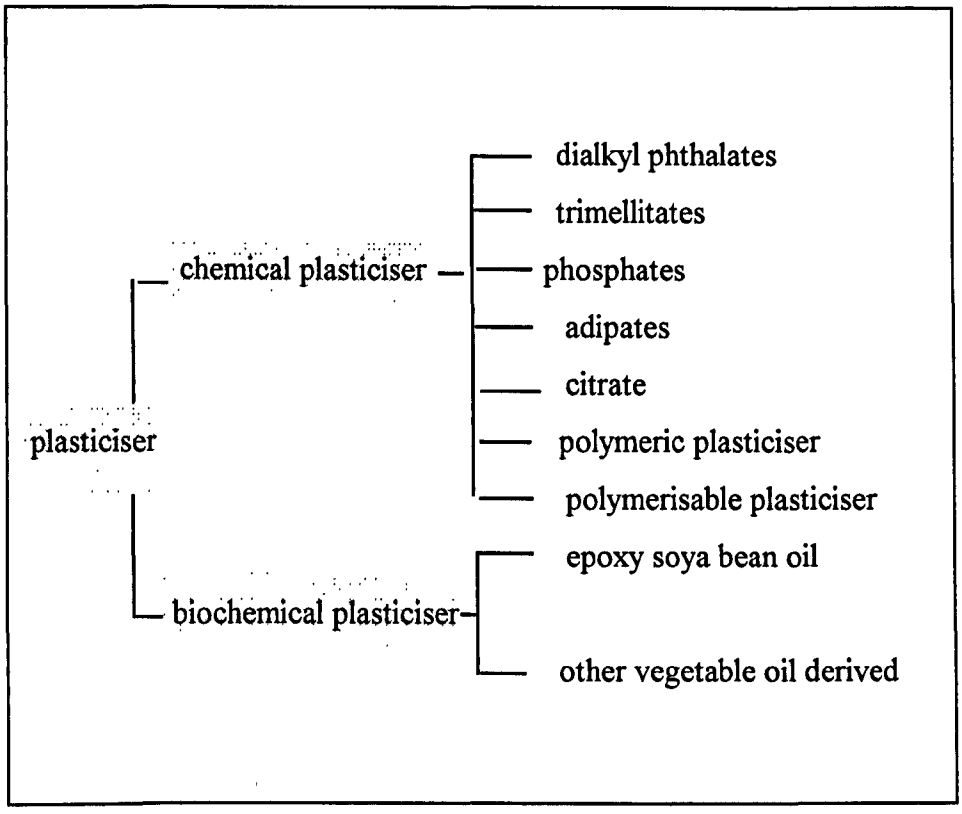


Fig 2.2 Category of plasticisers

Based on the chemical nature or molecular structure, plasticisers can be categorised as shown in Fig 2.2.

Dialkyl phthalate

Phthalate esters, particularly dialkyl phthalates, have dominated the plasticiser market since the 1930s. Presently, about 1 million tons of plasticisers are used annually in Western Europe. Some 92% of the total is used to plasticise PVC and about 95% of these PVC plasticisers are phthalate ester (ECPI, 1996).

The phthalate plasticisers are esters of ortho-phthalic acid and they are manufactured from phthalic anhydride via a straightforward esterification process with selected alcohols. The great majority of phthalate consumption is of the 'big three' general purpose PVC plasticisers, i.e.:

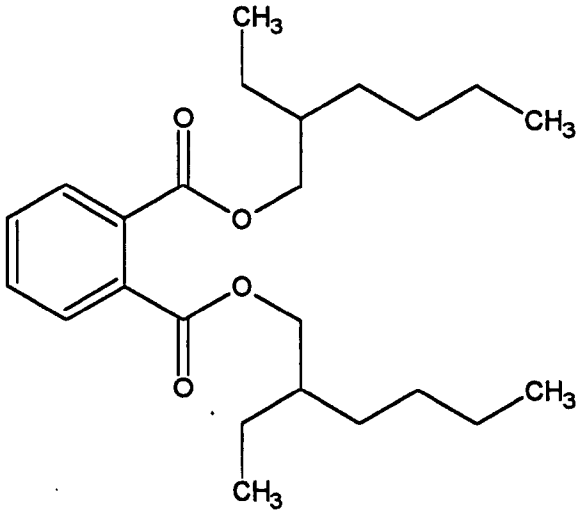
DEHP (DOP): di-2-ethylhexyl phthalate (or dioctyl phthalate)

DINP: di-isononyl phthalate

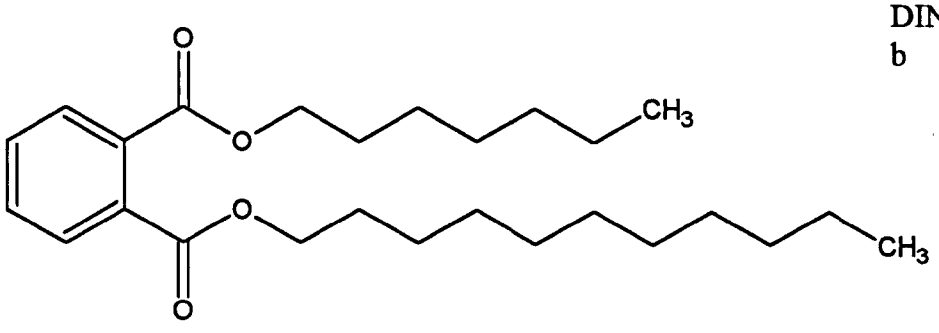
DIDP: di-isodecyl phthalate

Di-2-ethylhexyl phthalate (DEHP) is almost unique among the phthalates for PVC because of its simple chemical structure (Fig 2.3a). For many years, DEHP has been the accepted industrial standard general purpose plasticiser for PVC and is the most commonly utilised plasticiser. Its all-around performance, e.g. compatibility with PVC, plasticising efficiency, low temperature property and low volatility are so good that it alone has accounted for a fourth of the total plasticiser production (Kirk-Othmer, 1982).

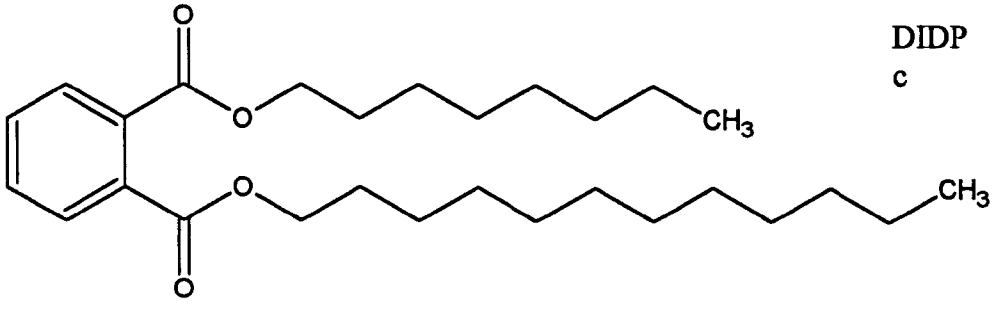
Various di-isoalkyl phthalates, such as DIDP and DINP, have accounted for another fourth of the market. They helped satisfy the growing need for lower volatility but with some sacrifice in plasticising efficiency. Their typical chemical structures are shown in Fig 2.3 b, c.



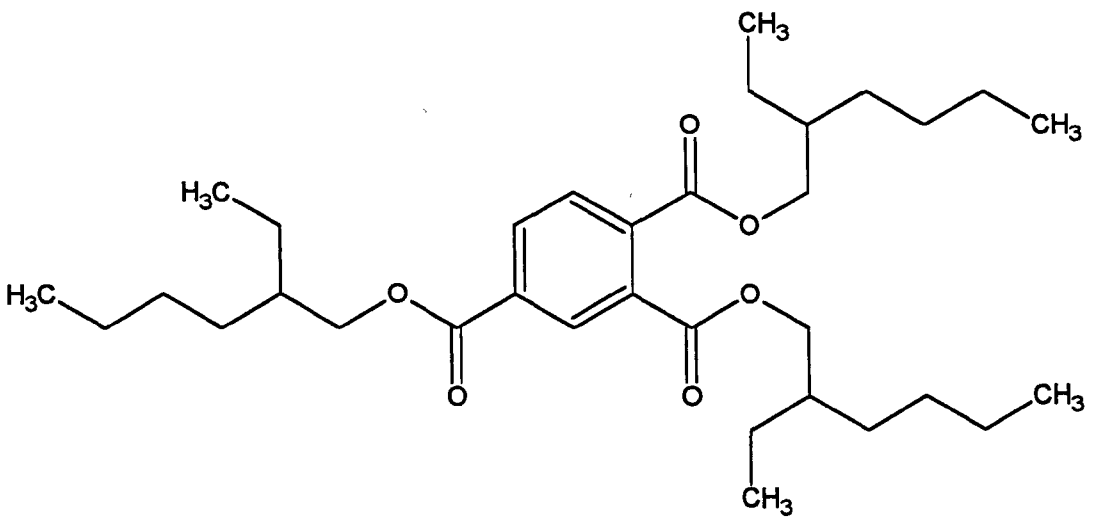
DEHP
a



DINP
b



DIDP
c



TEHTM
d

R-O-OC-$(CH_2)_4$-OC-$OCH_2CH(CH_3)$-OCO-$(CH_2)_4$COOR

e Adipate (R: alkyl)

Fig 2.3 (Continued) Chemical structures of plasticisers

The linear dialkyl phthalates account for about another fourth of the total market. For example, diundecyl phthalate (DUP) represents the upper useful limit of chain length for linear phthalate plasticisers. They are manufactured from alcohol with C11 content close to 100% containing between 50 and 70% of straight chain isomers. DUP has been found to be able to increase the gas permeability of the platelet storage bag (Suzuki et al, 1995).

Trimellitates

Trimellitates, e.g., tri-(2-ethylhexyl)trimellitate (TEHTM) and the mixed esters of almost completely linear heptyl and nonyl alcohols, were developed to provide very low volatility and maintain a good all round balance of performance, much like the phthalates. TEHTM is especially used in the situations where lower levels of migration are required than can be obtained with DEHP. The TEHTM molecular structure is shown in Fig 2.3 d

Adipates

The chemical structures of adipates are shown in Figs.2.3 e.

The same range of monohydric alcohols used as phthalate feed stocks is available for adipates. The flexible linear molecular structure of adipates gives them the common characteristics of low viscosity and good low temperature plasticising performance.

Phosphate

Phosphate plasticisers are esters of phosphoric acid. They have a long history of use as plasticisers dating from the early part of the twentieth century, when tricresyl phosphate was one of the first products to be substituted for camphor in nitro-cellulose. They are now mainly used as speciality plasticisers to provide PVC with fire resistance.

Citrates

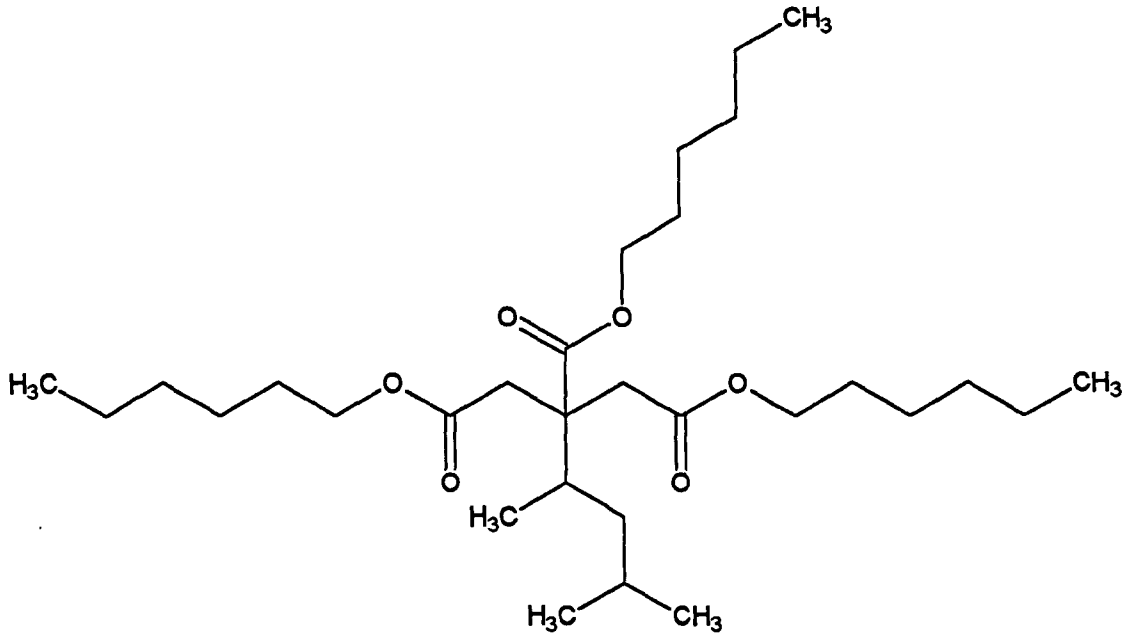


Fig 2.4 Chemical structure of BTHC

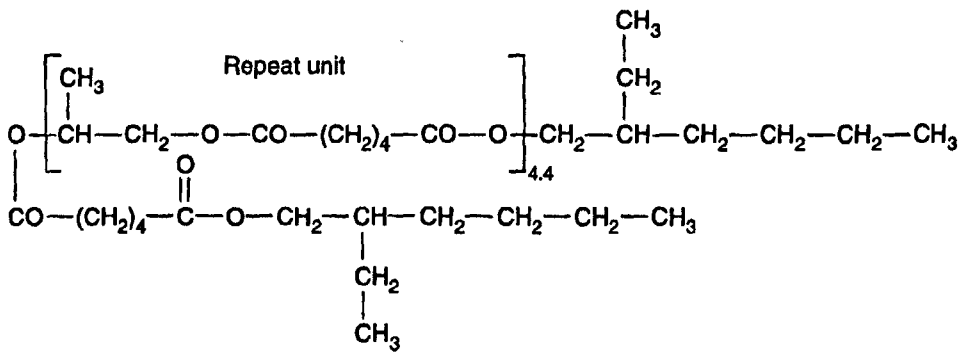


Fig 2.5 Chemical structure of polyadipate (PA) (from Wilson, 1995)

Citrates are esters of citric acid, a raw material manufactured from sugars by enzymatic reactions. Citrates are relatively expensive and whilst some of them show a useful balance of performance characteristics, they do not display any outstanding technical advantages over phthalate plasticisers. Commercially, the most important citrates are acetyl tributyl citrate (ATBC), butyryl tri-n-hexyl citrate (BTHC) and acetyl tri-2-ethylhexyl citrate (ATEC). The chemical structure of BTHC is shown in Fig 2.4. The particular attention to citrate owes much to the common knowledge that they are derived from citric acid, a natural product of low toxicity, occurring in citrus fruits and as human metabolite of carbohydrates. However, in comparison to the huge programmes studying the toxicology of DEHP, the various citrate esters have been relatively little investigated (Wilson, 1995).

Butyryl tri-n-hexyl citrate (BTHC) (Fig 2.4) has received particular attention following its evaluation as a non-phthalate plasticiser or an alternative to DEHP in PVC medical devices, particularly in blood-contacting materials.

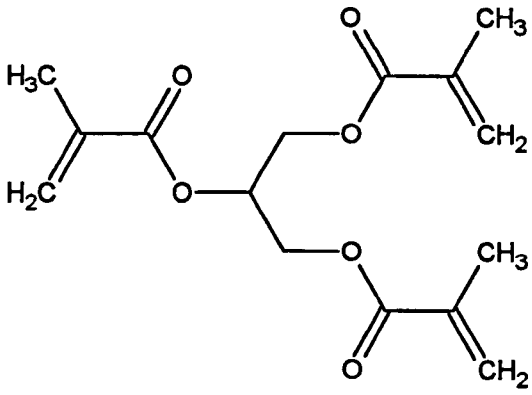
Polymeric plasticiser

Polymeric plasticisers, mainly polyesters, have about 2% of the total plasticiser market and are used in applications where specifications impose limits on levels of migration into solvents, oils and oily media. Fig 2.5 gives the chemical structure of typical polyester (polyadipate, PA).

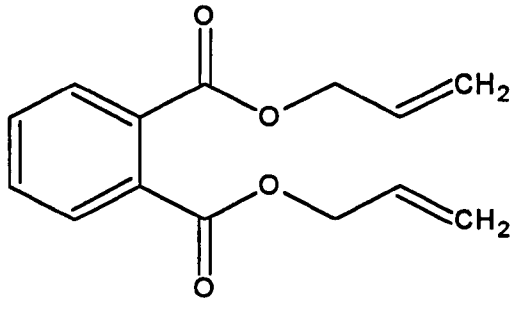
Some other types of polymeric plasticisers in current use are shown in Table 2.1

Table 2.1 Some examples of polymeric plasticisers (Modified from Wilson, 1995)

Trade name	Supplier	Chemical Composition
Elvaloy series	Dupont	Ethylene/vinyl acetate/carbon monoxide
Elvaloy HP series	Dupont	Ethylene/acrylate/carbon monoxide
Baymod L2418	Bayer	Ethylene/vinyl acetate copolymer (68% vinyl acetate)
Baymod PU	Bayer	Aliphatic polyester urethane
Chemigum P83	Goodyear	Partially crosslinked nitrile elastomer



Tri-glycidyl mathacrylate



Di-(1,2-proplenyl) phthalate

Fig 2.6 Some examples of polymerisable plasticisers

The molecular weight of adipate polyester (PA), terminated with alcohols, is commonly about 2000, with a range of ca 800-6000. The high MW results in exceptionally good resistance to extraction, migration and volatile loss. Unlike other polymeric plasticisers or high MW phthalate (trimellitate) with relatively low plasticising efficiency, PA acts almost segment by segment, which results in a good plasticising efficiency (see Table 2.2).

Certain high MW ethylene copolymers have been found to plasticise PVC. Typically, from Table 2.1, this ethylene copolymer is ethylene/vinyl acetate copolymer containing a high level of vinyl acetate, and terpolymers of ethylene, vinyl acetate or an alkyl acrylate, and carbon monoxide. The strongly polar nature of the carbon monoxide enhances the miscibility with PVC, which reducing the other comonomer content required for miscibility. These ethylene copolymers are soft but essentially non-fluid at ambient temperature (Hofmann, 1995).

Chlorinated polyethylene (CPE) with 36-48 wt% chlorine is polyblended with PVC as a polymeric plasticiser. Consequently, it can replace part of the PVC resin and part of the conventional plasticiser. Thus, a 50/50 blend of PVC/CPE-36%Cl with 30 phr of a conventional polyester plasticiser may exhibit much the same tensile properties achieved with 60phr of polyester with pure PVC (Dow Chemical Co., 1975).

Polymerisable plasticiser

The so-called polymerisable plasticisers only act for plasticisation at the process stage. In their monomeric state, they are liquid and compatible with PVC. During processing to the end product, polymerisation of the monomer occurs, resulting in the formation of a crosslinked-interpenetrating network not involving any reaction with the PVC. This gives the composition-reduced flexibility but the enhanced toughness required for specific end uses. Fig 2.6 gives two examples of polymerisable plasticisers.

Biochemical plasticisers

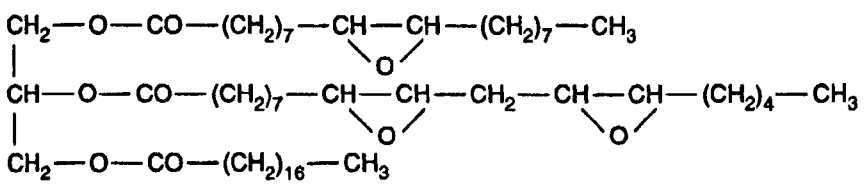


Fig 2.7 Chemical structure of ESBO (from Wilson, 1995)

In addition to the plasticisers derived from petroleum industry, there is another class of environmentally benign plasticisers, which are derived from vegetable oils. They are named biochemical plasticisers. One of the most significant biochemical plasticisers is epoxidised soybean oil (ESBO), which holds 43% of the vegetable oil derived plasticiser market. The typical structure of ESBO is shown in Fig 2.7. Another type of vegetable-based plasticiser is an ester, which is derived from the reaction of an alcohol with a fatty acid. Fatty acids are the main component of vegetable oils and sebacic acid, a component of castor oil, is the most commonly used fatty acid as a plasticiser for PVC.

Most biochemical plasticisers are suitable for use only as secondary plasticisers. At higher levels, they may not mix properly into the plastic formulation, or may cause PVC formulations to become brittle. At current levels of technology, the markets for vegetable oil-derived plasticisers are settled, and are likely to experience growth only with the growth of PVC market. In the future, the vegetable oil derived plasticisers may achieve improved properties to replace DEHP as primary plasticisers. This may provide a solution to those public concerns about the environment and potential health risks of chemical plasticisers, particularly in the area of food packaging and medical applications.

Other additives

Additives used in plastics formulation are normally classified according to their specific function, rather than on a chemical basis (Mascia, 1974). In PVC-P formulation, commonly applied additives other than plasticisers include: heat stabiliser, lubricant, antioxidants, colorants, fillers, flame retardant and smoke suppressers, fungicides, bactericides and pesticides, optical brighteners, surfactants and other surface property modifiers. Here, only stabilisers and some additives, which affect the surface properties of PVC-P will be reviewed.

Practical stabilisation of poly (vinyl chloride) has been investigated since the 1930s. Stabilisers are added to protect PVC against thermal decomposition during processing. The commonly applied PVC stabilisers include inorganic metal salts, such as basic lead carbonate (white lead) and tribasic lead sulphate (TBLS); metal soaps such as the soaps of lead, barium, cadmium, zinc, calcium and magnesium; metal complexes such as barium/cadmium, barium/cadmium/zinc and calcium/magnesium/zinc, organotin compounds and epoxy compounds. A good combination, which is non-toxic, specifically designed for food packaging or medical application uses calcium stearate, zinc stearate and their mixture with epoxidised soybean oil. This stabilising system is widely accepted in PVC-P formulation for medical application.

Lubricants are added to PVC formulation to avoid excessive sticking on the processing mill, and have a great influence on the surface properties of PVC-P (See section 2.1.3). The common lubricants for PVC-P formulation are stearic acid, waxes such as paraffin and microcrystalline waxes, low molecular weight polyethylene, natural and modified natural waxes, fatty-acid amides, silicones, as well as those "lubricating type stabilisers" (Sears&Darby, 1982).

2.1.2.3 PVC-P formulation

Selection of plasticiser

The ease of PVC processing, physical properties of a PVC formulation, and its bio-related performance are dependent to a large degree on the chemical structure and level of incorporation of the plasticiser if the PVC resin employed has already been selected. Molecular mass, polarity and linearity of the plasticisers are the three key molecular properties to determine the final properties of PVC-P (Wilson, 1995).

Chemicals with MW below 300 are likely to be too volatile for use in PVC and values above 800(except some polymeric plasticisers) suggest low compatibility, difficult processing and low efficiency but better extraction resistance. If the chemical

structure is predominantly cyclic or branched, the material will show poor low temperature performance.

Table 2.2 gives typical physical properties of Shore A 74 PVC compounds. The values indicate that there is a reduction in plasticising efficiency with TEHTM and PA in comparison with DEHP. A higher level of these DEHP alternative plasticisers is required in order to achieve the same softness and flexibility characteristics, although PA exhibits excellent extraction resistance to some extractants. (Blass, 1990)

For applications involving particular toxic risks in food contact, medical products or children's toys, the selection is based on a small group of approved plasticisers listed in Tables 2.3 and 2.4.

Table 2.2 Data on plasticisers and typical physical properties of their plasticised PVC compound (Shore A 74) (Blass, 1990, modified)

Plasticiser	**DEHP**	**TEHTM**	**PA**
MW	390	547	2000 approx.
Density (kg/m^3)	983	986	107.5
Refractive index	1.487	1.485	1.467
Liquid appearance	colourless	colourless to very pale yellow	colourless to very pale yellow
Relative Cost	1.0	3.2	3.5
PVC-P	**PVC-DEHP**	**PVC-TEHTM**	**PVC-PA**
Plasticiser (%)	31.7	35.8	33.8
Density (kg/m^3)	1230	1220	1260
Tensile strength (N/m^2)	$1.9x10^7$	1.89×10^7	1.93×10^7
Elongation (%)	355	400	365
Cold flex (°C)	-20	-20	-10

Table 2.3 List of plasticisers acceptable in food-contact applications

Plasticiser	Max. level of use(% W/W)	General food type	Countries
DBP	40	any	UK
DIBP	40	any	
DIDP	40	any	UK
DEHP	40	aqueous	UK
DEHP	28	fatty	UK
DIOP	40	non-fatty	UK USA
BBP	33	any	UK USA
DBS	40	any	UK USA
DEHA	40	any	UK USA
DEHS	30	any	UK USA
ESBO	11	any	USA, Europe
ATBC	38	any	USA Europe

Table 2.4 List of plasticisers acceptable in medical application

Plasticisers	Comments
DEHP	only plasticiser listed in Eur. Pharmacopoeia IV,1.2.1.1 and 1.2.1.2
TEHTM	some use in medical applications with better resistance to migration
BTHC	medical applications e.g. Baxter licence blood bags
PA	some medical applications with non-migration
ESBO	medical applications as a secondary plasticiser

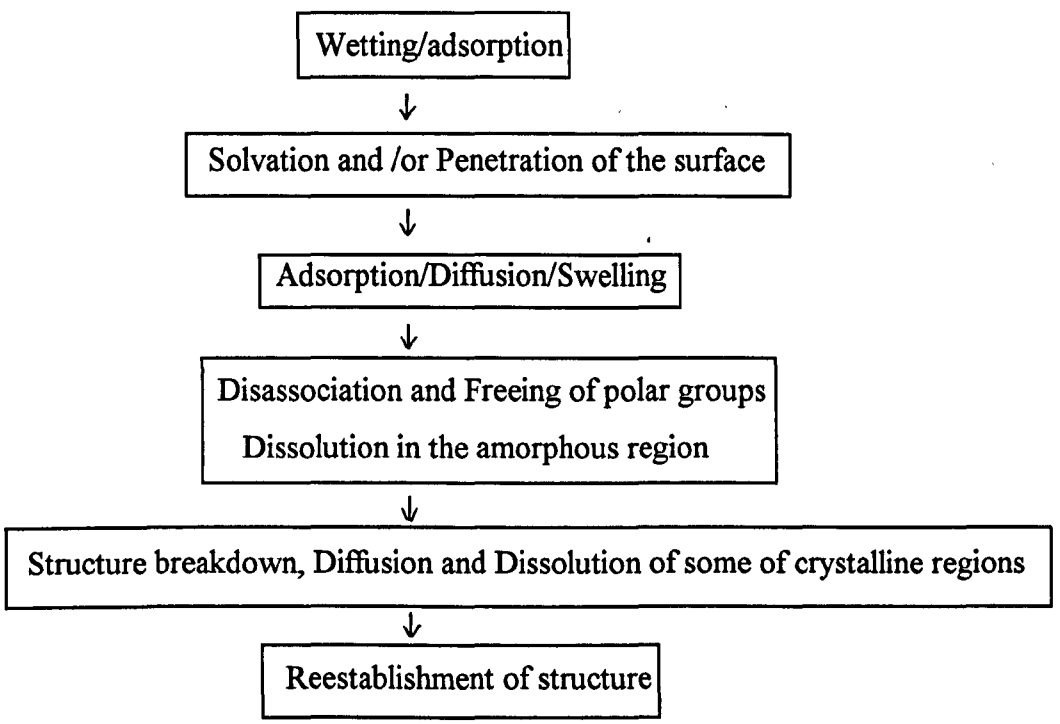


Fig 2.8 The overall procedures of plasticisation (from Mattews, 1996)

Historically, the main plasticiser for PVC food packaging film has been di-2-ethylhexyl adipate (DEHA) used in conjunction with a proportion of epoxy soyabean oil (Wilson, 1995), while DEHP is the most widely accepted and commonly used in medical grade PVC formulation. With respect to the concern over the migration of DEHP, TEHTM and PA have been used as an alternative of DEHP in haemodialysis tubing and blood storage containers (Blass, 1990).

N-Butyryltri-n-hexyl citrate (BTHC), a type of citrate plasticiser, was first introduced by Hull and Mathur to medical grade PVC formulation (Hull & Mathur, 1984). The data on BTHC are shown in Table 2.5, which is based on the BTHC manufacturer data sheet from Morflex, Inc., Greensboro, NC 27403.

Table 2.5 Data on BTHC

Product name	citroflex B-6
Chemical Name	n-Butyryltri-n-hexyl Citrate (BTHC)
Molecular Weight	514
Molecular formulae	$C_{28}H_{50}O_8$
Appearance	Clear, oily liquid
Odour	Mild, characteristic
Freezing point	-55°C
Specific gravity (25 °C)	0.991
Evaporation rate	<1 (Butyl acetate =1)
Toxic effects: Oral-mouse	LD50: > 48 g/kg
Oral-rat	LD50: > 20 g/kg

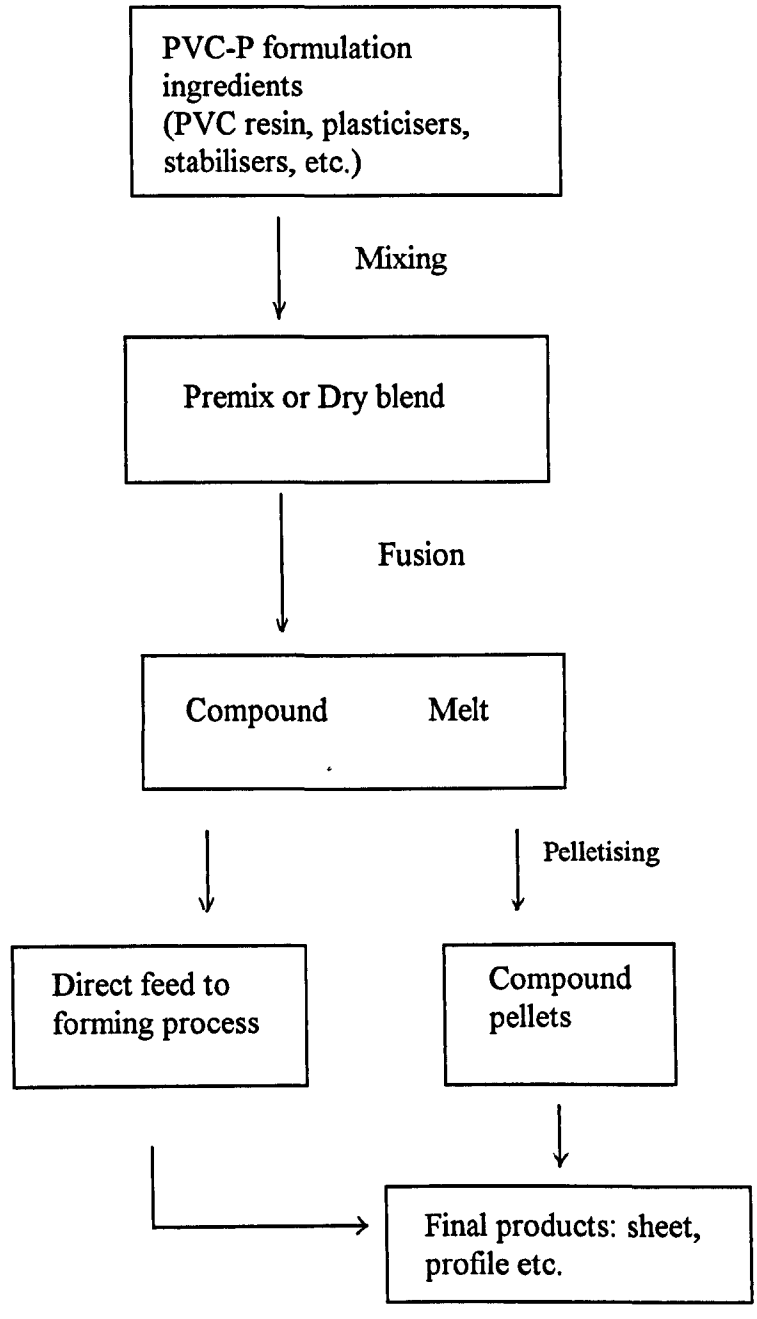


Fig 2.9 PVC-P compounding by melting process (from Mattews, 1996)

Plasticisation

The overall picture of the plasticisation of PVC by plasticisers is shown in Fig 2.8.

PVC-P compounding

The process of preparation of a PVC-P compound is defined as compounding, which involves a mixing procedure as shown in Fig.2.9. PVC-P compounding can be achieved with dry blending via compounding machines such as two-roll mills, internal mixers, single-screw and twin-screw.

Two-roll mills are extensively used in laboratories to examine the compounding behaviour of different components of PVC formulations, and for the preparation of specimens. Owing to the rather low output rates and high labour usage of compounding, they are now rarely employed for production purpose (Matthews, 1996).

Batch hot melting and mixing of PVC composition can be achieved in an internal mixer, which contains a well designed mixing chamber with a heating system. The advantage for using internal mixers is the achievement not only of a reduction in labour because of the provision automatic control system, but also of a more uniform repetition from batch to batch (Barclay, 1962).

Continuous compounding of PVC composition has been developed based on the modification of extruders with screws designed to ensure that adequate homogenisation is achieved. Usually, the single-screw extruder is inadequate to homogenise any PVC dry blend in a single pass unless an additional homogenisation process is introduced with a suitable adaptation and modification. In an extruder with two or more screws, there exists the possibility of increasing homogenisation of PVC composition. The whole operation of mixing and compounding, and indeed also of extrusion to finished product can be carried out continuously.

PVC composition can be dissolved in a suitable organic solvent to obtain a homogeneous solution as coating material. The solution can also be cast as a film. The structures and physical properties of films are strongly dependent on the nature of solvent employed, evaporation rate of solvent, residue of solvent, but mainly dependent on the compatibility among PVC, plasticiser and other ingredients.

According to the PVC processing as shown in Fig 2.9, the PVC compound can be further processed into a final product such as flexible sheet, film and tubing by injection moulding or extrusion, or calendering etc (Mattews, 1996).

2.1.3 Properties of PVC-P

Mechanical Properties

Generally, PVC-P differs from PVC-U most markedly in flexibility or rigidity with a much lower tensile strength and much higher elongation (%). Table 2.6 shows the range of mechanical properties of PVC-P compared with those for PVC-U.

Table 2.6 A comparison of the mechanical properties of PVC-P and PVC-U (Penn et al, 1971)

properties	ASTM test method	PVC-P	PVC-U
Tensile strength(Pa)	D638,D651	$1.03\sim2.41 \times 10^7$	$3.45 \sim 6.21 \times 10^7$
Elongation(%)	D638	$200 \sim 450$	$2.0 \sim 40.0$
Tensile modulus 10^{19} (Pa)	D638	----	$2.41\sim 4.14$
Compressive strength (Pa)	D695	$6.21\text{x } 10^6$ $\sim 1.17 \times 10^7$	5.52×10^7 $\sim 8.96 \times 10^7$
Flexural yield strength (Pa)	D790	-----	6.89×10^7 $- 1.1 \times 10^8$

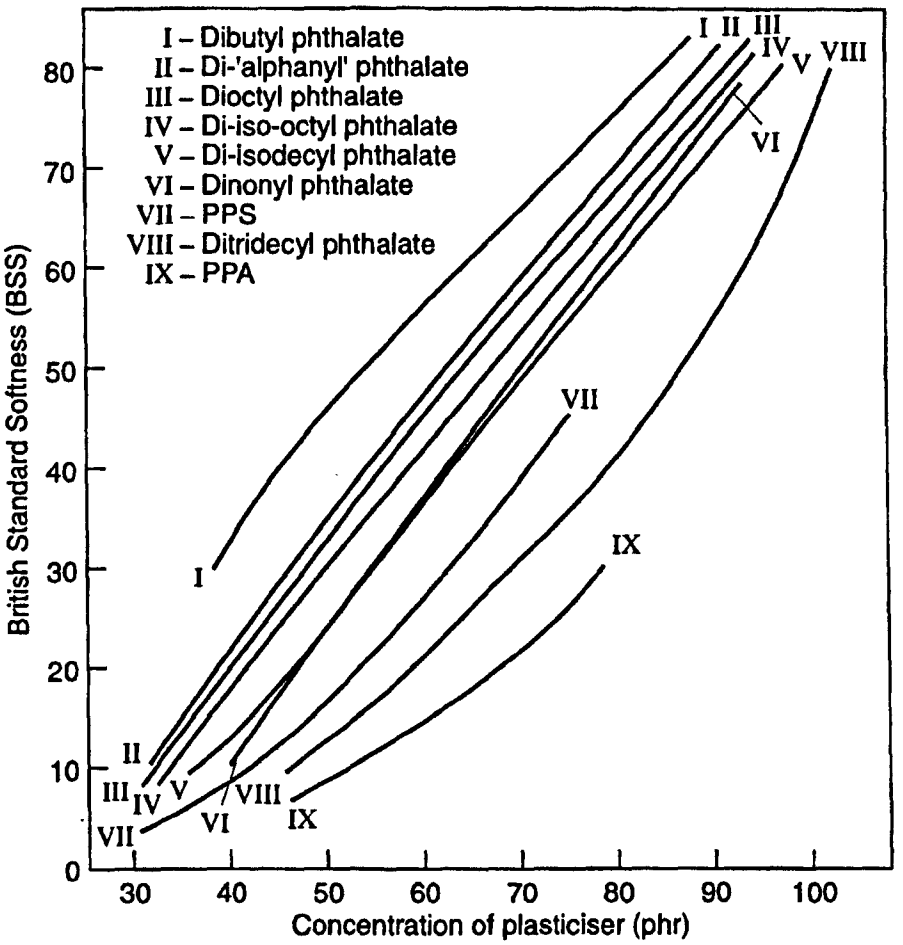


Fig 2.10 Influence of concentration of plasticiser on the softness of PVC-P (from Matthews, 1996)

The tensile strength and modulus decrease and elongation at break increase with increase in plasticiser content, with the pattern dependent on the particular plasticiser (Wilson, 1995).

Since different plasticisers have different efficiencies, there is little point in comparing them at a given constant plasticiser content and it is far more meaningful to compare them at such concentrations as yield the same flexibility, or other property which might be of importance in a particular set of circumstances. For example, with respect of the British Standard Softness (BSS) of PVC-P, the plasticiser with a lower efficiency will need a higher plasticiser level to reach the same BS softness, as shown in Fig.2.10.

Low temperature properties

Low temperature properties, as denoted by the cold flex temperature, are also affected by the selection of plasticiser and the concentration incorporated. Normally, linear plasticisers, such as adipates, have a good low temperature property while the high molecular weight polyester shows poor flexibility at low temperature.

Electrical properties

Insulating properties in terms of volume resistivity (VR) are strongly influenced by plasticiser content, type and temperature. The increase of a particular plasticiser concentration will reduce the volume resistivity markedly and TEHTM plasticised PVC appears to have a higher VR than that of DEHP plasticised PVC (Wilson, 1995).

Surface properties

Plasticisation normally lowers the critical surface tension (γc) of PVC. While γc for pure PVC is about 3.8×10^{-2} to 3.9×10^{-2} N/m, the γc for PVC-DBP (10-20 phr) falls to 2.4×10^{-2} N/m. However, when the surface was etched by solvents such as detergent or dimethylformamide (DMF), the γc of various plasticised PVCs increased to 3.4×10^{-2} N/m or more, suggesting plasticiser, lubricant, or stabiliser was removed and rigid PVC remained (Nakamura et al, 1972).

Surface friction is another important property related to wear and abrasion resistance. It is influenced by the deformation properties of PVC-P, which in turn are influenced by plasticisers and other additives (Owens, 1964). As the concentration of plasticiser increases, the amount of deformation for a given load increases, and the coefficient of friction also increases (Decoste, 1969). That is why tack and blocking action increase with increased plasticiser content.

Long-term properties

Volatility is the first long-term property requiring to be considered for application of PVC-P. The mobility of a plasticiser, which enables it to soften, impart flexibility and toughen PVC, also permits it to leave the PVC and enter other media which are in contact with it. The degree of migration will clearly depend on

a. The type of plasticiser
b. The type of material with which the PVC-P is in contact

In general, small molecules migrate faster than large ones, linear molecules migrate faster than bulky, branched ones, and highly solvating ones that produce an open gel structure migrate faster than those that are "frozen in" to isolated pockets (Sears&Pabby, 1982). For the contacted materials, the resistance to migration increases according to the order: polyethylene>rubber>cellulose nitrate, which depends on the compatibility between plasticisers and these materials.

Plasticisers may be extracted from PVC-P by liquid media, such as solvents, lipid, blood and detergent. The extraction may theoretically be controlled by rate of loss from the surface or by rate of diffusion inside the PVC, but the true extraction process becomes much more complex because of the nature of extractant (Sears & Barby, 1982). When a diffusing liquid has no solvent action on a polymer supermolecular structure, diffusion coefficients are independent of concentration of the liquid in the polymer. However, if the liquid does show some solvent or swelling action on the polymers, the diffusion coefficients may vary widely with solvent concentration (Laurence & Slattery, 1967)

Alcohol and alcohol-water blends may at times extract plasticiser from PVC. The extraction by 50% ethanol in water is much more sensitive to plasticiser concentration than extraction by pure water (Brighton, 1968), and the extraction should be more severe with increasing concentration of alcohol (Sears & Darby, 1982).

It is found that DEHP can diffuse to the surface faster than it can be "solubilized" into blood, but the polyester (polymeric plasticiser) can be "solubilized" faster than it can diffuse to the surface from inside the PVC sheet. So the extraction of DEHP was surface controlled, while extraction of the polyester was diffusion controlled. (Sears & Darby, 1982).

The problems concerning migration and extraction of plasticiser into blood or the human body during medical applications and recent approaches for overcoming these problems are discussed in section 2.4

2.1.4 Application of PVC-P

PVC is used in a broad range of applications. The major uses today are shown in Table 2.7. The applications of PVC-P are mainly cables, floor covering, wall covering, roof-sheeting, packaging, medical application, toys, footwear, adhesive sheet and tapes, and other calendered or extruded products (Wilson, 1995). In this thesis, the focus is on the medical application of PVC-P, particularly, as a blood-contacting biomaterial.

Table 2.7 Application of PVC

Application	Typical products	Percentage
pipes & fittings	water mains and sewer pipes	28%
rigid profiles	window frames	17%
rigid film and sheet	thermoformed trays and containers; credit cards	11%
cable insulation and sheathing	power, data and telephone cables	9%
bottles	mineral water and cooking oil containers	8%
flexible film and sheet	blood bags, stationary and roofing membranes	7%
flooring	domestic, commercial and hospital floors	5%
coating	leather cloth, car seating and wall covering	4%
flexible tubes and profiles	blood transfusion tubing and catheters drainage tubes	4%
footwear	shoes soles	2%
other uses	toys, clothes	5%

2.2 PVC-P as a biomaterial

2.2.1 Introduction

Flexible, soft plasticised poly (vinyl chloride) (PVC-P) is widely applied as a biomaterial for medical applications. The earliest medical application of PVC-P was to replace the traditional metal and glass packaging materials for the packaging of pharmaceutical products, such as blood components and sterilised sugars and electrolytes for intravenous infusion and peritoneal dialysis during World War II. As the increased need for flexible, disposable, biocompatible plastics for medical devices evolved over 50 years, PVC-P became by far the most commonly used polymer in the medical plastics industry. In 1990, the estimated market share was around 25% of all the polymeric materials used in medical devices (Blass, 1992). By 1995, it was estimated that PVC represented 37% of all medical plastics used in United States, with world-wide percentages believed to be even higher (Brookman, 1998).

According to Webster's New Collegiate Dictionary, a biomaterial is defined as a material used for or suitable for use in prostheses that come in direct contact with living tissues. More briefly, a biomaterial can be defined as a non-viable material used in a medical device intended to interact with biological system (Gurland et al, 1994). In more detail, a biomaterial is a substance which is used in prostheses or in medical devices designed for contact with the living body for the intended method of application and for the intended period (Piskin, 1992).

Synthetic polymers are the most diverse class of biomaterials. As an ideal biomaterial, a synthetic polymer needs to meet some criteria (Lyman, 1972)

- The polymer should be one that can be reproducibly obtained as a pure material.
- The polymer should be one that can be fabricated into the desired form without being degraded or adversely changed.
- The polymer should have the required chemical, physical and mechanical properties for performing its function.
- The polymer should be biocompatible.

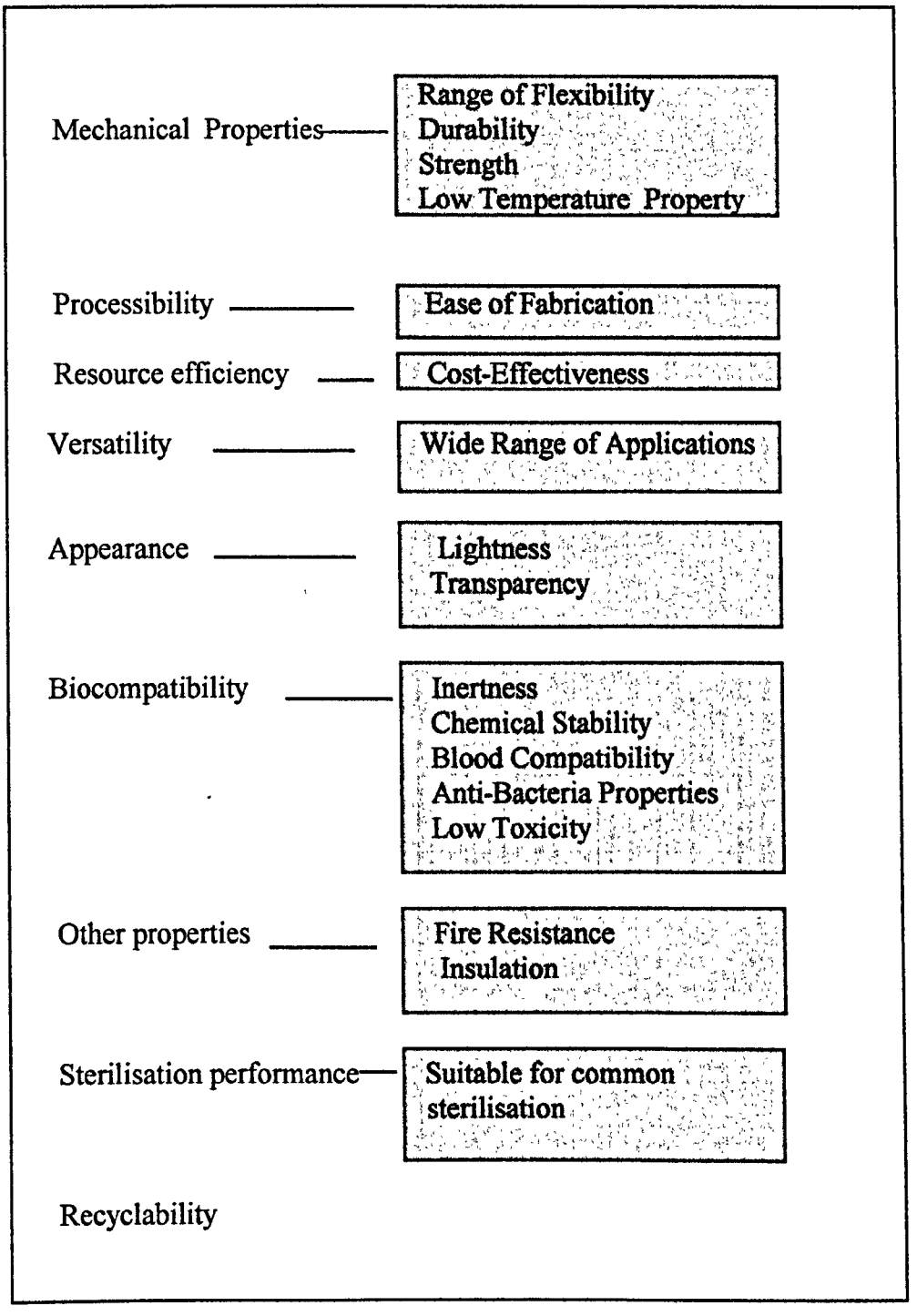


Fig 2.11 Advantages of PVC-P as a biomaterial

The following sections will discuss the advantages and disadvantages of PVC as a biomaterial.

2.2.2 Advantages of PVC-P

PVC-P based film, sheet and tubing are used in numerous medical products. The typical requirements for tubing, in the intravenous (IV) set, for example, include clarity, flexibility, kink resistance, toughness, scratch resistance, ease of bonding with common solvents or adhesives, and suitability for gamma, ETO (ethylene oxide) or E-beam sterilisation. As a biomaterial, PVC-P has achieved its prominent role in the medical plastics industry by virtue of a unique combination of desirable properties.

PVC can be used to produce a variety of medical products ranging from rigid components to flexible sheeting. The type and amount of plasticiser used determine the compound's Tg, which in turn defines its flexibility, and low temperature properties, thereby establishing its versatility. Flexible or rigid PVC can be easily processed to shaped end-products. They can be readily assembled by solvent bonding or sealed by heat or radio frequency (RF) sealing. As a biomaterial for medical products, PVC-P can be sterilised by most of commonly employed sterilisation methods, such as steam, ethylene oxide or gamma radiation. Plasticised PVC can have a Tg as low as -40°C and still be suitable for steam sterilisation at 121°C. PVC-P has excellent biocompatibility, very low toxicity and chemical stability. Additional characteristics that make PVC attractive include its low cost, high transparency, wide range of gas permeability, thermoplastic elastomer-like material properties, fire resistance and good insulation properties. Medical products made from PVC have passed many critical toxicological, biological and physiological tests according to national or international standards. In summary, PVC-P is one of the best medical materials in terms of cost and function. No other single material has such broad advantages (Fig 2.11).

2.2.3 Disadvantages

According to the criteria that an ideal biomaterial should meet, as previously mentioned, a polymer should be pure enough without any influence on biocompatibility due to unintentional additives such as monomer residues, LMW polymers and other reaction residues, and intentional additives such as plasticisers, stabilisers, lubricants and fillers etc. For PVC-P, however, it is the additives that make PVC versatile and useful, while at the same time continuously receiving criticism (Goodman, 1994).

The most commonly cited shortcomings involve toxic effluents such as VCM produced during manufacture and the generation of hydrogen chloride (HCl) during incineration. Other concerns related to PVC-P depend largely on the type and amount of plasticisers used.

Plasticiser such as DEHP, has a very similar solubility parameter [18.2 $(J/M^3)^{1/2}$]to that of PVC [19.1-20.67 $(J/M^3)^{1/2}$], therefore, DEHP is an excellent solvent for PVC. Plasticisers have been found to leach into medical solutions (Smistad et al, 1989), the human body during long-term dialysis (Ono et al, 1982, Nassberger et al, 1987), stored human blood (Jaeger et al, 1972) and foodstuffs (Till et al, 1982, Petersen et al, 1997). PVC-P pharmaceutical packaging bags have been found to cause various drug loss during storage period. For example, the drugs such as diazepam, isosorbide dinitrate, nitroglycerin, and warfarin sodium can be adsorbed by PVC-P with 55%, 23%, 51% and 24% loss respectively during a 24 h study period (Martens et al., 1990). Kowaluk et al (1981) have studied the interaction between 46 injectable drugs and PVC-P infusion bags. They found that the drug loss is due to a diffusion controlled-sorption process.

With regard to the leaching of the plasticiser DEHP, the most commonly applied plasticiser for medical application, however, there are many divided opinions. It seems that no definite proof has been found that DEHP is toxic or is a carcinogenic initiator, whilst its beneficial effects on red blood cell survival is a valued property.

- **cannulae**
- **devices for the collection of blood**
- **devices for the storage and administration of blood products**
  e.g. tubing and bags
- **catheters**
- **haemodialysis sets**
- **cardiopulmonary bypass systems**
- **extension sets**

Fig 2.12 Applications of PVC-P as a blood-contacting biomaterial

2.2.4 PVC-P as a blood-contacting biomaterial

The advantages of PVC-P have led PVC-P to be widely applied in a single-use, presterilised and disposable blood-contacting devices. Generally, blood-contacting devices are categorised in the ISO 10993-4 Standard into " external communicating devices" and "implant devices" (Braybrook, 1997). For PVC-P, the major applications are in the first area as external communicating devices, as shown in Fig 2.12.

The blood products collected and packaged using PVC-P include whole blood, red blood cells, and platelet concentrates. PVC-DEHP is currently the most widely used packaging material for the storage of whole blood, while for red blood cells and platelets, PVC-BTHC has been shown to be able to maintain these cells under optimum conditions (Turner et al, 1995).

Blood tubing made of PVC-P is widely used in blood extracorporeal circulating devices, such as haemodialysis equipment and lung-heart bypass sets. Medical tubing made of polyurethane and silicone have been tried, but both are relatively expensive.

2.2.5 Other applications of PVC-P as a biomaterial

The applications of PVC-P as a biomaterial other than blood-contacting use are summarised in Table 2.8.

Table 2.8 Applications of PVC-P as a non-blood contacting biomaterial

Applications	Examples
Pharmaceutical solution packaging or delivery sets	Intravenous solution pack, IV sets
	Peritoneal dialysis solution pack
	Endotracheal tube (Watson, 1980)
	Connectors
Medical disposable	Gloves , Syringes,
	Drainage tubing or bags
	Urinary bags and tubing
	Other surgical products
Medical building products	Waterproof mattress sheets
	Wall-coverings, Floor-coverings
	Electrical systems
	Appliances and furnishings
	Oxygen tents
Tissue-contacting biomaterial	Burn dressing (Milner et al, 1988)
	Artificial skin (Sekachev et al, 1982)
	Other surgical dressing (Bajda et al, 1976; Takagi et al, 1977)
Biosensor or enzyme electrodes	Glucose biosensors (Yang et al, 1995)
	Protamine-sensitive polymer membrane electrode (Yun, et al, 1995)
	Ion-sensors (Cha, et al, 1991)
Drug-delivery system	Prostaglandin-releasing polymers (McRea et al, 1981)
	Fungicidal and bactericidal additives-releasing PVC (Matthews, 1996)

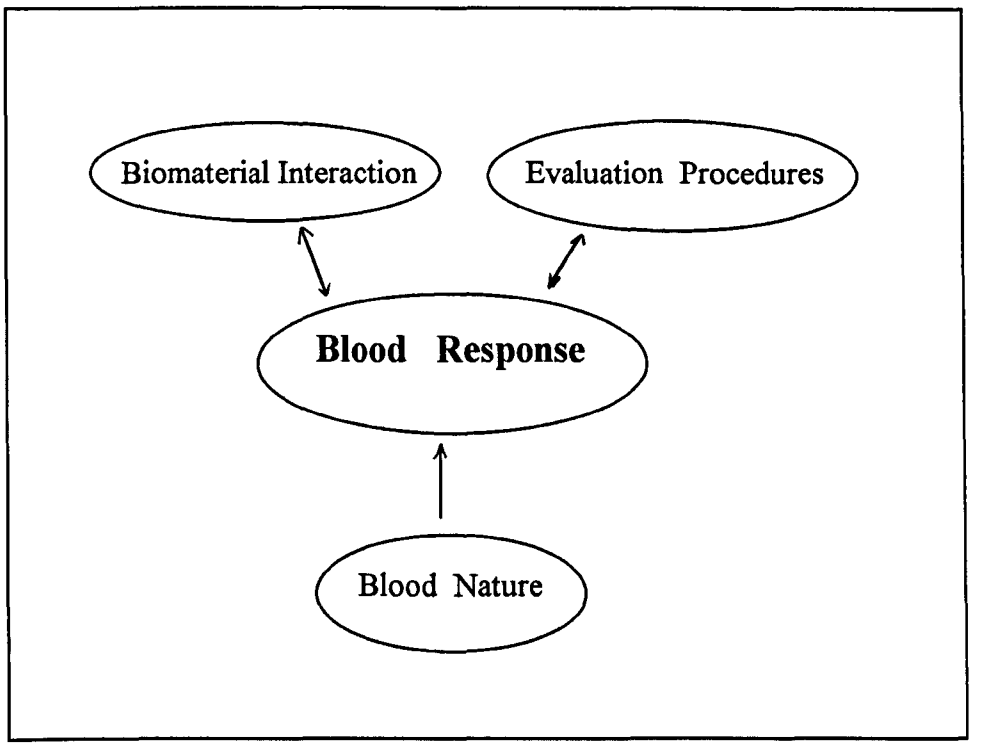


Fig 2.13 Blood response of a biomaterial
(Courtney et al, 1994)

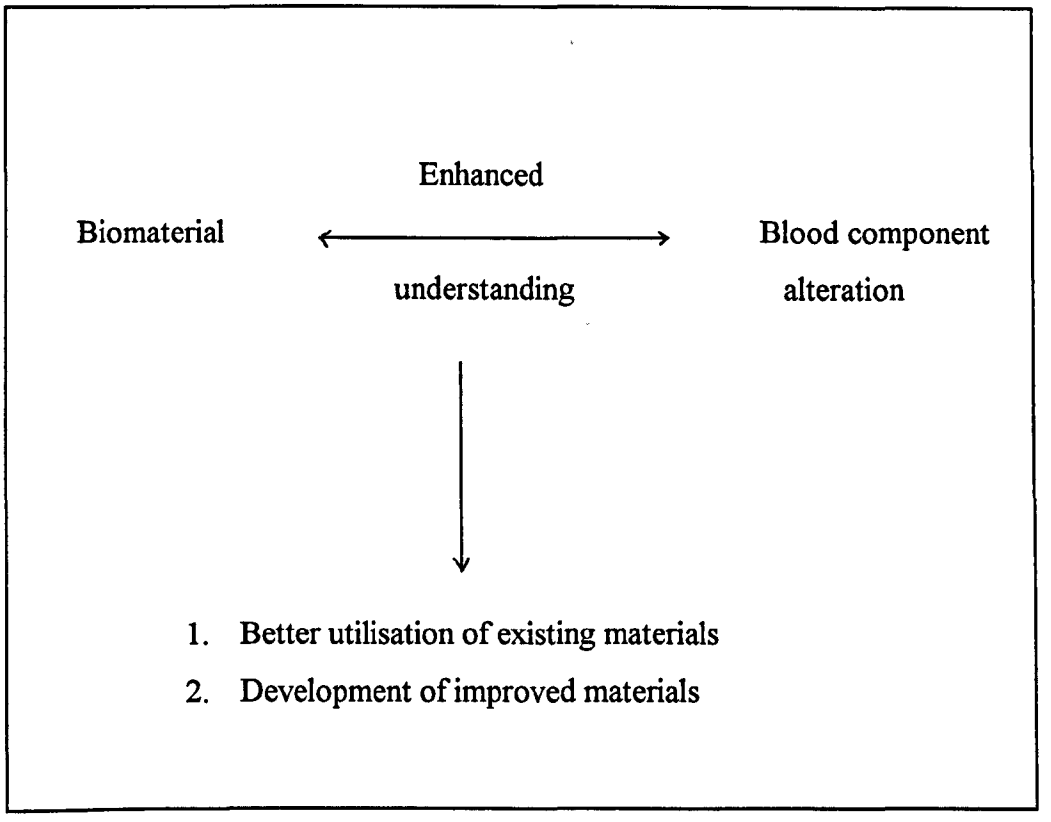


Fig 2.14 Objectives of studying biomaterial-blood interactions (Courtney et al, 1994)

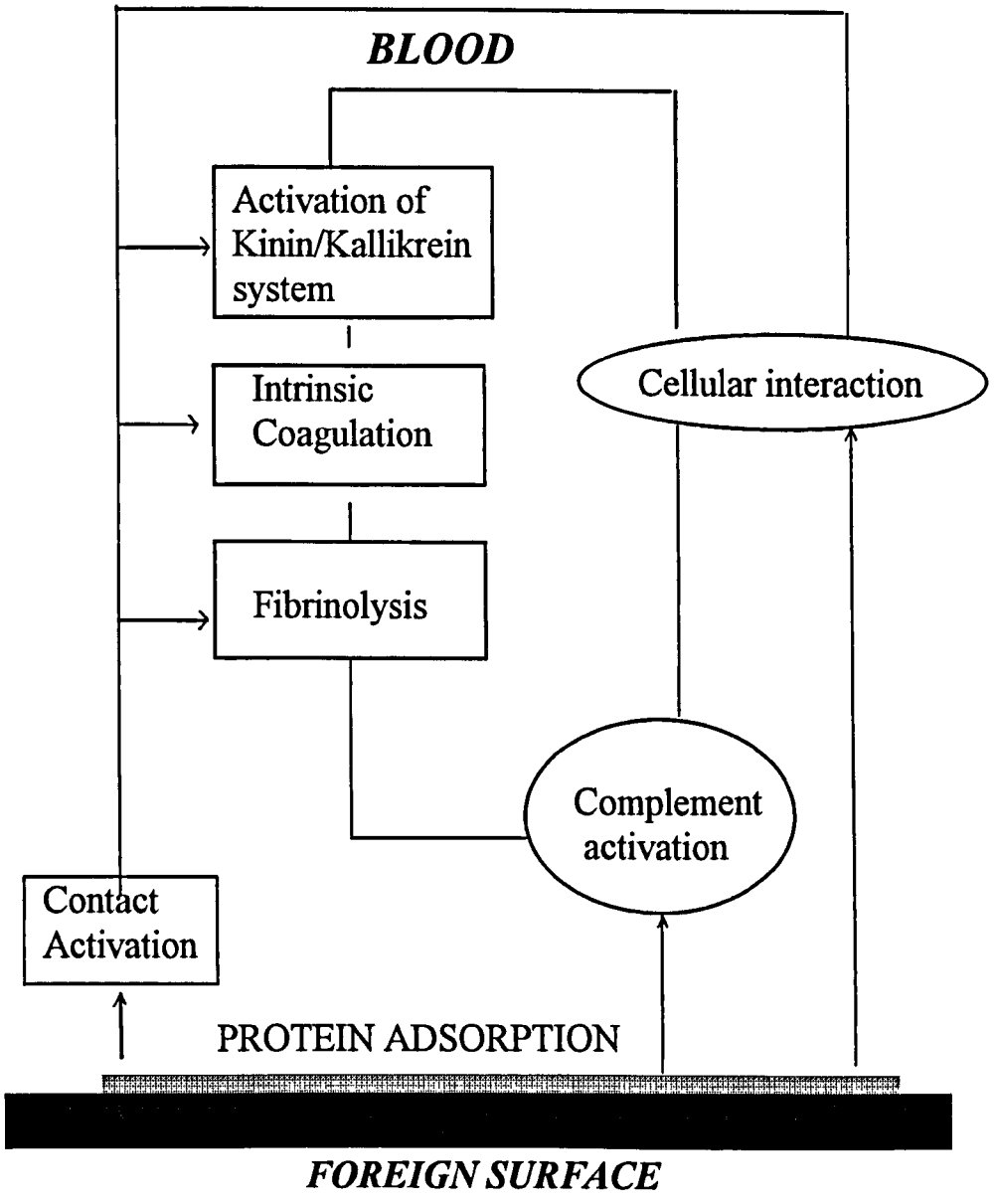


Fig 2.15 Schematic representation of blood-foreign materials surface interaction (from Hennink, 1983)

2.3 Blood compatibility of PVC-P

2.3.1 Introduction

There has been a long-standing interest in the relationship between blood and biomaterials for those blood-contacting applications (Forbes & Courtney, 1994). Although the blood response of biomaterials is complicated, the influences of blood response of a biomaterial can be schematically summarised as shown in Fig 2.13. In the case of PVC-P as one of the most conventional blood-contacting biomaterials, it is convenient to review its blood compatibility in terms of blood-biomaterial interactions, factors influencing the blood response and evaluation procedures (Courtney et al, 1994). Consequently, the objective of an improved understanding of the relationship between the biomaterial and the alteration to blood component could be achieved which would promote a better utilisation of this existing biomaterial and the development of improved materials (Courtney et al, 1995) (Fig 2.14).

2.3.2 Blood-biomaterial interactions

A definition of the blood-biomaterial interaction is as follows: any interaction between a biomaterial (device) and blood or any component of blood, resulting in effects on the biomaterial (device), or on the blood, or on any organ or tissue. Such effects may or may not have clinically significant or undesirable consequences. (ISO/TC 194,1991; Missirlis, 1992)

The high complexed "blood-biomaterials" interaction is of a multi-variable character, (Courtney et al, 1994). When a blood biomaterial interface is established, a rapid sequence of processes occurs. It is now generally accepted that the processes can be divided arbitrarily into the following groups of events (which partly occur simultaneously) (Dawids, 1993, Courtney et al, 1994), as shown in Fig 2.15.

1. Adsorption of plasma proteins onto polymer surface.
2. Activation of the systems of complement system, kinin/kallikrein system (Murabayashi & Nosê, 1986), blood cells and intrinsic coagulation initiated by the adsorbed proteins from the system.

Protein	mg/100 ml serum
Albumin	3500—5500
IgG	800—1800
Fibrinogen	200—450
α_1-Lipoprotein	290—770
α_1-Antitrypsin	200—400
β-Lipoprotein	200—650
Transferrin	200—400
IgA	90—450
IgM	60—250
IgE	~300
α_2-Macroglobulin	160—370
Haptoglobin 1-1 type	100—220
2-1 type	160—300
2-2 type	120—260
At least 100 known minor plasma proteins	<100

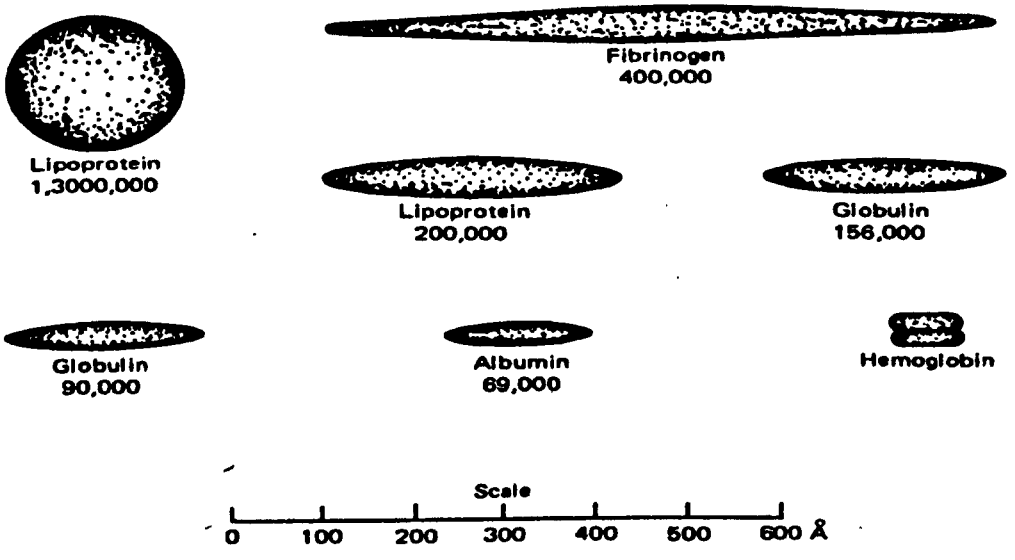


Fig 2.16

Molecular weight and shapes of some plasma proteins (from Weiss, 1983)

3. Adhesion of cell components (thrombocytes, granulocytes and monocytes) to the protein coating.
4. Formation of fibrin onto the surface and also possible activation of the fibrinolytic system (Sundaram et al, 1993).

Protein Adsorption

It is commonly stated that the first observable event at the interface between a foreign material surface and blood is adsorption of blood proteins onto the material (Baier & Dutton, 1969). This occurs in a few seconds when blood comes into contact with the material (Sawyer & Pate, 1953; Sharma, 1981). The proteins adsorbed initially are those present in relatively high concentration in the plasma, including species such as albumin, fibrinogen, γ-globulin and IgM. However, these adsorbed proteins will eventually be replaced by trace proteins such as the coagulation proteins of the intrinsic and extrinsic pathways, complement proteins and fibrinolytic proteins (Anderson et al, 1990), by high molecular weight kininogen (HMWK) and factor XII (Hageman factor). This is called the " Vroman effects" (Vroman, 1988). It is also believed that the adsorption rate, quantitative composition of sorbed protein layer, protein conformation changes in the spatial architecture and protein globular structures on the polymer surface define the subsequent events (Weiss, 1983; Andrade & Hiady, 1986).

Proteins are complex macromolecules with molecular weight ranging from thousands to millions (Fig 2.16) (Weiss, 1983).

Fibrinogen is a soluble blood component that comprises 0.2% by volume of whole blood. It plays a physiological role in the mechanism of haemostasis by forming a dense fibrin network in the presence of thrombin as part of the intrinsic and extrinsic

blood coagulation cascades (Mosesson, 1990). Also, it has long been realised that the adhesion of platelets is promoted by surfaces having adsorbed fibrinogen (Packham et al, 1969; Zucker & Vroman, 1969), and that platelet adhesion is reduced when pre-adsorbed albumin is present on the surface (Lee & Kim, 1979). It is considered the

information on fibrinogen and albumin adsorption is relevant for blood-biomaterial interactions (Forbes & Courtney, 1994). There has been considerable work in attempting to correlate the blood compatibility of biomaterials with the adsorption of fibrinogen, albumin and other proteins (Magnani et al, 1994; Sheppard et al, 1994; Anderson et al, 1996). The adsorbed fibrinogen has been utilised in monitoring the blood response to PVC-P tubing and is regarded as an index for blood compatibility evaluation (Yin et al, 1999a,b).

The forces for protein adsorption onto the surface are mainly due to the hydrophobic interaction, electrostatic interactions and conformational entropy of proteins (Norde, 1996). It has frequently been reported that hydrophobic surfaces adsorb more protein than hydrophilic ones. For instance, it was found that fibrinogen adsorption to surfaces with a hydrophobicity gradient decreased with decreasing water content angle (Elwing et al, 1987). With respect to surface charge, its effect on adsorption is not straightforward. Proteins that carry the same net charge at the surface might still bind to the surface. The driving force in this case was suggested by Norde (1996) to be an increase in entropy, due to conformational changes of the protein resulting in loss of secondary structure.

For most of globular proteins as shown in Fig 2.16, the amount adsorbed to most solid surfaces is usually around or below that which would correspond to a close-packed monolayer of protein (Baszkin & Lyman, 1980; Chan & Brash, 1981; MacRitchie, 1986) and the estimation of the amount of human blood protein adsorbed as a monolayer is shown in Table 2.9 (Iordanskii et al ,1994).

Polymers such as polyethylene, polystyrene and PTFE have demonstrated a pseudo-Langmuir type of adsorption, in agreement with monolayer adsorption (Lee & Kim, 1974). However, the occurrence of di- and multilayers has also been reported (Lee & Kim, 1974; Young et al, 1988).

Table 2.9 Geometric parameters of human blood (Iordanskii et al, 1994)

Protein (ig/cm ^{2})	MW x10 $^{-3}$	Globular Size (nm)		Monolayer coverage	
		diameter	length	Theory (Side-on packing)	Experiment $^+$ (PDMS)
Albumin	67.5	4.0	11.5	0.66	0.6
γ-globulin	169	4.4	23.5	1.85 *	1.6
Fibrinogen	340	6.5	47.5	0.18	0.17

* " end-on" packing ; + Protein adsorption on polydimethyl siloxane (PDMS)

Since proteins are large heterogeneous copolymers, their adsorption behaviour might be modified by temperature and buffer, suggesting that protein adsorption is also temperature and buffer media dependent (Slack & Horbett, 1992; Matat et al, 1997). Other factors, such as adsorption time and the blood hydrodynamic conditions also influence protein adsorption, in particular, the adsorption mode, for example, reversible or irreversible binding to polymer surface. Even for one type of protein, the ratio between reversibly and irreversibly sorbed molecules changes over time.

Surface modification by controlling protein adsorption for improving blood compatibility is one of the most practical approaches (Engbers & Feijen, 1991) and it is discussed in Chapter 3.

Platelet reaction

Platelet deposition on artificial surfaces is a characteristic feature of platelet function *in vitro* and *in vivo* (Whichter & Brash, 1978; Wilson et al, 1985; Courtney et al, 1994). The attachment and activation of platelets are associated with the adsorption of adhesive proteins, which are capable of binding to the platelet glycoprotein IIb-IIIa

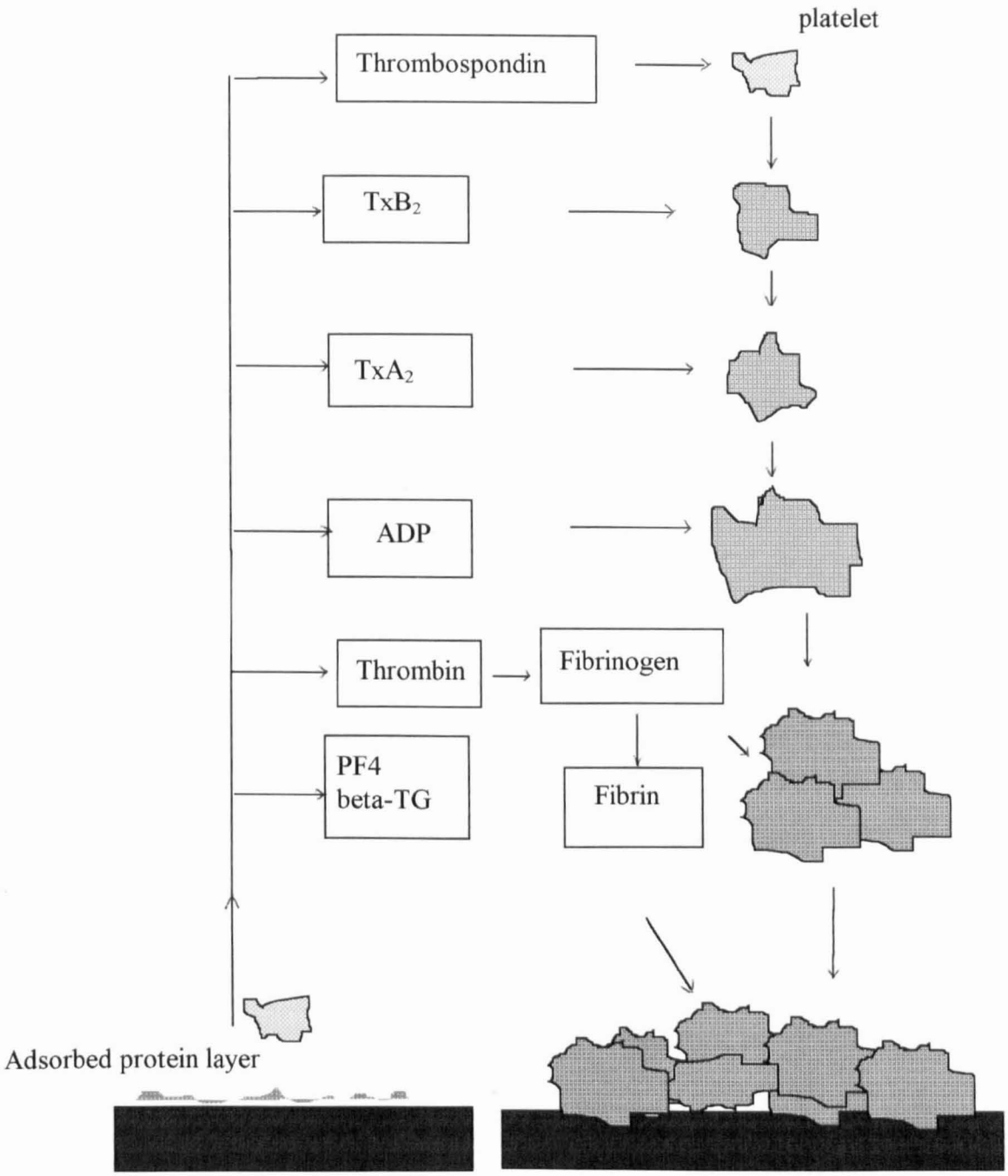


Fig 2.17 Platelet reaction to artificial surfaces (modified from Bantjes, 1978)

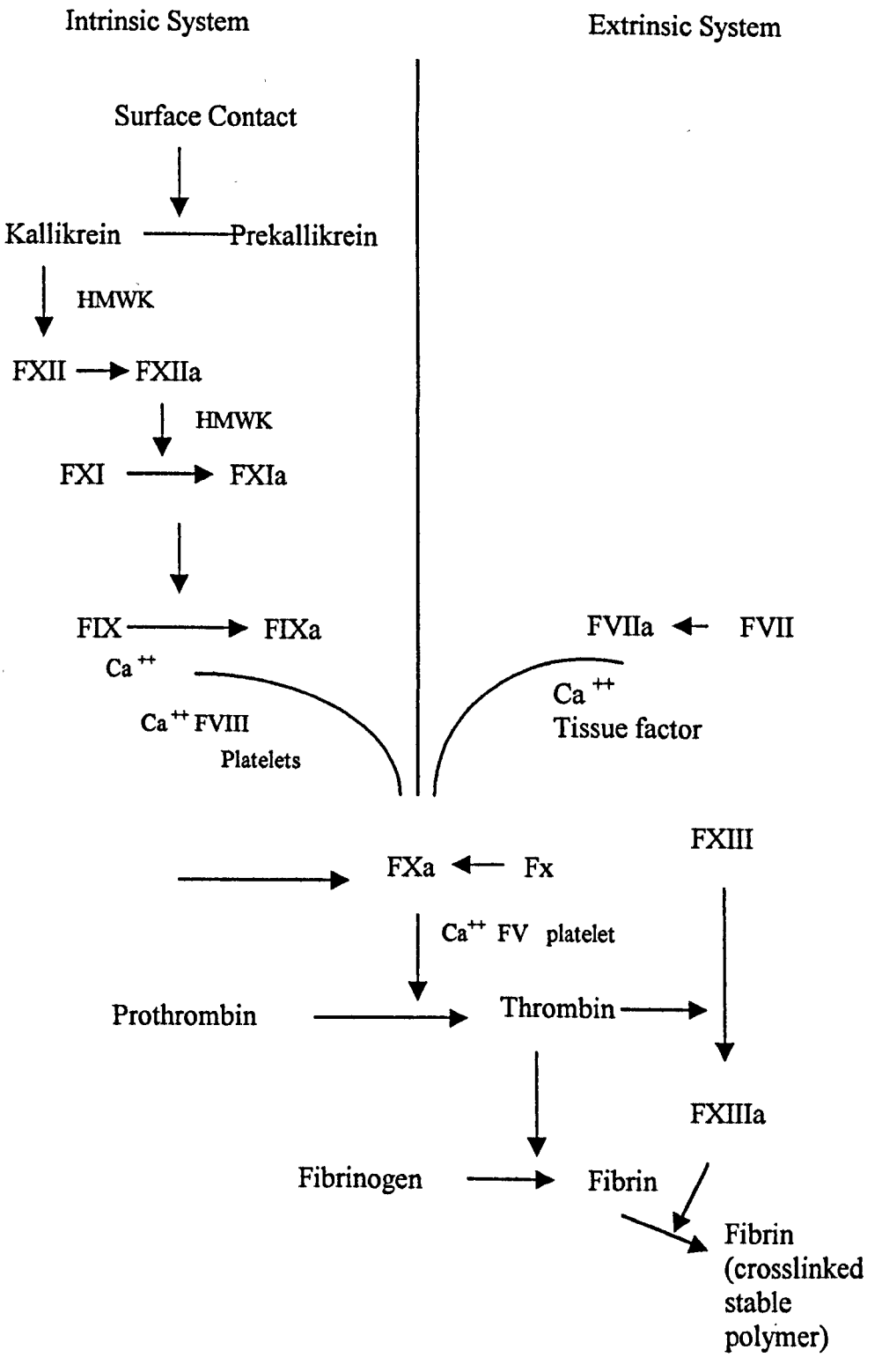


Fig 2.18 Mechanism of clotting factor interaction
(from Yin, 1996)

(GPIIb-IIIa) complex and other receptors of stimulated platelets. This complex is able to specifically recognise the Arg-Gly-Asp(RGD)-tripeptide sequence of adhesive proteins (Ruoslahti & Pierschbacher, 1987), such as fibrinogen (FGN), fibronectin (FN), vitronectin (VN) and von Willebrand factor (vWF) (Fabrizius-Homan & Cooper, 1992). After deposition, platelets undergo morphological changes and contraction (Allen et al, 1979; Lelah et al, 1984), and the platelet constituents such as adenosine diphosphate (ADP), serotonin and other substances are excreted. These released different constituents will affect further platelet adhesion and aggregation to the surface.

Fig.2.17 schematically describes the platelet reaction to artificial surface. It can be seen that ADP causes more platelets to stick to those already adhered on the surface, leading to aggregation (Bantjes, 1978). The procoagulation activity of platelets will induce the fibrin production through FXIIa, which results in a further promotion of platelet adhesion and aggregation (Waugh & Baughman, 1969; Chuang et al, 1979) and the secretion of PF4, TXB_2 and thrombospondin, which may be important in mediating platelet aggregation on a polymeric surface (Courtney et al, 1994).

Coagulation

Thrombus formation occurs either intrinsically by surface-mediated reactivity, or extrinsically through factors derived from tissues. The two systems converge upon a final common path, which leads to the formation of an insoluble fibrin gel when thrombin acts on fibrinogen. For blood-biomaterial interactions, the system of intrinsic coagulation is the most important part (Courtney et al, 1994).

From the biochemistry point of view, there are at least 12 plasma proteins, which interact in a series of reactions leading to blood clotting. Their biochemical properties are summarised in Table 2.10, and the clotting mechanism is shown in Fig 2.18.

Table 2.10 Human proteins of coagulation system

Protein (clotting factor)	Molecular weight (No. of chains)	Normal plasma concentration (μg/ml)	Active form
Intrinsic system			
Factor XII	80,000 (1)	30	serine protease
Prekallikrein	85,000(1)	50	serine protease
High MW kininogen	105,000(1)	70	cofactor
Factor XI	160,000(2)	4	serine protease
Factor IX	68,000 (1)	6	serine protease
FactorVIII	265,000 (1)	0.1	cofactor
vWF	1-15,000,000	7	cofactor for platelet adhesion
Extrinsic system			
Factor VII	47,000 (1)	0.5	serine protease
Tissue factor	46,000 (1)		cofactor
Common pathway			
Factor X	56,000(2)	10	serine protease
Factor V	330,000 (1)	7	cofactor
Prothrombin	72,000 (1)	100	serine protease
Fibrinogen	340,000(6)	2500	clot structure
Factor XIII	320,000 (4)	15	transglutaminase

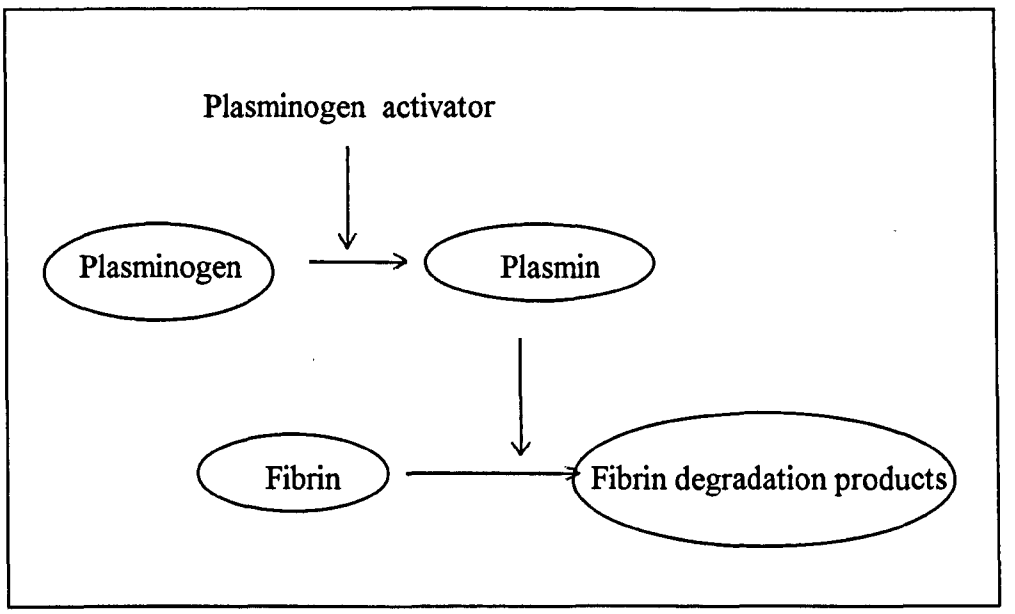


Fig 2.19 Schematic representation of fibrinolytic sequence

(from Yin, 1996)

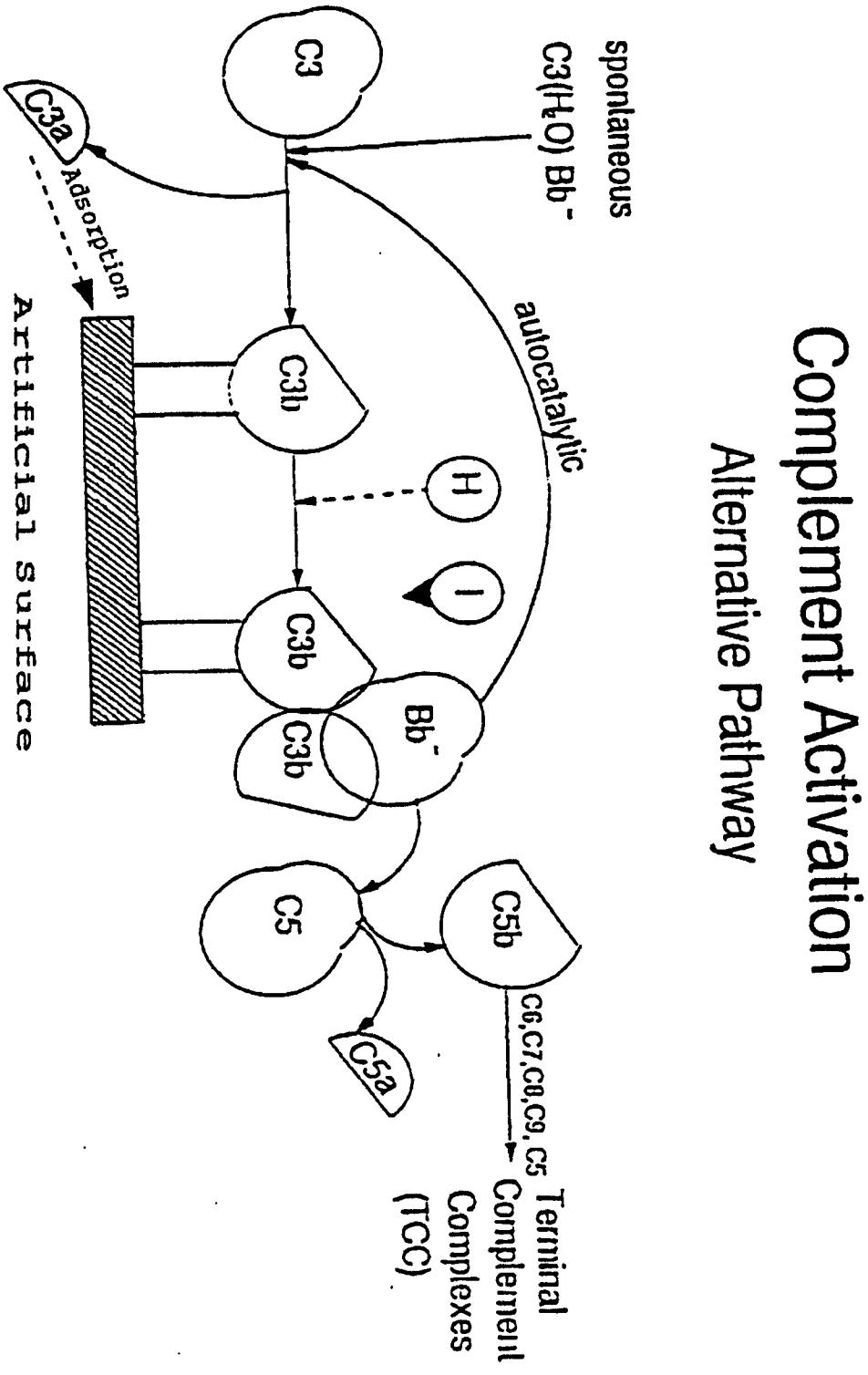


Fig 2.20 Scheme of the alternative pathway of complement activation.
Complement activation starts with the binding of C3b on the surface of biomaterial
(From Diamantoglou & Vinken, 1996)

Fibrinolysis

The fibrinolytic system removes unwanted fibrin deposits and facilitate the healing process after injury and inflammation. It is a multicomponent system composed of precursors, activators, cofactors and inhibitors, and has been studied extensively (Forbes & Courtney, 1987; Coleman et al, 1994). A simplified scheme of the fibrinolytic pathway is shown in Fig 2.19.

Erythrocytes and Leucocytes

Erythrocytes (red cells) make up 96% of the total blood cell volume but in discussing blood cell-foreign material surface interactions, relatively little attention has been paid to erythrocytes. It is known that this interaction may result in the release of several lipids and fatty acids from the red cell membrane, causing erythrocyte adhesion, a significant change in cell (membrane) metabolism, or haemolysis (Buck et al, 1969). The adhesion of red cells to a foreign material surface is mainly determined by the nature of surface, e.g. chemical structure and roughness, and blood flow parameters, e.g. shear rate, along the surface (Mohandas et al, 1974).

Reaction of leucocytes, also called as white blood cells, is directly related to the host defence of the human body towards foreign material surfaces. The interaction of white blood cells and foreign material surfaces is mediated by the adsorbed protein layer at the surface and depends also on the type of white blood cell. In blood-contacting applications, the leucocyte response is often linked to the relationship between leucocytes and complement activation (Courtney et al, 1994).

Complement activation

Activation of the complement system caused by blood-material interaction is believed to occur through the alternative pathway (Kazatchkine & Carreno, 1988). The complement system consists of about 20 proteins, which can be stimulated by bacteria

and endotoxins, but also by a foreign material surface such as a dialysis membrane. It is part of the immune system in response to a foreign material. For dialysis membranes, the decision to develop and use new membranes has followed their reduced capacity to induce complement activity in clinical dialysis (Diamantoglou & Vienken, 1996) (Fig 2.20).

Triggering of complement activation only depends on the chemical composition of the foreign material surfaces which are relevant to the affinity to C3b. In principle, a hydrophilic hydroxyl group gives rise to low coagulation and platelet adhesion, but considerably stimulates complement activation. Cationic surfaces trigger platelet adhesion, but show little influence in the activation of complement and coagulation (Diamantoglou & Vienken, 1996).

Interrelationships

As expected, blood-biomaterial interactions are very complicated, and there are many interrelated reactions and feedback networks (Piskin, 1992). For example, platelet reactions are interrelated with the coagulation system to promote thrombin formation while it can interact with the fibrinolytic system by binding of plasminogen to the GPIIb-IIIa complex (Adelman et al, 1988). It is found that leucocytes are involved in the intrinsic coagulation, fibrinolysis and complement activation. The leucocyte membrane contains phospholipids, which may play a role in blood clotting via the intrinsic pathway (Miller & Anderson, 1988). Marchant (1984) showed that leucocyte adhesion is complement-mediated through the complement protein C3b and Bb. This interrelationship is very important for extracorporeal blood-contacting applications (Courtney et al, 1994).

In summary, when artificial surfaces are exposed to blood, interrelated blood response systems occur in order to make quickly a balance between the processes of activation and inhibition of these systems. Although a great deal is known about the blood response to blood-contacting biomaterials or devices, important interrelationships are not fully defined in many instances. A compromise has to be made for blood-contacting biomaterial development (Diamantoglou & Vienken, 1996).

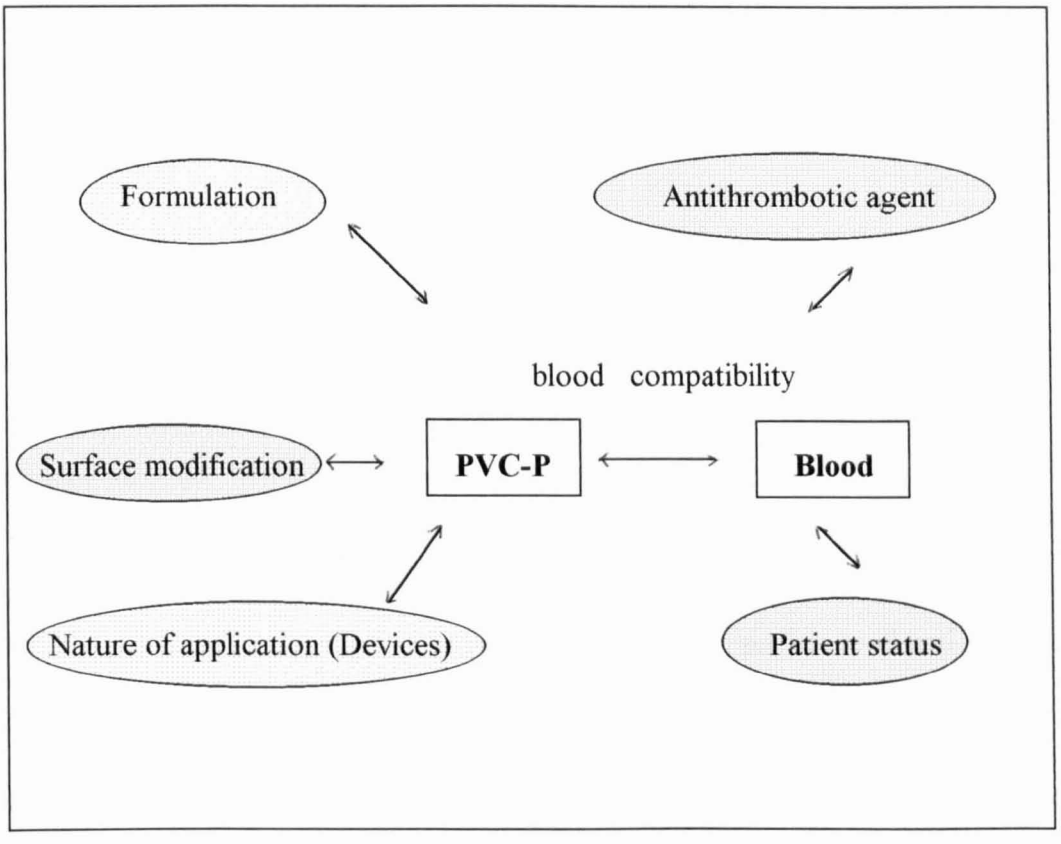


Fig 2.21 Factors influencing blood compatibility of PVC-P

2.3.3 Factors influencing blood response to PVC-P

Similar to other blood-contacting biomaterial such as polyurethane, the blood interactions with plasticised PVC (PVC-P) will lead to protein adsorption, coagulation activation, platelet reactions, fibrinolysis, complement activation and other cellular responses. The blood compatibility of PVC-P is dependent on many factors, as summarised in Fig 2.21.

PVC formulation

The final properties of PVC-P are mainly determined by plasticiser type and concentration incorporated. The correlation between plasticiser selection and end-product properties such as mechanical properties, low temperature properties, surface properties, permanence, electrical properties and cost-effectiveness has been discussed in previous sections. With respect to the blood compatibility of PVC-P for blood-contacting applications, the PVC formulation in terms of plasticiser selection and plasticiser concentration is considered the most important. The surface characteristics of PVC-P, such as plasticiser surface distribution, plasticiser surface level and surface morphology are also dependent on the formulation.

Selection of plasticiser

When PVC-P is applied as blood and blood components packaging material, the blood response is strongly affected by the selection of plasticiser.

From the original introduction of PVC-P into medical applications until the early 1980s, all PVC blood bag plastics contained the plasticiser DEHP (Carmen, 1993). It is found that DEHP can interact with the red cell membrane (Estep et al, 1984; Aubuchon et al, 1988) and improve the survival time of erythrocytes and their osmotic fragility and flexibility after prolonged storage, both in vitro (Rock et al,

1984; Horowitz et al, 1985) and in vivo (Aubuchon et al, 1982). DEHP has been found to induce reduced platelet function as defined by hypotonic shock recovery (HSR) (Ishikawa & Sasakawa, 1984) and aggregation (Ishikawa et al, 1983).

In recognising that DEHP is extracted into the stored blood or blood components, alternative plasticisers have been developed during the last decade, mainly for the storage of platelet concentrates. An example was a PVC formulation plasticised with TEHTM (Champion et al, 1987; Simon et al, 1983).

PVC-TEHTM was found to be unsuitable for red cell storage because this polymer had no stabilising effect on red cell membranes (Estep et al, 1984; Rock et al, 1984) and reduced in vivo survival time (Aubuchon et al, 1988). The whole blood stored in PVC-TEHTM and other non-PVC materials with no DEHP always had greater haemolysis (Carmen, 1993). This seems to imply that the blood response to PVC-TEHTM is more reactive than that to PVC plasticised with DEHP.

The most prominent advantages for PVC-TEHTM are its low extraction and improved gas exchange capacity (Simon et al, 1983; Heaton, 1986; Walker et al, 1984). The increased gas exchange rate or O_2 permeability is beneficial for platelet survival, which can be achieved by increasing the plasticiser concentration, resulting in a decreased PVC resin. In the utilisation of TEHTM, this is the case (Heaton, 1986) while the PVC-DEHP with a high level of DEHP is not preferred because of its poor compatibility with platelets (Carman, 1993).

Other than TEHTM, some phthalates have been reported to be able to improve O_2 permeability with physicochemical properties that are quite similar to those of DEHP. These are di-n-decylphthalate (DnDP) (Shimizu et al, 1989) and diundecyl phthalate (DUP) (Ito et al, 1995). DnDP is reported to be the most desirable plasticiser for increasing gas diffusion. This is achieved not by increasing the plasticiser concentration and it is related to the nature of DnDP (Shimizu et al, 1989). The selection of DUP for PVC formulation is strongly dependent on the selection of PVC resin. A highly porous PVC resin had to be employed for the formulation with DUP. It was claimed that PVC-DUP may be used not only for platelet storage but also for the storage of erythrocytes at low temperature or for storing plasma in a frozen state (Ito et al, 1995).

One US patent (Barnes & Mahal, 1987) reported using PVC plasticised with a blend of plasticisers, comprising a plasticiser resistant to extraction by blood such as TEHTM, and a blood extractable plasticiser such as DEHP or DEHA for storage of red blood cells and platelets. The nature and amount of DEHP present in the PVC were sufficient to allow at least 21-day storage of red blood cells and the total amount of plasticiser blend was able to enhance the gas permeability to allow at least 5-day storage of platelets. The important advantage of this invention is that a combination of benefits could be achieved both from DEHP and TEHTM.

Polymeric adipate (PA) plasticiser is developed for reduced extraction by blood or other body fluids. The influence of PVC formulations with DEHP, PA and TEHTM on the platelet release reaction and complement activation has been studied (Jones et al, 1989) Results indicate that plasticiser selection influences the blood response.

Ideally, the plasticiser selection should be able to support the storage of red cells, stabilising membranes, whilst causing few, if any, deleterious effects by any leaching of plasticiser into blood or blood components. In the meantime, gaseous exchange should be at least as good as that of PVC-TEHTM for platelet storage. PVC plasticised with BTHC may be such a choice (Kevy et al, 1985; Seidl et al, 1991; Gulliksson et al, 1991; Turner et al, 1995).

Since Hull and Mathur (1984) suggested that citrates might be useful as replacement for DEHP plasticiser in medical grade PVC formulations, citrates such as BTHC and ATHC with low toxicity have received attention. BTHC has been shown to have a stabilising effect on red blood cell membranes similar to DEHP (Buchholz et al, 1989), resulting in good autologous in vivo survival (Hogman et al, 1991). Most importantly, there were no demonstrable toxic effects of BTHC on livers of rats fed with the plasticiser, unlike DEHP (Jacobson & Kevy, 1988). In addition, PVC-BTHC has been found to be suitable for storage of platelets for 5 days, which is very similar to PVC-TEHTM (Turner et al, 1995).

Acetyl-tri-n-butyl citrate (ATBC) was shown to have the membrane-protective effect similar to BTHC. There is no significant difference between the values for cell stored in PVC-ATBC and PVC-DEHP containers (Carman, 1993).

Plasticiser concentration

The blood compatibility of PVC-P is very much dependent on the plasticiser concentration or level of PVC. Labow et al (1987) found that the blood cell deformability changes were reversed by addition of DEHP, and there was a direct correlation between DEHP concentration during storage and RBC membrane flexibility. The increased DEHP concentration might be able to enhance gas exchange rate, but it is limited by the processibility and the blood reactivity to the surface with a high plasticiser level (Fayz et al, 1977; Fishbein, 1984; Ljungren, 1984).

Kicheva et al (1995) investigated the effect of DEHP concentration on the biocompatibility of PVC-DEHP. They found that the amount of total protein adsorbed on PVC-DEHP increases with the increased DEHP concentration. A surface coated layer of paraffin was utilised to decrease the protein adsorption.

Plasticiser surface level

Efforts to determine the effect of surface plasticiser level on biocompatibility of PVC-P have been made by Kim et al (1976) and Spilezewski et al (1988). It has been shown that the removal of DEHP from PVC-P surface alters the blood compatibility. An attempt to bring plasticisers to the PVC-P catheter surface by pretreating at 37°C for 24 h in PBS solution caused the highest level of inflammation compared to polyurethanes. The high plasticiser surface level in the PVC can alter the inflammatory response to the material and thus affect its relative biocompatibility.

Plasticiser surface distribution

Plasticiser surface distribution has been found to have great influence on blood compatibility. Table 2.11 lists blood response to three types of haemodialysis blood lines with different surface plasticiser distribution and the blood compatibility, in terms

of C3a desarg concerntration *in vivo*, is strongly dependent on the surface composition (Branger et al, 1990). High quality medical grade PVC-DEHP, polyurethane coated PVC-DEHP and polyurethane coated PVC-TEHTM blood tubing were selected for connecting to an extra-corporeal circuit with heparin administration. This circuit consisted of an arterial bloodline, connected to the venous bloodline. The total length of the tubings was 8m. Blood flow was kept constant (250-300 ml/min). The C3a desarg generation rate was expressed as μg/min.

Table 2.11 Correlation of surface composition with C3a generation rate (Branger et al, 1990)

Blood lines	Surface plasticiser distribution	C3a generation rate (μg/min)		
		5 min	15 min	30 min
PVC-DEHP	DEHP mainly	53 ± 48.6	12 ± 13.8	8 ± 14.6
*PVC-PU-DEHP	PU mainly	10 ± 21.9	9 ± 16.4	7 ± 10.8
*PVC-PU-TEHTM	TEHTM/PU	50 ± 42	23 ± 26.1	35 ±32.5

It was also found that a higher TEHTM surface distribution will lead to a stronger blood response in terms of fibrinogen adsorption and the generation of C3a than that of surface plasticised with DEHP (Yin, et al, 1999b;)(Table 2.12). However, theoretically, if it is simply to increase the DEHP plasticiser level by 1.25 fold to the same level as TEHTM at PVC-TEHTM surface, the so-calculated fibrinogen adsorption and C3a values are found the approximately same as those obtained by experiment on PVC-TEHTM (Table 2.13).

Table 2.12 Correlation of Surface composition with *in vitro* fibrinogen adsorption and C3a measurement

Samples	Plasticiser distribution (%)	Blood response (*in vitro*) Fibrinogen adsorption (ng/cm^2)	C3a generation (ng/cm^2)
PVC-DEHP	68	4.1	1309
PVC-TEHTM	85	5.8	1671

Table 2.13 Theoretically evaluation of the effects of plasticiser surface distribution on blood compatibility based on *in vitro* assessment

Samples	Plasticiser distribution (%)	*In vitro* blood test Fibrinogen adsorption (ng/cm^2)	C3a (ng/cm^2)
PVC-DEHP	***85*** (68 x 1.25*)	***5.1*** (4.1 x 1.25*)	***1636***(1309x1.25*)
PVC-TEHTM	***85***	***5.8***	***1671***

***Note: normalisation of plasticiser surface distribution with *in vitro* blood compatibility evaluation.**

It might imply that TEHTM and DEHP have the same chemical nature which will induce the similar effect on the blood compatibility.

Surface contamination other than from plasticisers has also been found. By using Attenuated Total Reflectance (ATR)-FT-IR, a layer of an amide wax at the inner surface of PL-146 blood bag was found. The bag was made of PVC-DEHP with wax as an anti-tack agent in plastic materials. It was believed the surface contamination will have great effects on blood compatibility (Kasemo & Lausmao, 1988; Chawla & Hinberg, 1991).

will have great effects on blood compatibility (Kasemo & Lausmao, 1988; Chawla & Hinberg, 1991).

In addition, surface roughness is of great influence on blood response of PVC-P. It was found the blood compatibility of the PVC-DEHP coextruded with PU got worse after 6 months implantation mainly due to a change of surface morphology (Branger et al, 1990).

Surface modification

The surface of a material (the outermost few atomic layers) is the only part of the material that can interact with blood. Modification of the surface will alter its blood response and it is the most common approach to improving the biomaterial influence on blood (Jacobs et al, 1988; Courtney et al, 1994). Surface modification can be achieved by an increase in hydrophilicity, chemical modification, attachment of antithrombotic agents, treatment of surfaces with protein and preparation of biomembrane-mimetic surfaces (Courtney et al, 1994). Modification of PVC surface is considered in more detail in Chapter 3.

Nature of application as devices

PVC-P has found wide applications as a blood-contacting material to form a device, which will be used for the patient. It could be used for a relatively short time (minutes to hours), such as a catheter, a haemodialyser, a blood oxygenator, blood tubing in the extracorporeal devices etc. or for a relatively long time (days to months), such as a blood or blood components storage bag, or even could be incorporated in the cardiovascular systems for rather long time (years) such as an artificial vessel and an artificial heart component etc (Rosato, 1983). Therefore the dynamic flow conditions of blood (shear rates, turbulence, secondary flows), duration of contact, size of the contact surface area, and actual placement site in the cardiovascular system are very

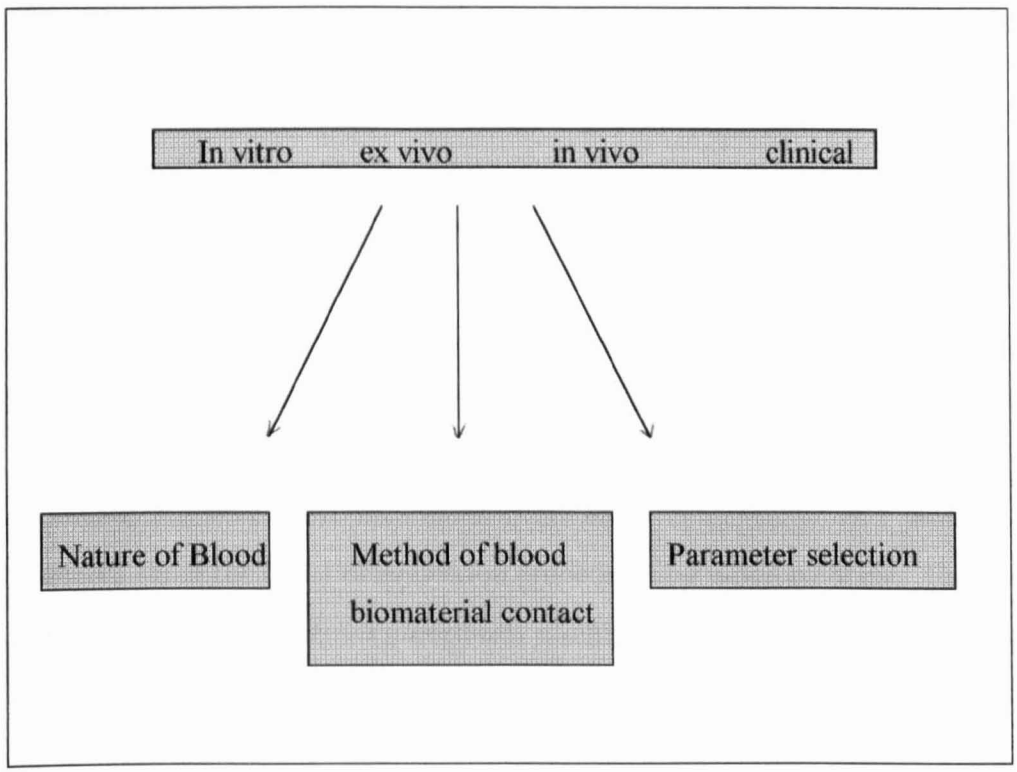


Fig 2.22 The options and features of evaluation procedures (Courtney et al, 1994)

important parameters which are related to the nature of application (Missirlis, 1992; Sundaram et al, 1993).

Blood nature and evaluation procedures

The clinical application of PVC-P as blood tubing or blood bag generally requires the administration of an anticoagulant or antithrombotic agent such as citrate, heparin and prostacyclin (PGI_2) etc. The presence of these agents, for instance, heparin, will influence blood compatibility of PVC-P tubing with a reduced TAT levels and increased C3a values as found *in vitro* assessment (Yin et al, 1999 a). In addition, the blood response to a PVC-P biomaterial is influenced by the blood condition of an individual patient (Courtney et al , 1994), which makes the evaluation of blood compatibility in clinical conditions even more complicated and leads to a great concern about the effect of evaluation procedures on blood response. The options and basic features of evaluation procedures on the blood compatibility of biomaterials can be shown in Fig 2.22 (Courtney et al, 1994).

2.4 Plasticiser migration

2.4.1 DEHP migration and extraction

Although the aqueous solubility of DEHP is very low (< 0.04mg/ml at 20°C) (Jaeger&Rubin, 1973), it is not covalently bound in the PVC matrix and may therefore migrate out of the polymer into contacting media. Since 1970, it has been known that DEHP is present in blood stored in PVC bags (Jaeger & Rubin, 1970a,b; Marcel & Noel, 1970) and is released into patients given blood transfusions (Jaeger & Rubin, 1972; Marcel, 1973; Hillman et al, 1975). The varied extraction rates have been reported ranged from 50 to 70 mg/l in blood (Jaeger & Rubin, 1970 b) and 20mg/per pack in platelet concentrates (Jaeger & Rubin, 1973; Valerietal, 1973). These observations led to the publication of hundreds of articles and reviews on this subject (Peck & Zuck, 1977; Racz et al, 1993) and related toxicology study of DEHP (Autian, 1973; Thomas et al, 1978; Woodward, 1988; Rubin & Ness, 1989).

The migration of DEHP into the human body from haemodialysis blood tubing has also been found since the early 1970s (Nergagard, 1971; Ono et al, 1975; Ono et al,

1976; Nassberger et al, 1987; Flaminio et al, 1988). These findings caused great public concern about the toxicity of DEHP.

2.4.2 Toxicity of DEHP

DEHP has an extremely low acute toxicity with LD_{50} in excess of 30,000 mg/kg. Putting this into perspective, ethanol has an acute toxicity an order of magnitude higher (LD50 = 3,300 mg/kg) (Wilson, 1995). The tests of chronic toxicity of DEHP carried out before 1978 showed no evidence of chronic toxic effects. But some long-term animal feeding studies later suggested adverse effects on several major organ systems, such as the liver and the reproductive system.

The most important finding causing a great concern of DEHP toxicity is that carried out by the National Toxicity Program (NTP)/ National Cancer Institute (NCI) Bioassay Program of America in 1978. It was concluded that DEHP was carcinogenic in Fischer 344 rats and $B_6C_3F_6$ mice and caused a significant increase in liver tumours (Srivastava et al, 1978). The dose levels were extremely high, which corresponding to a human intake of ¼ litre per day (on rats) and ½ litre per day (on mice) respectively.

However, many later experiments indicate that DEHP is a tumour promoter rather than a tumour initiator and MEHP (mono-(2-ethylhexyl)-phthalate), a major hydrolysis product of DEHP, was much more toxic than the parent compound and shown to be effective for tumour promotion at a lower dose (Ward et al, 1986).

In Europe, the main work on this subject was co-ordinated by European Council for Plasticiser and Intermediates (ECPI). This detailed research has drawn the following conclusion (Wilson, 1995):

1. DEHP is not genotoxic, that is to say unlike direct carcinogens, it does not react with genetic material.
2. The mechanism by which it causes tumours after repeated high dosing of rodents is believed to be peroxisome proliferation, which is the same case as some safely used hypolipodaemic drugs.
3. The results are specific to rodents. Feeding of DEHP to species metabolically closer to humans does not cause peroxisome proliferation or liver tumours.
4. There is no significant difference in effect between DEHP and the alternative general purpose phthalate plasticisers.

The above conclusions do not imply any restriction on the research and development of other alternatives of DEHP towards a lower migration property in order to ease the increasing concern about the leaching of DEHP into the human body. In addition, the loss of plasticiser will alter the mechanical properties of PVC-P to make the plastic useless and even dangerous to human body. For instance, a linear relationship was demonstrated between hardness and released amount of DEHP per surface area during extraction by Ljunggren (1984). Loss of plasticiser causes the PVC device to become more rigid and, for example, in the case of nasogastric feeding tubes or wound drainage tubes, removal from the body after 21 days can be painful and difficult, possibly involving surgery (Hayhurst & Wyman, 1975; Jones, 1989). Meanwhile, the loss of plasticiser must affect the surface properties of the plastic, which will alter the blood compatibility. For the relatively tong term application, the search for alternatives to DEHP and PVC-P or the modification of PVC are continuing.

2.4.3 Alternatives to DEHP

In section 2.2, three types of alternatives to DEHP, which have been approved for medical practices, are reported. They are a trimellitate such as TEHTM (Jacobson et al, 1980; Flaminio et al, 1988), a polymeric plasticiser such as PA (Graham, 1978; Biggs & Robson), a citrate such as BTHC (Gulliksson et al, 1991).

With respect to plasticiser migration or extraction, many studies indicate that less TEHTM is apparently leached from PVC bags or haemodialysis tubing than DEHP. For example, TEHTM migration into stored blood components is 1/100 or less than that of DEHP. Platelet concentrates stored for 7 days at room temperature in PVC-TEHTM bags contain only 0.2 mg TEHTM per pack. TEHTM is generally not detectable in refrigerated red cell products, even after a 42-day storage period (Carmen, 1993).

Polymeric adipate (PA) is a type of extraction resistant plasticiser which has been permitted for use in flexible PVC for food contact applications and for many medical devices throughout Europe (Biggs & Robson, 1984).

From the viewpoint of migration, citrates such as ATHC and BTHC are not extraction resistant plasticisers because of their relatively lower molecular weight than those of TEHTM and PA. It was found that platelet concentrates stored for 7 days in CL-4093 plastic (a PVC-BTHC container) contain about 20mg of BTHC per unit. This is the same order of magnitude as DEHP, and it seems reasonable to assume that RBA products would extract BTHC and DEHP at roughly the same rates (Carmen, 1993). However, these esters provide a low order of toxicity when compared to DEHP or other phthalate esters. They are the most promising alternatives to DEHP and have replaced DEHP plasticiser for many blood-contacting applications including:

1. Bags for the storage of whole blood and blood components such as red blood cells, platelets and plasma.
2. Intravenous tubing for the transportation of blood, blood products and crystalloid fluids.
3. Indwelling intravenous and intra-arterial catheters.
4. Blood tubing contact with blood as used in: haemodialysis devices, oxygenators as used in open heart surgery, membrane oxygenators for pulmonary support, apheresis for the collection of platelets and leucocytes for transfusions and intensive plasma exchange devices.

Recently, some new citrates named as acetyl-tri-n-(hexyl/octyl/decyl) citrate are produced and have been found useful as medical-grade plasticisers in PVC compositions with improved extraction resistance, particularly in soapy water extraction tests in a simulated blood fluid situation (Hull & Frappier, 1991).

The 70s' findings related to the leaching and toxicity of DEHP also encouraged the development of internally plasticised PVC to replace DEHP at least partly to a level where plasticiser migration problems are eliminated (Laverty & Gardlund, 1976). PVC could be copolymerised with poly (ethylene oxide) to form a ABA block copolymer wherein the A parts are PVC segments. PEO serves as an internal plasticiser. It was also possible to produce a thermoplastic PVC block copolymer in AB type and the B block is flexible linear aliphatic polyester or polyether which serves as an internal plasticiser (Laverty & Gardlund, 1981).

In addition, some new polymeric plasticisers have been developed, such as a carbon monoxide-propylene copolymer (Aaronson et al, 1992) and a polyester derived from glutaric acid and a diol (Vyvoda, 1994). These polymeric plasticisers were claimed to be able to replace DEHP partly or totally for application in medical devices.

In summary, although numerous alternatives to DEHP have been developed during last 30 years, the detailed evaluation of their suitability for the blood-contacting applications, especially, the blood compatibility is relatively lacking. Their all-round performances have to be compared with those of DEHP, the most extensively studied plasticiser, for their future development.

2.4.4 Alternatives of PVC-P as a blood-contacting biomaterial

PVC-P can provide a wide array of functional performance characteristics at a low cost and any potential replacement material will need to provide similar performance at a comparable total system cost. A list of alternative materials to PVC for blood tubing applications has been given by Blass et al (1992), covering polyurethane/PVC

coextrusion material, polyurethanes, and silicone rubbers. Obviously, the high cost of these polymers has prevented the application as a replacement for PVC blood tubing.

Recently, some alternatives to PVC-P have been developed. Among them are metallocene polyolefins (polyethylene and polypropylene) (Shang & Woo, 1996; Lipsitt, 1997,1998), ethylene-vinyl acetate (EVA) (Drago & Kuhlemann, 1996) and polyether-ester plastic (Spencer, 1998). Metallocene polyolefins such as metallocene polyethylene (mPE) are produced with metallocene as a catalyst. This metallocene technology makes it possible to precisely control the molecular architecture and get a narrow molecular weight distribution of polyethylene. mPE has demonstrated enhanced toughness, sealability, clarity and elasticity with low extractables and has created many great opportunities for medical and health-care industries. It was concluded that mPE materials might be able to provide a high-performance, practical and cost-effective polymer to replace PVC-P materials (Lipsitt, 1998).However, before deciding to use metallocene polyolefins in their medical products, manufacturers will need to consider the material, design, processing, and the product performance characteristics of the compounds as they relate to every phase of product development. It still has long way to go (Shang & Woo, 1996).

Ethylene-vinyl acetate (EVA) film as an alternative to PVC-P film has been promoted as combining toughness and low temperature sealability with clarity, flexibility and impact and puncture resistance (Elaay, 1997). They have been accepted for blood-contacting application in many countries.

A recently developed ionomeric modified polyether-ester blended with PVC was reported to be used as a substitute for PVC in the conventional use of PVC as blood-contacting biomaterial while having advantages over such PVC material (Spencer, 1998). This might suggests that one of the most promising approaches for obtaining

improved extraction resistance, while maintaining other properties is to modify PVC-P either through PVC formulation or surface modification.

2.4.5 New development of PVC-P biomaterials

As one of the oldest commodity polymers, which have already achieved their prominent role in the medical plastics industry, PVC-P has been developed by many new technologies including new PVC resins, enhanced compounding technologies, PVC modification and novel alloys of PVC. These advanced PVC materials in many instances are replacing higher-priced plastics such as polyurethanes, silicones and other thermoplastic elastomers and finding a new way into medical device industry.

UHMW PVC resin

Flexible PVC has been recognised as a material for a long time with a notorious poor compression recovery properties, which are less easily resolved. If flexible PVC was available with a rubber-like recovery property, a number of new applications would become potentially available to this polymer as an alternative to silicones, polyurethanes and other rubber-like biomaterials.

Conventional PVC resin usually is of lower molecular weight (30,000~75,000) with short chains and is highly branched, while the rubber-like polymers are for most part highly crosslinked or are very high molecular weights (long chains). Therefore, one approach to develop new PVC with high elasticity is to increase the molecular weight of PVC resin. The ultra-high-molecular weight (UHMW) PVC is a PVC resin with a number molecular weight as high as 150,000 (K value > 100) (Brookman et al, 1993). Compared with the traditional PVC resin, these materials are more linear and have a higher degree of crystallinity. The formed flexible compounds based on UHMW PVC are superior to conventional types with improved compression recovery properties. They have found their way into the automotive industry and currently, a variety of uses of these polymers have been found in medical device industry (Carmen et al, 1998).

Further study of processing techniques due to their high melt viscosity, high cost and limited compatibility is still lacking.

New crosslinked compounding technology

A characteristic feature of PVC is that it does not contain sites of suitable reactivity to enable it conveniently to be crosslinked by reaction with common reagents. This means a highly plasticised PVC body often suffers from excessive creep and stress relaxation, when subjected to sustained stress or strain, and also exists the plasticiser migration problem.

PVC-P produced by a crosslinked compounding technology might be able to solve such problems. The formulation contains PVC plastisol, a polyisocyanate and diol or diamine or their mixtures. After compounding, the PVC-P polymer is produced with a network-like structure, which gives the resulting product with many improved properties (Petit & Ladang, 1995).

PVC modification

For improving the plasticiser migration property, PVC modification through PVC surface modification and PVC formulation has been approached.

Hatada & Kobayashi (1982) patented a PVC sheet for blood or infusion bag which are modified so that the diffusion of plasticiser to the surface is suppressed and compatibility of PVC with respect to the contents of the bag is improved. The key technique is using glow discharge treatment with fluorine gas. The formed thin fluorinated crosslinked layer on the PVC surface acts as a good barrier with respect not only to plasticiser such as DEHP diffusion to the surface but also to the diffusion of stored contents. Levin (1989) introduces some functional groups to PVC surface, which can be crosslinked under the influence of heat. Such crosslinking provides a

thin coating which prevents leakage of plasticiser and additives from the PVC-P substrate when it is contacted with extractants. Jayakrishnan et al (1995) coated PVC-P with crosslinkable PVC resin to reduce the migration of the plasticiser to potential organic extractants such as hexane.

Radiation grafting of hydrophilic monomers onto PVC-P surface has been found to be able to reduce the plasticiser migration. The hydrophilic monomers including 2-hydroxylethyl methacrylate (HEMA) and N-vinyl pyrrolidone (NVP) (Krishnan & Jayakrishnan, 1990; Krishnan et al, 1991). In addition, chemically binding of polyethylene glycol (PEG) to the PVC-P surface was reported to be able to make a migration-resistant and blood-compatible biomaterial (Lakshmi & Jayakrishnan, 1998).

Modification of PVC formulation using some specific additives can also reduce plasticiser migration. An example is the use of cyclodextrins. It was reported that a film cast from the mixed solution of PVC, DEHP and beta-cyclodextrin (β-CD or B-CD) had a reduced DEHP migration property (Sreenivasan, 1996).

Novel alloys of PVC

PVC, as a slightly polar material, has wide compatibility with many other polymers either synthetic polymers or natural polymers. In addition, researchers have developed materials known as compatibilisers that allow some polymers, normally not miscible with PVC - for example, polyethylene, polypropylene, butyl rubber, or natural polymers such as starch and cellulose to form useful alloys. Cyclodextrin is an example of such a compatibiliser applied for improving the mutual compatibility of polymers, including PVC with starch (Videau, 1997).

Some of the more interesting current alloys include PVC/Nylon, for enhanced physical and high temperature properties; PVC/urethanes, for high abrasion resistance, reduced

extractables (Ljunggren, 1983); PVC/polyolefin, especially suitable for applications requiring soft, oxygen-barrier materials (Wickson,1993).

Other new technology in the future (Brookman, 1998)

Advances in PVC technology will soon bring about a new generation of PVCs formulated to function in specific medical application to replace the conventional ones (Brookman, 1998). One is to mimic the successes of metallocene catalytic chemistry in polyolefins, a new PVC resin composed of blocks of syndiotactic, atactic, or isotactic resin could be designed for different applications.

Another approach is to produce PVC using non-free-radical polymerisation technique, which leads to PVC products with improved resistance to heat degradation and ionising radiation by reducing the defects in the structures due to the free radical polymerisation.

2.4.5 Summary

The migration problem of DEHP promotes the search for alternatives to both DEHP plasticiser and PVC-P and considerable progress has been made in last two decades. However, plasticised PVC, the plastic used in the first blood bags introduced by Carl Walter over 40 years ago, remains the material of choice today. It would seem to be promising in the future if PVC technology is directed to the development of new synthesis technology, new PVC formulation and PVC surface modification.

CHAPTER 3

SURFACE MODIFICATION OF PLASTICISED POLY(VINYL CHLORIDE) FOR IMPROVED BLOOD COMPATIBILITY

3.1 Introduction: Importance of surface

The selection of biomaterials for use in medical devices and artificial organs involves consideration of both surface and bulk property requirements. The bulk properties, mainly mechanical properties, such as strength, toughness, fatigue resistance and stability, often influence the durability of biomaterials for long-term applications. In the meantime, the bulk properties have great influences on the surface properties. For instance, the migration and leaching of surface active additives from the bulk phase or reorientation of bulk molecules will dramatically change the surface properties (Ward, 1995)

Obviously, the surface plays a very important role in determining its blood compatibility, since, as a blood-contacting biomaterial, only the surface contacts blood. The blood-foreign surface interaction determines the blood compatibility, which is strongly correlated to the surface characteristics. It is well recognised that understanding the nature of the surface of a biomaterial is essential both for understanding the interaction between materials and blood and for fabricating biomedical and medical devices (Ratner, 1992).

The rationales for developing a new biomaterial can be grouped as polymer synthesis, polymer formulation and polymer surface modification (Courtney et al, 1999). During the last 50 years, many polymeric biomaterials have been investigated for biomedical applications because of their favourable mechanical and processing properties, such as polyurethane, silicone rubber, EVA, PVC, polycarbonate, polyester, polyacrylonitrile, cellulose acetate, hydrogels and biodegradable polymers. Due to the difficulties of finding a novel synthetic polymeric biomaterial, many researchers have been focusing on maintaining the bulk properties of those conventional materials, and only try to modify the surface of the polymer and/or the blood/polymer interface.

This chapter presents an overview of the hypotheses, proposed for correlating the surface properties with blood compatibility. Based on these hypotheses, numerous blood compatible surfaces have been designed. The final focus will be on the modification of PVC-P for improving its blood compatibility.

3.2 Hypothesis on correlation of blood compatibility with polymer surface characteristics

The hypothetical correlation of blood compatibility with surface properties can be traced back to 1863 when Lister (1863) first experimented a jugular vein bypass in a sheep using a rubber tubing bypass and a glass one respectively. He found that blood clotted more slowly in rubber than in glass. In 1885, Freund (1885) found glassware coated with petroleum jelly such as Vaseline delayed blood-clotting time. About 18 years later, Bordet & Gengou (1903) discovered that the blood clotting time was increased when glass was covered with paraffin wax. These above observations and experiments have led to the conclusion that the nature of the polymer surface affects the clotting process. Since then, many more hypotheses trying to correlate the polymer surface with blood compatibility have been proposed.

Neubauer & Lampert (1930) were the first to outline a rule to describe the inverse relationship between blood clotting time and surface wettability, which was called Lampert rule of blood clotting time.

In 1953, Sawer pointed out that a blood compatible surface should be of a net negative charge and i.e., negatively charged surfaces tend to be nonthrombogenic. This hypothesis was supported by the fact that heparin, a common anticoagulant, and many sulphonated carbohydrate heparin-like substances are highly negatively charged, and the both vein inner walls and formed bodies of the blood bear, in physiological conditions, are of a net negative charge. Indeed, early studies by Lovelock & Porterfield (1951) on the sulphonation of polystyrene to produce sulphonic acid

groups analogous to those on heparin showed that such a surface increased static blood coagulation times.

In 1963, Gott et al (1963) first reported a heparinised artificial surface as thromboresistant. This finding stimulated the development of nonthrombogenic polymer surface using antithrombotic agents.

Later on, many contradictory results were found against the "surface charge hypothesis". For example, some uncharged hydrophobic surfaces such as silicones, showed excellent blood compatibility. Amine-rich surfaces and polyamines have also been used to improve the blood compatibility of materials (Oja et al, 1969; Tsuruta, 1987). This indicates that the simplistic correlation of blood compatibility with surface charge could not satisfy the complexity of the interface between proteins and surface. The surface free energy was then considered to be very important.

Zisman (1964) proposed that a material with minimal critical surface tension will be blood compatible ($\gamma c \rightarrow 0$) in the range of 1.0~1.5 x 10^{-2} N/m and Lyman et al (1965) suggested a relationship between surface free energy and blood compatibility, i.e. the lower the surface free energy or critical surface tension, the better the blood compatibility of the material. Baier (1967) eventually led to the hypothesis that surfaces with a critical surface tension in the range of 2.0~2.5 x 10^{-2} N/m have optimal blood compatibility. While Nyilas (1975), however, hypothesised that the thrombogenicity increases as the polar contribution to the surface free energy increases, which might cause protein conformational change. It was the first time, the possible conformational change due to surface adsorption was mentioned.

In contrast to the surface energy concept, Andrade (1973) postulated that the blood compatible biomaterial must have the minimal interfacial free energy, i.e., as the interfacial-free energy goes to zero, the driving force for protein adsorption goes to zero, and the adsorption cannot occur. This might be able to explain the protein-

resistant property of a hydrophilic surface. However, this hypothesis could not be rigorously tested because of the difficulty in evaluating the difference in hydrophilicity of a surface ranging from about 40% water to over 95% water. Later on, Andrade(1986) admitted that the attribution of the blood compatibility to only one surface parameter was too simplistic to reflect the complexities of blood compatibility (1986).

Kaelble et al (1977) found that an implant surface with strongly adsorbed plasma protein film provided the best blood compatibility and low thrombogenic effects. They claimed that this could be due to materials with high dispersion and low polar surface free energy.

Bruck (1979) suggested that the electrical conduction and semiconduction may have a relationship with blood compatibility. The importance of "structured water" was described as a type of "shield", which allows the prevention of the neutralising effect of the ionic components of the blood on the electrical properties of biopolymers. The intrinsic conduction properties of natural and synthetic polymers may be involved in blood compatibility. In the same year, due to the discovery that a highly hydrated poly(hydroxyl ethyl methacrylate) (PHEMA) was not as blood compatible as they thought, Ratner & Hoffman (1979) hypothesised that an optimum balance of polar and apolar sites on a surface may be important for its blood compatibility.

The late 60's clinical practice on improvement of blood compatibility by pretreatment of artificial kidneys and blood oxygenators with albumin solutions led to the hypothesis by Lee & Kim (1979) on that the degree of albumination should be used as a criteria for surface blood compatibility. Conversely, those surfaces with albumin coating while reducing platelet adhesion to the materials still have a significant tendency to initiate thrombosis *in vivo*.

In 1981, Munro et al (1981) hypothesised that surfaces which provide a dynamically renewable, natural albumin layer between the surface of the device and the blood would show a good thromboresistance. This was supported by the fact that polyurethane grafted alkyl chains demonstrated improved blood compatibility because of the preferential adsorption of albumin.

In addition to attention paid to effects of surface chemical structure and their relevant surface energies on blood compatibility, Lyman et al(1975) and Hecher and Edwards (1981) postulated the importance of surface morphology and hypothesised that the smoother the polymer surface, the more antithrombogenic it is . This hypothesis promoted the development of biomaterial surfaces with low-friction or slippery characteristics.

Since the early 1980's, it has been realised that a surface with microphase structure might be beneficial for the preferential adsorption of albumin. It was Okano (1981) who first explained that within the microphase system, the high affinity to serum albumin is due to the hydrophilic phase while the hydrophobic phase refers to fibrinogen and γ-globulin.

In addition, Barenberg et al (1981) proposed that the surface mobility of the hydrophilic segment could be correlated with blood compatibility. In contrast, Yeh et al (1988) hypothesised that a stable surface configuration is required for good blood compatibility, which is supported by the observations that those polymers which are rather well behaved towards to blood are constituted by rotationally symmetric macromolecules, thus their configuration is stable even if their polymer chains are mobile. This hypothesis was also supported by the observation of a substantial reduction in thrombus accumulation when side-chain motions at the polymer-blood surface was restricted by irradiation.

In 1984, Ikada (1984) proposed that the polymer surface, which does not adsorb any plasma protein, must be a blood compatible surface, and this could be achieved by introducing a super-hydrophilic diffuse surface. They claimed that this type of surface appears to be more promising for long-term blood compatibility.

Also in 1984, Ruckenstein and Gourisankar (1984) believed that a compromise between adhesive and non-adhesive properties of the surface is required for blood compatibility. While the driving force for the adsorption of blood components should be minimised, a certain degree of mechanical stability of the interface is also required. They postulated that an interfacial tension of 1~3 dyne/cm will satisfy blood-compatibility.

Based on the well-accepted concept that the conformational change of adsorbed protein will alter the subsequent blood response of material surface, Lin (1984,1985) proposed a hypothesis of maintaining a protein's normal conformation for blood compatible biomaterials. This hypothesis was supported by evaluation of the blood compatibility of a novel segmented polyurethane-siloxane copolymer and polyimide-siloxane copolymer (Lin et al, 1992; 1994).

With the discovery of a surface containing polyethylene oxide (PEO), which can act by repelling macromolecules such as proteins from the interface by steric exclusion, Nagaoka et al (1984) have studied the effect of PEO side-chain length and surface mobility on the platelet adhesion minimisation. They concluded that the longer PEO side chain shows a better blood compatibility.

In 1987, Andrade et al (1987) summarised that the hydrated dynamic surface formed by longer chains of PEO grafting onto the surface is blood compatible. However, when summarising the hypothesis and mechanism suggested for blood compatibility, Andrade (1986) only made some uncontroversial statements, such as protein

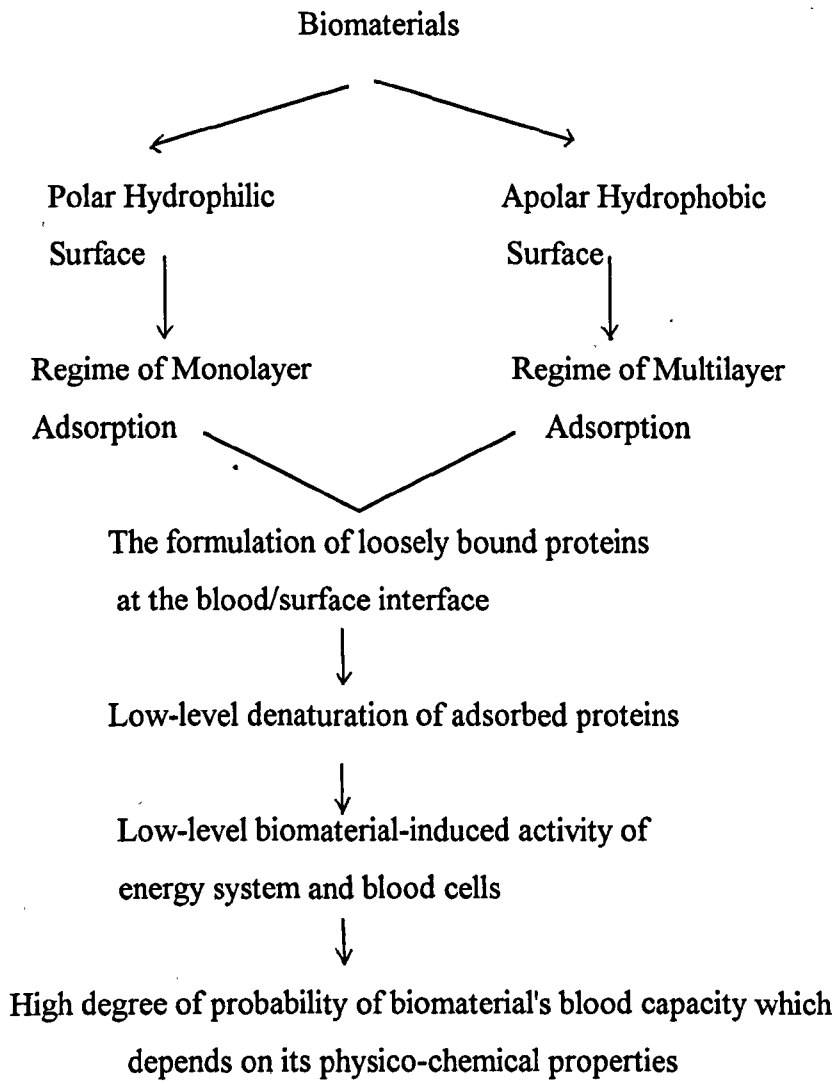


Fig 3.1 One route concept for development of blood-compatible surface (from Sevastianov, 1988)

adsorption is indeed important in the blood compatibility process, and any simplistic hypothesis and mechanism are generally not very applicable.

However, Pitt et al (1986) found certain hydrophobic surfaces, which possessed a high adsorption, and low desorption rates of fibrinogen, showed better compatibility. Horbett et al (1986) postulated that this was due to the rapidly forming fibrin film from fibrinogen. They thought this might be another approach for designing blood compatible materials.

In 1988, Sevastianov (1988) proposed an "one-route Concept", trying to deal with these complexities as shown in Fig 3.1 and claimed that two opposite approaches are actually based on one concept.

In 1989, Ito et al (1989) developed the previous " Concept of Correspondence" into a " Concept of Complementarity", that is, for a high blood compatible biomaterial, the "Complementarity" between hydrophobic and hydrophilic regions of the surface should be satisfied. In other words, ideal blood-compatible materials should possess an amphiphilic surface.

By simulating the external surface of blood cells, which are inert in coagulation assays, Chapman & Hayward (1984) proposed the development of new biomaterials with a biomembrane-like surface composed of polymer and phospholipids. This proposal was supported and confirmed by Imanish (1986) who found that the platelet adhesion onto the polyamide beads coated with lipid bilayer membrane was significantly suppressed.

Nakabayashi et al (1978), however hypothesised that if a polymer surface possesses phospholipid-like structure, then a larger amount of natural phospholipids in plasma can be adsorbed on the surface by their self-assembling character. Thus, a well-structured lipidsome will form on the surface. This might be able to simulate blood

cell membrane properties. Based on this idea, Nakabayashi designed a methacrylate monomer with a phosphorylcholine (MPC) and synthesised for the first time and Ishihara et al (1990, 1992) developed an improved method to prepare MPC and perfected the "biomembrane-mimetic hypothesis".

In 1991, Kim et al (1991) suggested surfaces with a random network structure would be blood compatible. This hypothesis was supported by a PEO-linked surface, in which PEO serves either as a spacer or a modifier, is blood compatible. Okkema et al (1991) applied sulphonated PEO to modify polyurethane. Based on the low platelet adhesion and other blood compatibility results, they postulated a "fibrinogen retention hypothesis", i.e., the sulphonic ions of the surface could inactivate fibrinogen, causing it to be unrecognisable by platelets. Han et al (1993) also proposed a similar mechanism to explain how the blood compatibility of fibrinogen adsorbed sulfonated surface can be improved. These concepts were similar to that suggested by Horbett about conformational change of fibrinogen due to adsorption might be an approach for designing a blood compatible surface, as previously stated.

So far, many basic concepts and hypotheses for development of blood compatible surface are described. It is not surprising to find different views and contradictory results in the literature due to the multivariable nature of the blood compatibility of biomaterials. It is also difficult to regulate blood-foreign surface interaction by any simple hypothesis. However, reviewing these hypotheses reflects the great achievement people have made in the past. Based on these concepts, it is believed that many new concepts emerge in the future to promote the development of new surface modified biomaterials with improved blood compatibility.

3.3 Molecular design of surface for improved blood compatibility

The prediction that surface properties are related to blood compatibility has been made from very early times (Lyman 1975). Ideally, the properties of an artificial

surface designed for blood-contacting application should be as similar as possible to those of a natural blood vessel surface. It has many physical characteristics such as highly hydrated, multiphase and flexible in structure, functioning biologically with response, secretion and metabolism due to the presence of endothelial cells on the inside surface (Gebelein, 1985).

In this chapter, the molecular design of a polymeric surface for improved blood compatibility is focused on increased hydrophilicity, increased hydrophobicity, microphase separated (microdomain) structure (balance of hydrophobicity and hydrophilicity), and biomimetic surface design, including bioactive surface.

3.3.1 Increase in Hydrophilicity

The minimal protein adsorption on a surface is important for blood contacting devices, which could be a choice by an increase in hydrophilicity (Engbers & Feijen, 1991; Amiji & Park, 1993; Courtney et al, 1994). In this section, three types of surface with increased hydrophilicity are reviewed, eg. polyethylene oxide (PEO) modified surface, nonionic surfactant modified surface and hydrogel modified surface.

PEO modified surface

A poly (ethylene oxide) (PEO) modified surface is considered capable of simulating the natural blood vessel surface in terms of the hydrophilic nature and highly mobility. For preventing protein adsorption on the polymeric surface, utilisation of PEO is effective (Merrille & Salzman, 1983). There are many possible factors involved in the protein-resistant character of the PEO surface in aqueous media. These can be summarised as: minimum interfacial free energy with water(Andrade, 1973., Coleman et al,1982), steric stabilisation effect (Lee et al., 1995) and its unique solution properties which differ from those of other hydrophilic polymers. PEO shows complete water solubility among the related polyethers because its segments fit nicely in the water structure without any distortion of water lattices. The PEO modified surface in aqueous media would exhibit considerable flexibility or mobility due to this unique water solubility.

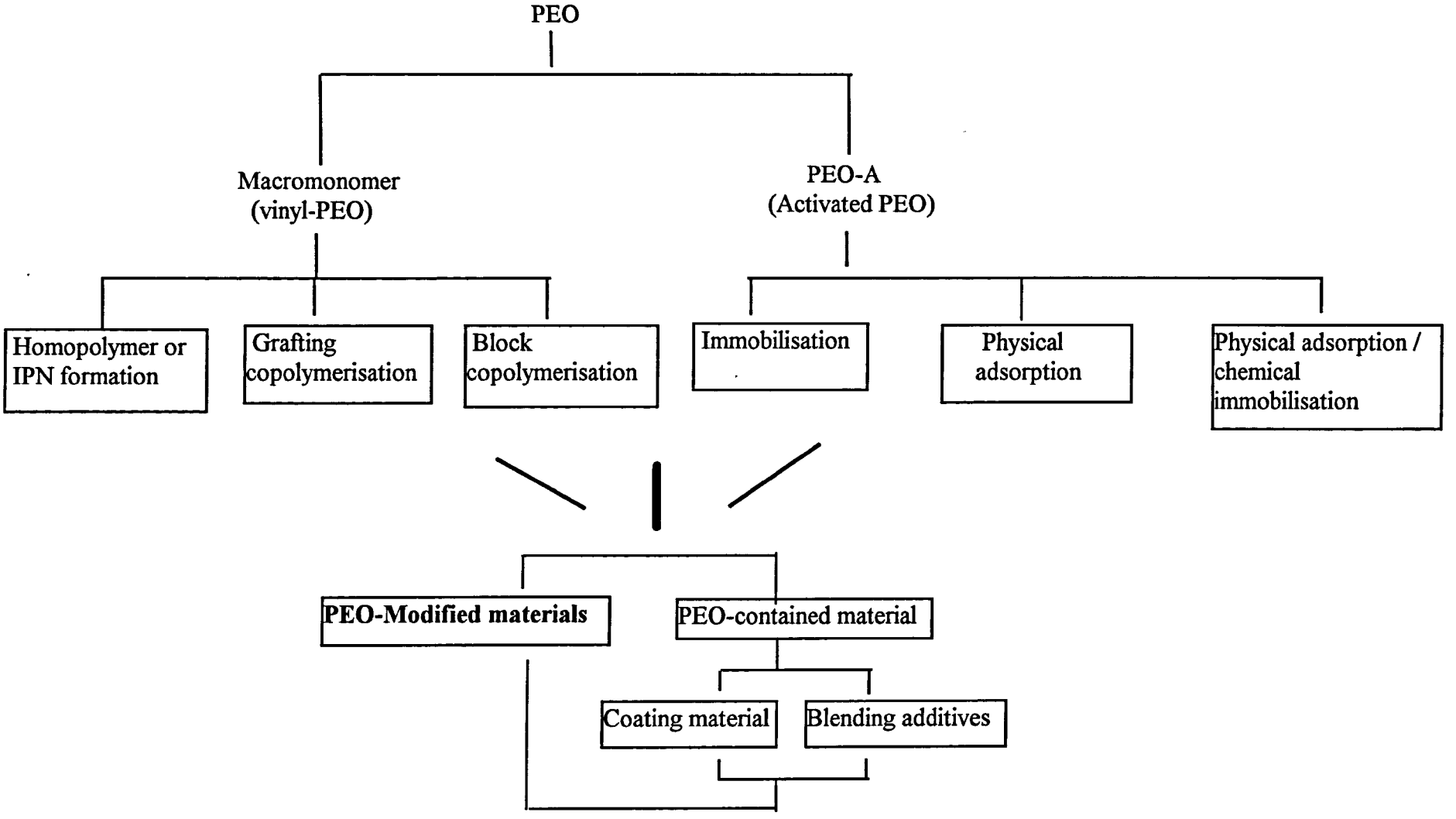


Fig 3.2 Molecular design of PEO-modified surface

The highly miscibility of PEO with water causes a large excluded volume in water and thus is very effective for steric repulsion of any protein. Meanwhile, the surface mobility of the PEO chains is very effective in preventing stagnation of the proteins on the surface probably because the contact time is shortened. The longer PEO chains are more effective than shorter chains (Nagaoka et al, 1984). Molecular design of a PEO-modified surface is schematically described in Fig3.2.

Methoxy-poly(ethylene glycol)methacrylate is the most commonly used PEO macromonomer with controlled chain length. Yamada et al (1990,1991) grafted PEO-macromonomer onto PVC with PEG as a side chain. An *in vitro* test indicated PEO modified surface exhibited excellent blood compatibility. PEO can also be grafted chemically to many polymeric surfaces such as polyethylene (PE), polyurethane (PU) (Brickman et al, 1990; Han et al, 1993), haemodialysis cellulose membrane (Akizawa et al ,1989) and polytetrafluroethylene (PTFE) (Allmer et al., 1990). PEO modified haemodialysis membrane surface has been found to reduce complement activation (Akizawa et al, 1989).

PEO-containing polymers can be built by block copolymerisation. Lee et al (1989,1990) synthesised copolymers of alkyl methacrylates with methoxy PEO methacrylates. The formed material can be used as a coating material. The PEO surface prepared by adsorption of this synthesised PEO-grafted copolymer showed efficient protein-resistant character. Bergström et al (1992) applied PEO to coat a polystyrene surface to achieve a reduced fibrinogen adsorption. For a strong retention of PEO at the modified surface, Lens et al (1997) developed alkyl-PEO surfactants, which contain a terminal hydroxyl, sulfate, or carboxylate group. By surface coating these surfactants and following with argon plasma treatment, a PEO strongly bound surface can be achieved.

PEO has been widely applied as a soft segment for polyurethane biomaterials and the PEO-PU segmented hydrogels have been shown to possess improved blood

compatibility in terms of protein adsorption and complement activation (Yu et al 1991).

PEO can be immobilised to surfaces by the Williamson reaction (Litauszki et al., 1997) and this has been employed for PEO modification of PVC (Lakshmi & Jayakrishnan., 1998) A PEO-linked surface is usually considered to be a protein-resistant surface. However, some PEO-derivatives, such as sulphonated PEO, have been found to show a very strong affinity to fibrinogen and cause a conformational change. Polyurethane surface grafted with sulfonated PEO has achieved improved blood compatibility due to this possible inactivation of fibrinogen (Han et al, 1996). Nelson et al (1996) also investigated the high affinity for albumination due to PEO attachment.

Polymer blending has always been considered to be industrially relevant. Surface modification by this approach could be achieved based on segregation phenomenon, i.e., surface active additives prefer to accumulate at surface. Wesslen et al (1992,1994) modified segment PU surface through the use of PEO-containing block copolymers as additives. They showed that adsorption of fibrinogen is significantly reduced by these additives to levels similar to those obtained for PU surface grafted with PEO. A PEO-polysiloxane copolymer can be used as a melt additive to achieve a PEO-enriched surface in polymer blends while contacting physiological solution (Nohr & Macdonald, 1995).In addition, PEO has been reported to blend with other polymers such as PE, PVC by melting to achieve a PEO-riched surface(Ding et al., 1996).

Surfactant modified surface

Amphiphilic polymers and surfactants containing PEO have been used to render surfaces hydrophilic (Lee et al, 1989; Han etal, 1991). Commercially available surfactant is poly (oxyethlene)-poly (oxypropylene) (PEO-PPO), for example,

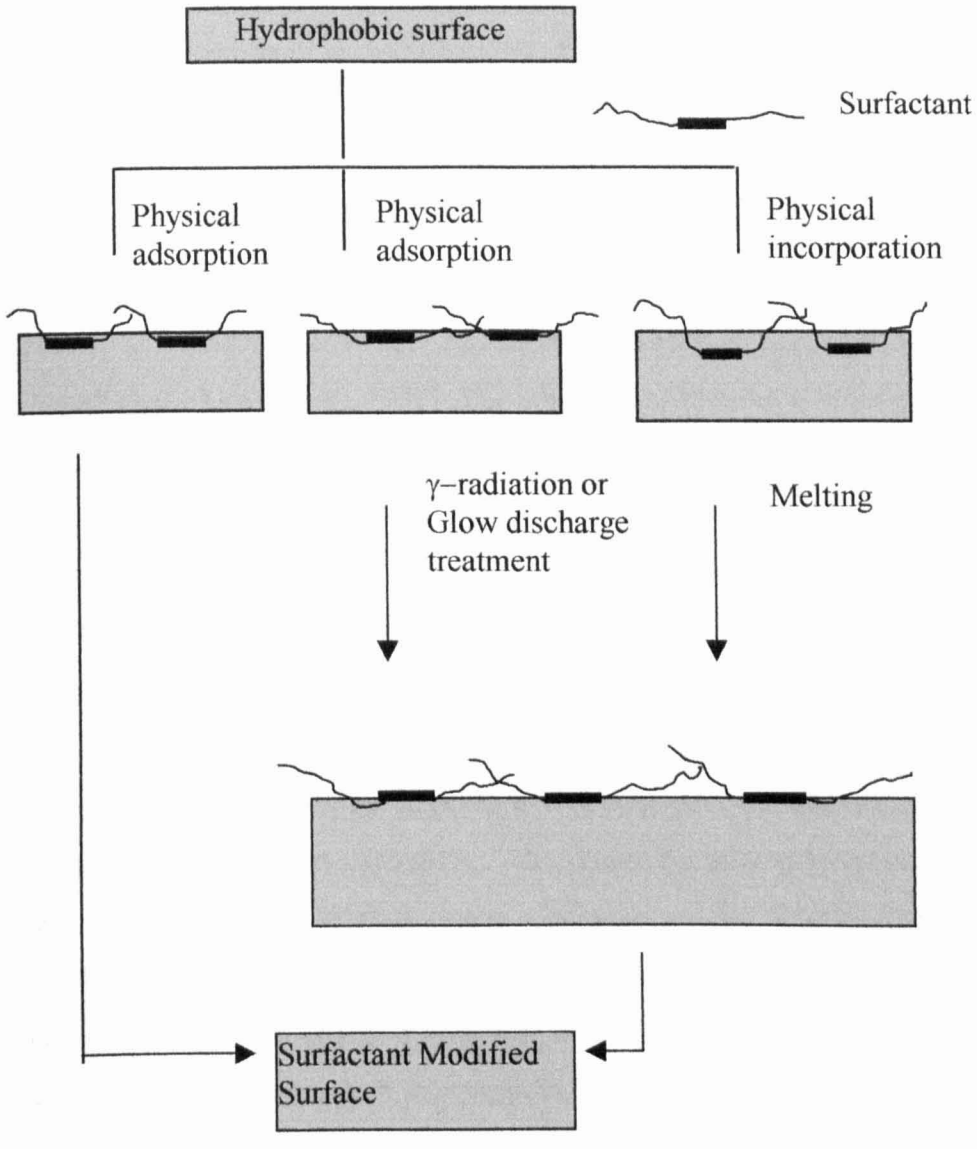


Fig 3.3 Molecular design of surfactant modified polymeric surface

Pluronics, is the most widely investigated (Table 3.1). They have been investigated for use in biomedical applications for reduction of the adsorption of proteins (Lee etal, 1989) and adhesion of cells (Amiji & Park, 1992). It has been applied as a stabiliser of fluorocarbon emulsions for use *in vivo* oxygen delivery during percutaneous transluminal coronary angioplasty (PTCA) (Lowe, 1997), and as a pharmaceutical stabiliser for formulating applications (Sweetana & Akers, 1996).

The effect of surfactant on haemolysis of human red blood cell has been extensively investigated. The amount of haemolysis induced by nonionic surfactant formulations is shown to be the relatively low and to increase only slightly with contact time (Al-Assadi et al., Lowe et al., 1995). The influence of Pluronic F-68, a commonly utilised PEO-PPO surfactant, on platelet aggregation in human whole blood has been studied. It was found that the surfactant inhibited platelet aggregation significantly.

Physical adsorption, polymer blending, and physical adsorption/chemically binding (Fig3.3) can achieve a PEO-PPO-PEO surfactant modified surface.

For modification involving physical adsorption, the hydrophobic nature of PPO is responsible for such an adsorption. The adsorption of a segment of the hydrophobic middle PPO block will promote the adsorption of the neighbouring segments, and finally the whole PPO block will be attached at a hydrophobic surface (Freij-Larsson et al, 1996). The block copolymer may adsorb in more regular tail-train-tail conformations (Takahashi & Kawagguchi, 1982).

Table 3.1 Physical properties of some selected Pluronics

Trade Name	Mw	PEO/PPO/PEO (repeat unit number)	HLB(hydrophile -lipophile balance)
Pluronic P105	6,500	37/56/37	12~18
Pluronic F68	8,400	76/30/76	>24
Pluronic F88	11,400	104/39/104	>24
Pluronic F108	14,600	129/56/129	>24

The effects of the Pluronic preadsorbed surface in suppressing protein adsorption are related to PEO block length when the PPO block is kept nearly constant in length and the layer thickness. Although increasing the PEO block length increases the protein resistance of the modified surface, a surfactant with a given PEO-block size appears to be more effective as a protein repellent when a less concentrated and thin surface layer is formed (Li et al., 1996).

The adsorption of albumin and fibrinogen at the surface preadsorbed with this surfactant was significantly reduced as compared to the bare hydrophobic surface at a 10-fold reduction rate(Freij-Larsson et al, 1996). Although surface coating with PEO-PPO surfactant might be an effective approach to achieve a blood compatible surface, the possible leaching or desorption of the surfactant may reduce the long-term effectiveness. To overcome this problem, a technique using the combination of physical adsorption and covalent immobilisation was developed (Terlingen etal., 1992). Sheu et al (1993) applied this concept successfully to prepare non-fouling surfaces on biomaterials. They developed a novel radio-frequency glow discharge (RFGD) process. The surfactant is first" anchored" on the polymer surface via physical adsorption from a solvent which swells the substrate polymer. Then the solvent is evaporated and the adsorbed surfactant is bound covalently to the surface by a gas discharge treatment, which causes "crosslinking" of the surfactant with the

polymer molecules in the surface. The modified surfaces exhibit a significant reduction of fibrinogen adsorption.

As previously stated, a material that is surface active in a condensed phase will have a higher concentration in the surface than it has in the bulk. In fact, even in metal alloys the component of lowest surface tension will enrich the air-facing surface if sufficient time is available for that component to diffuse to the surface(Somorjai, 1981). For instance, lubricants have been added to avoid excessive sticking on the processing mill to give good mould-release surface properties for PVC processing. PEO surfactants are such surface active agents and have been reported as antifogging agents added in small amounts (2 phr to 5 phr) to the PVC formulation to modify the surface wettability. The modified surface causes the condensed water to wet the surface and leaves the film cleaner.

In theory, the best way to achieve a hydrophilic surface is by a covalent bonding process, but such methods are generally difficult and too costly for commercialisation. One alternative has been reported which involves melt blending of the water soluble polymer into the base polymer, accompanied by shear processing to drive the water-soluble polymer towards the surface. This technique was considered much easier and more economical than covalent bonding, provided that the resulting surface modification can be made sufficiently permanent. PEO, PVA, PNVP have been selected for surface modification to obtain low-protein-adsorption biomaterials (Ding et al, 1996). In this thesis, Pluronic surfactant has been considered as a surface modifier in PVC formulations. The utilisation of Pluronic surfactant, PEO and their combination with cyclodextrins to modify PVC-P surface properties is reported in chapters 6 and 7.

Hydrogel modified Surface

Hydrogels are water-swollen, crosslinked polymeric materials produced by the single reaction of one or more monomers or by association bonds such as hydrogen bonds

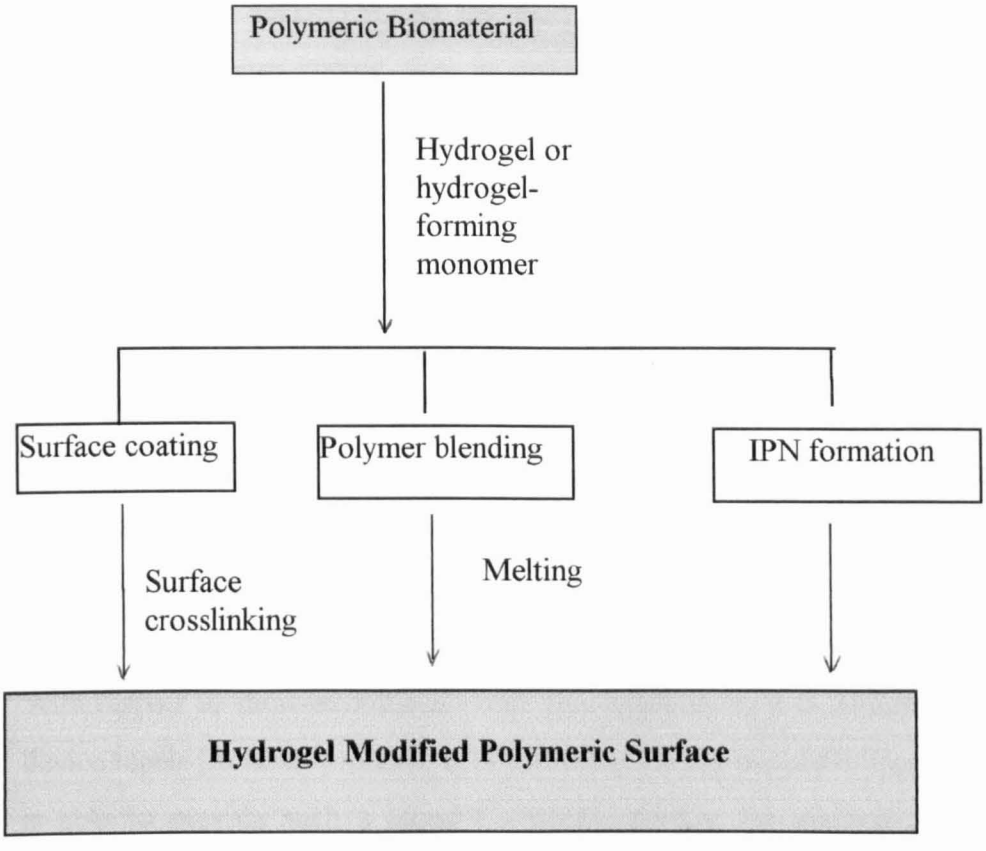


Fig 3.4 Molecular design of hydrogel modified surface

and strong Van der Waals interaction between chains (Peppas, 1987). Hydrogels can be derived from natural biopolymers or synthetic hydrophilic polymers. The most widely used synthetic hydrogels are crosslinked poly (hydroxyethylmethacrylate) (PHEMA), poly(vinyl alcohol) (PVA), polyacrylamide, poly(vinyl pyrrolidone) (PVP), poly(methacrylic acid) (PMAA) and their copolymers among them (Ratner et al, 1996). Natural biopolymers, such as collagen, albumin, alginate, chitosan and many other polysaccharides, have also been employed to produce hydrogels for biomedical applications.

The utilisation of hydrogels to modify a polymer surface could achieve a lubricious surface with reduced frictional resistance or increased slipperiness due to the high water content of hydrogels (Ikada, 1994). Additionally, hydrogels provide the base for improving blood compatibility in active ways by incorporation of bioactive substances or cell seeding. The molecular design of a surface by utilisation of hydrogels is shown in Fig 3.4.

With respect to short-term blood-contacting application, it is important only that the device repels platelets, proteins, cells and other fouling materials. Coating of hydrogel is able to provide such a blood compatible surface for medical devices, such as catheters or introducers, which also require a lubricious surface. A hydrogel coating can be achieved by photo-initiated polymerisation of hydrophilic monomer at the catheter surface. Studies have shown that platelet aggregation and clot formation through adherence of blood components to the coated catheters is less than with uncoated catheters (Anderson et al, 1996). Albumin or photoactivated albumin can be coated to a hydrophobic surface followed by crosslinking with glutaraldehyde (Kottke-Marchant et al, 1989) or by photo-initiated crosslinking (Matsuda & Inoue, 1990). Poly (vinyl pyrrolidone) (PVP) crosslinked with an isocyanate has been employed for coating polyurethane catheter surfaces. The PVP coating induced a hydrophilic surface with nonadhesive surface properties, which would minimise difficulties during their insertion into the vessels of the patient (Nurdin et al, 1996).

Glutaraldehyde-crosslinked PVA hydrogel with and without heparin (Sefton et al, 1987) and PEG-PVA-Heparin hydrogel (Gerard et al, 1992) were reported to modify the surface of a substrate to obtain improved blood compatibility.

Polysaccharides are very promising modifiers of biomaterials because of their abundant availability, structural diversity, high hydrophilicity, degradability and likely biocompatibility (Zdrahala, 1996). By coating polysaccharides such as dextran (Õsterberg et al, 1995) and dextran-containing copolymer (Marchant et al, 1998), the modified surfaces show resistance to protein deposition.

In addition to the hydrogel coating approach, the surface modified with hydrogel can be achieved by polymer blending and interpenetrating network (IPN) formation. Lee et al (1995) modified PET textile vascular graft using a semi-IPN formation technique to achieve an alginate modified surface. Gutowsky et al (1997) reported an IPN coating technique using a thermosensitive hydrogel for controlled delivery of heparin. According to this report. a hydrophobic surface was immersed into the monomer/solvent system initially. After polymerisation, a portion of the hydrogel was formed within the hydrophobic to get an IPN. The remainder of the hydrogel, which polymerises above the interface, forms a new hydrogel surface. Inoue et al (1997) modified polyurethane catheters by utilisation of IPN formation with polyacrylamide hydrogel. The formed hydrogel-modified surface showed excellent blood compatibility. As the PEO-polymer blending technique, other hydrogels such as PVA and PVP were reported to blend with polyethylene (PE), plasticised PVC and many other polymers to achieve an increase in hydrophilicity and a protein resistant surface (Ding et al, 1996).

3.2.2 Increase in Hydrophobicity

According to the very classical recognition of the potential benefit of a hydrophobic surface in minimising blood response, which was reported by Bordet and Gergou

(1903), the use of hydrophobic polymer surfaces for blood contacting application, was developed. The principal surfaces of interest are silicones, polytetrafluoroethylene (PTFE) and diamond-like surfaces.

In general, hydrophobic surfaces possess relatively low surface free energies. It has been proposed that the clotting time for human blood increased linearly with the logarithm of the critical surface tension (Lyman et al, 1965) and this suggested that the minimisation of surface energy is a valid approach to obtain nonclotting surfaces. In practice, the lowest critical surface tension is that obtained from a PTFE surface, which was found to exhibit less adhesion and shape change of platelets when it was grafted on a polyurethane surface (Han et al, 992). The results indicate that this type of hydrophobic surface is significantly blood compatible and it is also interesting to find that the enhanced blood compatibility of very hydrophobic PU-PFDA (perfluorodecanoic acid) was equivalent to hydrophilic PU-PEO.

By utilisation of surface modifying additives (SMAs) such as triblock copolymer: polycaprolactone-polydimethylsiloxane-polycaprolactone, a polymer blend with a silicone-enriched surface can be formed and improved blood compatibility was achieved (Tsai et al, 1994).

The mechanism of blood compatibility of a hydrophobic surface has also been extensively investigated. It is generally accepted that proteins and other coagulation factors adsorb considerably on such a surface. However, the preferential adsorption of albumin from plasma can protect soluble thrombin from inactivation by the hydrophobic surface and retained less fibrinogen than when the PTFE was incubated with a pure fibrinogen solution of the same concentration (Schlosser et al, 1993). Similarly, the expanded PTFE (e-PTFE) generally seems to exhibit a better blood compatibility compared to other materials that were used in the past for partial replacement of blood vessels in vascular surgery (Silver & Doillon, 1989).

Diamond-like coatings (DLCs) have been exploited to build a highly hydrophobic surface with a lowest coefficient of friction values for biomedical applications. The coating could be achieved via a technology called chemical-vapour-deposition (Oleary et al, 1995).

Protein adsorption on a carbon surface has been studied. It was found that there was no preference on adsorption of proteins but with a very high rate and high concentration of adsorption. It implies that the carbon surface accomplishes its blood compatibility through a passivating film of strongly adsorbed bland proteins, which do not interact with platelets nor participate in blood coagulation (Feng & Andrade, 1995).

Although it is predicted that for the future the best coating material would be diamond-like carbon (DLC) or crystalline diamond coating, the coating technology and blood compatibility assessment for DLCs-modified polymers lack detailed investigation.

3.2.3 Microdomain structured surface

It is known that all of the cells and tissues in the living organism are built up with a microdomain structured surface (Ishihara, 1993). The normal vascular endothelium, which possesses ideal antithrombogenic properties, has a microphase separated structure composed of hydrophilic and hydrophobic microdomains (Sawyer et al, 1964). In order to achieve a blood-compatible surface mimicking these bio-surfaces, many synthetic polymers with microdomain structured surfaces have been designed, mainly through segmented block and graft copolymerisation.

The polyurethanes are the most widely investigated biomaterials with microphase separated surface structures (Lelah & Cooper, 1986). The relatively good blood compatibility was suggested by Lyman et al (1974) who assumed it was because of

the similar domain size as the size of globular proteins. In addition to the common polyether-urethane and polyester-urethane, Yu et al (1985) developed polydimethylsiloxane-polyurethane elastomers and they have been found to have favourable blood-contacting properties compared to a polyetherurethane (Lim et al, 1994).

Block copolymers with hydrophilic and hydrophobic segments are reported to be antithrombogenic. Okano et al (1978) synthesised a triblock (A-B-A) copolymer consisting of HEMA (A) and styrene (B), which forms a typical domain structure. The antiththrombogencity of these A-B-A triblock copolymers was proposed to have arisen from their microphase separation, which in turn affects protein adsorption and thereby influences platelet adhesion and activation (Okano et al., 1981).

By grafting hydrophobic groups to a hydrophilic polymer, it is also possible to produce a microdomain structured surface which exhibits blood compatibility (Methew & Kodama, 1992; Matthew et al, 1992). Grainger et al (1990) reported a poly(dimethylsiloxane)-poly(ethylene oxide)-heparin block copolymers, which possesses a microphase separated surface as well as exhibiting antithrombogenic activity.

Kawahito et al (1995) synthesised a new microdomain structured copolymer called fluorine-acryl-styrene-urethane-silicone (FASUS). This new copolymer may be effective in preventing thrombus formation *in vitro*, *ex vivo* and in clinical situations.

From the above examples, it is clearly shown that equilibrium between hydrophilicity and hydrophobicity is necessary to build a microdomain-structured surface, which exhibits blood compatibility. (Akers et al, 1997).

3.3.4 Bioactive surface

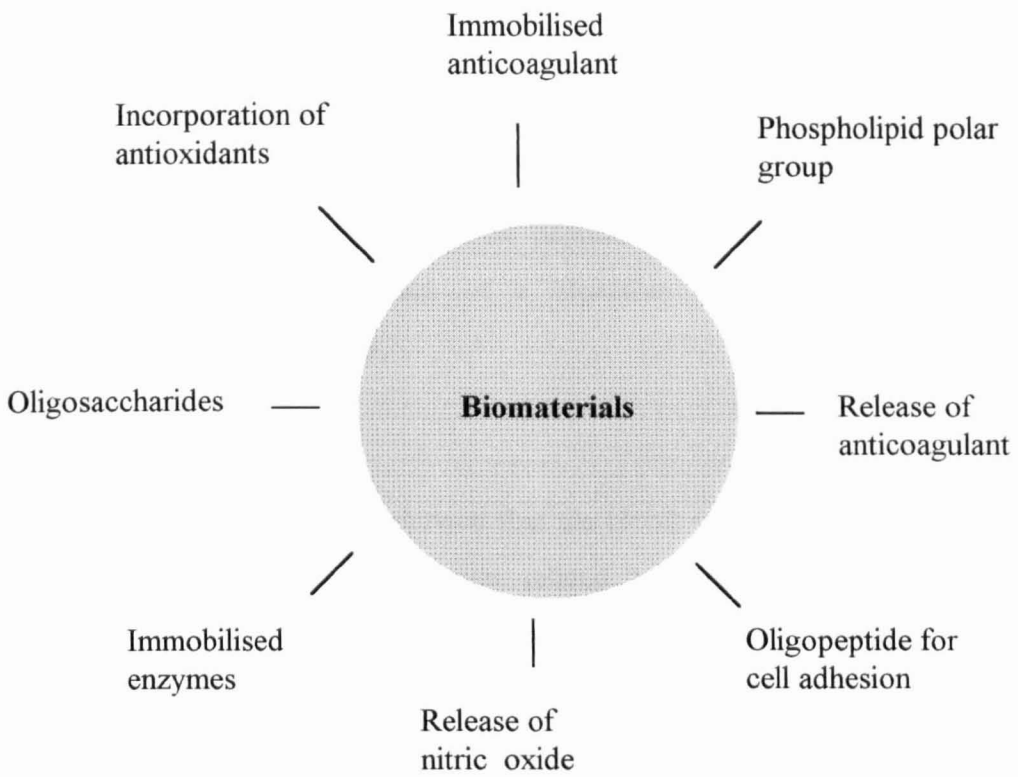


Fig 3.5 Molecular design of bioactive surfaces with improved blood compatibility

A bioactive surface is a surface that is able to simulate or mimic the function of a blood vessel surface to secrete a bioactive substance, to function metabolically, and to respond to any physiological effects. In summary, biomimetic character is the main feature of a bioactive surface.

Bioactive surfaces can be prepared by surface immobilisation of bioactive substances, incorporation of bioactive additives to a blend system, utilisation of oligosaccharides, phospholipid substances and biospecific peptide, or/and the combination of above methods. The molecular design of a bioactive surface for improved blood compatibility is shown in Fig 3.5.

Heparinised surface

Recently, Lindahl et al (1994) showed that the blood vessel wall contains substances that are structurally and functionally related to heparin, which led to further acceptance of the concept of immobilisation of the heparin anticoagulant activity. In fact, the heparinised surface is the most commonly applied nonthromogenic surface for blood-contacting medical devices today. The design of such a surface has been well reviewed by Kim et al (1996 a,b) and Plate & Valuev(1986), covering (1) heparin-releasing surfaces (Gutowska et al ,1997; Kashiwagi et al, 1993) (2) heparin-immobilised surfaces (Larm et al, 1983; Lin et al, 1991) and (3) Coating surfaces with copolymers of heparin (Park et al, 1991; 1992)or organic solvent soluble heparin complexes (Hsu et al, 1995).

Other anticoagulants or platelet aggregation inhibitors modified surface

Surface immobilisation or incorporation of other bioactive substances with antithrombogenic activities is another approach. Many such active substances as urokinase (Oshiro & Kosaki, 1980), lumbrokinase (Ryu et al, 1993), hirudin (Phaneuf et al, 1998), and human thrombomodulin (Kishida et al, 1994; 1995) or inhibitors for activation and aggregation of platelets such as prostacyclin (Ebert et al, 1982; McRea & Kim, 1983) and dipyridamole (Aldenhoff et al, 1997), or inhibitor of complement

activation such as human decay accelerating factor (hDAF) (Watkins et al, 1997) have been used to modify polymeric surfaces. In the approaches involved the substances are either tightly bound to the surface or simply blended into the polymer system for controlled release of them. Recently, nitric oxide (NO) has been found to show a strong inhibitory activity of platelet aggregation (Yin et al, 1995; Sly et al, 1995). Several nitric oxide-releasing polymers have been developed to coat medical devices to deliver nitric oxide *in vivo* to treatment sites (Stamler et al., 1998) or incorporate into vascular grafts (Pulfer et al, 1997).

Surface containing phosopholipid polar groups, oligosaccharides and peptides

Modifying polymeric surfaces by introducing phospholipid polar groups, oligosaccaride chains, and specific oligopeptides for promotion of cell growth have been shown many successes in developing new biomimetic biomaterials. Ishihara et al (1992, 1993) have been studying blood-compatible surfaces with phospholipid polar groups. Their idea was to synthesis a polymer possessing a strong affinity for phospholipids from blood, which could be organised to form a biomembrane-like assemblage on the polymer surface. Based on this idea, many phosphorylcholine (PC) modified surfaces have been developed, including poly (MPC-CO-BMA) (2-methacryloyloxyethyl phosphorylcholine-co-butyl methacrylate) (Ueda et al, 1992). MPC-co-cyclohexyl methacrylate and MPC-co-2-ethylhexyl methacrylate (Ishihara et al, 1996) . The incorporation of phosphorylcholine-containing moieties into a polymer is an effective method for imparting nonthrombogenicity (Yu et al, 1994; Ishihara et al, 1996; Zhang et al, 1998; Vander Heiden et al, 1998). In addition, some other phospholipid-containing materials have been investigated using phosphatidylcholine analogous as chain extender for polyurethane synthesis. No platelet adhesion was observed for all new phospholipid polyurethane casting films (Li et al, 1997).

Polysaccharides, such as dextran sulphate, have been used as an anionic modifying agent to modify haemodialysis membrane. The modified membranes resist

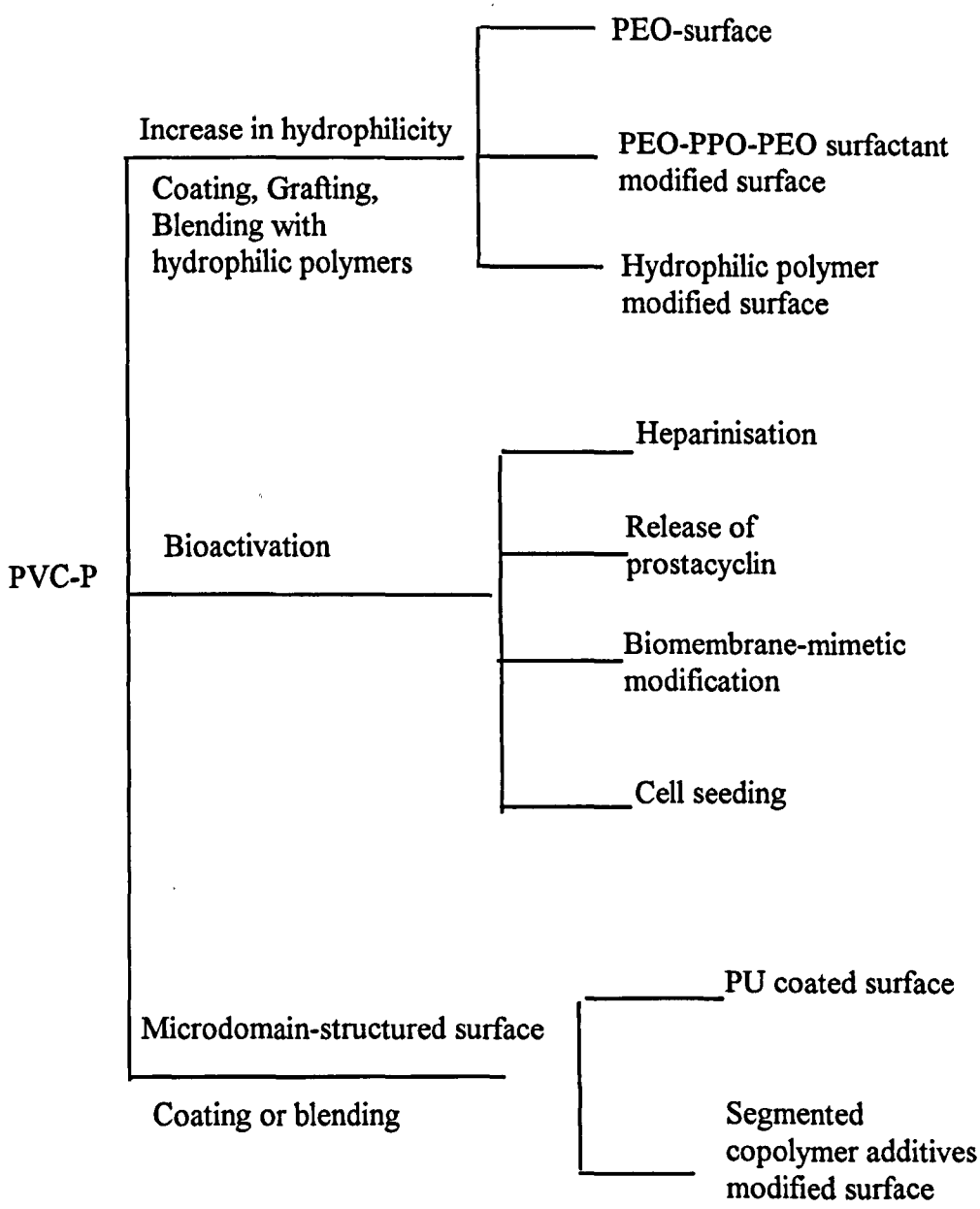


Fig3.6 Surface modification of plasticised PVC for improved blood compatibility

complement activation and platelet adhesion and activation (Amiji, 1996). The surface modified with dextran has been shown to behave as a glycocalyx-like interface in aqueous environment. It was claimed that this biomimetic surface was effective in suppressing protein adsorption from human plasma protein solution (Holland et al, 1998).

Endothelial cell seeding of small calibre vascular prostheses has been shown to reduce long-term platelet deposition, thrombus formation and thus graft failure. (Simon et al, 1996). There are many approaches that have been explored to facilitate the achievement of endothelialisation. An effective method of promoting the integration and adhesion of the cells onto the device is to immobilise agents such as extracellular matrix (ECM) protein and oligo-peptides such as RGDs directly onto the device surfaces. (Pierschbacher & Ruoslahti, 1984; Sugawara & Matsuda, 1995).

For enhancing the recognition of cell by the RGD-ligand modified surface, the combination of Pluronic surfactant/RGD containing hexapeptide has shown an effective way to promote cell attachment to hydrophobic substrates (Neff et al, 1998).

Recently, a haemocompatible surface-modifying additive has been invented for modifying polyurethane or polyurethane urea substrate. The additive has a urethane hard block and a silicone soft block, an optional hydrophilic spacer and a RGD peptide. It can be used as an additive to blend with polyurethane in order to promote cell adhesion (Riffle, 1998).

In summary, there are many approaches to modify a polymeric surface for improved blood compatibility on the basis of different hypotheses, including increase in hydrophilicity, increase in hydrophobicity, forming a microdomain structured surface, and building a bioactive surface which functioning as a living cell. It is believed that mimicking the blood vessel surface represents the future for developing long-term blood-contacting medical devices.

3.4 Modification of PVC-P surface for improved blood compatibility

Utilisation of hydrophilic polymers, surface heparinisation, incorporation of bioactive substances and biomimetic modification can achieve surface modification of plasticised PVC for improved blood compatibility (Fig 3.6).

Based on the hypothesis that hydrophilic and negatively charged surfaces are blood compatible, many hydrophilic polymers such as poly (2-hydroxyethyl-methacrylate) (PHEMA) (Yamauchi, 1975), poly(N-vinyl-N-methylacetamide) (Paul et al, 1982), negatively charged poly(methacrylic acid) (Singh et al, 1990a,b), combination of two different hydrophilic polymers (Krishnan & Jayakrishnan, 1990) and PEO (Mori, 1982 ; Golander & Kiss, 1988; Lakshmi & Jayakrishnan,1998) have been grafted onto PVC-P surface. A PVC-P surface grafted with hydrophilic polymers has exhibited improved blood compatibility as well as the ability to prevention of plasticiser migration. In addition, it is also possible to produce low-friction PVC-P catheter by such grafting hydrophilic polymer technology (Uyama et al., 1991).

Heparinisation of PVC-P is also a well-accepted approach to obtain improved blood compatibility. It can be achieved by ionic complexation. Tridodecylmethylammonium chloride (TDMAC)/ heparin complex is soluble in common organic solvents. Brenner et al (1974) used its solution to coat PVC-P bypass tubes. Miyama et al (1977) introduced a photoactive group onto PVC and then dimethylamino-containing monomer was photografted onto PVC-P surface, which being capable of ionically bonding heparin. Similarly, poly (amido-amines) have been found to be able to form stable complexs with heparin. By grafting such moieties, a heparinisable PVC-P surface was achieved with powerful heparin retention ability. (Ferruti et al, 1982; 1984).

Covalent end-point immobilisation of heparin onto a PVC-P surface has been investigated extensively (Larm et al, 1983; Riesenfeld, 1995) and the blood compatibility of so-formed heparinised PVC-P has been studied to show an improved blood compatibility (Yin et al, 1999). The influence of heparin coating by end-point attachment technology on *in vitro* bacterial adherence on PVC-P has also been investigated (Zdanowski et al, 1997).

Hsu et al (1995) employed many other hydrophobic cationic substances such as polyethyleneimine, dimethylstearylamine, benzalkonium, stearylkonium and tridodecylmethlammonium to form a complex with heparin. The formed complex is soluble in lower alcohol such as isopropyl alcohol. By coating the complex on PVC-P and following by γ-radiation sterilisation, the heparin moiety can be bound to PVC-P surface.

By incorporation of prostacyclin into PVC-P blending system (McRea & Kim, 1983), a controlled delievery of prostacyclin can lead to an improved surface blood compatibility. In another approach, albumination of PVC surface has also been shown to effectively suppress the adhesion and activation of platelets when it contacts whole blood (de Queiroz et al, 1997).

Biomimetic modification of PVC-P has been approached by coating MPC/lauryl methacrylate copolymer to incorporate phosphorylcholine polar groups onto the surface to achieve biomembrane-like surface properties (Yianni, 1995; Zhang et al, 1998). The coated surface properties are hypothesised to be mainly influenced by the underlying material surfaces (Zhang et al, 1998). In addition to those modifications based on MPC copolymer, some different phospholipids have been bound onto PVC tubing (von Segesser et al, 1994) to achieve improved blood compatibility. PVC-P surface is also improved for cell adhesion (Klee et al, 1994).

Surface modification of PVC-P is also achieved by PVC surface coating and blending with surface-active additives such as PEO-PPO surfactant and HEMA-Styrene copolymer having a microdomain structure. The resulting materials show high anticoagulant activity and inhibition action of platelet loss (Iguchi et al, 1998). The blending of PVC-P with hydrophilic polymers such as PEO, PVA and PVP has also been employed to prepare a protein-resistant surface as previously stated (Ding et al, 1996).

In this thesis, a novel approach for surface modification of PVC-P for improved blood compatibility is undertaken by utilisation of oligosaccarides such as cyclodextrin and cyclodextrin/ PEO, cyclodextrin/PEO-PPO combination. One of the commercialised cyclodextrins, β-Cyclodextrin has been utilised to modify the surface of biomedical adsorbents for biospecific adsorption in blood purification (Zhao & He, 1994 a, b). The amphiphilic nature of cyclodextrins and their ability of forming inclusion complex with many bioactive substances were of interest for us to exploit their potential applications for modification of polymers. Cyclodextrin-modified PVC-P and possible mechanism for improved blood compatibility will be investigated in Chapter 6 and Chapter 7.

In summary, surface modification of PVC-P can be achieved by means of those common approaches for other biomaterials. However, before undertaking these approaches, surface pre-treatment might be necessary due to the presence of high levels of plasticiser at the surfaces, which differ from other biomaterials. Consequently, polymer blending for modification of PVC-P might be more industry-relevant and suitable for the development of PVC related biomaterials.

CHAPTER 4

SELECTED MATERIALS AND ASSESSMENT PROCEDURES

4.1 Introduction

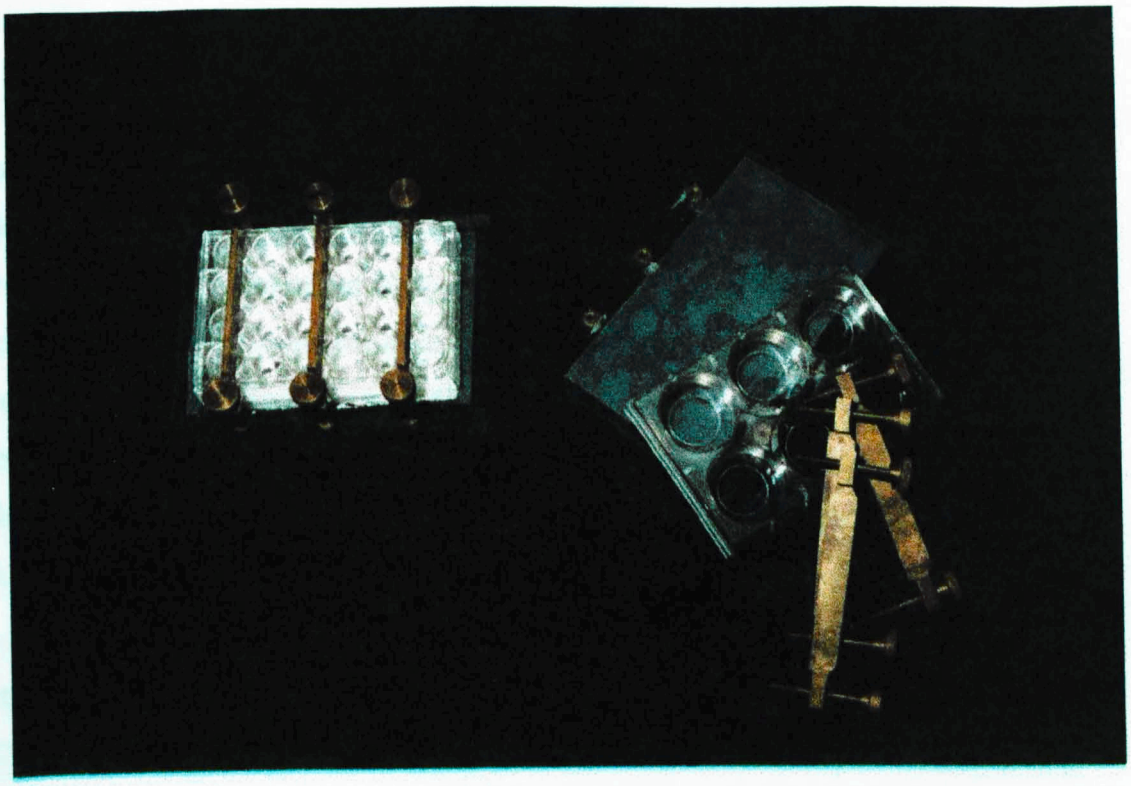
The objective of this project was to study the potential blood compatibility in terms of the influence on protein adsorption onto plasticised poly (vinyl chloride) (PVC-P) of plasticiser selection and surface modification. With respect to plasticiser selection, three types of PVC-P plasticised with 2-di(ethylhexyl)phthalate (PVC-DEHP), tri-(2-ethyhexyl)trimellitate (PVC-TEHTM), and n-butyryltri-n-hexyl citrate (PVC-BTHC) were selected. Protein adsorption was studied using radiolabelled human I^{125}-fibrinogen and bovine serum I^{125}-albumin. The surface modification was achieved using surface methanol extraction and cyclodextrin (CD) modification by casting and blending respectively.

The combination of CDs with polyethylene oxide (PEO) and poly (ethylene oxide)-poly(propylene oxide) (PEO-PPO) triblock copolymer to modify PVC-P was also investigated. The effects of possible inclusion complex formation between CDs and PEO-PPO-PPO or PPO-PEO-PPO copolymer on their modified PVC-P surface in respect to protein adsorption was studied. The β-CD/PEO-PPO-PEO inclusion complexes were prepared. Protein adsorption of these cyclodextrin-inclusion-complexes (CICs) modified PVC-P was compared with those of the simple physical mixture modified PVC-P.

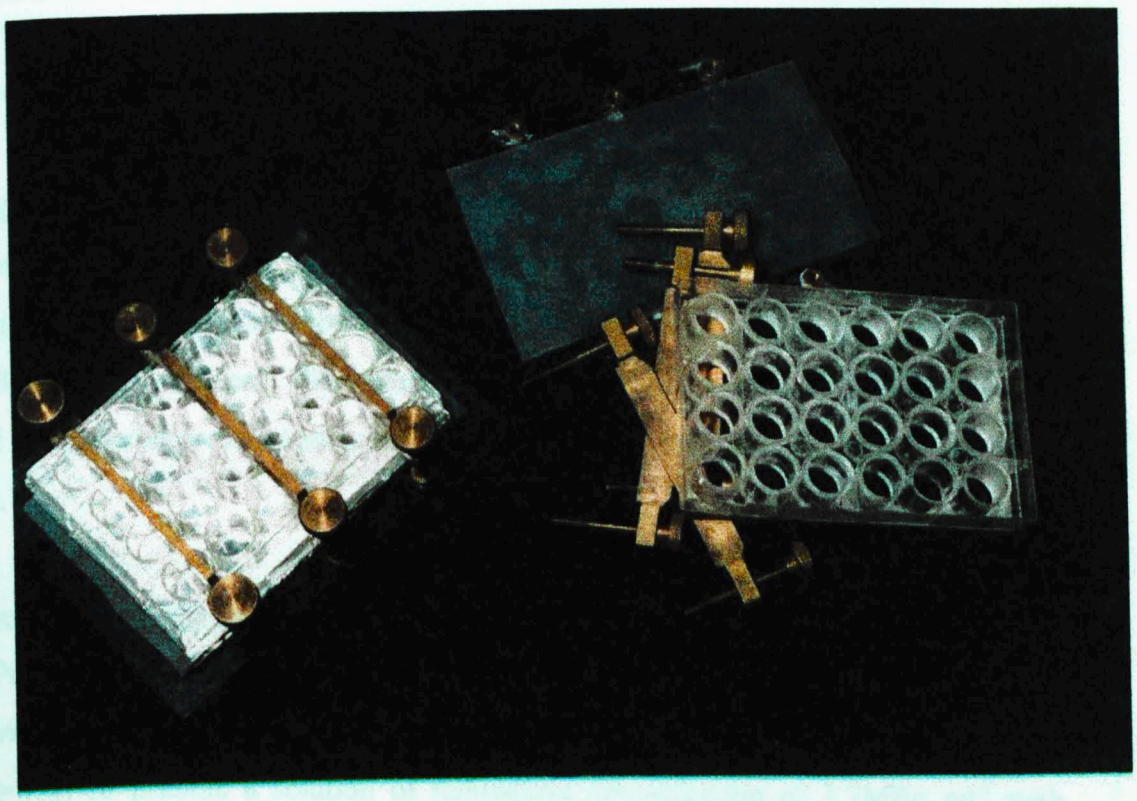
4.2 Plasticised poly (vinyl chloride): Influence on protein adsorption of plasticiser selection and plasticiser surface level

4.2.1 Materials

The selected medical grade PVC-P materials plasticised with DEHP, TEHTM and BTHC were supplied by Ellay Inc., City of Commerce, California, USA, in flat sheet form with a similar softness. Unplasticised poly(vinyl chloride) (PVC-U) powder, DEHP and TEHTM plasticiser were supplied by Hydro Polymers LTD., Newton Aycliffe UK. A PVC-U sheet, as a control, was produced by polymer melting and compressing and the process is detailed in section 4.3.3. Cuprophan haemodialysis



A



B

Fig 4.1 In vitro protein adsorption set-up

membrane 13μm in thickness was obtained from Akzo Faser AG, Wuppertal, Germany , and also served as a control material for evaluation of the effectiveness of protein adsorption assessment using a modified 24-well incubation cell plate. All other reagents, such as methanol for surface treatment and phosphate-buffered saline (PBS) were of analytical grade from Aldrich-Sigma company, Dorset, UK. Human ^{125}I-fibrinogen was purchased from Amersham International PLC., Buckinghamshire, UK. The product is supplied lyophilised from a solution containing sodium chloride (0.65%), trisodium citrate (0.75%) and bovine serum albumin (29mg/ml). Despatched in silanised glass vials containing 4.07MBq (110μCi) of ^{125}I-fibrinogen with specific activity 5.7 MBq/mg (155μCi/mg). Bovine Serum ^{125}I-albumin was purchased from ICN pharmaceuticals, Inc. Radiochemical Division, 2727 Campus Drive, Irvine, California 92715, USA. ^{125}I-albumin is in 0.1M Kphos (pH=7.5) with specific activity 764 μCi/mg and 250 μCi in total activity for each kit.

4.2.2 Protein adsorption assessment

Material-Protein Contact

Protein-material contact was achieved with the modified 24-well incubation test cell (Yu, 1993). Fig 4.1(a,b) shows the protein adsorption set-up either in 6-well or 24-well incubation test cell. The volume of each well is about 4ml for 24-well test cell and the material-protein contact area in each well is 2cm^2 for 24-well incubation test cell.

Assessment procedure

1. Preparation of ^{125}I-fibrinogen and ^{125}I-albumin stock solution

A vial of ^{125}I-Fibrinogen (4.07MBq) was made up to 1.1ml using 0.05M PBS (pH=7.4) to provide a solution of radioactive concentration of 3.7MBq ml^{-1} with 0.649 mg/ml concentration as stock solution. All work was carried out in the fume-hood at room temperature. A vial of ^{125}I-albumin (250μCi) was made up to 2.5ml using 0.05M PBS (pH=7.4) to provide stock solution of radioactive concentration of

100μCi/ml with 130.8μg/ml as stock solution. The stock solution was kept at 4°C in a refrigerator.

2. Preparation of protein bulk solution for adsorption study

Ten to sixty microliters of the fibrinogen stock solution were added to a vial containing 20 ml phosphate-buffered saline (PBS) buffer solution (0.05M, pH7.4) and stirred at 25°C to provided an initial protein solution concentration varying from 0.325 μg/ml to 1.95μg/ml as shown in Table 4.1. Similarly, twenty to one hundred microliters of the albumin stock solution were added to a vial containing 10 ml PBS solution and stirred at 25°C to provide an initial protein solution concentration in the range 0.2616 to 1.295 μg/ml as seen in Table 4.2.

Table 4.1 Preparation of I^{125}-Fibrinogen bulk solution for adsorption test

No.	1	2	3	4	5
Volume of fibrinogen stock solution taken(μl)	10	20	40	50	60
Bulk solution concentration (μg/ml)	0.325	0.650	1.30	1.625	1.950

Table 4.2 Preparation of I-125 albumin bulk solution for adsorption test

No	1	2	3	4	5	6
Volume of albumin stock solution taken(μl)	20.0	30.0	40.0	50.0	80.0	100.0
Bulk solution concentration (μg/ml)	0.262	0.391	0.521	0.651	1.038	1.295

3. Protein adsorption study: time dependence:

1ml of protein solution with 1.3μg/ml for fibrinogen and 0.521μg/ml for albumin were added to a well using a modified 24-well incubation test cell plate and contacted with the polymer at 25°C for a selected time period (5min, 10 min, 15 min, 20 min, 30 min, 45 min and 60 min). On completion of the contact period, the protein solution was removed, 1ml of buffer solution added to the well, and rinsing performed for 20s. The polymer was removed, placed in a vial, and the radioactivity counted 3 times with a standard gamma well counter. With each polymer, the procedure was repeated three times. The level of protein adsorption was calculated by dividing the retained radioactivity on the polymer sample, corrected for background, by the specific activity of the initial protein bulk solution and the contacted area of the sample.

4. Protein adsorption study: bulk solution concentration dependence

The same procedure was adopted as above for time-dependence protein adsorption study with a varied initial bulk solution concentration, as shown in Tables 4.1 and 4.2 for fibrinogen and albumin respectively. The adsorption time period was 20 minutes and the adsorption temperature was at 25 °C.

5. Protein adsorption on 24-well incubation test cell wall (polystyrene)

This was calculated from the substraction between the fibrinogen concentration before test and after test plus the fibrinogen uptaken by PVC-P samples.

4.2.3 Surfaces plasticiser removal using methanol surface extraction

The modified 24 well incubation cell was employed again with a polymer contact area of $2cm^2$. Four millilitres of methanol were added to a well and contact with PVC-P for a selected time period (5,10,15,20,25,30,45,60,90 minutes). The overtime extraction will damage PVC-P material and change its properties, which is not acceptable. At the end of the contact period, the methanol was removed, the surface cleaned material was assessed for protein adsorption. The surface treatment was performed in a fume-hood.

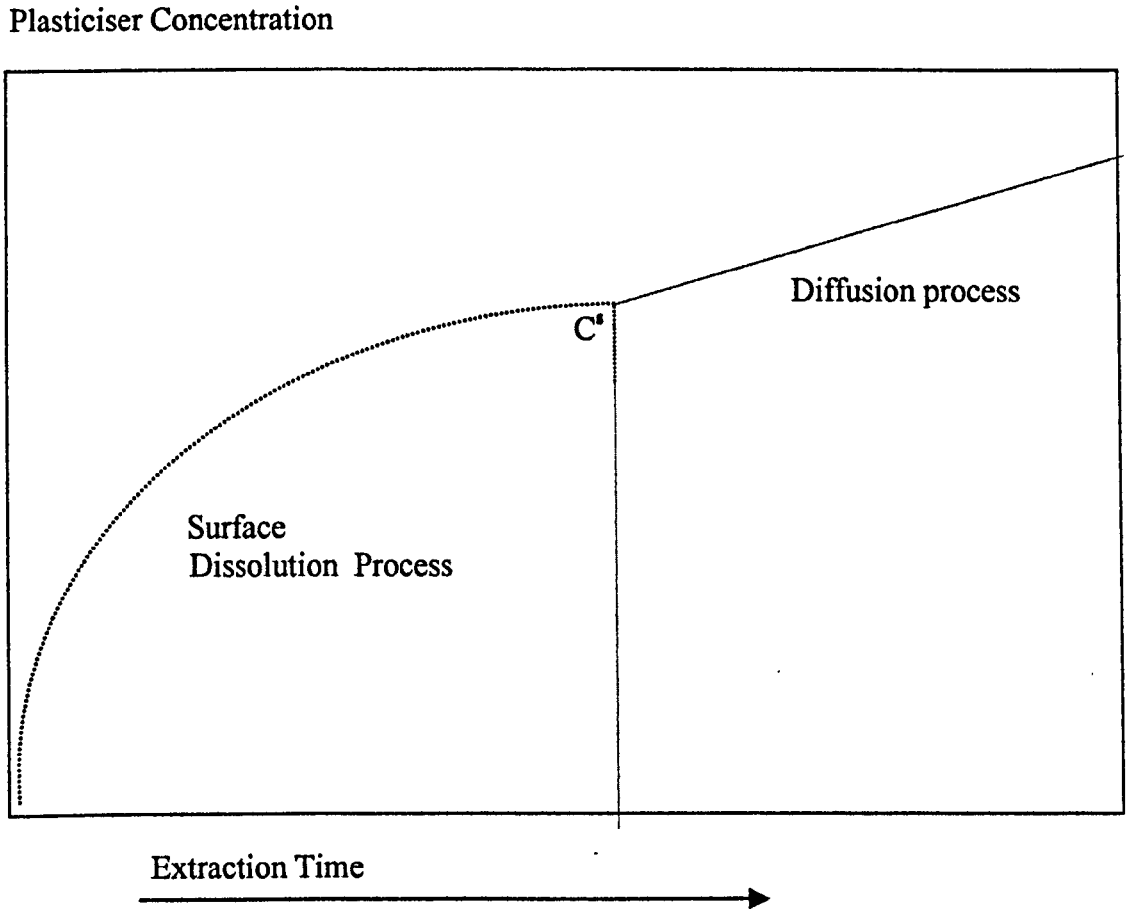


Fig 4.2 Illustration of plasticiser migration behaviour using organic solvent as an extractant (from Kim et al, 1976)

4.2.4 Surface characterisation

Evaluation of the plasticiser surface level using UV-Spectrophotometer

Evaluation Principle

Surface extraction of plasticiser by an organic solvent such as methanol can be divided into two processes, covering an initial surface dissolution and plasticiser diffusion from the bulk through the surface into contact media. The amount of extracted plasticiser increases at the first dissolution stage. On completion of the removal of surface plasticiser, the amount of extracted plasticiser increases according to a diffusion mechanism (Kim et al, 1976)(Fig 4.2). There is an overlapping point between dissolution and diffusion process and the overlapping extraction time can be theoretically regarded as the time when the surface is free of plasticiser. The amount of extracted plasticiser measured by UV-Spectrophotometer at this time is considered to be the original surface plasticiser level. Hence, the plasticiser surface level during extraction period can be evaluated using the following formula:

$Ct^s=Co^s-Ct^e$ ------ (4.1)

where Ct^s represents the surface level of plasticiser at selected extraction time., Co^s is the original surface plasticiser level; Ct^e stands for the concentration of extracted plasticisers at selected time period which is determined by UV-spectrophotometer according to a standard method(Europe Pharmacopoeia, 1997).

Procedure of evaluation plasticiser surface level

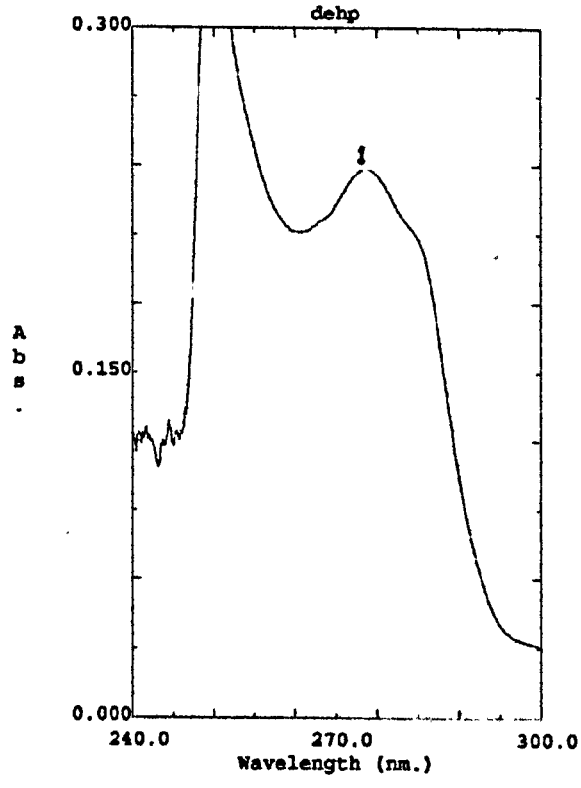
1. Calibration of DEHP and TEHTM plasticiser

Stock solution preparation

Dissolve 0.100g of DEHP and TEHTM in the methanol and dilute to 100.0ml with the same solvent to get stock solution.

Plotting of calibration curves

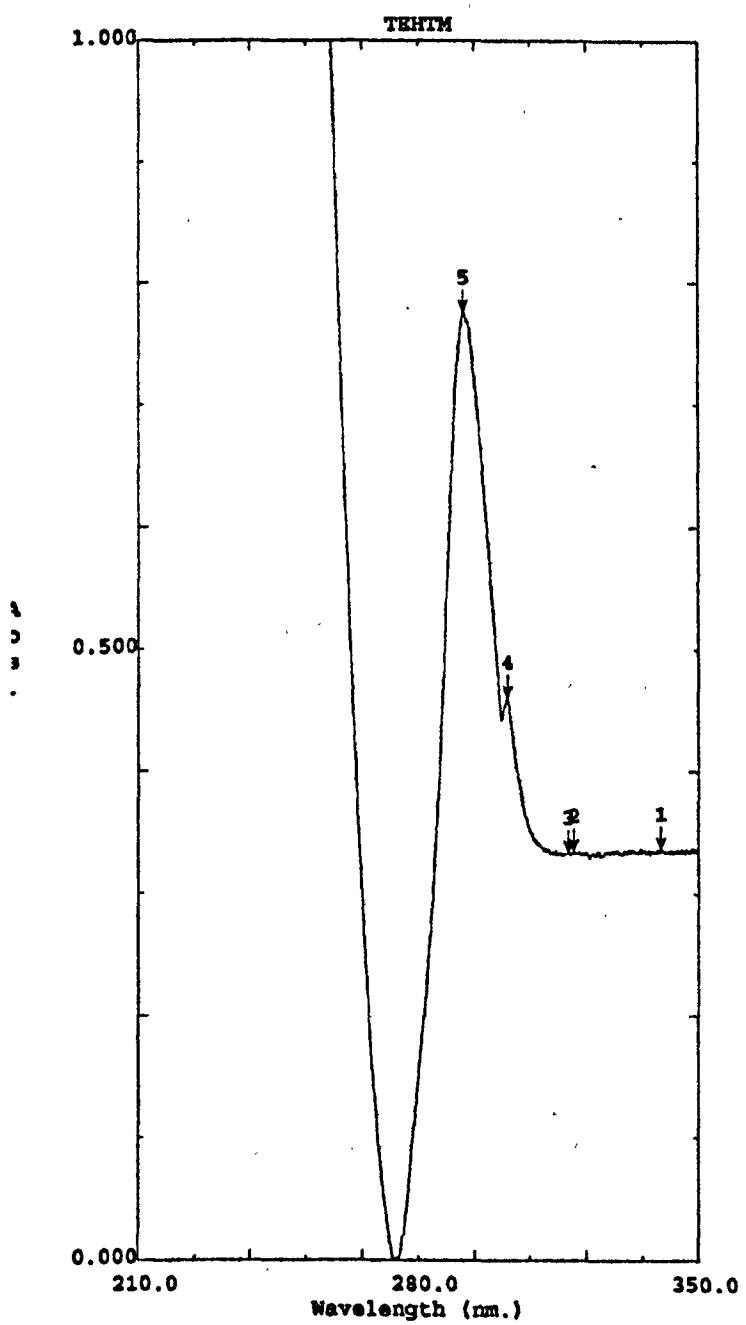
Dilute 100μl, 200μl, 500μl, 1ml and 2ml of above stock solution respectively to 10.0ml with methanol to get standard solutions. The absorbancies of the standard solution were measured at 274nm and 290nm using an UV-spectrophotometer



Peak Pick

No.	Wavelength (nm.)	Abs.
1	274.50	0.239
2	251.50	0.349
3	241.90	0.126
4	238.90	0.127
5	230.90	0.119
6	225.90	0.131
7	220.80	0.124
8	212.60	0.133

Fig 4.3 UV-spectrum of DEHP



Peak Pick		
No.	Wavelength (nm.)	Abs.
1	340.70	0.337
2	318.90	0.336
3	317.60	0.334
4	302.30	0.461
5	291.30	0.777

Fig 4.4 UV-spectrum of TEHTM

(Model1201, Shimadzu, Japan); where DEHP and TEHTM have a characteristic absorption band obtained using a UV-vis scanning spectrophotometer, Shimadzu UV-2101 PO, Japan (Fig 4.3 and 4.4). The methanol was used as compensation liquid.

The calibration curves for DEHP and TEHTM were shown in Appendix 1, 2 and Table 4.3.

Table 4.3 Calibration of DEHP and TEHTM

CONCENTRATION(MG/ML)		0.01	0.02	0.05	0.1	0.2
Absorbance	DEHP	0.086	0.212	0.343	0.575	1.162
	TEHTM	0.122	0.303	0.516	0.849	1.649

2. Evaluation of plasticiser level at PVC-P surface

PVC-DEHP and PVC-TEHTM samples (2.5x10cm) were stored in 100ml of methanol for selected time periods to permit plasticiser extraction. The quantity of plasticiser extracted into the methanol (Ct^e) was monitored by UV-spectrophotometer and calculation using previously obtained calibration plots. From the extraction curves of DEHP and TEHTM by methanol, the overlapping points were found. Thus the plasticiser level of original surface (Co^S) can be obtained and the plasticiser surface level at selected extraction time period (Ct^s) can be calculated using Formula 4.1.

Surface characterisation of PVC-P using Fourier Transform Attenuated Reflection Infrared Spectroscopy (ATR-FT-IR)

Infrared spectroscopy gives information about chemical groups and molecular structure. FT-IR combined with ATR can be used to study molecular surface composition qualitatively and in some cases quantitatively (Nocentini & Barbucci, 1993).ATR-FT-IR analysis in this study was performed on a Mattson 3000 FT-IR spectrometer, UNICAM. Spectra were recorded on an average of 25 scans at a resolution of $2cm^{-1}$. Slices of polymers with a smooth side were pressed against 45-degree Zinc selenide(ZnSe). The sampling depth at $1000cm^{-1}$ band is about 0.6μm using ZnSe. The background spectrum of the crystal was recorded firstly. Then the

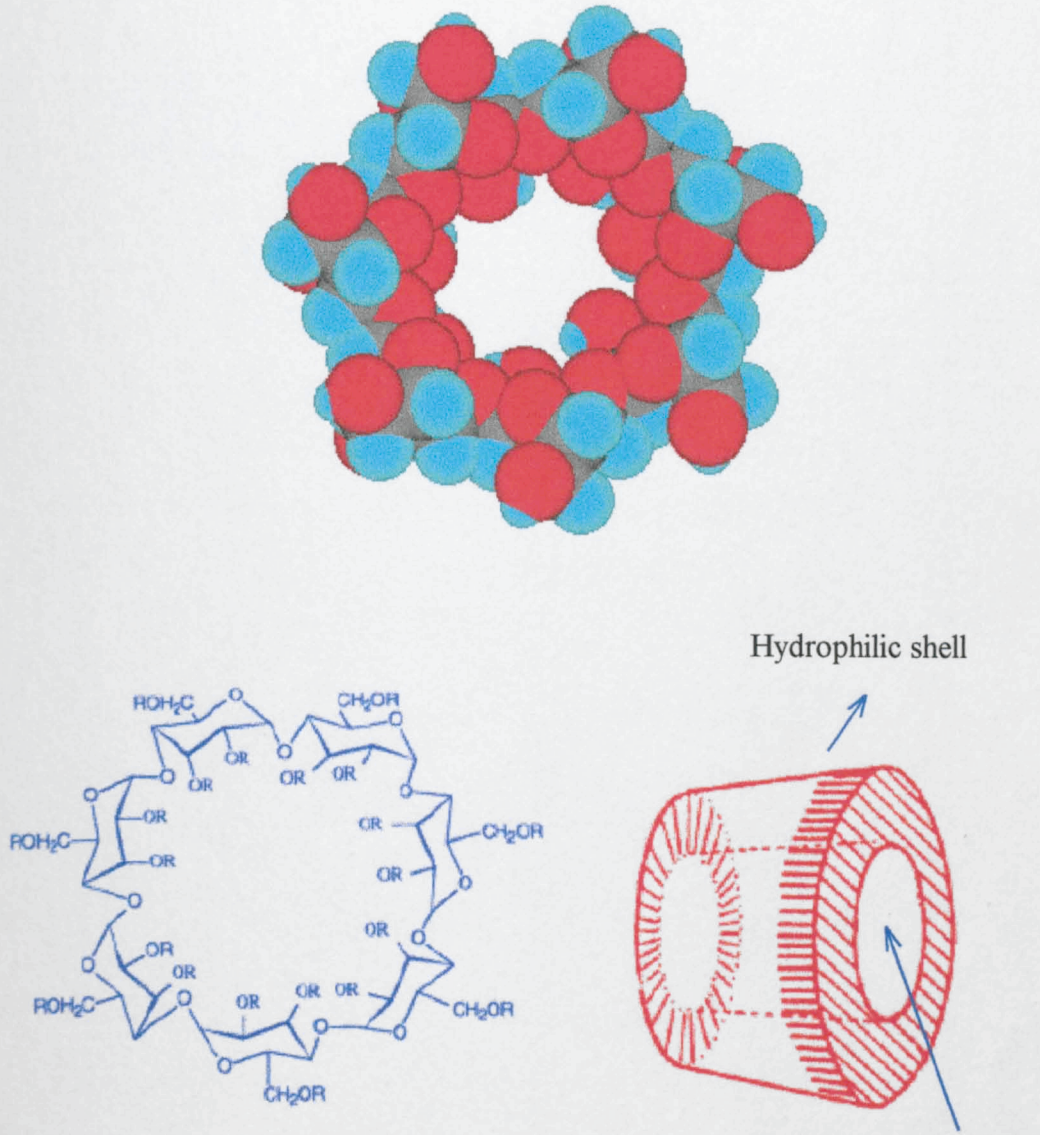


Fig 4.5 Chemical Structure of β-Cyclodextrin
(3D structure, cavity structure)

sample spectra were recorded and the background subtracted. The integration of peaks, the subtraction of background spectrum or between two spectra and baseline correction or smoothing to reduce the noise level were manipulated using a computerised programme.

4.3 Cyclodextrin modified PVC-P

4.3.1 Materials

β-Cyclodextrin was purchased from Guanddong Yuenan Cyclodextrin Factory, China as food additive grade. α-Cyclodextrin, γ-cyclodextrin and hydroxypropyl-β-cyclodextrin were provided by American Maize-Products Company, Hammond, IN, USA with purity of 99.8%. The chemical structure of one example of cyclodextrins, β-cyclodextrin (β-CD) is shown in Fig 4.5 and the molecular formula and molecular weight of cyclodextrins are summarised in Table 4.4

Table 4.4 Some basic chemical information on cyclodextrins

Cyclodextrins	Molecular Formula	Molecular Weight	Cavity Diameter Å
α-CD	$C_{36}H_{60}O_{30}$	973	4.7-5.3
β-CD	$C_{42}H_{70}O_{35}$	1135	6.0-6.5
γ-CD	$C_{48}H_{80}O_{40}$	1297	7.5-8.3
HP-βCD	$\{(C_{42}H_{70-n}O_{35}).(C_3H_7O)_n\}$ n=5-8	1425-1600	6.0-6.5

PVC-DEHP, PVC-TEHTM and unplasticised PVC raw materials in powder form were provided by Hydro Polymers Ltd, Newton Aycliffe, UK. Poly(ethylene oxide)(PEO)(MW=100,000), PPO-PEO-PPO (MW=2,000; 50% wt of PEO) and low density polyethylene(LDPE)(Mw=10,000) were purchased from Aldrich. The triblock copolymer of PEO-PPO-PEO Pluronic F68 was purchased from Sigma and Pluronic

L81 was provided by BASF Wyandotte Corp, Parsippany, NJ, USA respectively. All substances were used without further treatment and other organic solvents used were in HPLC analytical grade.

4.3.2 Incorporation of cyclodextrins into PVC-DEHP by polymer solution casting

The preparation of cyclodextrin modified PVC-DEHP film was performed according to the method by Sreenivasan (1996) and detailed in the following procedures.

Preparation of plasticised poly(vinyl chloride) solution (solution 1)

5.0gram of compounded plasticised poly(vinyl chloride) (PVC-DEHP) were dissolved using 50.0ml of tetrahydrofuran(THF) or DMF at room temperature under stirring condition to get a clear solution with 10.0% concentration.

Preparation of cyclodextrins (CDs) solution

2.0gram of cyclodextrins were dissolved in 20ml of THF/DMF (1:2) mixed solvent at room temperature to get clear solution.

Mixing two solutions

According to Table 4.5 , a mixed solution of CDs and PVC-DEHP was prepared with various CDs concentration.

Table 4.5 Preparation of CD-modified PVC-DEHP polymer solution

NO	1	2	3	4	5	6	7	8
solution 1(ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
solution 2(μl)	0.0	50	75	100	125	150	175	200
CDs(w%)	0.0	2.44	3.61	4.76	5.88	6.98	8.05	9.09

Casting and PVC-DEHP-CDs film preparation

0.5ml (50μl) of mixed solution was added to a 24-well test cell plate sandwiched with a aluminium foil (2cm^2 area) and dried at room temperature for 48 hrs, 50°C for 24 hours and room temperature 24hours in vacuum dessicator. All procedures were carried out in a fume- cupboard. The casting set-up was shown in Fig 4.6.

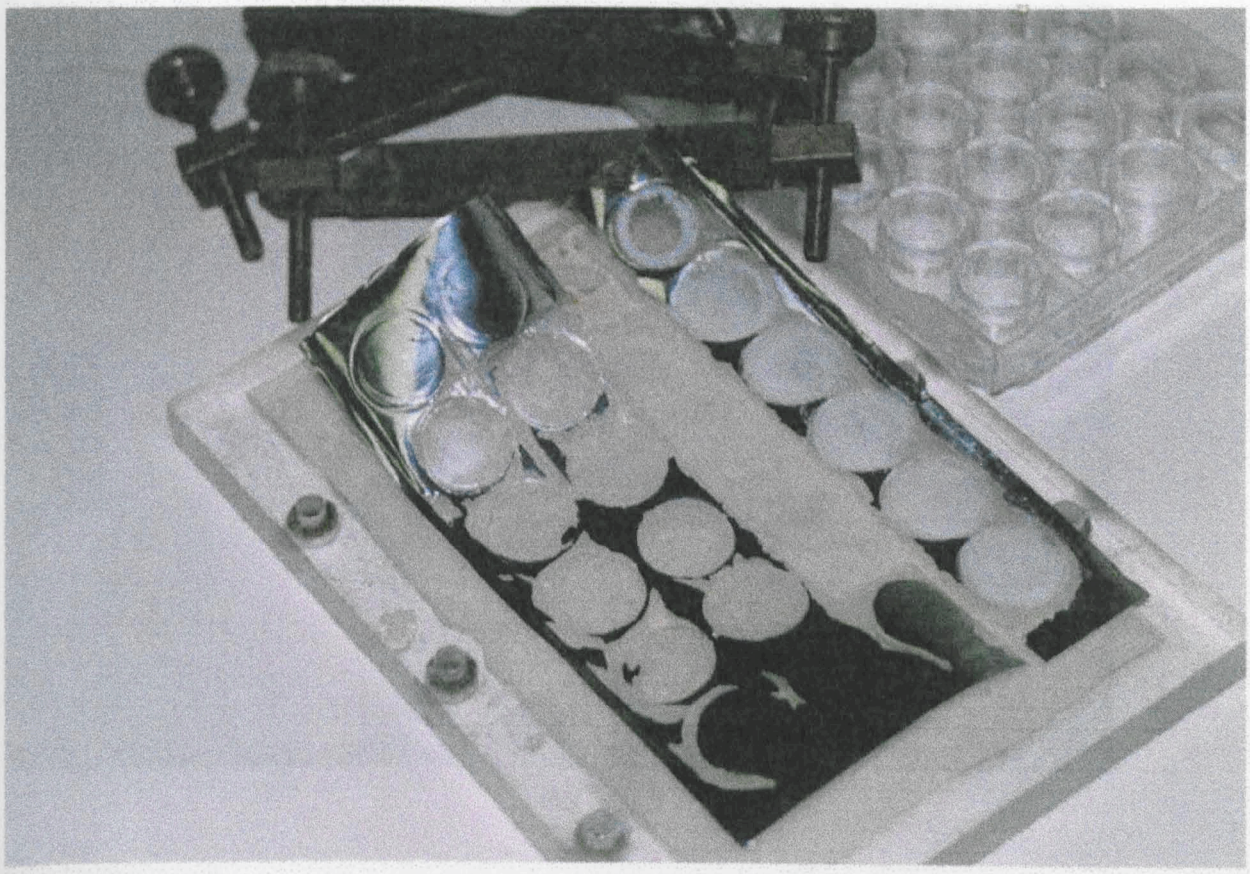


Fig 4.6 PVC casting set-up

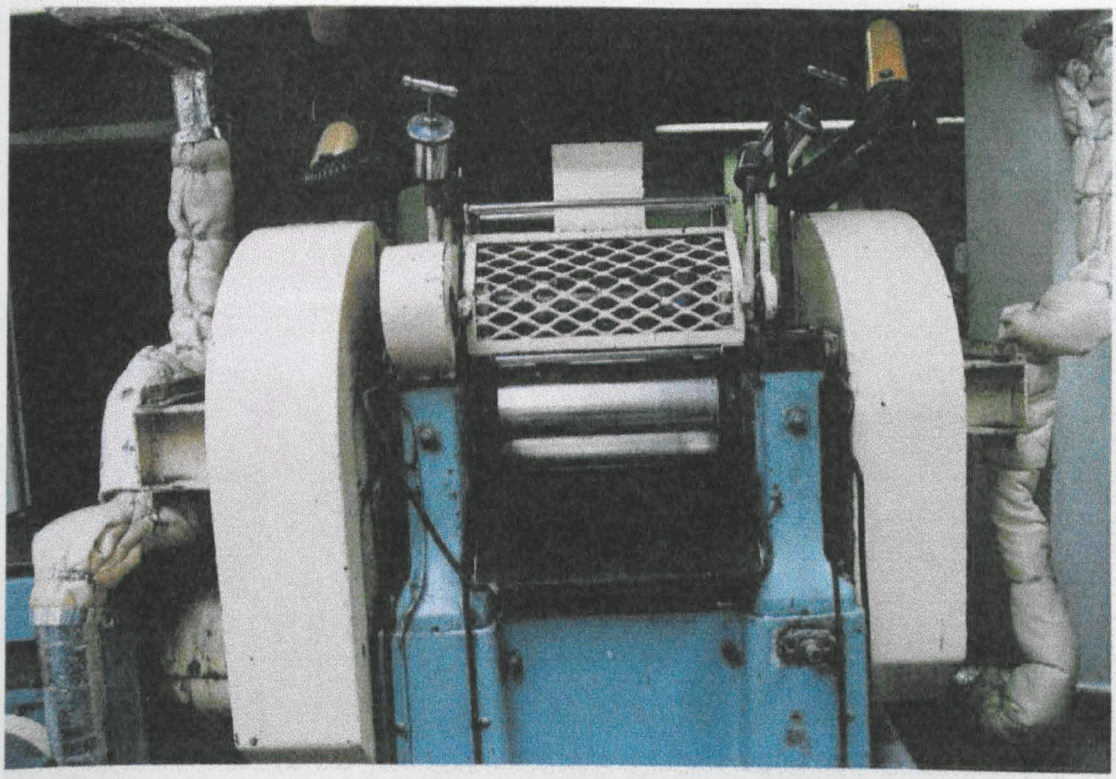


Fig 4.7 Two roll mill for PVC blending

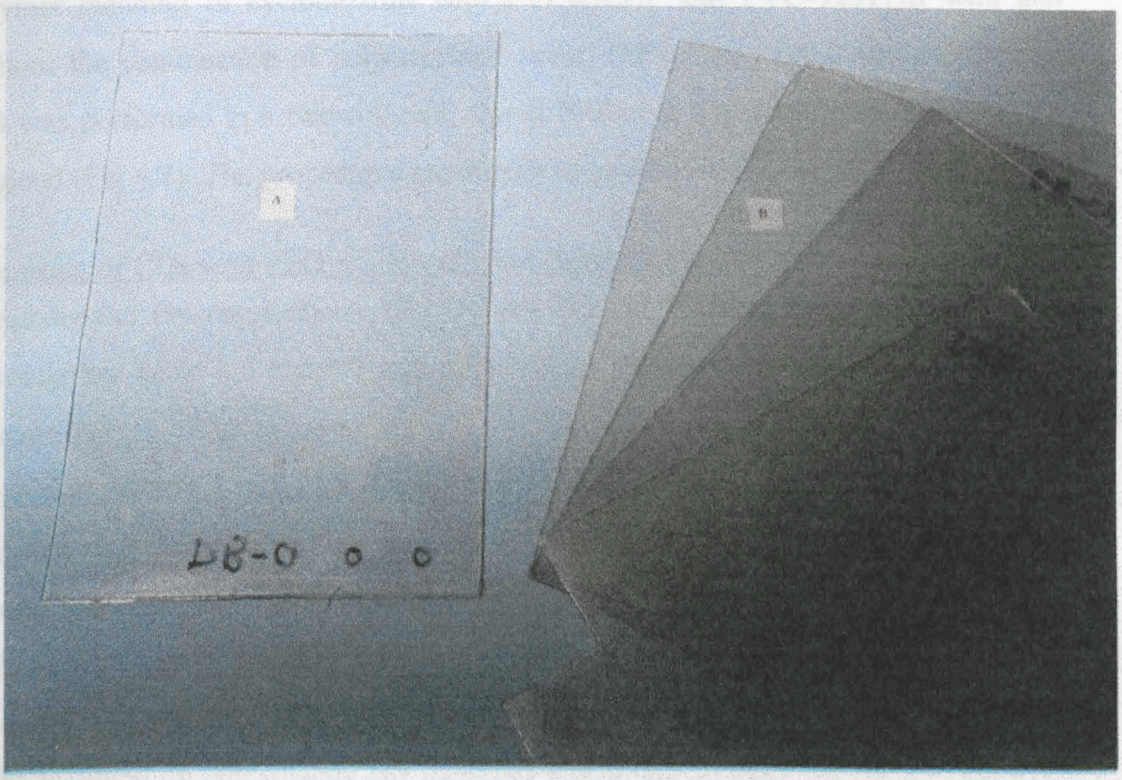


Fig 4.8a Modified PVC-P samples compared to unmodified PVC-P

A: unmodified PVC-DEHP; B: modified PVC-DEHP

4.3.3 Incorporation of cyclodextrin by polymer blending

Polymer blending of cyclodextrins with PVC-DEHP and PVC-TEHTM with and without the combination of poly(ethylene oxide) (PEO, Mw=100,000) and Pluronic F68 was performed in a two-roll mill, David Bridge & Co.Ltd, Castleton Rochdale, England (Fig 4.7). The procedures are described as follows:

Premixing of CDs with PEO or Pluronic F68 (Mixture 1)

Weighing out the requirements of CDs and PEO or Pluronic F68 according to the Formulation Table 4.6 and 4.7 and physically premixing these ingredients to obtain mixture 1.

Premixing mixture 1 with PVC-DEHP or PVC-TEHTM

Weighing out the required mixture 1 and PVC-DEHP or PVC-TEHTM and physically mixing them according to the formulation (Tables 4.6 and 4.7).

Polymer Blending by melting

The mill rolls are warmed to about 140-145°C and the PVC mixture was fed onto the rolls, brushing up immediately any materials which falls through the nip and adding it back to the bank of compound. During the mixing, the temperature was allowed to build up to 150-155°C and the compound should be milled for between 5 and ten minutes with cutting about continually and finally up-ending several times. After milling, the flexible sheet was removed and cooled down at room temperature. 20~25g milled sheet were weighed out and loaded into a mould (15cm x 12cm x 00.9cm) and compressed for 5 minutes at 150°C. The compressive load is of 10 tons on 41/2"Ram.

A final cyclodextrin modified PVC-P sheet (15cm*12cm*0.09cm) with a smooth surface, which was achieved using a well-polished stainless steel plate as a top cover, as shown in Fig 4.8 a.

Incorporation of CDs into LDPE by melting was achieved using an Inter-Mixer, Brabender, Engelmann & Buckham Machinery Ltd (Figs. 4.9). The mixing was undertaken at 140 °C for 5minutes and finally the mixture was pressed into a sheet

Fig 4.8 Internal mixer for LDPE modification

using the same technology of PVC-P processing. The prepared CDs modified LDPE materials were selected as control materials for comparison.

4.3.4 Protein adsorption studies

Protein adsorption studies on cyclodextrins modified PVC-P were carried out using the same procedure as previously described in 4.2.2. The polymers were soaked in 0.05M PBS (pH=7.4) overnight before protein adsorption assessment.

Table 4.6 Polymer blending modification of PVC-DEHP by cyclodextrins or their combination with PEO or PEO-PPO-PEO triblock copolymer

Samples	PVC-DEHP(g)	Mixture 1 (PHR)			
		β-CD	PEO	Pluronic F68	HP-βCD
DB-0	100.0	0			
DB-3	100.0	3.1			
DB-5	100.0	5.3			
DB-8	100.0	8.7			
DBP-03	100.0	0	3.1		
DBP-33	100.0	3.1	3.1		
DBP-35	100.0	3.1	5.3		
DBP55	100.0	5.3	5.3		
DBPP-03	100.0	0		3.1	
DBPP-53	100.0	5.3		3.1	
DBPP-55	100.0	5.3		5.3	
DH-3	100.0	0			3.1
DH-5	100.0	0			5.3
DHP-53	100.0	3.1			5.3
DHP-55	100.0	5.26			5.3

Fig 4.9 Migration test set-up using cottonseed oil as extractant

Table 4.7 Polymer blending modification of PVC-TEHTM by beta-CD and its combination with Pluronic F68

	PVC-TEHTM(g)	Mixture 1 (PHR)	
		β-CD	Pluronic F68
TB-0	100.0	0.0	0.0
TB-3	100.0	3.1	0.0
TB-5	100.0	5.3	0.0
TBPP-03	100.0	0	3.1
TBPP-33	100.0	3.1	3.1
TBPP-35	100.0	3.1	5.3

4.3.5 DEHP migration test of β-cyclodextrin modified PVC-DEHP

Migration studies of DEHP in methanol, ethnaol/H_2O (10%) mixture and cotton seed oil were carried out at room temperature for different time periods.

Methanol, as extractant

Polymers (1cm x 2cm x 0.09cm) were immersed into 25ml methanol at room temperature for 5 minutes. The absorbance of solution was immediately measured at 274nm using Ultraviolet-visible spectrophotometer and the extractant solution was used as a compensation solution.

Cottonseed Oil as extractant (Krishnan et al, 1990)

Selected polymer (2cm x 2cm x 0.09cm) was placed into a 6-well incubation cell plate and 10 ml cotton seed oil (Sigma Chemical Company) was added to each well (Fig 4.9). The extraction was performed at room temperature for time period varied from 24 hours, 48 hours, 96 hours, 120 hours and 1 month. The pre-weighed samples were removed at such intervals, washed with 0.5% soap solution for 5min, rinsed with

methanol for 15 seconds to remove the surface adhered oil, dried in an oven at 60°C for 60 minutes to a constant weight. The amount of plasticiser migrated into cottonseed oil was determined by calculating the percentage loss of weight after specific intervals.

4.3.6 Studies of interaction of CDs with DEHP using UV-spectrophotometer

Many studies have found that CDs would change the absorption of spectroscopy of a guest molecule by enhancement of absorption intensity or causing band shift due to the formation of inclusion complex. In order to understand the possible inclusion complex formation between CDs with DEHP plasticiser, UV-spectrophotometer was employed for this study. According to Table 4.8, the solutions of α-CD/DEHP, β-CD/DEHP, γ-CD/DEHP in water/methanol with different molar ratio were prepared. The UV-absorption at 274nm of these solutions was measured using a UV-spectrophotometer as mentioned in 4.2.4. The scanning of β-CD/DEHP solution was achieved using a UV-vis scan spectrophotometer.

Table 4.8 Preparation of CDs/DEHP solution for UV-Spectrophotometry measurement

NO	1	2	3	4	5	6	7	8	9	10
*β-CD/DEHP	0	0.32	0.48	0.64	0.80	1.28	1.60	2.41	3.2	3.36
α-CD/DEHP	0	0.38	0.76	1.14	1.52	1.9				
γ-CD/DEHP	0	0.28	0.84	1.4	1.68	2.24	2.8			

* Molar ratio. 1.0 ml 2.742 μmol/ml DEHP methanol solution + varied volume (ml) of 4.4μmol/ml β-CD, 5.1μmol/ml α-CD and 7.6μmol/ml γ-CD aqueous solution in a 10 ml volumetric flask and methanol/water was used as compensation solution.

4.3.7 ATR-FTIR surface characterisation of cyclodextrin modified PVC-P

The same procedure was taken for ATR-FTIR surface characterisation of cyclodextrins modified PVC-P as described in 4.2.4

4.4 Cyclodextrin Inclusion Complexes (CIC) modified PVC-P

4.4.1 Introduction

Cyclodextrins have been incorporated into PVC-P in combination with poly (ethylene oxide) (PEO) and poly(ethylene oxide)-poly(Propylene oxide) copolymer surfactant(PEO-PPO-PEO) to modify PVC-P surface. However, it is not very clear whether there is a complexing occurring between CDs and other additives including plasticisers, PEO and PEO-PPO-PEO surfactant. In order to clarify the modification mechanism, cyclodextrin inclusion complexes prepared from cyclodextrins and PEO-PPO-PEO or PPO-PEO-PPO were utilised to modify PVC-DEHP and PVC-TEHTM and surface protein adsorption properties were assessed.

4.4.2 Preparation of CD-PEO/PPO/PEO (Pluronic L81) Complex

10 mg L81 (Mw=2800) was dissolved in 0.2 ml distilled H_2O and added to a saturated aqueous solution of β-CD at room temperature under stirring. The solution became turbid and complexes were obtained as crystalline precipitates. The precipitated products were collected by centrifugation, dried at 60°C to obtain CIC-1. The yield ratio is 60%.

4.4.3 Preparation of CD-PPO/PEO/PPO Complex

20.0 mg PPO/PEO/PPO (MW=2000) were put into tubes. A saturated aqueous solution of β-CD (5.00ml) containing 92.5 mg of β-CD was added at room temperature, and the mixture was agitated for 10 mins and then allowed to stand overnight at room temperature. The precipitated products were collected by centrifugation, dried at 60°C to obtain CIC-2. The yield is 17%.

4.4.4 Polymer blending of CIC with PVC-P

Polymer blending of CIC with PVC-DEHP carried on using the same procedure as described in section 4.3.3. The blending formulation is shown in Table 4.8.

Table 4.8 The PVC formulation of CIC modified PVC-DEHP in comparison with physical mixture modified samples

SAMPLES	PVC-DEHP	PVC-TEHTM	CICS (PHR)	MIXTURES(PHR)
DB-0	100	/		
DB-5	100	/		5 (B-CD)
DBPP-35	100	/		3/5 (B-CD/F68)
D-CIC1	100	/	5 (CIC1)	
D-M1	100	/		4.42/0.58(B-CD/L81)
D-CIC2	100	/	5 (CIC2)	
D-M2	100	/		4.1/0.9 (B-CD/PPG-PEG-PPG)
D-M3	100	/		5 (L81)
D-M4	100	/		4/1(L81/B-CD)
D-M5	100	/		3/2 (L81/B-CD)
D-M6	100	/		2/3 (L81/B-CD)
D-M7	100	/		5 (PPG-PEG-PPG)
TB-0	/	100		
TB-5	/	100		5 (B-CD)
TBPP-35	/	100		3/5(B-CD/F68)
T-CIC1	/	100	5 (CIC1)	
T-M1	/	100		4.42/0.58(B-CD/L81)

* the formulation for the physical mixtures was based on the theoretical molecular composition of an inclusion complex: 2 propylene glycol unit / 1β-CD.

Polymer blending by melting was performed as described in section 4.3.3. Samples with designed formulation were produced as shown in Table 4.8.

4.4.5 Protein adsorption

Protein adsorption was carried out according to the same procedure described in section 4.2.2.

4.4.6 Surface characterisation using ATR-FTIR

ATR-FTIR was employed for surface characterisation of CIC modified PVC-P materials. The procedure was the same as described in section 4.2.4.

4.5 Statistical analysis

Statistical analysis was performed with the Minitab package (version 8.0). Comparisons of different groups were carried out by analysis of variance. All statistical significant differences are reported at 95% confidence intervals ($p<0.05$).

CHAPTER 5

PLASTICISED POLY(VINYL CHLORIDE): INFLUENCE ON PROTEIN ADSORPTION OF PLASTICISER SELECTION AND PLASTICISER SURFACE LEVEL

5.1 Introduction

The high level of plasticiser in plasticised poly (vinyl chloride) (PVC-P) ensures that plasticiser selection has an important influence on the suitability of PVC to function in blood-contacting applications. In this chapter, three types of PVC-P in sheet form plasticised with di-(2-ethylhexyl)phathalate (PVC-DEHP or PD, D), tri-(2-ethylhexyl)trimellitate (PVC-TEHTM or PT, T) and n-butyryltri-n-hexyl citrate (PVC-BTHC or PB, B) were selected for study of the interaction with blood components such as proteins. DEHP, as the most commonly used plasticiser, has been found to leach into blood during blood-polymer contact. As an alternative, tri-(2-ethylhexyl)trimellitate (TEHTM) is less leachable than DEHP because of its relatively higher molecular weight (546 vs 390). It has been confirmed that plasticisers are accumulated at the PVC-P surface and the less extractable plasticiser, TEHTM, however, induced a more pronounced blood response because of its higher surface level than that of DEHP at the PVC-DEHP surface (Yin et al, 1999). This indicates that the blood response of PVC-P is influenced by plasticiser selection, plasticiser surface level and plasticiser surface distribution (Zhao & Courtney, 1999).

The objective of this chapter is to correlate the protein adsorption behaviour of PVC-P with plasticiser selection, plasticiser surface level, and the adsorption conditions. In addition, the adsorption mechanism is discussed with Freundlich and Langmuir adsorption modelling.

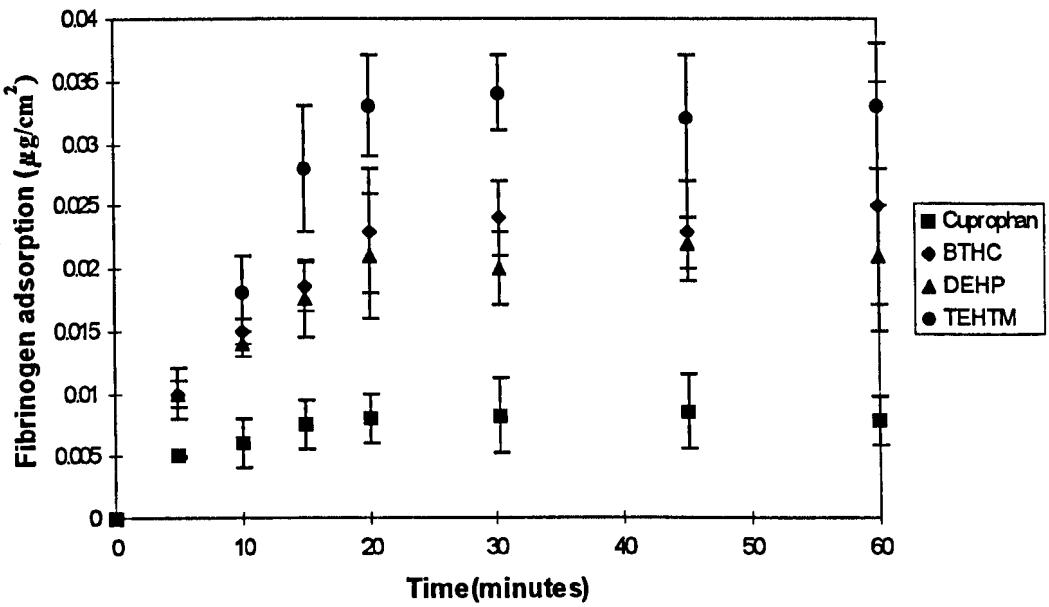


Fig 5.1 Time dependence of fibrinogen adsorption on PVC-P with Cuprophan as a control material

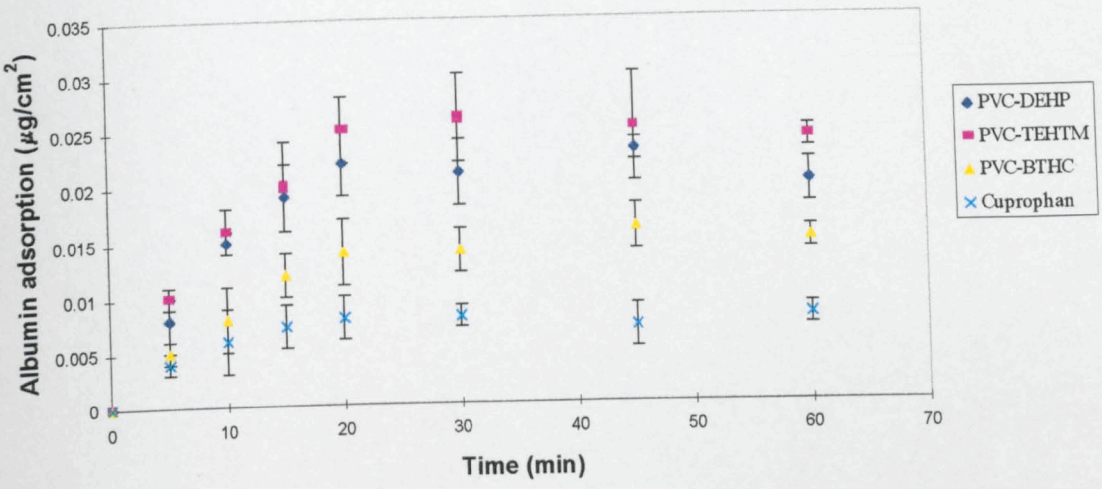


Fig 5.2 Time dependence of albumin adsorption on PVC-P with Cuprophan as a control material

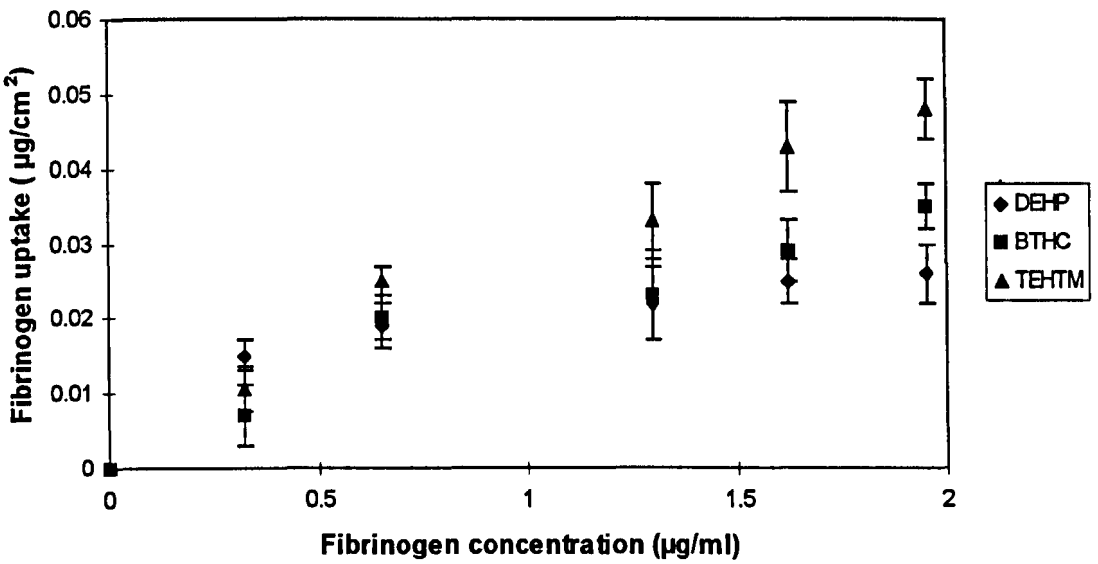


Fig 5.3 Influence of fibrinogen bulk concentration onto fibrinogen adsorption on PVC-P

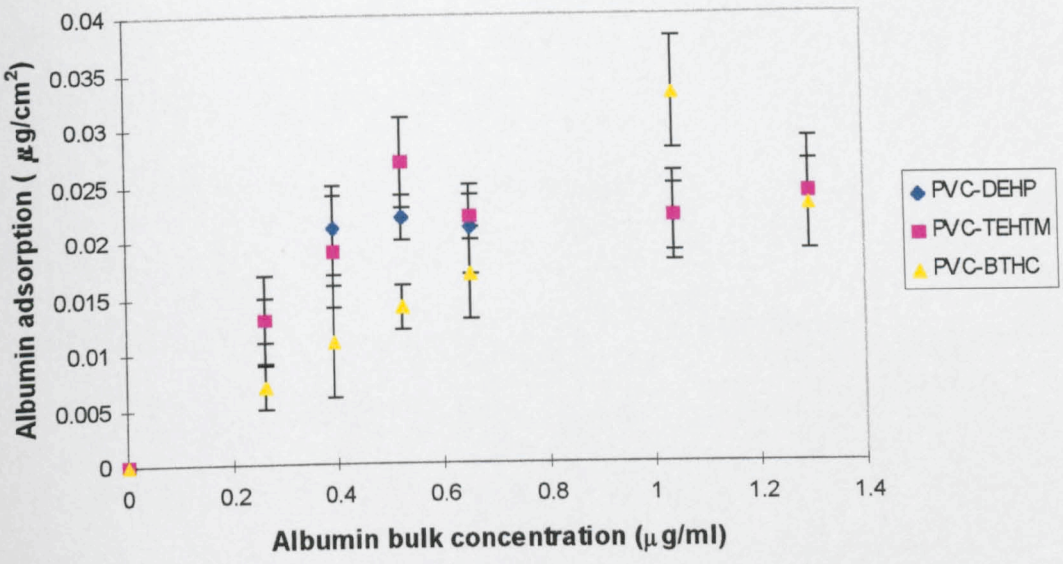


Fig 5.4 Influence of albumin bulk concentration onto albumin adsorption on PVC-P

5.2 Results

5.2.1 Influence on protein adsorption of plasticiser selection

5.2.1.1 Adsorption time dependence study

For obtaining the equilibrium adsorption time at which the adsorption reaches a steady level, the time dependence of fibrinogen and albumin adsorption on three types of PVC-P was investigated, as shown in Tables 5.1, 5.2, Fig 5.1 and 5.2. From these results, it can be found, in a very dilute protein bulk solution, the protein adsorbs at all three types of PVC-P surfaces in very fast adsorption kinetics. The levels of protein adsorption reach adsorption equilibrium after 20minute incubation and there is no significant difference between the interval at 20min and the later intervals. On this basis, the adsorption time as 20 minutes was selected for subsequent adsorption investigation.

5.2.1.2 Influence of protein bulk solution concentration onto protein adsorption

The adsorption isotherms of PVC-P in a range from 0.0 to 2.0 μg/ml for fibrinogen concentration, 0.0 to 1.3μg/ml for albumin are shown in Tables 5.3, 5.4, Fig 5.3 and 5.4. In this study, since the protein adsorbed by contacted material was not significantly changing the initial bulk concentration, the initial bulk concentration was used as the equilibrium concentration for data analysis empirically. From Fig 5.3, it can be seen that PVC-BTHC possesses the lowest adsorption capacity when the fibrinogen concentration is lower than 0.6 μg/ml. For DEHP plasticised PVC, it can reach the maximum adsorption capacity at 0.6 μg/ml of fibrinogen concentration, while the adsorption capacity for TEHTM plasticised PVC has a remarkable increase

with the increase in fibrinogen concentration. This indicates that fibrinogen concentration has a great influence onto the adsorption pattern of plasticised PVC with different plasticisers. For albumin adsorption, there is no significant difference between PVC-DEHP and PVC-TEHTM at any bulk concentration. However, there is a significant difference between PVC-BTHC and the PVC-DEHP/PVC-TEHTM, at a concentration below 0.5211μg/ml. In addition, it seems to have no significant difference for the albumin adsorption on PVC-DEHP and PVC-TEHTM after the bulk concentration is larger than 0.3912 μg/ml, while the albumin adsorption on PVC-BTHC increases significantly with the increase in bulk concentration.

5.2.1.3 Plasticiser selection

The fibrinogen adsorption on three types of PVC-P with and without PBS buffer solution soaking overnight is shown in Fig5.5, which illustrates the influence of plasticiser selection on fibrinogen adsorption. Results indicate that there is no significant difference between PVC-DEHP and PVC-BTHC but PVC-TEHTM shows the highest binding ability to fibrinogen. The hydrophilic Cuprophan served as a control for evaluating the effectiveness of the methodology, and also represented a protein-resistant material. For Cuprophan, the overnight soaking led to a reduction of fibrinogen adsorption, but for hydrophobic PVC-P, no significant difference was found between soaking and no soaking. This indicates that it is not necessary to soak PVC-P sheet overnight before protein adsorption.

Table 5.1 Fibrinogen adsorption isotherms of PVC-P and the adsorption capacity (ig/cm ^{2}) was expressed in Mean±SD, n=9

TIME (MIN)	0	5	10	15	20	30	45	60
PVC-DEHP (ig/cm ^{2})	0	0.010± 0.002	0.014± 0.001	0.018± 0.003	0.021± 0.005	0.020± 0.003	0.022± 0.002	0.021± 0.004
PVC-TEHTM (ig/cm ^{2})	0	0.009± 0.001	0.018± 0.003	0.028± 0.005	0.033± 0.004	0.034± 0.003	0.032± 0.005	0.033± 0.005
PVC-BTHC (ig/cm ^{2})	0	0.010± 0.001	0.015± 0.001	0.019± 0.002	0.023± 0.005	0.024± 0.003	0.023± 0.004	0.025± 0.001
Cuprophan (ig/cm ^{2})	0	0.005± 0.001	0.006± 0.002	0.0075± 0.002	0.008± 0.002	0.0082± 0.003	0.0085± 0.003	0.0078± 0.002

Table 5.2 Albumin adsorption isotherms of PVC-P and the adsorption capacity (ig/cm ^{2}) was expressed in Mean ± SD (n=9)

TIME (MIN)	0	5	10	15	20	30	45	60
PVC-DEHP (ig/cm ^{2})	0	0.008± 0.002	0.015± 0.001	0.019± 0.003	0.022± 0.006	0.021± 0.003	0.023± 0.001	0.020± 0.002
PVC-TEHTM (ig/cm ^{2})	0	0.010± 0.001	0.016± 0.002	0.020± 0.004	0.025± 0.003	0.026± 0.004	0.025± 0.005	0.024± 0.001
PVC-BTHC (ig/cm ^{2})	0	0.005± 0.001	0.008± 0.003	0.012± 0.002	0.014± 0.003	0.014± 0.002	0.016± 0.002	0.015± 0.001
Cuprophan (ig/cm ^{2})	0	0.004± 0.001	0.006± 0.003	0.0072± 0.002	0.0078± 0.002	0.0076± 0.001	0.008± 0.002	0.0079± 0.001

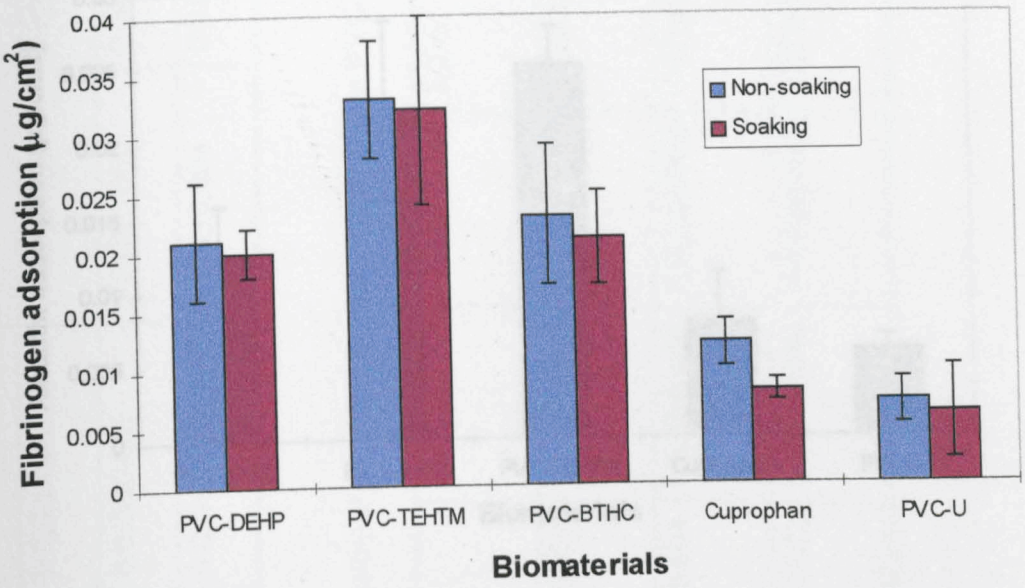


Fig 5.5 Influence on fibrinogen adsorption of PVC-P with respect to plasticiser selection. Adsorption conditions: Fibrinogen bulk concentration: 1.3 μg/ml; 25°C incubated for 20minutes.

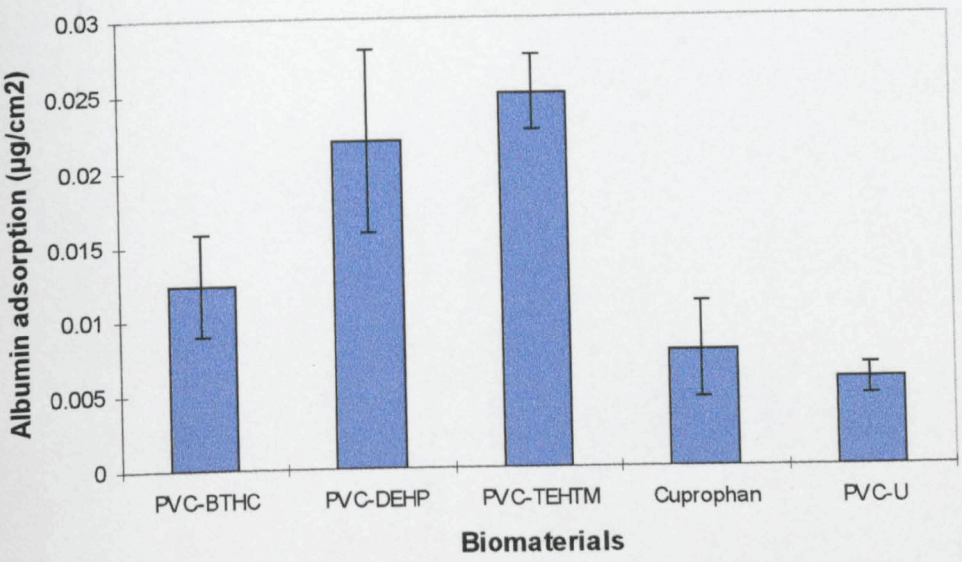


Fig 5.6 Influence on albumin adsorption of PVC-P with respect to plasticiser selection. Adsorption conditions: albumin bulk concentration: 0.5211 µg/ml; 25°C incubated for 20minutes.

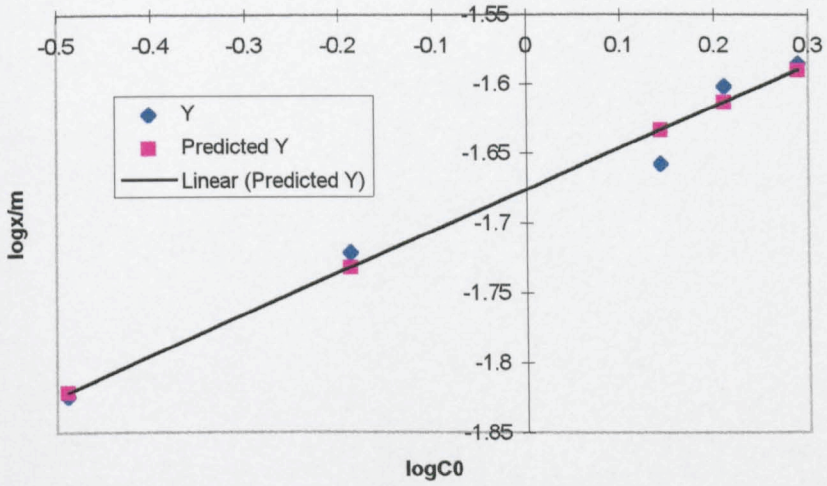
Table 5.3 Influence of fibrinogen bulk concentration on the amount of fibrinogen adsorption (ig/cm ^{2}) on PVC-P

BULK CONC. (µG/ML)	0	0.325	0.650	1.302	1.625	1.950
PVC-DEHP (ig/cm ^{2})	0	0.015±0.002	0.019±0.003	0.022±0.005	0.025±0.003	0.026±0.004
PVC-TEHTM (ig/cm ^{2})	0	0.011±0.003	0.025±0.002	0.033±0.005	0.043±0.006	0.048±0.004
PVC-BTHC (ig/cm ^{2})	0	0.007±0.004	0.020±0.003	0.023±0.006	0.029±0.004	0.035±0.003

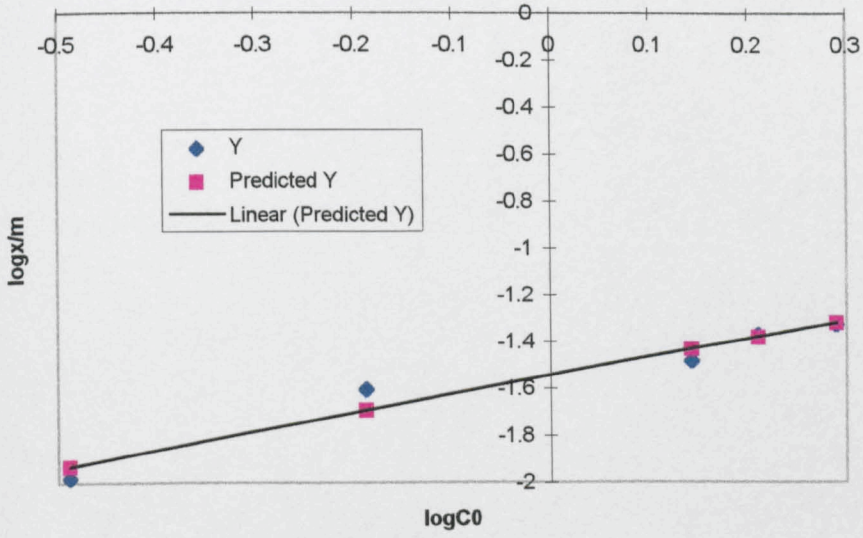
Table 5.4 Influence of albumin bulk concentration on the amount of albumin adsorption (ig/cm ^{2}) on PVC-P

BULK CONC. (µG/ML)	0	0.2616	0.3912	0.5211	0.6507	1.038	1.295
PVC-DEHP (ig/cm ^{2})	0	0.013±0.002	0.021±0.004	0.022±0.002	0.021±0.004	0.022±0.003	0.024±0.005
PVC-TEHTM (ig/cm ^{2})	0	0.013±0.004	0.019±0.005	0.027±0.004	0.022±0.002	0.022±0.004	0.024±0.005
PVC-BTHC (ig/cm ^{2})	0	0.007±0.002	0.011±0.005	0.014±0.002	0.017±0.004	0.033±0.005	0.023±0.004

A: PVC-DEHP



B: PVC-TEHTM



C: PVC-BTHC

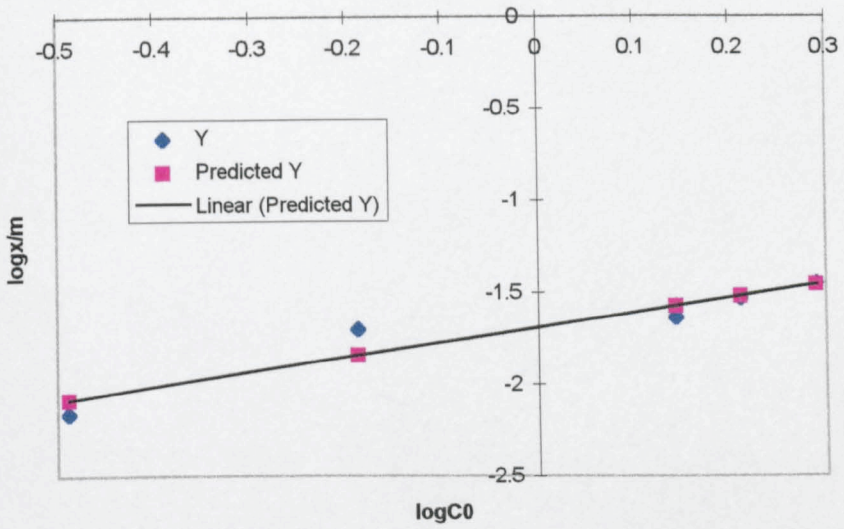


Fig 5.7 Freundlich modelling fibrinogen adsorption on PVC-P

a: PVC-DEHP; b: PVC-TEHTM ; c: PVC-BTHC

Surprisingly, the protein adsorption on PVC-U as a control shows a remarkable low adsorption capacity. There is a significant difference between PVC-U and PVC-P for both fibrinogen adsorption and albumin adsorption (Fig 5.6).

5.2.2 Investigation of adsorption mechanism

5.2.2.1 Fibrinogen adsorption

Freundlich adsorption modelling

According to the Freundlich adsorption isotherm (Equation 5.1 and 5.2), the modelling is represented by: $X/M = KC^{1/n}$ ... (5.1)

where x/m is the fibrinogen amount up-taken ($\mu g/cm^2$); K is the adsorption constant and n is the adsorption index; C is the corresponding fibrinogen concentration. The amount of protein uptaken by contacted material is very small compared to initial bulk concentration. The maximum difference between C and the initial bulk protein concentration (Cib) is in the range of 6%. Therefore, in this study, Cib was used for modelling. From Equation 5.1, the Freundlich adsorption isotherm can be derived as Equation 5.2: $\log X/M = \log K + 1/n \log C$ ... (5.2)

Plotting log X/M against log C, the Freundlich adsorption isotherms for PVC-P are shown in Figs 5.7 a,b,c and the adsorption parameters are presented in Table 5.5a,b.

Table5.5a Freundlich adsorption parameters of PVC-P for fibrinogen

NO	ADSORPTION PARAMETERS	
	$K_{25}°C$	n
PVC-DEHP	0.0257	4.47
PVC-TEHTM	0.0284	1.22
PVC-BTHC	0.0194	1.15

Table 5.5 b Regression data analysis results of Freundlich modelling fibrinogen adsorption on PVC-P

SUMMARY OUTPUT PVC-DEHP		PVC-BTHC		PVC-TEHTM	
Multiple R	0.988274	Multiple R	0.948835	Multiple R	0.948835
R Square	0.976686	R Square	0.900289	R Square	0.900289
Standard Error	0.017182	Standard Error	0.09929	Standard Error	0.09929
Observations	5	Observations	5	Observations	5

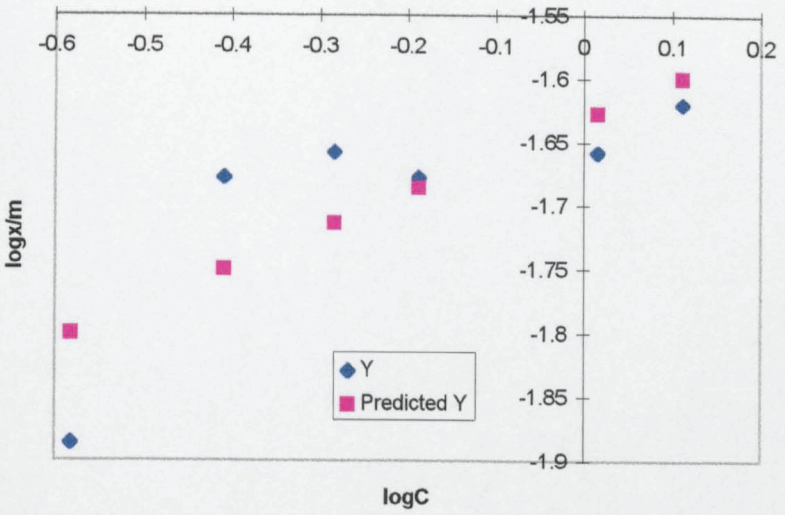
Langmuir modelling

Equation 5.3 gives the Langmuir modelling formulae:

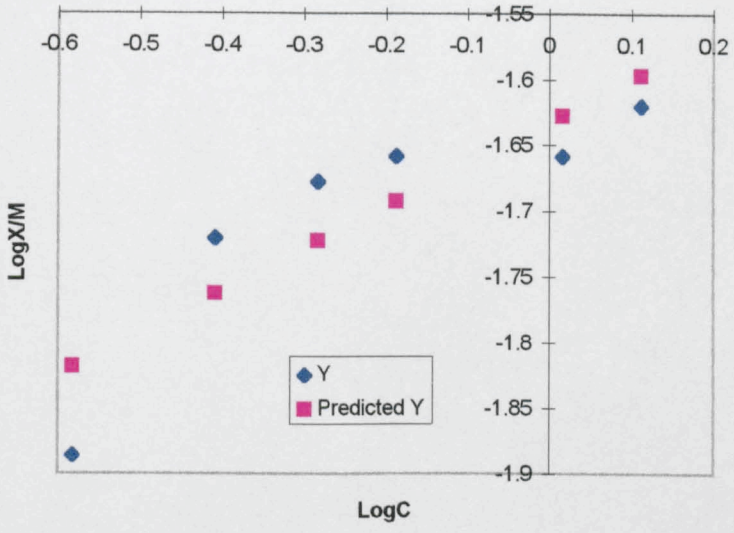
$$Cb/Cs = 1/KCm + Cb/Cm \quad \ldots \quad (5.3)$$

where Cs is the weight of protein adsorbed per unit area of surface, equivalent to x/m as shown in equation 5.1 and 5.2; K is an equilibrium adsorption constant, Cb is the bulk protein concentration in solution, equivalent to C shown in equation 1 and 2; Cm is the monolayer concentration. Plotting Cb/Cs with Cb, the Langmuir modelling isotherms for PVC-P are obtained, as shown in Fig.5.8 a,b,c and the regression data are shown in Table 5.6.

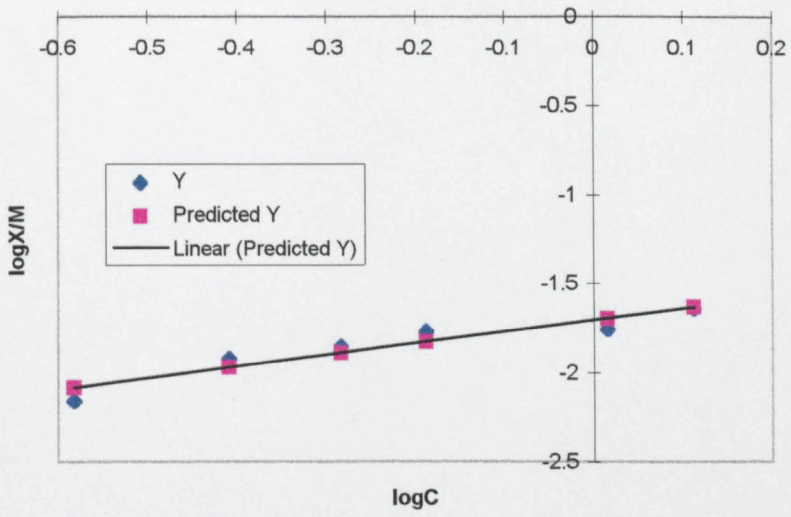
Results indicate that the three types of PVC-P fit Freundlich model while only PVC-DEHP fits the Langmuir model with regression square at 0.9896.



Fa: PVC-DEHP



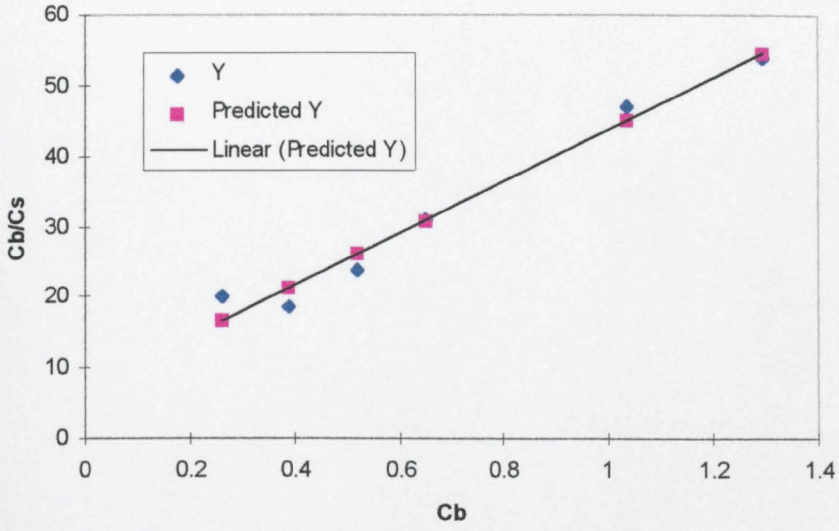
Fb: PVC-TEHTM



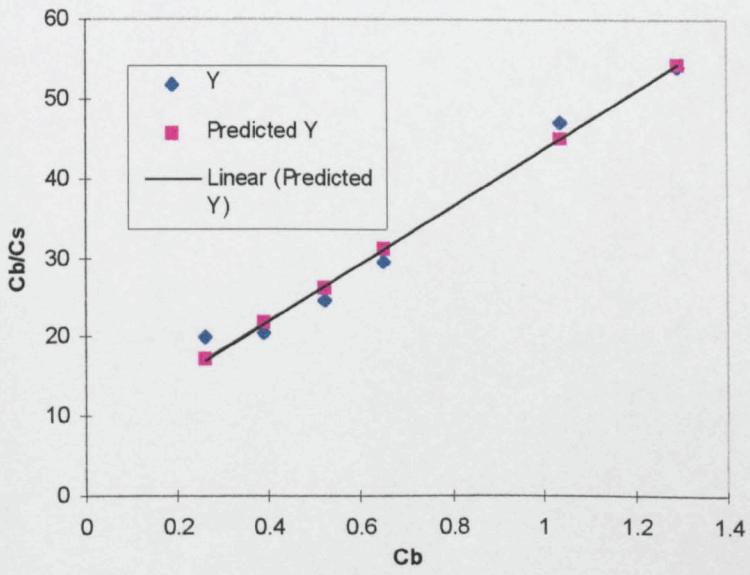
Fc: PVC-BTHC

Fig 5.9 Freundlich modelling of albumin adsorption on PVC-P

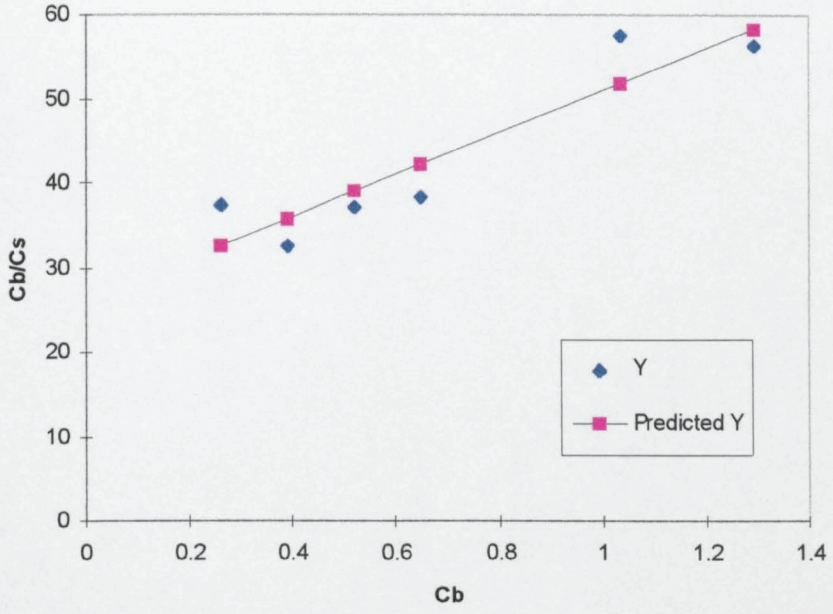
a: PVC-DEHP; b: PVC-TEHTM; c: PVC-BTHC



La: PVC-DEHP



Lb: PVC-TEHTM



Lc: PVC-BTHC

Fig 5.10 Langmuir plot of albumin adsorption on PVC-P

a: PVC-DEHP ; b: PVC-TEHTM ; c: PVC-BTHC

Table 5.6 Regression data analysis of Langmuir modelling fibrinogen adsorption on PVC-P

PVC-DEHP		PVC-BTHC		PVC-TEHTM	
Multiple R	0.994764	Multiple R	0.722281	Multiple R	0.851042
R Square	0.989556	R Square	0.52169	R Square	0.724272
Adjusted R Square	0.986075	Adjusted R Square	0.362253	Adjusted R Square	0.632363
Standard Error	2.625148	Standard Error	8.269282	Standard Error	3.788281
Observations	5	Observations	5	Observations	5

5.2.2.2 Albumin adsorption

As for fibrinogen, Freundlich and Langmuir isotherms were employed for modelling albumin adsorption on PVC-P. Results are shown in Fig 5.9a, b, c and Fig 5.10 a,b,c. Table 5.7 and 5.8 give the results of regression data analysis.

Table 5.7 Regression data results for Langmuir modelling of albumin adsorption on PVC-P

PVC-DEHP		PVC-TEHTM		PVC-BTHC	
Multiple R	0.986427	Multiple R	0.990353	Multiple R	0.920142
R Square	0.973039	R Square	0.980799	R Square	0.846662
Adjusted R Square	0.966298	Adjusted R Square	0.975998	Adjusted R Square	0.808327
Standard Error	2.721815	Standard Error	2.235564	Standard Error	4.747121
Observations	6	Observations	6	Observations	6

Table 5.8 Regression data analysis of Freundlich modelling of albumin adsorption on PVC-P

PVC-DEHP		PVC-TEHTM		PVC-BTHC	
Multiple R	0.790008	Multiple R	0.870477	Multiple R	0.948761
R Square	0.624113	R Square	0.757731	R Square	0.900147
Adjusted R Square	0.530141	Adjusted R Square	0.697164	Adjusted R Square	0.875183
Standard Error	0.065325	Standard Error	0.052424	Standard Error	0.062854
Observations	6	Observations	6	Observations	6

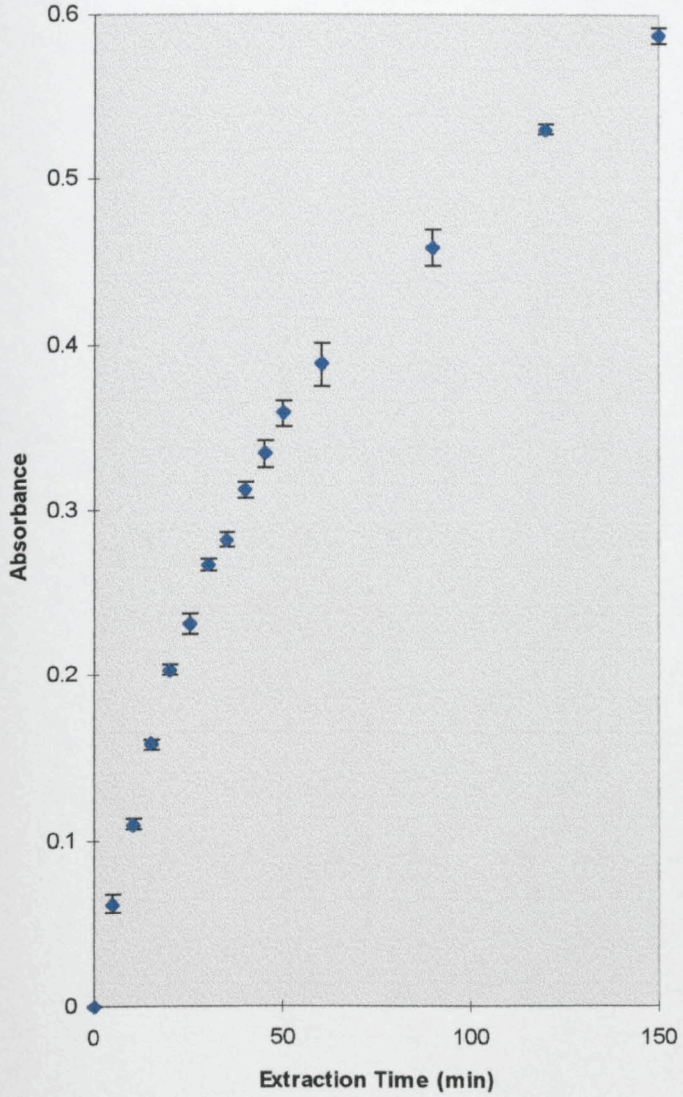


Fig 5.11 Migration test of PVC-DEHP using methanol as an extractant

Test condition: 2.5x 1.0 cm in 10 ml methanol. Diluted 2.5 times to measure UV absorbance at 274nm at varied extraction time.

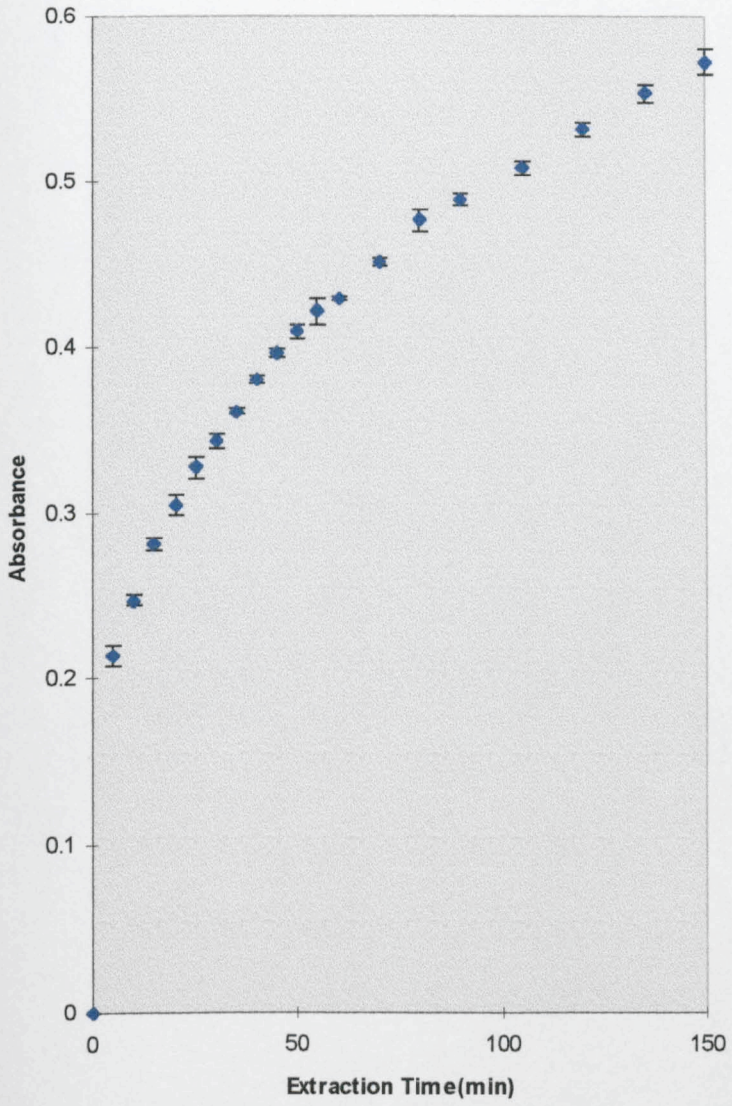


Fig 5.12 Migration test of PVC-TEHTM using methanol as an extractant

Test condition: 1.0 x 2.5cm in 10ml methanol. Diluted 3.0 times to measure UV absorbance at 290 nm.

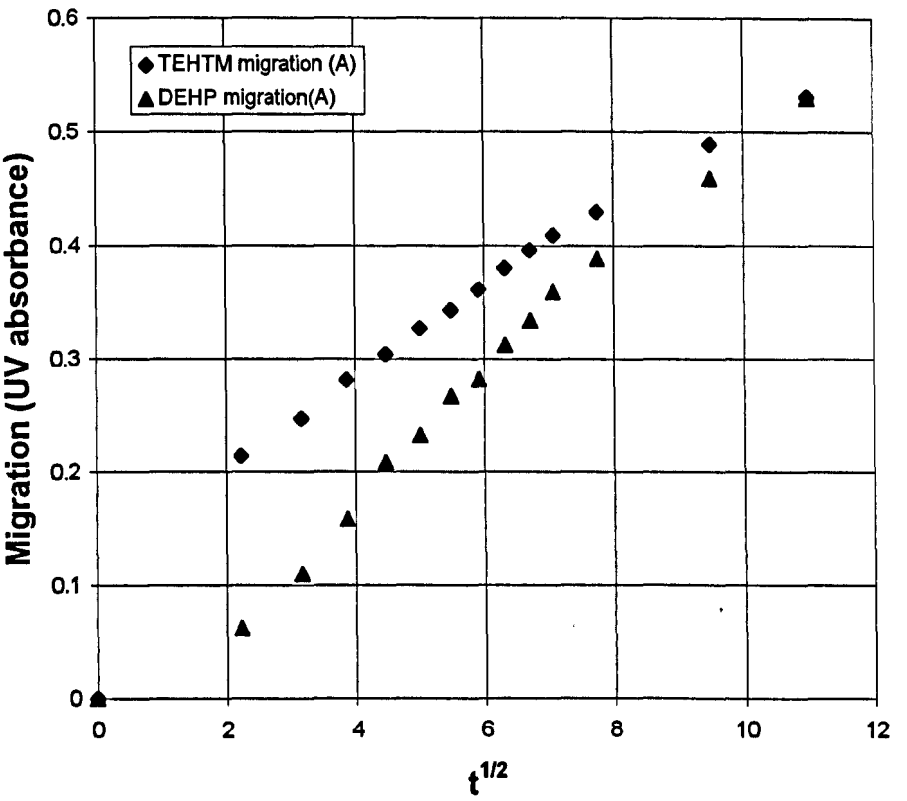


Fig 5.12 a Fickian diffusion behaviour of plasticiser

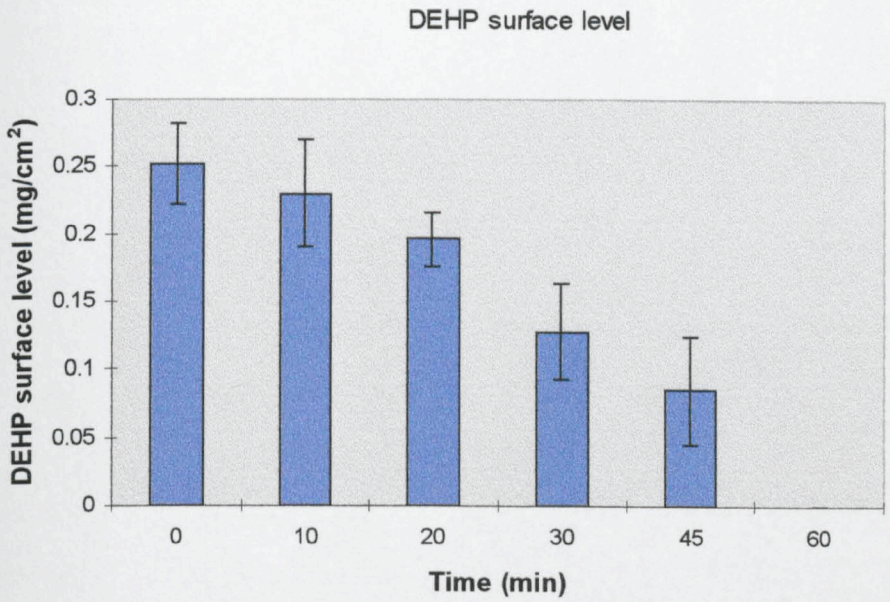


Fig 5.13 Dependence of DEHP surface level on surface methanol extraction time

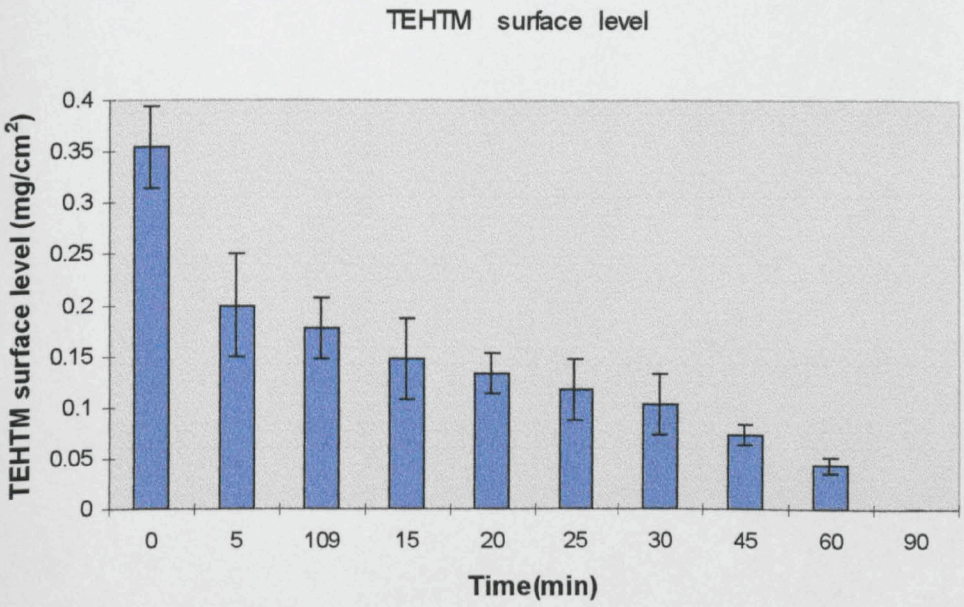


Fig 5.14 Dependence of TEHTM surface level on methanol extraction time

Results indicate that albumin adsorption on three types of PVC-P fits Langmuir model very well, while only PVC-BTHC fits Freundlich model.

5.2.3 Influence on protein adsorption of plasticiser surface level

5.2.3.1 Migration behaviour of PVC-DEHP and PVC-TEHTM in methanol and correlation of plasticiser surface level with methanol surface treatment time

Fig.5.11 and 5.12, Tables 5.9 and 5.10 demonstrate the DEHP and TEHTM migration behaviour from plasticised PVC in methanol, as which detected by UV-spectrophotometer at designated intervals. Results indicate that a surface dissolution process is reflected in the figures as an asymptotically increased curve at the initial stage, which removes the surface plasticiser. This process is followed by the bulk plasticiser diffusion through the surface. This migration follows Fickian Law of diffusion. The plots of plasticiser migration data against $t^{1/2}$ was shown in Fig 5.12 a , which are linear. The slope is greater for the DEHP, indicating that its diffusion coefficient is greater than that of TEHTM. This is due to the respective sizes of the molecules.

From Fig 5.11 and 5.12, the overlapping point crossed at the two processes is regarded as the end of surface removal of plasticisers by dissolution. At this point, the surface plasticiser level is regarded as zero and the extracted amount of plasticiser at this time represents the original surface plasticiser level. Based on this method, the surface plasticiser level at varied extraction times can be evaluated according to Equation 4.1, as shown in Chapter 4. The relevance of surface plasticiser level with methanol extraction time can be obtained as shown in Fig 5.13, 5.14 and Table 5.11 and the time for cleaning TEHTM is about 90minutes and 50 minutes for DEHP. The ATR-FTIR surface characterisation results in 5.2.4 also indicate a similar extraction time needed for cleaning the surface.

Table 5. 11 Dependence of plasticiser surface level on surface methanol extraction time (n=3)

TIME (MIN)	0	10	20	30	45	60
DEHP surface level(mg/cm^2) (Mean)	0.252	0.23	0.196	0.128	0.085	0
SD	0.03	0.04	0.02	0.035	0.04	0

TIME(MIN)	0	5	10	15	20	25	30	45	60	90
TEHTM surface level (mg/cm^2) (Mean)	0.355	0.2	0.177	0.148	0.133	0.118	0.104	0.074	0.044	0
SD	0.04	0.05	0.03	0.04	0.02	0.03	0.03	0.01	0.008	0

5.2.3.2 Relationship of protein adsorption with methanol surface treatment time

Table 5.12, 5.13 and Fig 5.15, 5.16 give the correlation of fibrinogen and albumin adsorption with surface methanol treatment time.

Table 5.12 Dependence of fibrinogen adsorption ((ì g/cm^2) on methanol surface extraction time (n=9)

TIME(MIN)		0	5	10	20	30	45	60	90	360
PVC-DEHP	mean	0.023	0.021	0.02	0.018	0.018	0.015	0.012	0.021	0.022
(ig/cm ^{2})	SD	0.006	0.005	0.004	0.003	0.004	0.003	0.005	0.004	0.004
PVC-TEHTM	Mean	0.032	0.03	0.025	0.018	0.016	0.014	0.032	0.04	0.045
(ig/cm ^{2})	SD	0.005	0.004	0.002	0.002	0.003	0.003	0.002	0.005	0.003
PVC-BTHC	Mean	0.024	0.026	0.02	0.018	0.016	0.015	0.013	0.018	0.021
(ig/cm ^{2})	SD	0.001	0.002	0.003	0.001	0.004	0.002	0.002	0.003	0.003

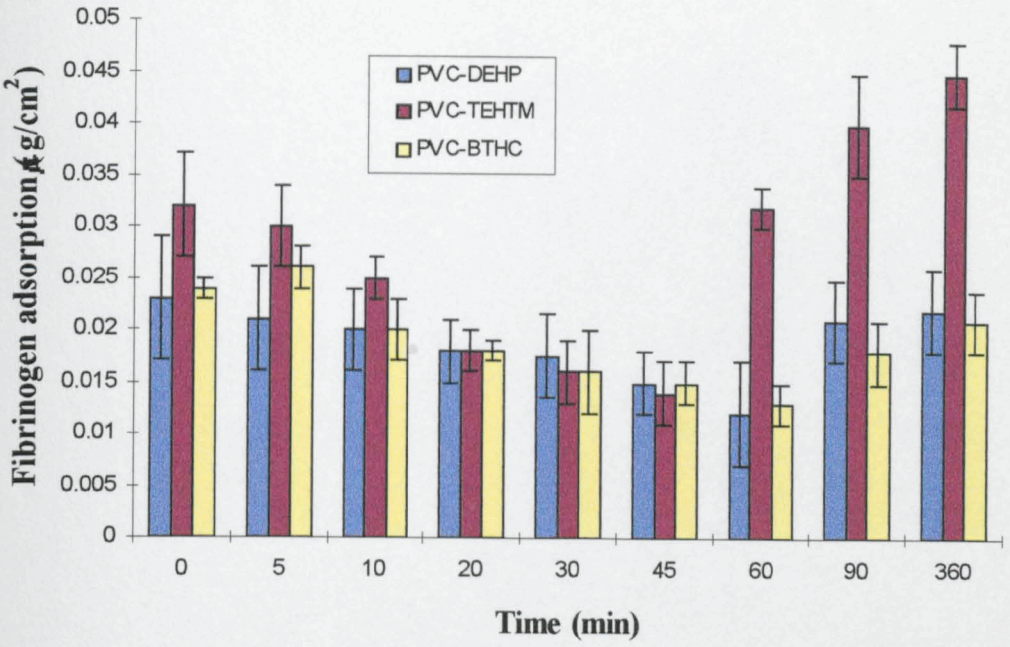


Fig 5.15 Relationship of fibrinogen adsorption on PVC-P with surface methanol extraction time

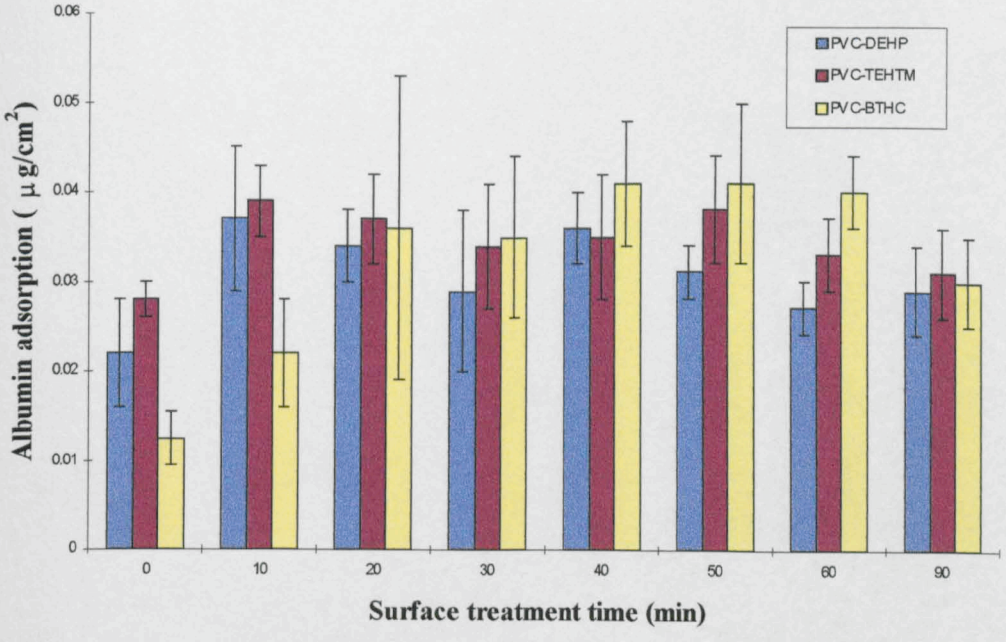


Fig 5.16 Relationship of albumin adsorption on PVC-P with surface methanol treatment time

Table 5. 9 Migration test of PVC-TEHTM (UV absorbance) using methanol as an extractant

TIME (MIN	0	5	10	15	20	25	30	35	40	45	50	55	60	70	80	90	105	120	135	150
A 1	0	0.208	0.25	0.278	0.301	0.321	0.342	0.363	0.38	0.395	0.408	0.418	0.429	0.449	0.484	0.494	0.513	0.535	0.56	0.58
2	0	0.215	0.247	0.279	0.3	0.324	0.34	0.36	0.379	0.394	0.406	0.415	0.428	0.453	0.486	0.486	0.506	0.528	0.548	0.565
3	0	0.219	0.245	0.285	0.31	0.335	0.348	0.36	0.382	0.398	0.413	0.43	0.429	0.45	0.470	0.487	0.505	0.53	0.55	0.571
Mean	0	0.214	0.247	0.281	0.304	0.327	0.343	0.361	0.380	0.396	0.409	0.421	0.429	0.451	0.480	0.489	0.508	0.531	0.557	0.572
SD		0.006	0.003	0.004	0.006	0.007	0.004	0.002	0.002	0.002	0.004	0.008	0.001	0.002	0.004	0.004	0.005	0.004	0.006	0.008

A: UV-absorbance value

Table 5.10 Migration test of PVC-DEHP (UV absorbance)using methanol as an extractant

TIME (MIN)	0	5	10	15	20	25	30	35	40	45	50	60	90	120	150
A 1	0	0.068	0.113	0.156	0.205	0.239	0.268	0.287	0.317	0.343	0.368	0.384	0.453	0.532	0.583
2	0	0.056	0.108	0.158	0.21	0.227	0.263	0.28	0.311	0.328	0.354	0.377	0.453	0.527	0.593
3	0	0.062	0.109	0.161	0.21	0.229	0.271	0.279	0.308	0.33	0.354	0.402	0.472	0.532	0.589
Mean	0	0.062	0.11	0.158	0.208	0.232	0.267	0.282	0.312	0.334	0.359	0.388	0.459	0.530	0.588
SD		0.006	0.003	0.003	0.004	0.006	0.004	0.004	0.005	0.008	0.008	0.012	0.011	0.003	0.005

A: UV-absorbance value

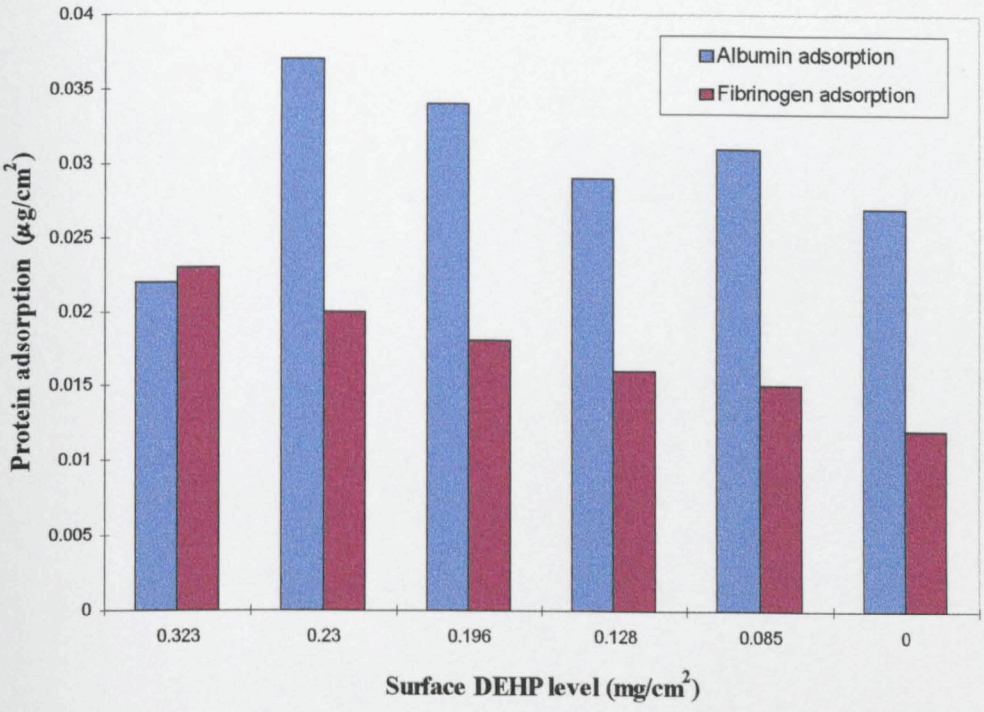


Fig 5.17 Relationship of protein adsorption with DEHP surface level

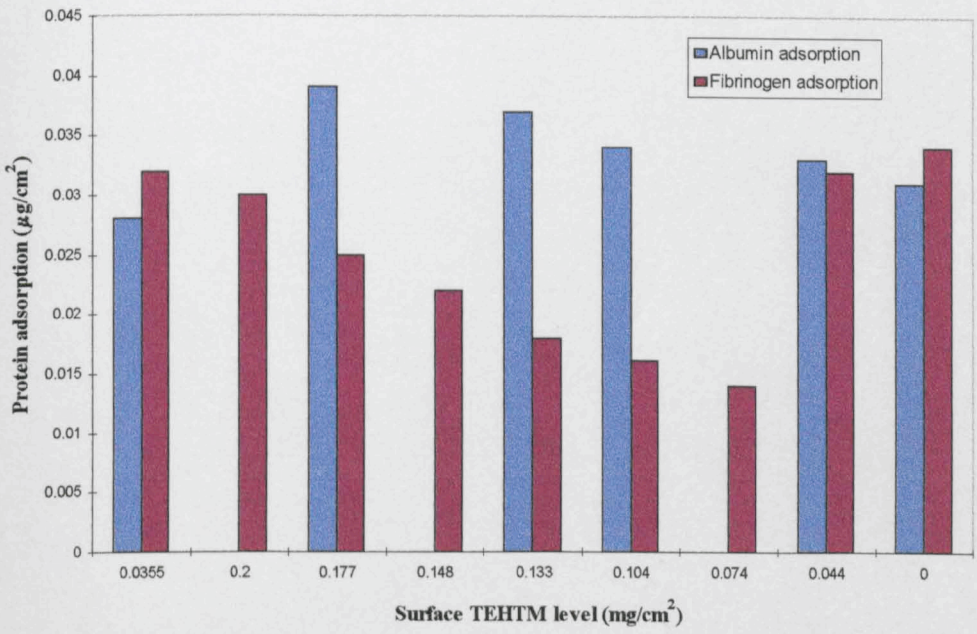


Fig 5.18 Relationship of protein adsorption with TEHTM surface level

Table 5.13 Relationship of albumin adsorption on PVC-P with methanol surface treatment time (n=9)

TIME(MIN)		0	10	20	30	40	50	60	90
PVC-DEHP	Mean	0.022	0.037	0.034	0.029	0.036	0.031	0.027	0.029
(ig/cm ^{2})	SD	0.006	0.008	0.004	0.009	0.004	0.003	0.003	0.005
PVC-TEHTM	Mean	0.028	0.039	0.037	0.034	0.035	0.038	0.033	0.031
(ig/cm ^{2})	SD	0.002	0.004	0.005	0.007	0.007	0.006	0.004	0.005
PVC-BTHC	Mean	0.0124	0.022	0.036	0.035	0.041	0.041	0.04	0.03
(ig/cm ^{2})	SD	0.003	0.006	0.017	0.009	0.007	0.009	0.004	0.005

5.2.3.3 Relationship of protein adsorption with plasticiser surface level

From the results of relevance protein adsorption with surface treatment time and the relevance of plasticiser surface level with surface treatment time, the relevance of protein adsorption with plasticiser surface level could be derived as shown in Table 5.14.

Table 5.14 Relationship of plasticiser surface level with protein adsorption

DEHP SURFACE LEVEL (mg/cm^2)	0.323	0.23	0.196	0.128	0.085	0
Albumin adsorption (μg/cm^2)	0.022	0.037	0.034	0.029	0.031	0.027
Fibrinogen adsorption (μg/cm^2)	0.023	0.02	0.018	0.016	0.015	0.012

TEHTM SURFACE LEVEL(mg/cm^2)	0.355	0.2	0.177	0.148	0.133	0.104	0.074	0.044	0
Albumin adsorption (μg/cm^2)	0.028		0.039		0.037	0.034		0.033	0.031
Fibrinogen adsorption (μg/cm^2)	0.032	0.03	0.025	0.022	0.018	0.016	0.014	0.032	0.034

Fig 5.17 and 5.18 show the influence of DEHP and TEHTM surface level on the fibrinogen and albumin adsorption on their plasticised PVC respectively. Results indicate that with the increase of surface plasticiser level, the fibrinogen adsorption

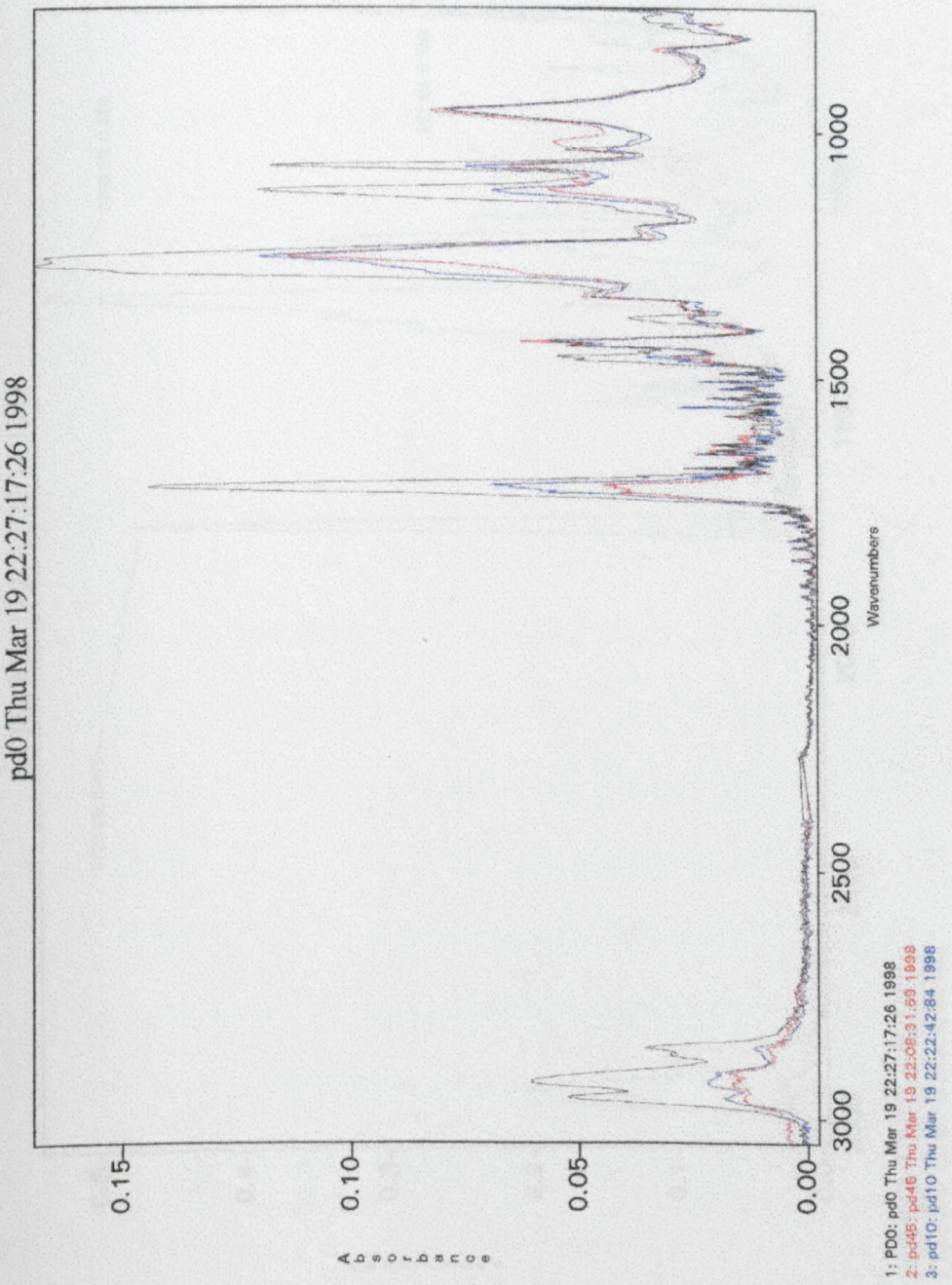


Fig 5.19 ATR-FTIR surface characterisation of PVC-DEHP with increased methanol surface treatment time (0,10 and 45minutes)

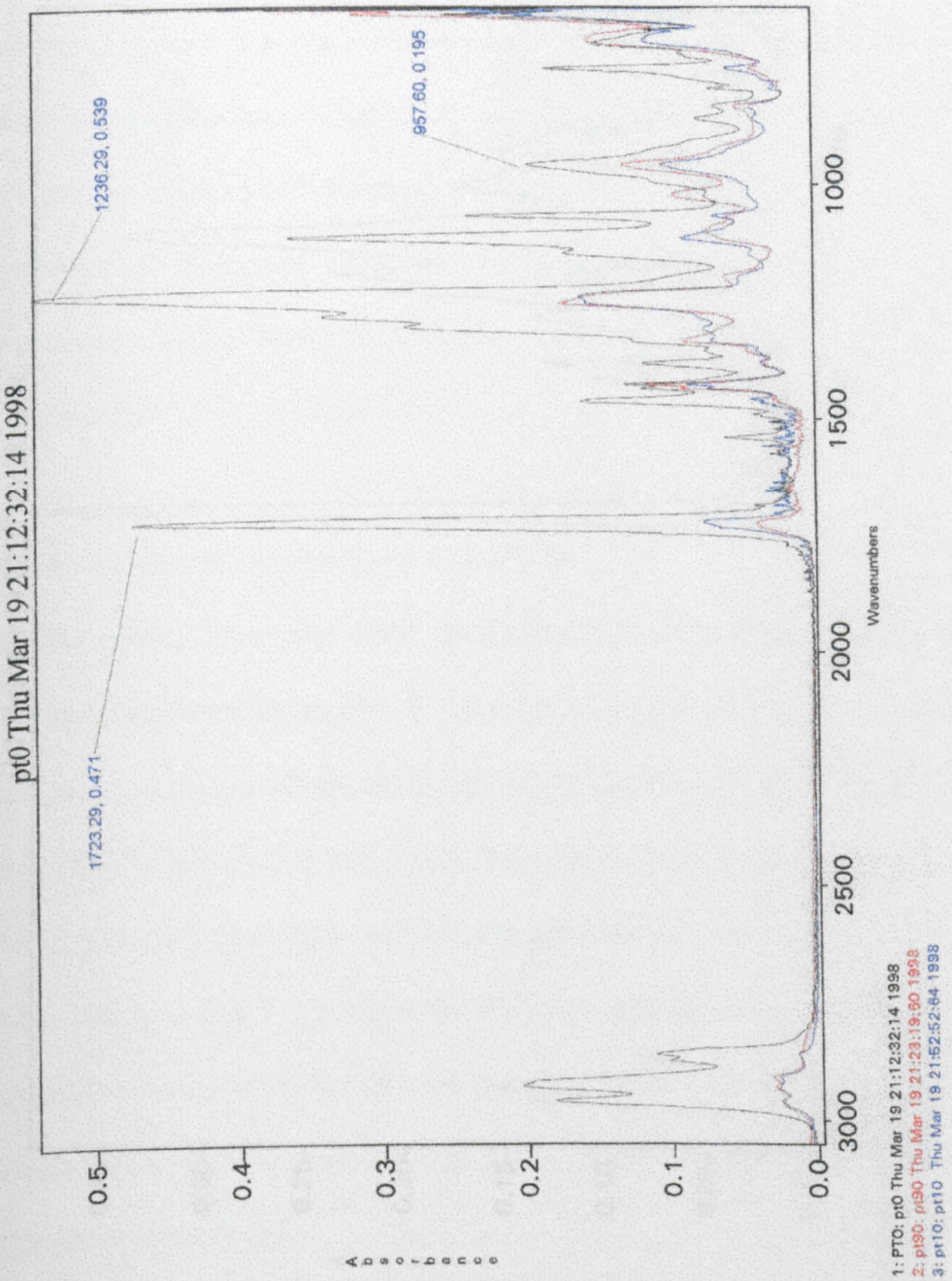


Fig 5.20 ATR-FTIR surface characterisation of PVC-TEHTM with increased methanol surface treatment time (0,10 and 90 minutes)

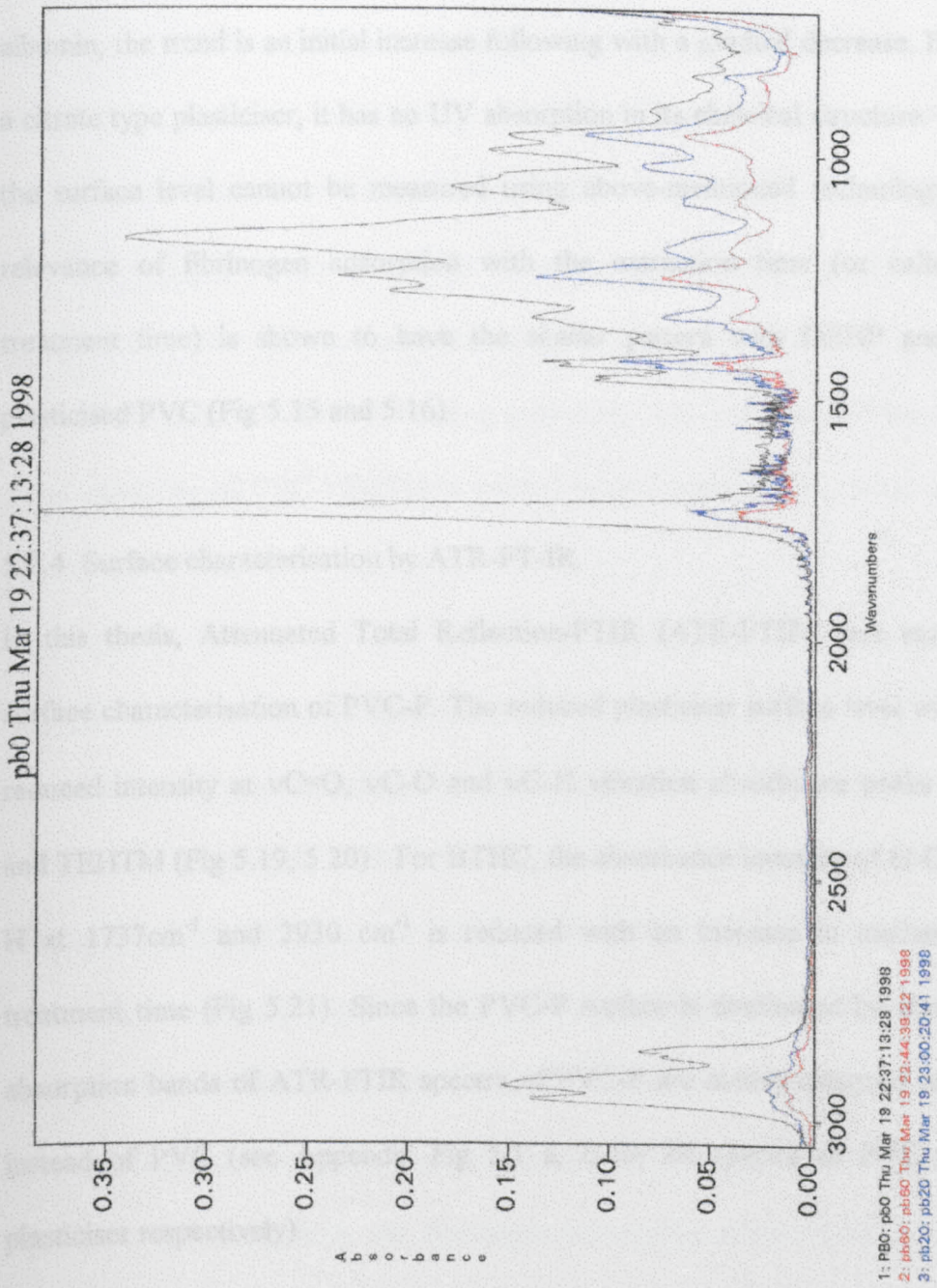


Fig 5.21 ATR-FTIR surface characterisation of PVC-BTHC with increased methanol surface treatment time (0, 20 and 60 minutes)

capacity increases almost linearly. For TEHTM, a long extraction time leads to an increase of adsorption, which might be due to the factor of surface roughness. For albumin, the trend is an initial increase following with a gradual decrease. For BTHC, a citrate type plasticiser, it has no UV absorption in its chemical structure. Therefore, the surface level cannot be measured using above-mentioned technology. But the relevance of fibrinogen adsorption with the extraction time (or called surface treatment time) is shown to have the similar pattern with DEHP and TEHTM plasticised PVC (Fig 5.15 and 5.16).

5.2.4 Surface characterisation by ATR-FT-IR

In this thesis, Attenuated Total Reflection-FTIR (ATR-FTIR) was employed for surface characterisation of PVC-P. The reduced plasticiser surface level will lead to a reduced intensity at νC=O, νC-O and νC-H vibration absorbance peaks for DEHP and TEHTM (Fig 5.19, 5.20). For BTHC, the absorbance intensity of O-C=O and C-H at 1737cm^{-1} and 2930 cm^{-1} is reduced with an increase in methanol surface treatment time (Fig 5.21). Since the PVC-P surface is dominated by plasticiser, the absorption bands of ATR-FTIR spectra of PVC-P are mainly assigned to plasticiser instead of PVC (see Appendix Fig 5.1 a, b for IR spectra of PVC and DEHP plasticiser respectively).

5.3 Discussion

5.3.1 The influence of plasticiser nature

In the selected plasticisers, DEHP and TEHTM have the same chemical nature, differing only in molecular weight. However, BTHC has a different chemical nature compared to DEHP and TEHTM. From the fibrinogen adsorption results, it is found that BTHC possesses the lowest adsorption capacity when the fibrinogen concentration is less than 0.6 μg/ml. For TEHTM and DEHP, TEHTM plasticised PVC has a higher reactivity to bind fibrinogen but it is hard to infer that this is contributed by their different molecular weight. Similar results have been observed in PVC-DEHP and PVC-TEHTM tubing previously and it is believed that the higher blood reactivity of PVC-TEHTM is related to the higher surface level of TEHTM (Yin et al, 1999a).

5.3.2 The influence of surface plasticiser level

For an enhanced understanding the relationship between fibrinogen adsorption and surface plasticiser level, surface cleaning treatment with methanol was utilised to achieve an alteration of surface plasticiser level which was determined using UV-spectrophotometer. It is very clear from the experimental results that PVC-P with a plasticiser-free surface, no matter which type of plasticiser it contained, has a similar lowest fibrinogen adsorption capacity (about 0.010-0.014 $\mu g/cm^2$) which has no significant difference from the fibrinogen adsorption value of PVC-U. With the increase of plasticiser level at the surface, fibrinogen adsorption capacity increases. For DEHP and TEHTM plasticised PVC, if the fibrinogen adsorption capacity is based on the surface level, there is no difference between them as shown in Table 5.15.

Table 5.15 Evaluation of influence of surface plasticiser level onto fibrinogen adsorption

*FU/SPL OF PVC-P(μg/mg)	EXTRACTION (MIN)		TIME	
samples	0	10	20	30
PVC-DEHP	0.092	0.089	0.094	0.117
PVC-TEHTM	0.093	0.133	0.105	0.120

* FU/SPL: Ratio of Fibrinogen uptake ($\mu g/cm^2$) to surface plasticiser level (mg/cm^2)

5.3.3 The migration behaviour of PVC-P

From Fig 5.11 and 5.12, it can be seen that TEHTM plasticised PVC needs a longer extraction time to reach the cleaning point than DEHP plasticised PVC. This indicates that TEHTM is less extractable than DEHP because of its higher molecular weight, and is in agreement with other extraction studies.

5.3.4 The adsorption mechanism

The adsorption modelling of fibrinogen adsorption on PVC-P reveals that the isotherms of adsorption on three types of PVC-P fit the Freundlich isotherm indicating a monolayer adsorption pattern. It also indicates that the PVC-P surface contains a set of sites with varying heats of adsorption, which is different from the Langmuir adsorption isotherm of PVC (Young et al, 1988 a). Furthermore, the Freundlich modelling emphasises the importance of plasticiser selection in determining the PVC-P fibrinogen adsorption pattern. The higher n value of PVC-DEHP indicates a decreased adsorptivity towards fibrinogen. The highest K value and lower n value

reveals PVC-TEHTM exhibits the highest fibrinogen adsorptivity. For PVC-BTHC, it has the lowest K value but with a similar n value to that of PVC-TEHTM, indicating that PVC-BTHC has a very similar surface hydrophobicity as PVC-TEHTM, which can be seen from the fact that the BTHC percentage presenting at the surface is the highest. In addition, that PVC-P fit Freundlich isotherm obtained from this study is in agreement with the report that Freundlich modelling can be employed for the situation, in which protein adsorption remains under diffusion control throughout the time of adsorption with a very dilute solution and relatively short sorption times (Young et al, 1988 b).

For albumin adsorption, PVC-DEHP and PVC-TEHTM fit the Langmuir isotherm but PVC-BTHC fits the Freundlich isotherm. In conjunction with the above-mentioned fibrinogen adsorption modelling, PVC-BTHC is proved again to be of a different plasticiser nature as reflected from adsorption modelling. In comparison with the monolayer concentration adsorbed at the PVC-P surface (Cm), i.e., 0.14~0.21 $\mu g/cm^2$ for albumin adsorption on PVC-DEHP and 0.44~0.60 $\mu g/cm^2$ for fibrinogen adsorption (Young et al, 1988 a), the protein adsorption value for PVC-P is far less than Cm, which indicates the adsorption is mono-layer adsorption.

5.3.5 ATR-FTIR surface characterisation of PVC-P

Tables 5.16 and 5.17 gives the assignment of absorption bands to specific chemical groups of PVC and plasticisers(Tabb & Koenig, 1975).

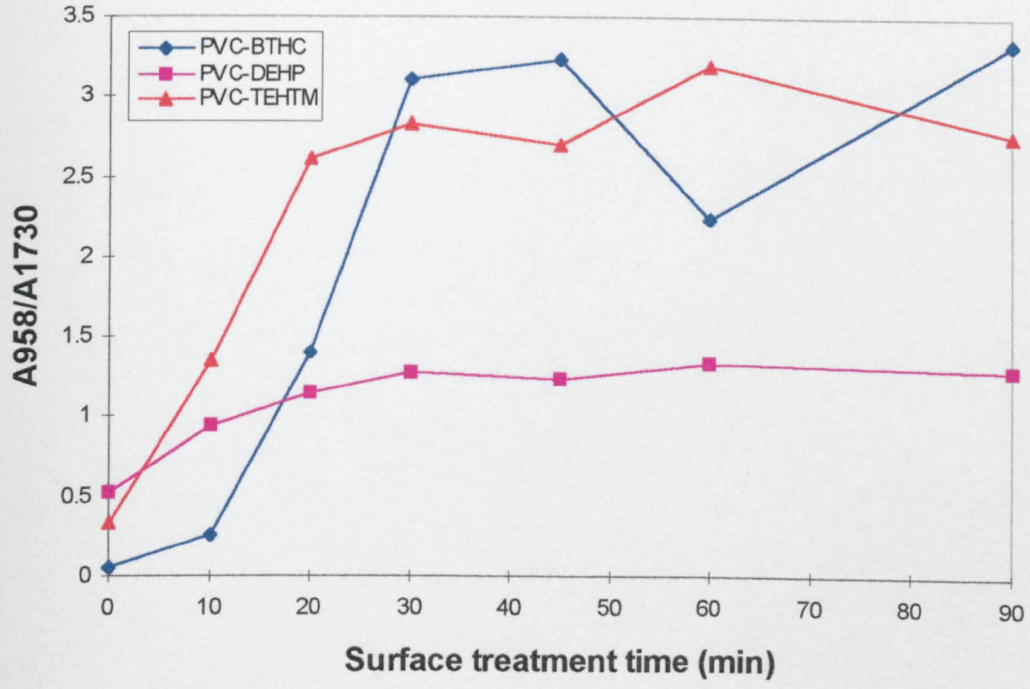


Fig 5.22 Correlation of A958cm^{-1}/ A 1726cm^{-1} with methanol surface treatment time

Table 5.16 Correlation of absorption band with specific chemical groups of PVC

C-H stretching vibration	
(-CH_2-, -CH-)	2900-2940 cm^{-1}
C-H deformation vibration	
-CH_2-	1425 cm^{-1}
-CH-	1330 cm^{-1}
C-Cl stretching vibration	1260 cm^{-1}
CH_2 skeletal vibration (γr CH_2)	958 cm^{-1}

Table 5.17 gives the assignment of IR absorption band to plasticisers.

Table 5.17 Correlation of characteristic adsorption band with chemical groups of Plasticisers (Tabb & Koenig, 1975)

C-H stretching vibration					
$-CH_3$	2975-2950 cm^{-1}	-CH-	2900-2880 cm^{-1}	CH_2-	2940-2845 cm^{-1}
C-H deformation					
$C-CH_3$		1385 cm^{-1}			
-CH_2-		1440 cm^{-1}			
-CH-		1330-1350 cm^{-1}			
C=O stretching vibration		1720 cm^{-1}			
C-O stretching vibration		1050-1100 cm^{-1}			

Obviously, from the spectra of ATR-FTIR, the decrease of intensity of absorption bands at 2900-2880 cm^{-1}, 1720 cm^{-1}, 1122 cm^{-1} and 1072 cm^{-1} indicate the reduced DEHP, TEHTM and BTHC level by methanol surface treatment. At the same time, the increase of intensity in 1425 cm^{-1} and 958 cm^{-1} indicates the increase of PVC concentration at the surface. The ratio of peak integrated area at 1726 cm^{-1} to that at 958 cm^{-1} ($A_{1726cm-1}/A_{958cm-1}$) can be calculated from the spectra, which indicates the

surface distribution of PVC and plasticiser. However, plasticised PVC is a polymer blending system which consisting of many ingredients, and for example, the heating stabilisers might also contribute a weak IR absorption at 1726 cm^{-1}. In this study, ATR-FTIR is mainly used to assess the surface chemical structure of PVC-P quantitatively, but the results of ($A_{1726cm-1}/A_{958cm-1}$) can be applied to evaluate the change of surface composition during the surface treatment period. From the results given in Table 5.18 and Fig 5.22, it is found that after surface treatment for 30-45 minutes, a clean surface was achieved for PVC-DEHP, since there was no intensity change at these two bands. For PVC-TEHTM, initially, there is a weak adsorption at 1425 cm^{-1}, indicating a higher TEHTM surface level and lower PVC level compared to the DEHP level in PVC-DEHP. After surface treatment for 60-90 minutes, the value of $A_{1726cm-1}/A_{958cm-1}$ tends to be steady, indicating a cleaned surface. For PVC-BTHC, the increase in intensity and the dramatically shaped band at 1260 cm^{-1} indicate the increased level of PVC at the surface due to surface treatment. It can be evaluated that it needs about 45 minutes to get a clean surface for PVC-BTHC. These results compared to those obtained from UV-spectrophotometry measurement show a very similar trend. However, it is very difficult to state accurately the degree of cleanness due to the difficulties of the definition of surface thickness by both technologies.

Table 5.18 Correlation of *A958cm^{-1}/A1730cm^{-1} with methanol treatment time

PVC-P	TIME (MIN)	A1730CM^{-1}	A958CM^{-1}	A958/A1730
PVC-BTHC	0	13.577	0.749	0.055
	10	3.953	1.03	0.26
	20	1.607	2.25	1.399
	30	0.4	1.246	3.108
	45	0.417	1.353	3.239
	60	0.622	1.397	2.244
	90	0.2	0.667	3.334
PVC-DEHP	0	4.18	2.19	0.524
	10	2.066	1.935	0.937
	20	1.456	1.664	1.142
	30	1.353	1.73	1.278
	45	1.404	1.689	1.23
	60	1.388	1.863	1.342
	90	1.393	1.808	1.297
PVC-TEHTM	0	14.209	4.672	0.328
	10	2.086	2.814	1.348
	20	1.393	3.644	2.614
	30	1.242	3.507	2.822
	45	1.232	3.327	2.699
	60	1.058	3.388	3.2
	90	1.068	2.949	2.76

* Areas of peaks at 958cm^{-1} and 1730 cm^{-1} were obtained by utilisation of computerised programme (integration operation)

5.4 Summary

This chapter reports the study of protein adsorption on three types of plasticised PVC in order to correlate this with plasticiser selection and surface properties. Results indicate that the plasticiser level at the PVC surface has a strong influence on both the fibrinogen and albumin adsorption. The migration behaviour of DEHP and TEHTM was assessed and UV-spectrophotometry was employed to evaluate the plasticiser level at the PVC surface, a straightforward approach for measurement of surface phthalate plasticiser level. In addition, ATR-FTIR was utilised for surface characterisation of PVC-P in order to obtain surface chemical information due to surface modification by methanol treatment. The Freundlich and Langmuir modelling of protein adsorption on PVC-P were utilised to reveal the influence of plasticiser nature and surface composition on protein adsorption. Obviously, BTHC has a different chemical nature from other two phthalates, which causes differences both in adsorption mechanism and adsorption capacities. Based on these results, an enhanced understanding of plasticised PVC as a blood-contacting biomaterial and its association with the nature of plasticiser, the plasticiser surface level and protein adsorption conditions were obtained. In addition, the study emphasises the importance of the surface, which is relevant for the utilisation of PVC-P and the development of improved PVC-P by PVC formulation and PVC surface modification.

CHAPTER 6

CYCLODEXTRIN MODIFIED PLASTICISED POLY(VINYL CHLORIDE)

6.1 Introduction

A noncovalently controlled phenomenon is well known to be crucial in biological systems. Nature uses this phenomenon of self-assembly, self-organization and self-replication to construct large molecular assemblies and supramolecules (Huff et al, 1996). This phenomenon is mainly based on molecular recognition. The specific recognition between enzyme and substrate, antibody and antigen, substrate and receptor and other host-guest pairs has driven modern chemical research to the design of molecular devices to match the sophistication of nature. For instance, chemists have developed molecular chemistry into supramolecular chemistry (Lehn, 1995), which may be defined as chemistry beyond the molecule, i.e. the chemistry of molecular assemblies and of the intermolecular bond. According to this concept, many receptors or host molecules have been synthesised as a building block of supramolecules. These hosts might be able to show some specific biomimetic functions, in which inclusion phenomena between host and guest is one example of these functions (Atwood, 1990).

Of all the hosts which can form inclusion complexes with specific guests, cyclodextrins (CDs), being of natural origin, organic biocompatible substances, seem to have unique status. They have found many applications in drugs formulation, food and cosmetics, adhesives and coatings, plastics and rubber, and biotechnology (Szejtli, 1997). Another important reason for synthetic chemists to pay interests on CDs is that CDs are chemically and physically stable and can be modified in a regioselective manner (Shieh &

Hedges, 1996). In addition, CDs are of great importance in the field of supramolecular chemistry. They can be used as models for studying intermolecular interactions between CDs and many guest molecules varying from ions, small organic substances to polymers (Harada, 1997) including biopolymers such as proteins (Cooper et al, 1996; Zhao et al, 1998) and bilirubin (Zhao & He, 1994a).

In our focus of interest, CDs might be as building blocks of a supramolecular structure and functional units, serving as an integral component of a biomaterial. Polymeric medical adsorbents containing CDs as an example have been well investigated in synthetic ways (Zhao & He, 1994b; He & Zhao, 1993). In this thesis, CDs have been employed as additives to be incorporated into PVC-P blending system for three objectives:

1. To improve blood compatibility using CDs or their combination with polyethylene oxide (PEO) or polyethylene oxide (PEO)-polypropylene oxide (PPO)-PEO surfactant based on the hypothesis that a balance of hydrophilicity / hydrophobicity at surface might be effective.
2. To retard DEHP migration due to possible inclusion complex formation between β-CD and DEHP and the increased hydrophilicity.
3. To develop a novel process for PVC-P with improved blood compatibility.

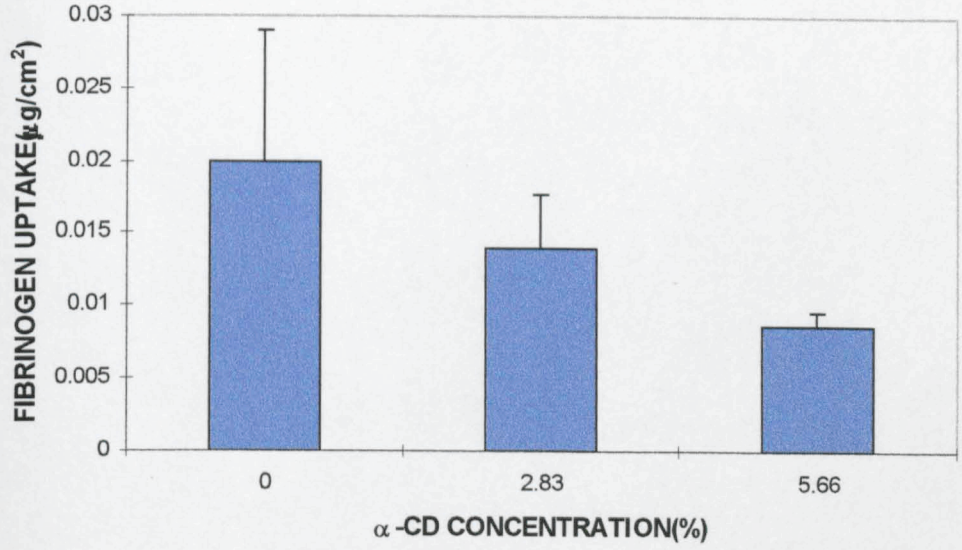


Fig 6.1 Fibrinogen adsorption on α–CD (A-CD) modified PVC-DEHP by casting

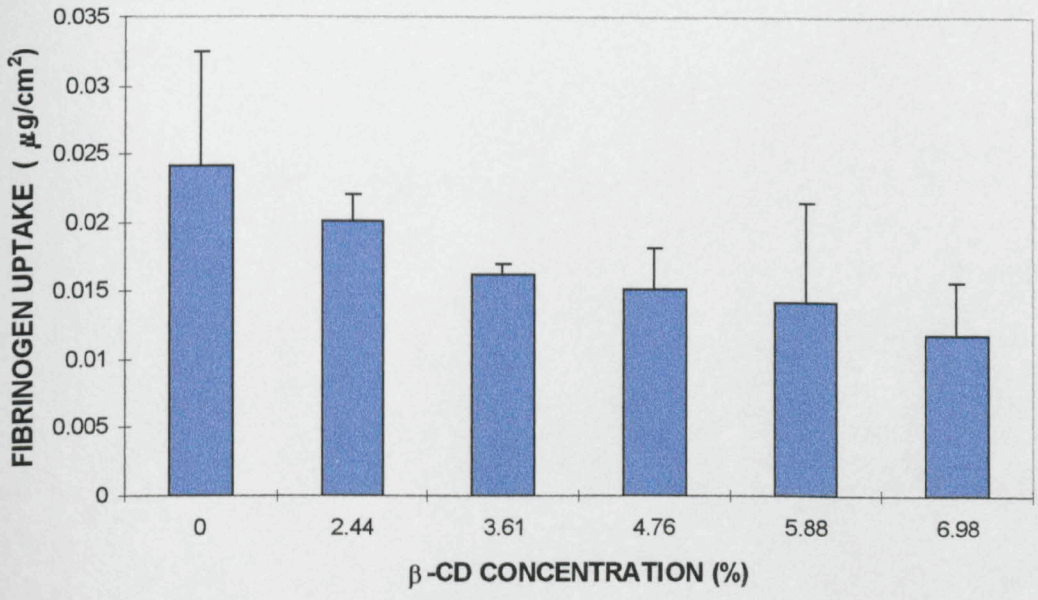


Fig 6.2 Fibrinogen adsorption on β-CD (B-CD) modified PVC-DEHP by casting

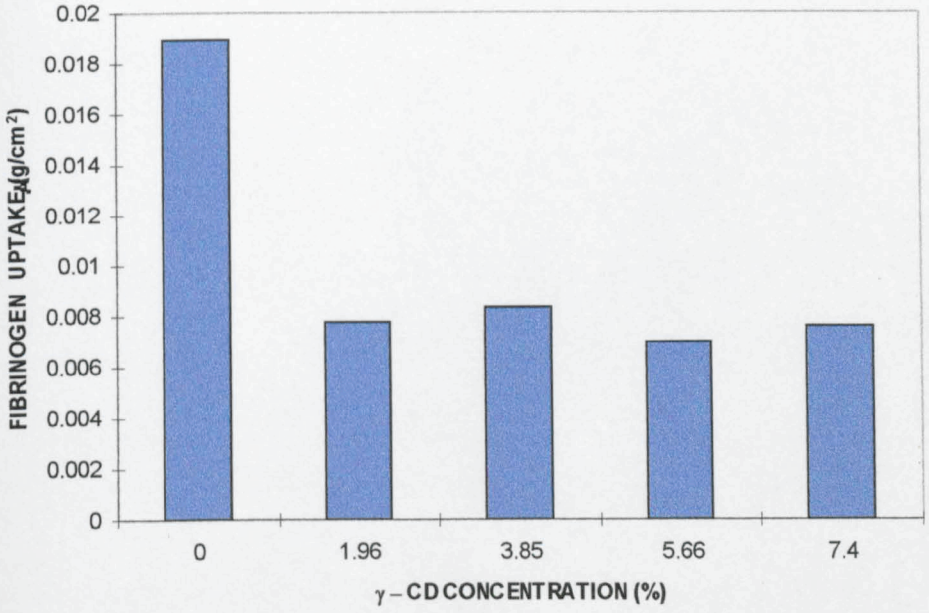


Fig 6.3 Fibrinogen adsorption on γ–CD (C-CD) modified PVC-DEHP by casting

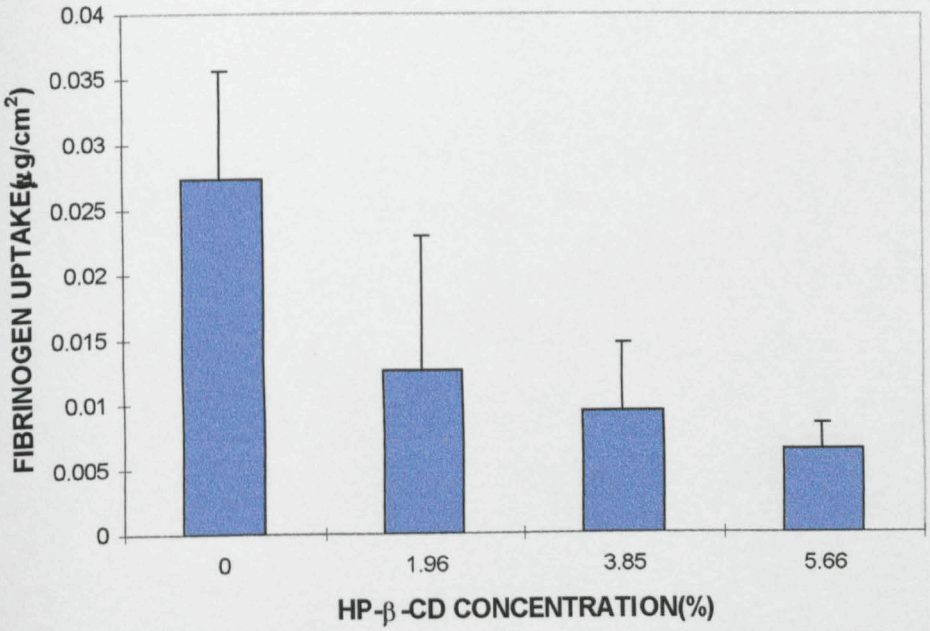


Fig 6.4 Fibrinogen adsorption on HP- β-CD (HP-B-CD) modified PVC-DEHP(casting)

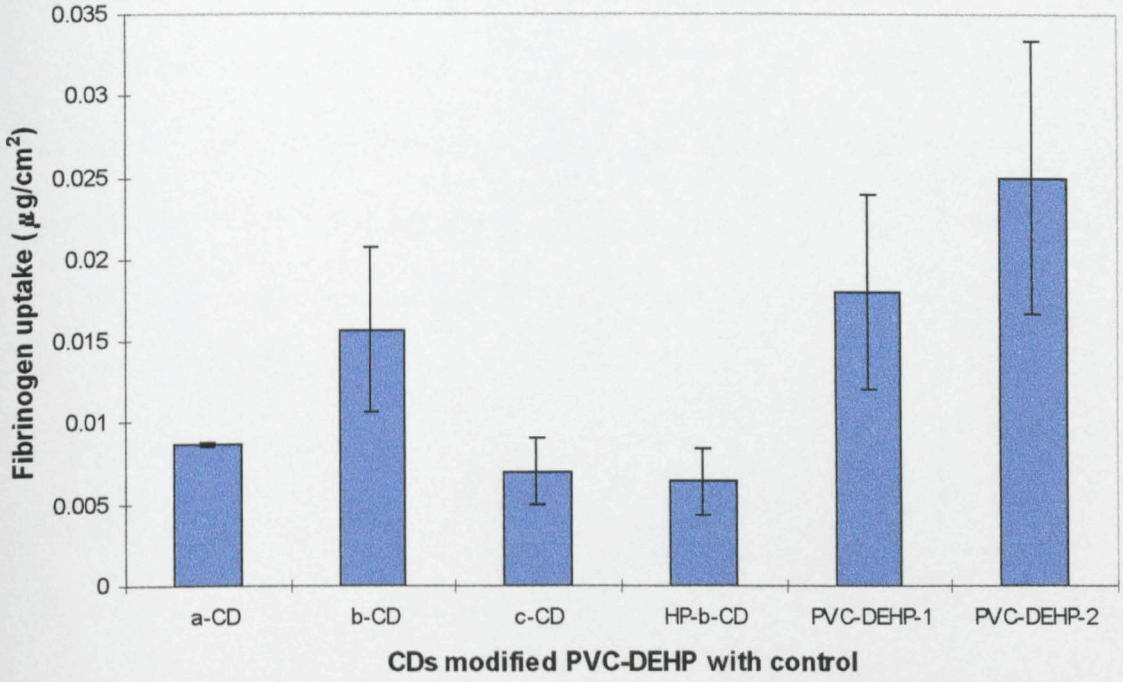


Fig 6.5 Summary of fibrinogen adsorption of CDs modified PVC-DEHP in comparison to PVC-DEHP-1(Ellay Inc.) and cast PVC-DEHP-2

The preliminary study focused on fibrinogen adsorption onto CDs modified PVC-DEHP, which was prepared by casting technology. In addition, polymer blending technology, which is more industry relevant, was applied to PVC-P modification by utilisation of CDs and their combination with PEO and PEO/PPO/PEO surfactant. The consideration of this combination was initiated by that these water-soluble polymers might be able to help CDs to move to PVC-P surface due to their possible inclusion interaction between CDs and these water-soluble linear polymers. The investigation results were shown as below.

6. 2 Protein adsorption results

6.2.1 Fibrinogen adsorption on CDs modified PVC-DEHP by casting

Table 6.1-6.5 list the results of fibrinogen adsorption on CDs modified PVC-DEHP by casting. The selection of different type of CD has great influence on the fibrinogen adsorption property. The incorporation of HP-β-CD and γ-CD can achieve a modified surface with a relatively lower fibrinogen adsorption than those of β-CD and α-CD modified PVC-DEHP at the same level of CD incorporation concentration. Fig 6.1~ 6.5 show the fibrinogen adsorption properties of these CDs modified PVC-DEHP. Results indicate that the hydrophilicity (water solubility) of CDs at the PVC-DEHP surface plays a very important role in modification of protein adsorption property of PVC-DEHP. HP-β-CD and γ-CD have higher water solubility than β-CD and α-CD. The increase in hydrophilicity would be effective to achieve a protein-resistant surface.

Table 6.1 Influence of α-CD concentration on fibrinogen adsorption (n=9)

α-CD CONCENTRATION (%)		0	2.83	5.66
Fibrinogen adsorption	Mean	0.02	0.014	0.0086
($\mu g/cm^2$)	SD	0.009	0.0037	0.0001

Table 6.2 Influence of β-CD concentration on fibrinogen adsorption (n=9)

β-CD CONCENTRATION (%)		0	2.44	3.61	4.76	5.88	6.98
Fibrinogen adsorption	Mean	0.0241	0.0201	0.0162	0.0152	0.0142	0.0117
($\mu g/cm^2$)	SD	0.0083	0.002	0.0008	0.003	0.0072	0.004

Table 6.3 Influence of γ-CD concentration on fibrinogen adsorption

γ-CD CONCENTRATION (%)	0	1.96	3.85	5.66	7.4
Fibrinogen adsorption (mg/cm^2)	0.019	0.0078	0.00836	0.007	0.0076

Table 6.4 Influence of HP-β-CD concentration on fibrinogen adsorption (n=9)

HP-β-CD CONCENTRATION (%)		0	1.96	3.85	5.66
Fibrinogen adsorption ($\mu g/cm^2$)	Mean	0.0273	0.0127	0.0094	0.0064
	SD	0.0084	0.0102	0.0053	0.002

Table 6.5 Summary of influence of CDs selection on fibrinogen adsorption of CDs modified PVC-DEHP (n=9)

	FIBRINOGEN ADSORPTION ($\mu g/cm^2$)					
CDs	α-CD	β-CD	γ-CD	HP-β-CD	PVC-DEHP-1*	PVC-DEHP-2*
Mean	0.0087	0.0157	0.007	0.0064	0.019	0.025
SD	0.0001	0.005	0.002	0.002	0.006	0.0084

* PVC-DEHP-1: from Ellay; PVC-DEHP-2: from casting

From the preliminary results, it was found that the incorporation of CDs to PVC-DEHP is an effective approach for modifying the surface to achieve a fibrinogen resistant surface. However, the polymer solution casting technology is limited for practical industry application. Therefore, in the following sections, polymer blending technology instead of casting was employed for PVC-P modification by utilisation of CDs or their combination with PEO and PEO-PPO-PEO surfactant. The adsorption of the blood components fibrinogen and albumin on the modified surfaces were assessed.

6.2.2 CDs modified PVC-P by polymer blending

6.2.2.1 Fibrinogen adsorption

Table 6.6 gives the fibrinogen adsorption results for β-CD, β-CD/PEO (P), β-CD/Pluronic F68 (PP), HP-β-CD and HP-β-CD/PEO modified PVC-DEHP (D) and PVC-TEHTM (T) with different incorporated amount in the PVC formulation. In addition, the fibrinogen adsorption on the test cell wall (polystyrene material) was calculated from the subtraction between the fibrinogen concentration before test and after test plus the fibrinogen uptake by PVC-P samples. Results indicate that β-CD and HP-β-CD alone can modify PVC-DEHP with reduced fibrinogen adsorption. The resistance to fibrinogen adsorption increases with the increase in the amount of CDs incorporated.

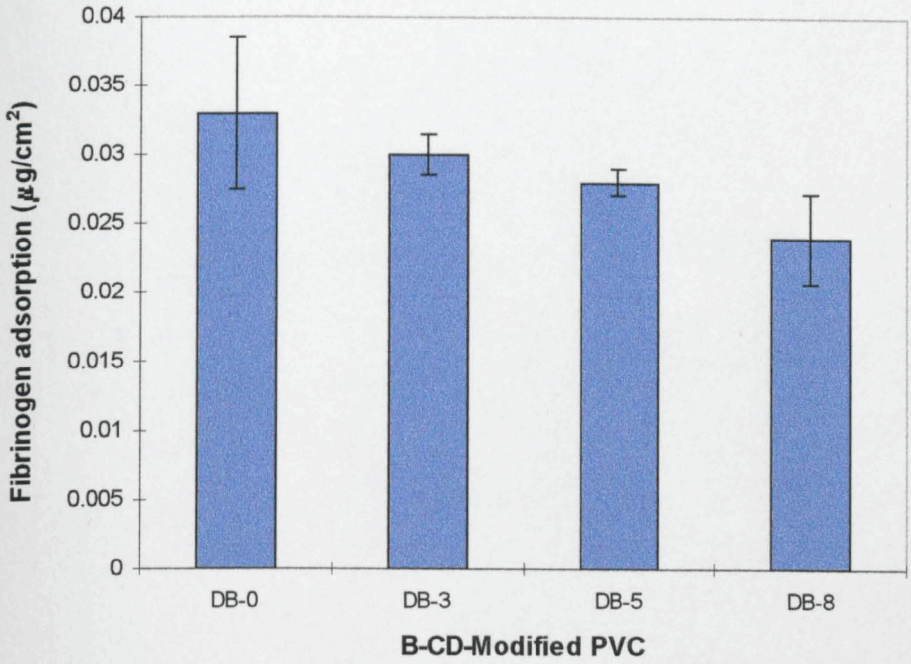


Fig 6.6 Fibrinogen adsorption on B-CD modified PVC-DEHP by blending

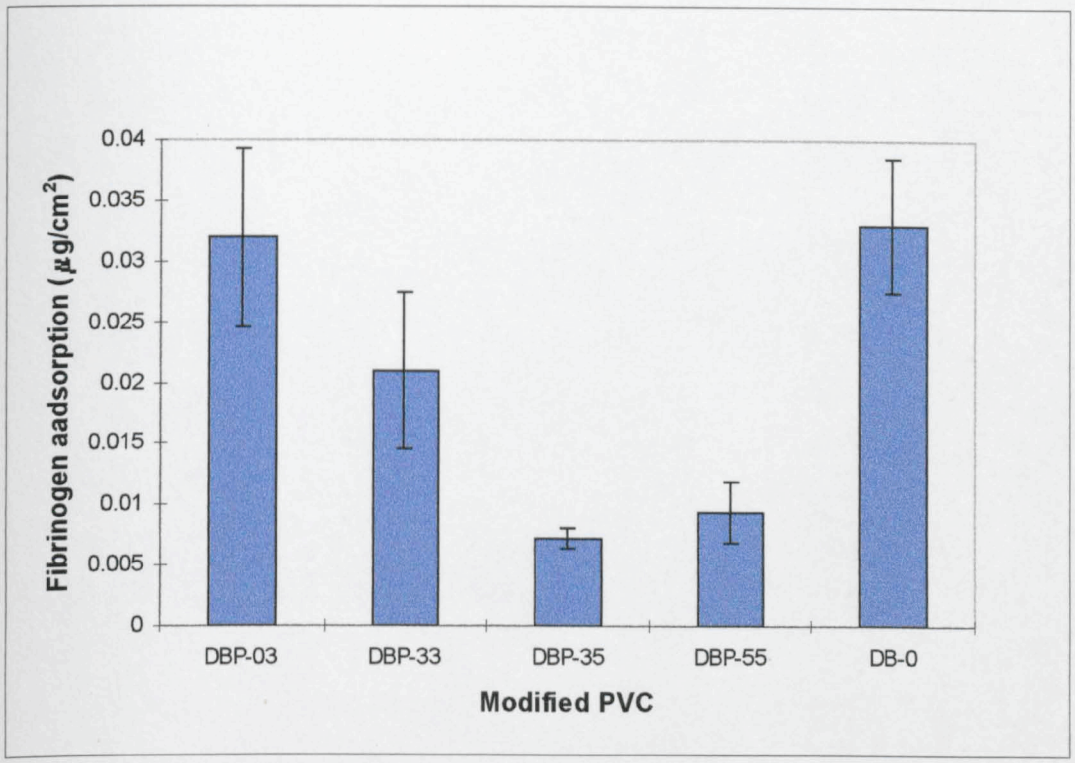


Fig 6.7 Fibrinogen adsorption on B-CD/PEO modified PVC-DEHP by blending

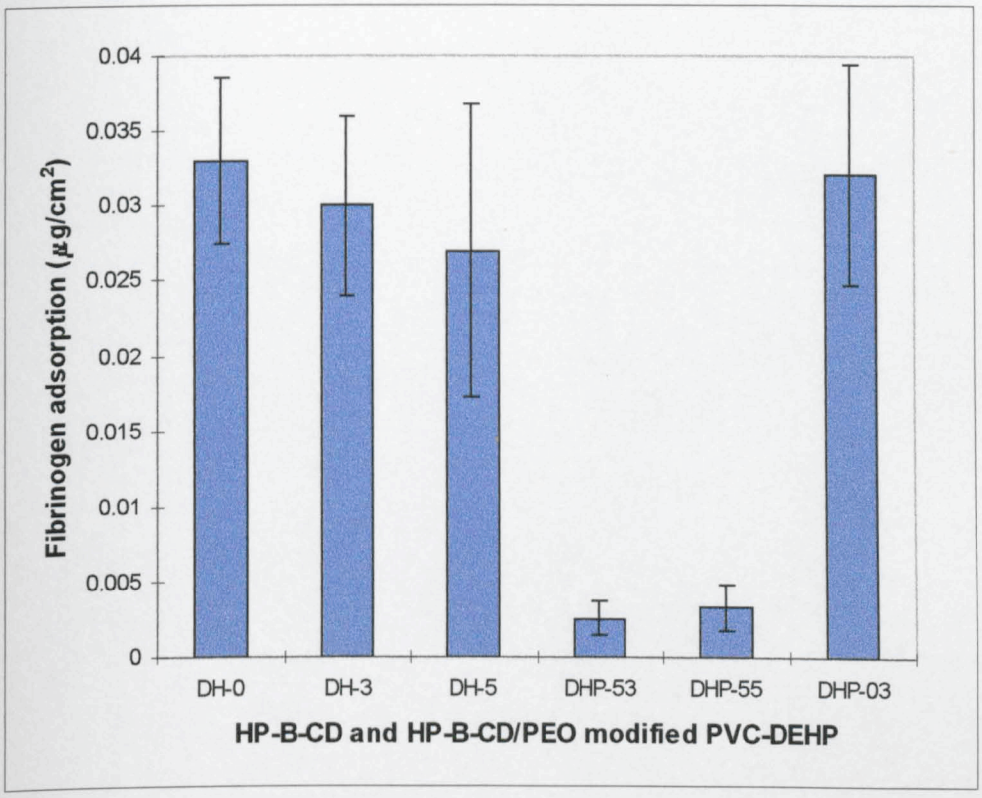


Fig 6.8 Fibrinogen adsorption on HP-B-CD and HP-B-CD/PEO modified PVC-DEHP by polymer blending

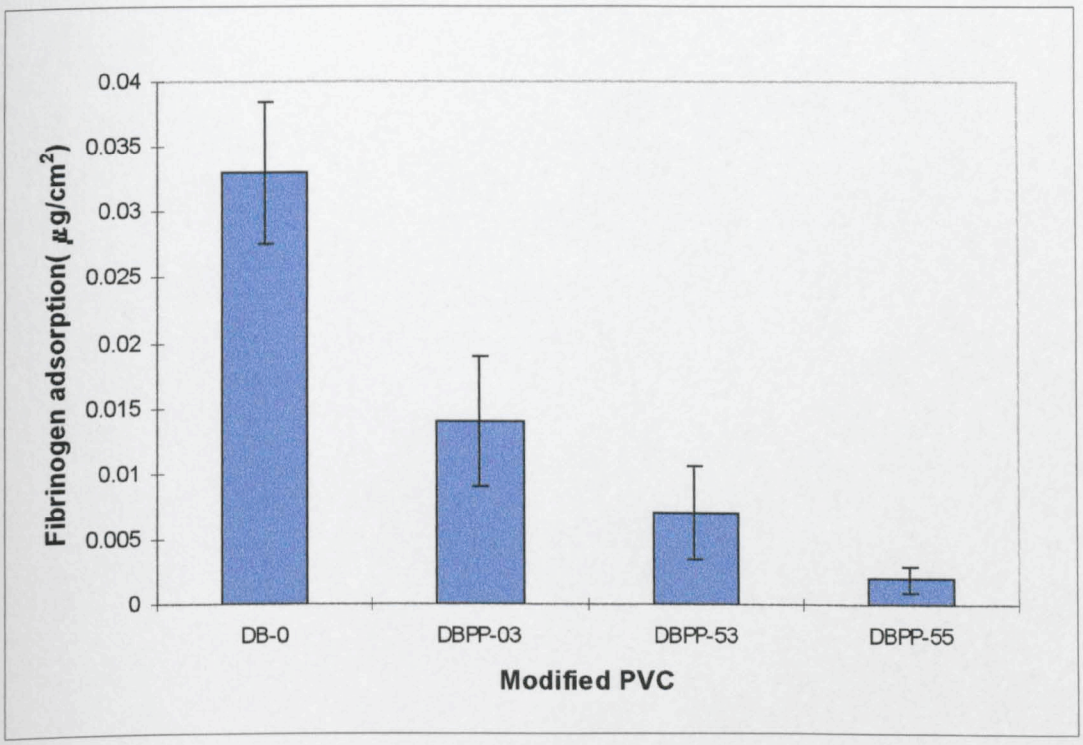


Fig 6.9 Fibrinogen adsorption on B-CD/Pluronic F68 modified PVC-DEHP by polymer blending

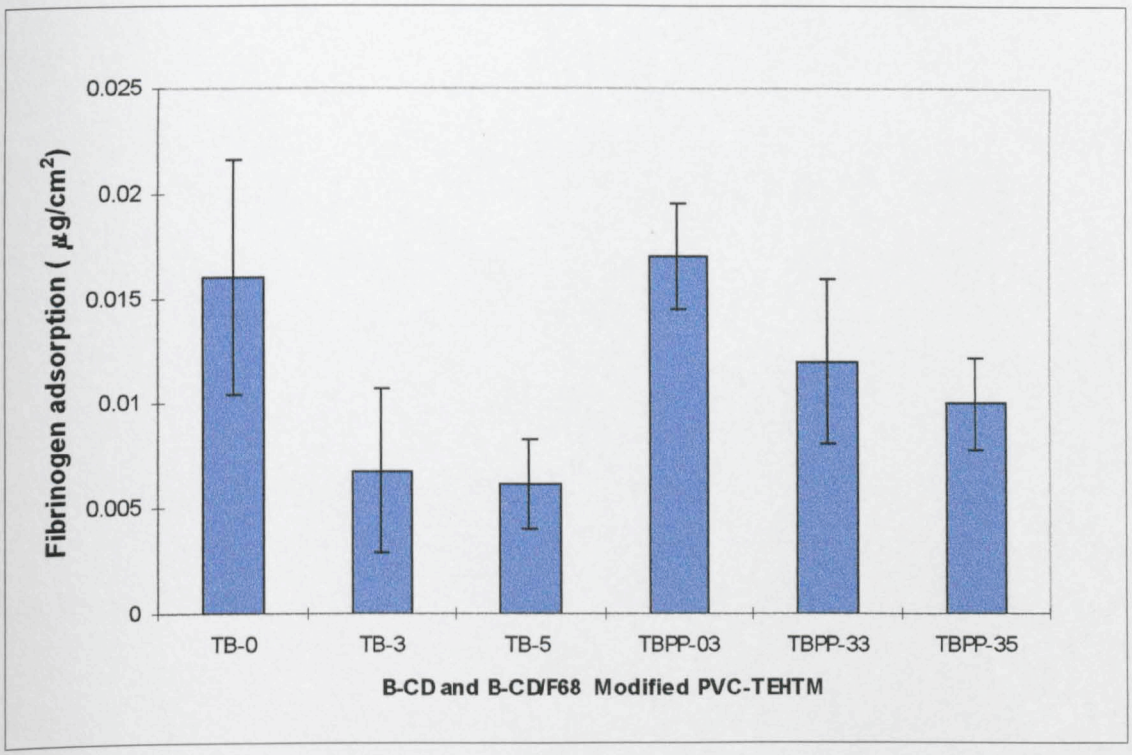


Fig 6.10 Fibrinogen adsorption on B-CD/ Pluronic F68 modified PVC-TEHTM by polymer blending

Also, the incorporation of PEO and PEO-PPO-PEO (Pluronic F68) alone is also able to modify PVC-DEHP with reduced fibrinogen adsorption. However, in a suitable incorporation ratio of β-CD/PEO and β-CD/F68, a remarkably high fibrinogen resistant surface was achieved as shown in Fig 6.6 ~ 6.9. The possible interaction of β-CD with PEO and PEO-PPO-PEO might be able to lead to a synergistic effect on the minimisation of protein adsorption on their modified PVC-DEHP. There is a different situation with PVC-TEHTM. The combination of β-CD with Pluronic F68 seems to be less effective in reducing fibrinogen adsorption than that of using β-CD alone, as shown in Fig 6.10. The possible answer can only be found from their surface characterisation, which is discussed in section 6.3. In addition, from the commercialisation point of view, the combination of relatively cheaper PEO or PEO-PPO-PEO to replace part of expensive CDs would reduce the production cost.

6.2.2.2 Albumin adsorption

Table 6.7 gives the albumin adsorption results for β-CD, β-CD/PEO and β-CD/Pluronic F68 modified PVC-DEHP. There is no significant difference in albumin adsorption, using β-CD alone to modify PVC-DEHP in a low concentration of β-CD less than 5% by weight. The incorporation of PEO and PEO-PPO-PEO alone can bring down the albumin adsorption level significantly compared to non-modified PVC-DEHP. The combination of β-CD with PEO in a suitable ratio such as 3/5 or 5/5 can achieve a low albumin adsorption level. More significantly, the utilisation of Pluronic F68 or its combination with β-CD can create an albumin resistant surface. However, there is no significant difference between albumin adsorption on Pluronic F68 modified PVC-DEHP and Pluronic F68/β-CD combination modified PVC-DEHP. This indicates that PEO-PPO-PEO (F68) is such a powerful surface modifier with respect to the reduction of albumin adsorption that it would mask the β-CD effect on albumin adsorption (Fig 6.11-13).

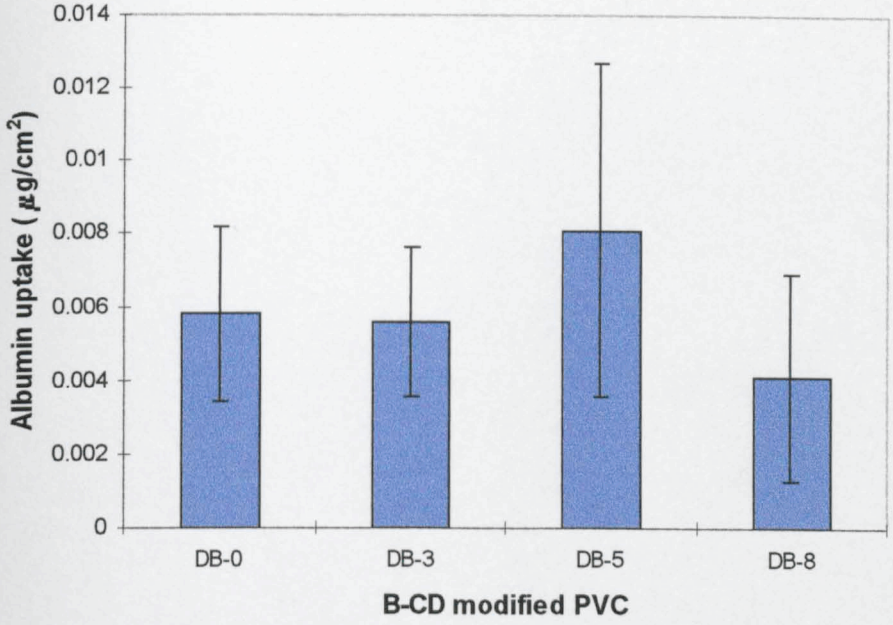


Fig 6.11 Albumin adsorption on B-CD modified PVC-DEHP by polymer blending

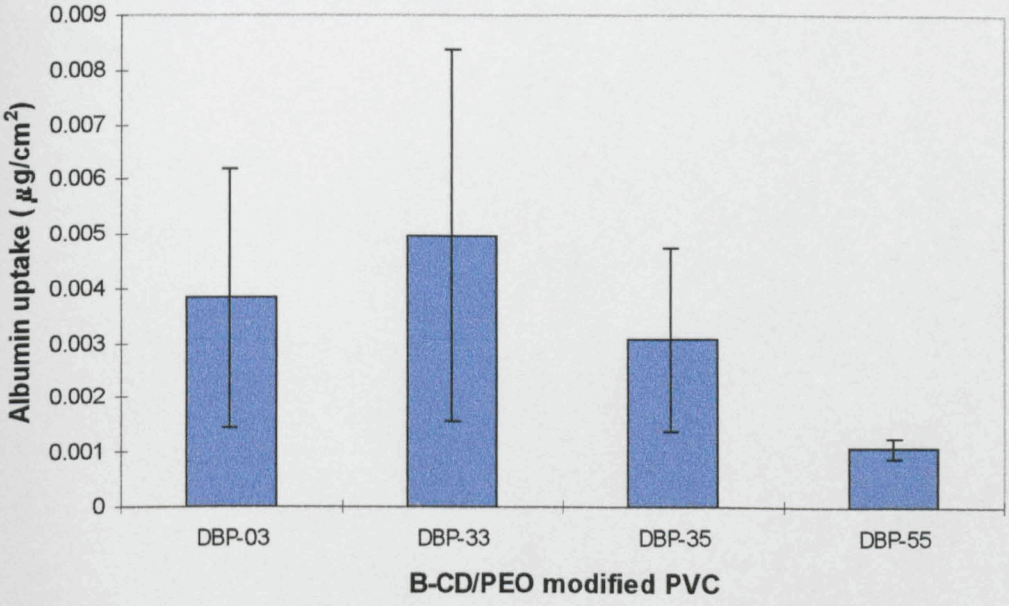


Fig 6.12 Albumin adsorption on B-CD/PEO modified PVC-DEHP

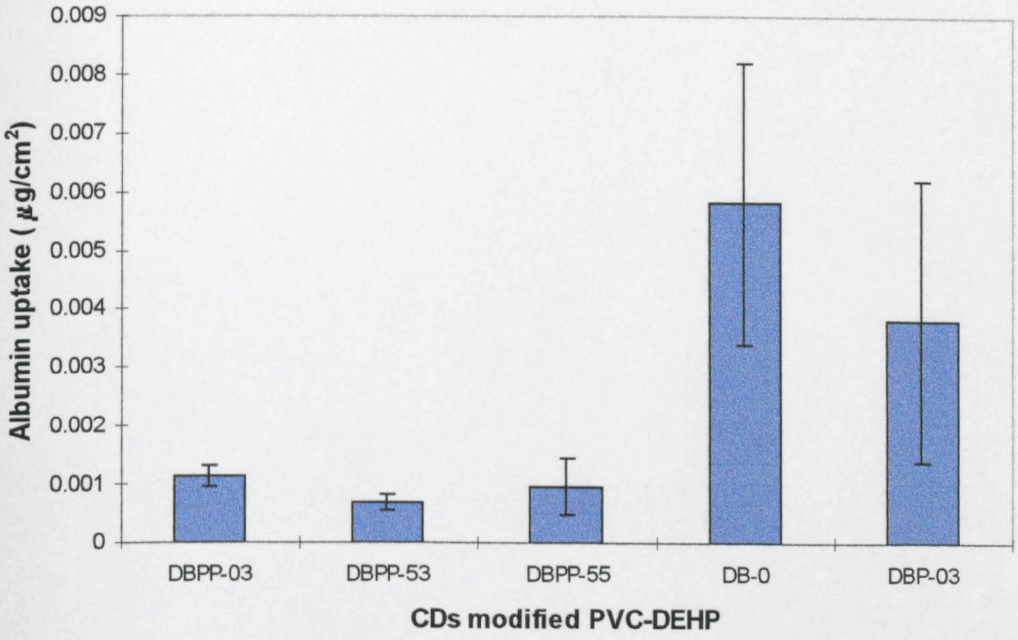


Fig 6.13 Albumin adsorption on B-CD/Pluronic F68 modified PVC-DEHP by polymer blending comparing to non-modified and PEO modified PVC-DEHP.

Table 6.6 Fibrinogen adsorption on CDs or CDs/PEO, and CDs/Pluronic F68 modified PVC-P

	FIBRINOGEN ADSORPTION ((μg/cm^2)				
Sample	one	two	three	Mean	SD
DB-0	0.039	0.029	0.03	0.032667	0.005508
DB-3	0.03	0.029	0.032	0.030333	0.001528
DB-5	0.029	0.028	0.027	0.028	0.001
DB-8	0.023	0.022	0.028	0.024333	0.003215
DBP-03	0.026	0.029	0.04	0.031667	0.007371
DBP-33	0.026	0.024	0.014	0.021333	0.006429
DBP-35	0.0074	0.0062	0.008	0.0072	0.000917
DBP-55	0.007	0.009	0.012	0.009333	0.002517
DBPP-03	0.013	0.019	0.009	0.003556	0.005033
DBPP-53	0.003	0.008	0.01	0.007	0.003606
DBPP-55	0.0014	0.0033	0.0014	0.002033	0.001097
DH-3	0.036	0.024	0.03	0.03	0.006
DH-5	0.03	0.035	0.016	0.027	0.009849
DHP-53	0.0037	0.0014	0.0026	0.002567	0.00115
DHP-55	0.0023	0.0025	0.005	0.003267	0.001504
TB-0	0.015	0.022	0.011	0.016	0.005568
TB-3	0.01	0.0025	0.0079	0.0068	0.003869
TB-5	0.004	0.0082	0.0064	0.0062	0.002107
TBPP-03	0.019	0.014	0.017	0.016667	0.002517
TBPP-33	0.009	0.0097	0.016	0.011567	0.003855
TBPP-35	0.0077	0.0089	0.012	0.009533	0.002219
PVC-U	0.0058	0.0083	0.0044	0.006167	0.001976
PS CELL	0.018	0.016	0.017	0.017	0.002

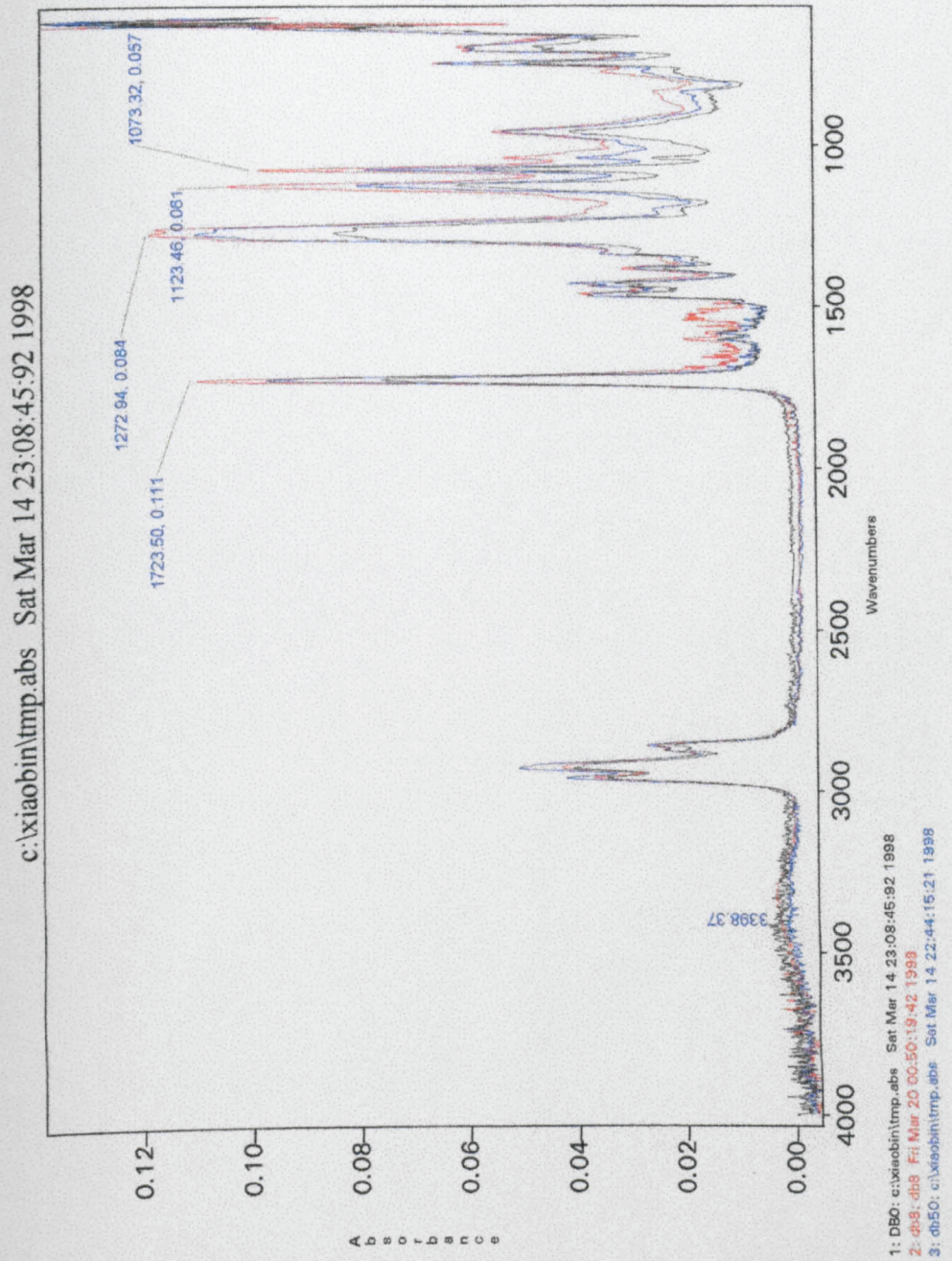


Fig 6.14 ATR-FTIR surface characterisation of B-CD modified PVC-DEHP (DB0: unmodified; DB5o:original 5% B-CD modified; DB8: 8%B-CD modified)

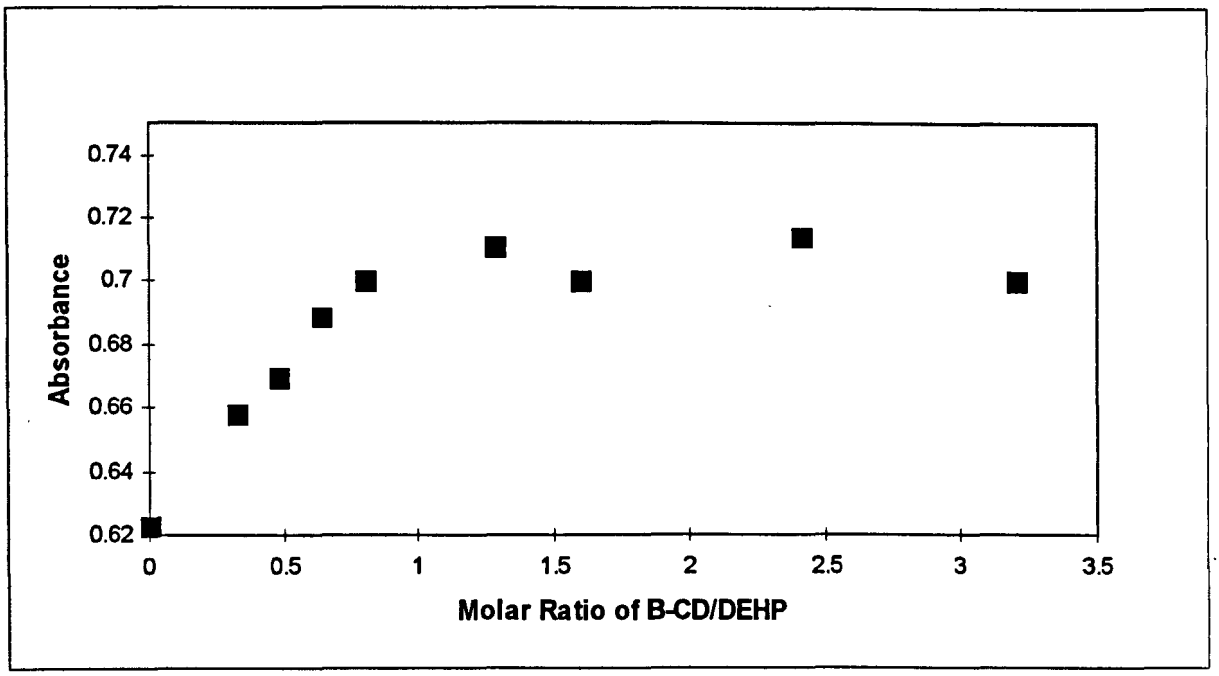


Fig 6.15 Influence of B-CD on UV absorption of DEHP

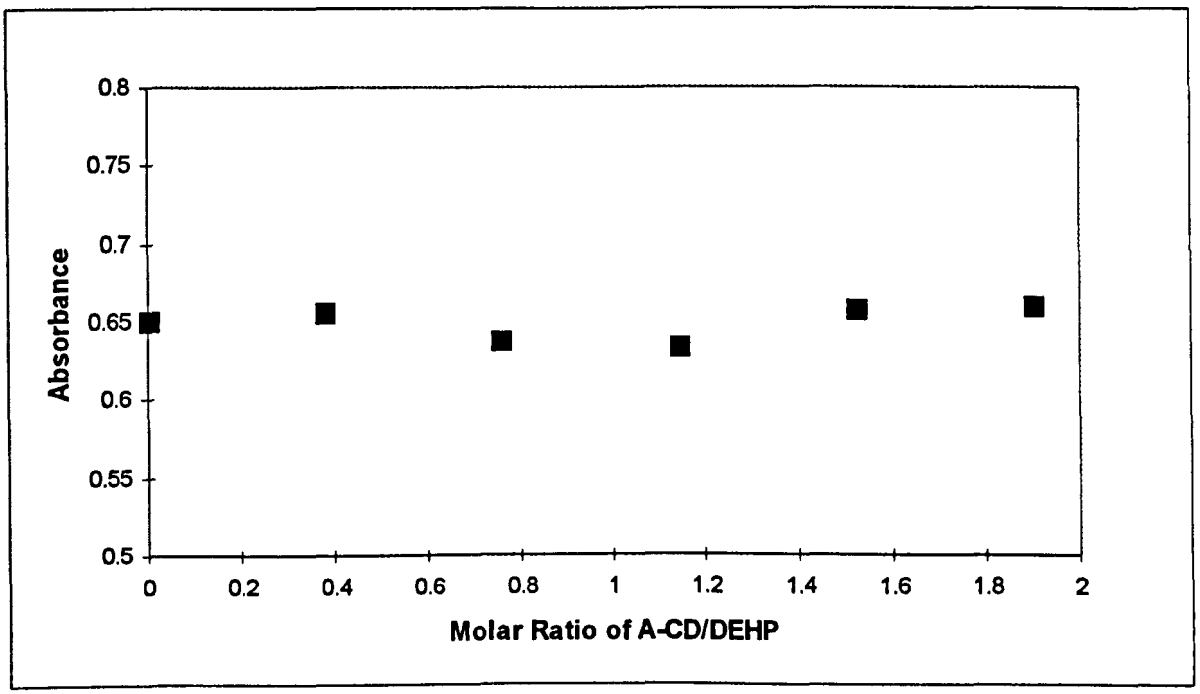


Fig 6.15 a Influence of A-CD on DEHP UV absorption

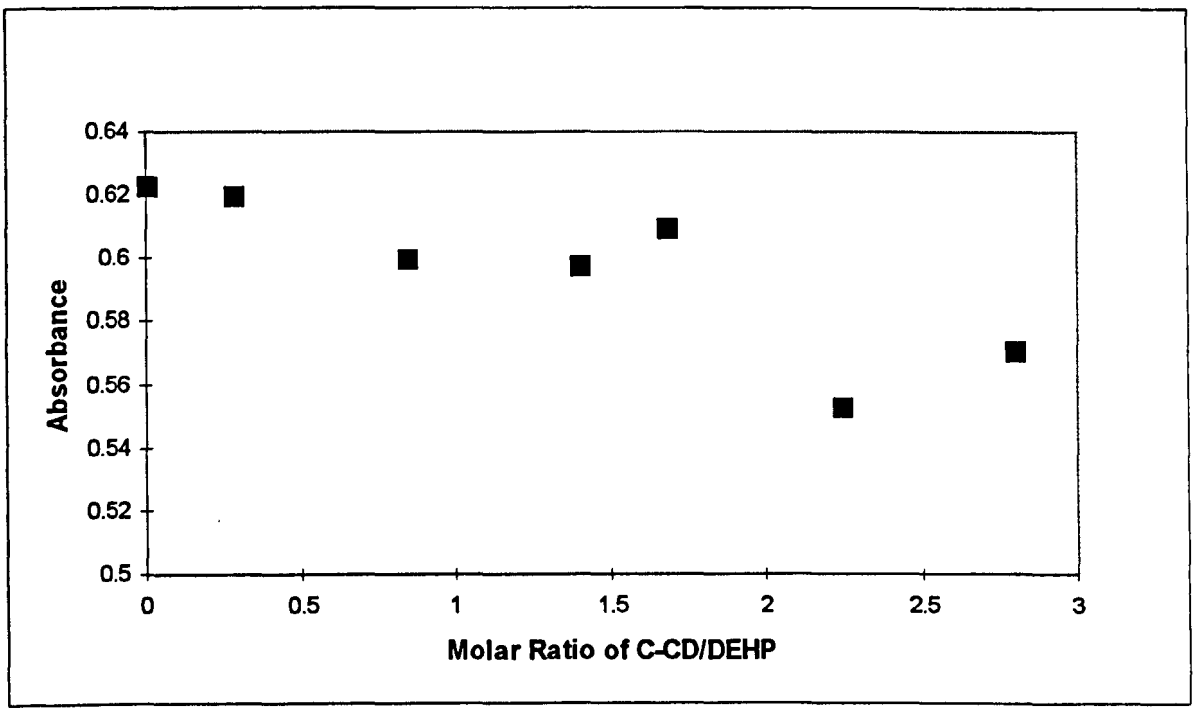


Fig 6.15 b Influence of C-CD on DEHP UV absorption

Table 6. 7 Albumin adsorption on CDs modified PVC-DEHP

SAMPLES	ALBUMIN ADSORPTION ($\mu g/cm^2$)				
	One	Two	Three	Mean	SD
DB-0	0.0047	0.0041	0.0085	0.0058	0.0024
DB-3	0.0041	0.007	0.0056	0.0056	0.0021
DB-5	0.0057	0.0053	0.0134	0.0081	0.0021
DB-8	0.0021	0.0061	0.004	0.0041	0.0028
DBP-03	0.0065	0.002	0.003	0.0038	0.0024
DBP-33	0.0033	0.0018	0.0098	0.005	0.0034
DBP-35	0.0022	0.005	0.002	0.0031	0.0017
DBP-55	0.0013	0.001	0.001	0.0011	0.00018
DBPP-03	0.0011	0.0014	0.001	0.0011	0.00018
DBPP-53	0.00074	0.00079	0.00052	0.00068	0.00014
DBPP-55	0.00063	0.00132	0.001	0.001	0.00049

6.3 ATR-FTIR surface characterisation of CDs modified PVC-P

As previously stated, one of the research objectives of using CDs is to provide the PVC-P surface with an improved blood compatibility in terms to protein adsorption. The altered protein adsorption properties of CDs modified PVC-P are mainly determined by the surface chemical structures and physical morphology. In this section, surface characterisation of CD modified PVC-DEHP and PVC-TEHTM is characterised by ATR-FTIR to reveal the chemical information of the surface.

Fig 6.14 shows the ATR-FTIR spectra of β-CD modified PVC-DEHP with different β-CD incorporated amounts. From the spectra, it is found that a broad band at 3400~3230

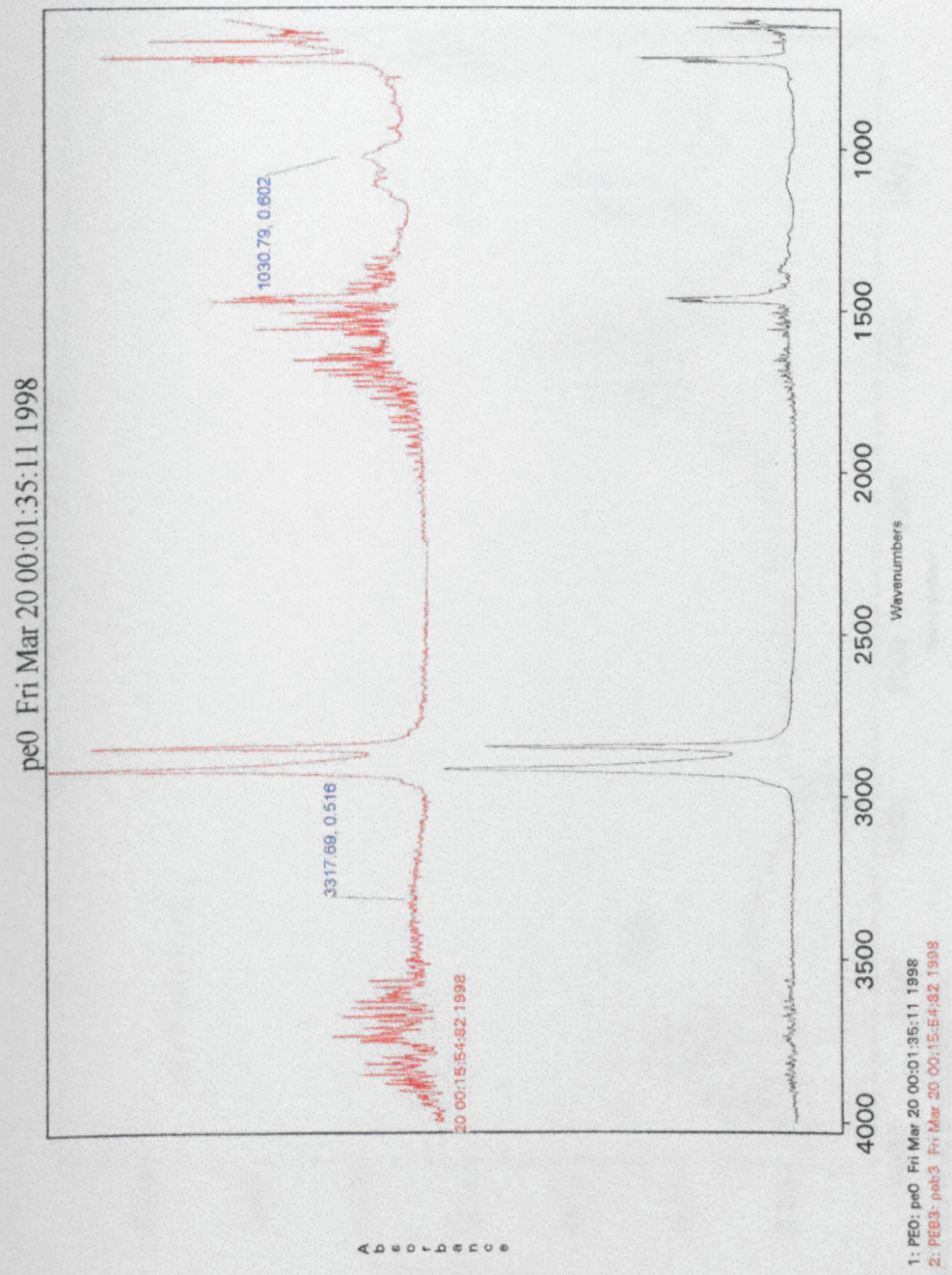


Fig 6.16a ATR-FTIR surface characterisation of B-CD modified LDPE (PE0: unmodified ; PEB3: 3% B-CD modified)

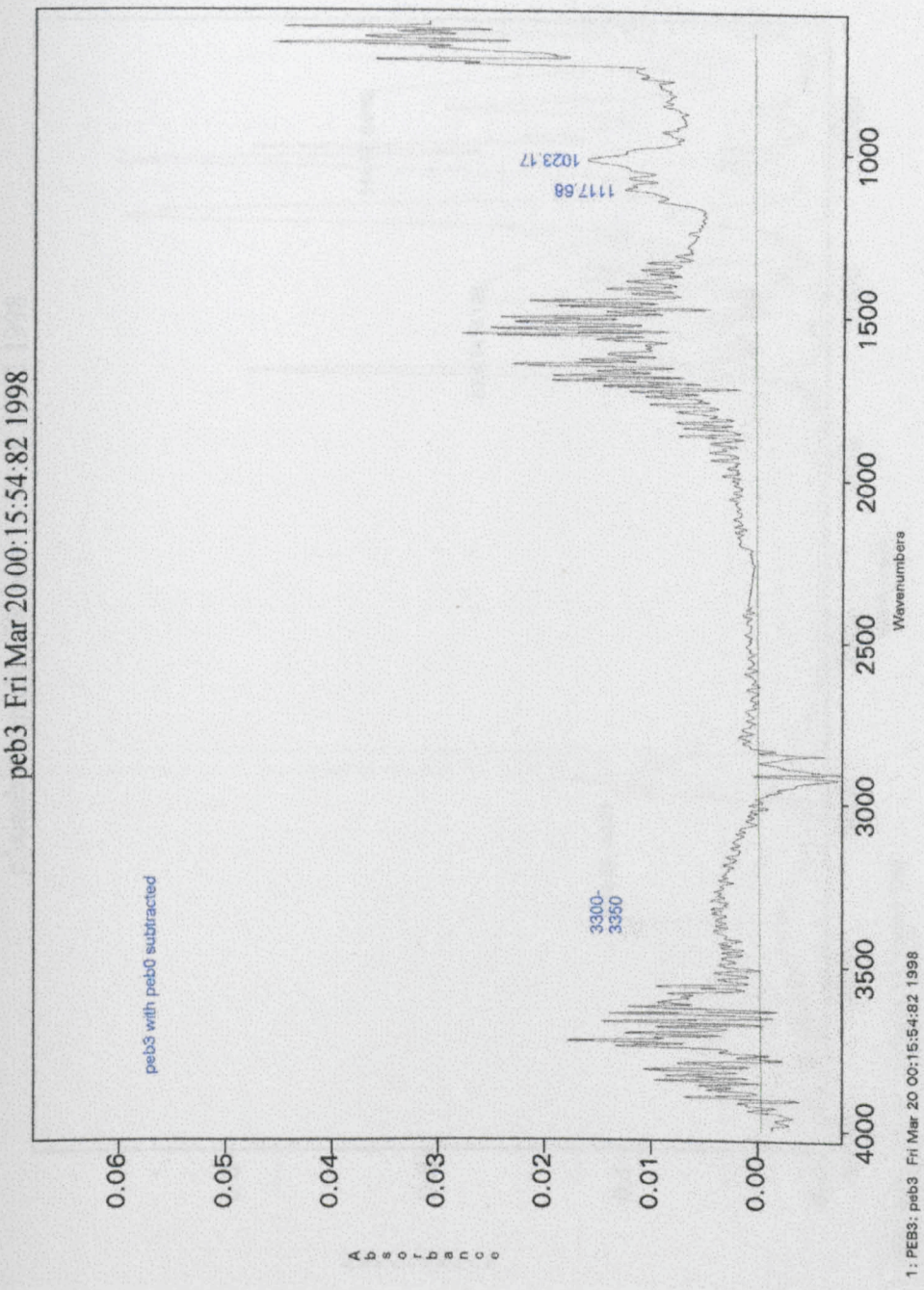


Fig 6.16b ATR-FTIR subtraction spectrum of B-CD modified LDPE with unmodified LDPE (PE0: unmodified; PEB3: 3% B-CD modified)

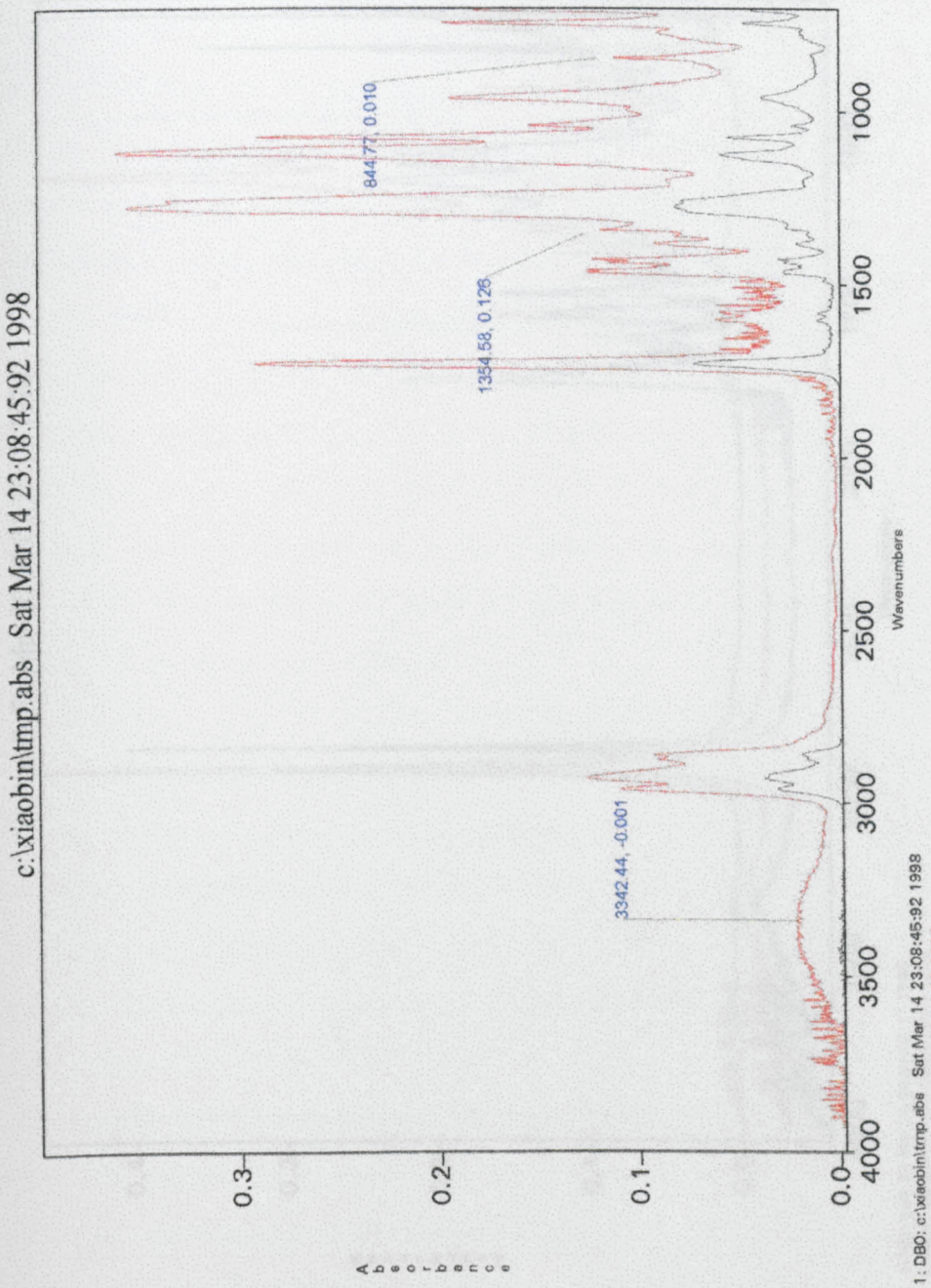


Fig 6.17 ATR-FTIR spectra of B-CD/PEO modified PVC-DEHP (DBP55: modified with 5%B-CD/5%PEO

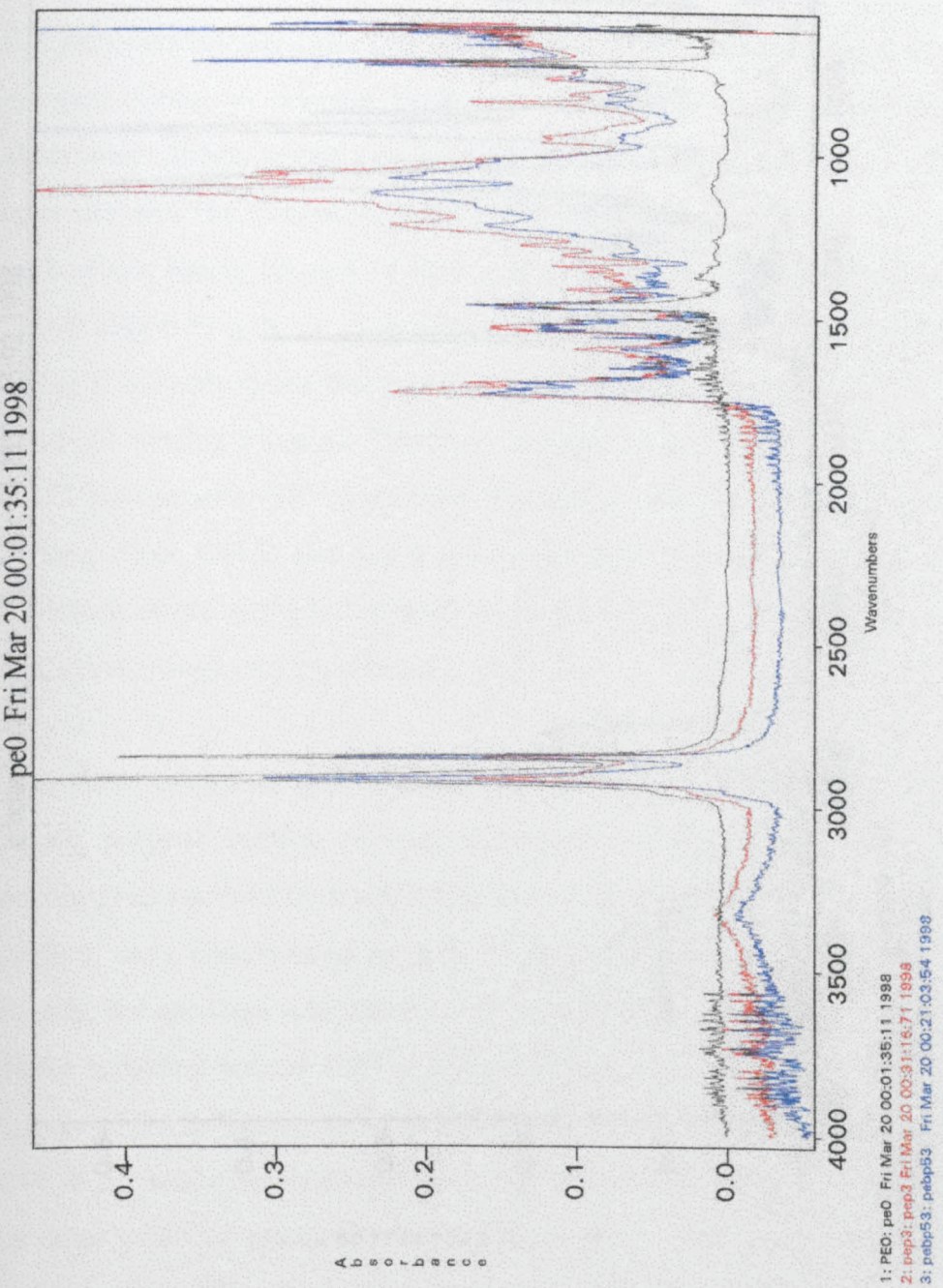


Fig 6.18 ATR-FTIR spectra of B-CD/PEO modified LDPE as control (PEP3 : 3% PEO modified; PEBP53: 5%B-CD/3%PEO modified)

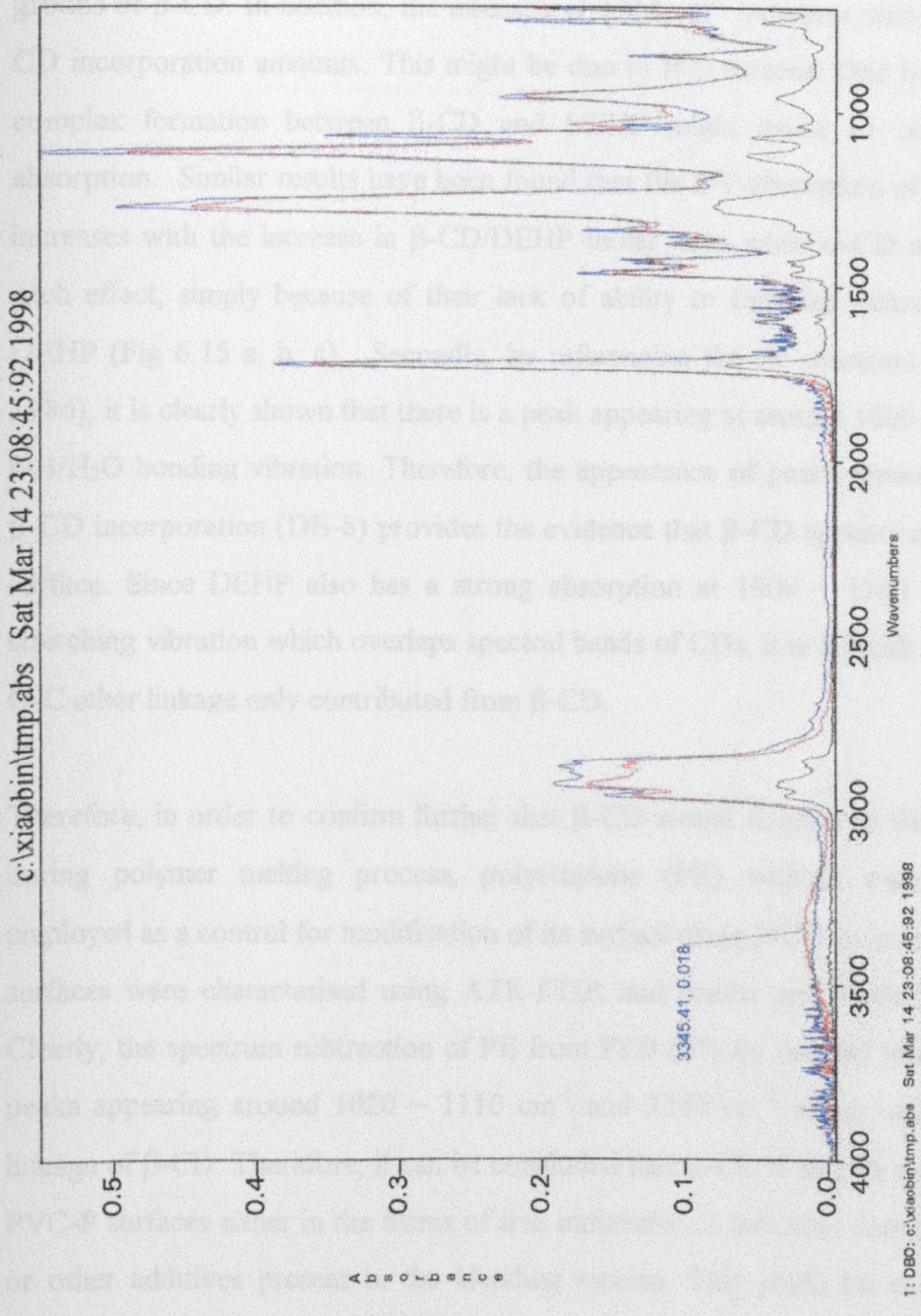


Fig 6.19 ATR-FTIR spectra of B-CD/F68 modified PVC-DEHP (DBPP03:3%F68 alone modified;DBPP53:5%B-CD/3%F68 modified)

cm^{-1} appears after the incorporation of β-CD, which is attributed to the OH functional groups of β-CD. In addition, the intensity at 1730 cm^{-1} increases with the increase in β-CD incorporation amounts. This might be due to two reasons. One is that the inclusion complex formation between β-CD and DEHP might cause an enhancement in IR absorption. Similar results have been found that the UV-absorption of DEHP at 274 nm increases with the increase in β-CD/DEHP molar ratio while α-CD and γ-CD have no such effect, simply because of their lack of ability to form an inclusion complex with DEHP (Fig 6.15 a, b, c). Secondly, by referencing the IR spectrum of β-CD (Szejtli, 1986), it is clearly shown that there is a peak appearing at around 1600~1730 cm^{-1} due to OH/H_2O bonding vibration. Therefore, the appearance of peaks around 1600 cm^{-1} after β-CD incorporation (DB-8) provides the evidence that β-CD appears at the PVC-DEHP surface. Since DEHP also has a strong absorption at 1000 ~ 1360 cm^{-1} due to C-O stretching vibration which overlaps spectral bands of CDs, it is difficult to indicate the C-O-C ether linkage only contributed from β-CD.

Therefore, in order to confirm further that β-CD would migrate to the polymer surface during polymer melting process, polyethylene (PE) without major additives was employed as a control for modification of its surface using β-CD by polymer melting. The surfaces were characterised using ATR-FTIR and results are shown in Fig 6.16 a, b. Clearly, the spectrum subtraction of PE from PEB (3% by weight) leaves a pronounced peaks appearing around 1020 ~ 1110 cm^{-1} and 3340 cm^{-1} which indicates the C-O-C linkage of β-CD. Therefore, it can be concluded that β-CD is able to move to the PE and PVC-P surfaces either in the forms of free molecules or inclusion complexes with DEHP or other additives present in the blending system. This could be evidenced from the appearance of OH stretching vibration and OH/H_2O bonding vibration at the FT-IR spectra.

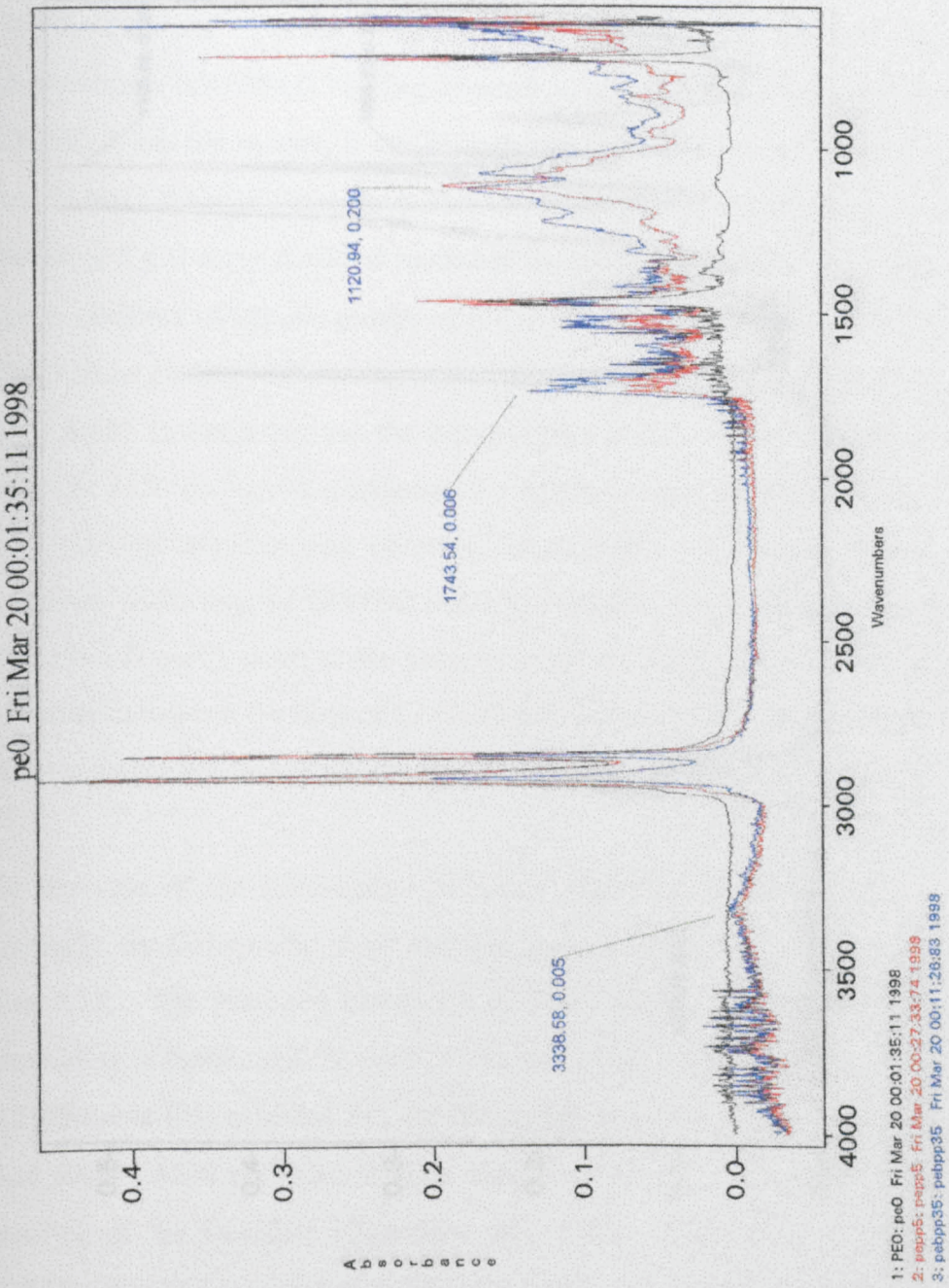


Fig 6.20 ATR-FTIR spectra of B-CD/F68 modified LDPE as controls (PEPP5: 5% F68 alone modified; PEBPP35: 3%B-CD/5%F68 modified)

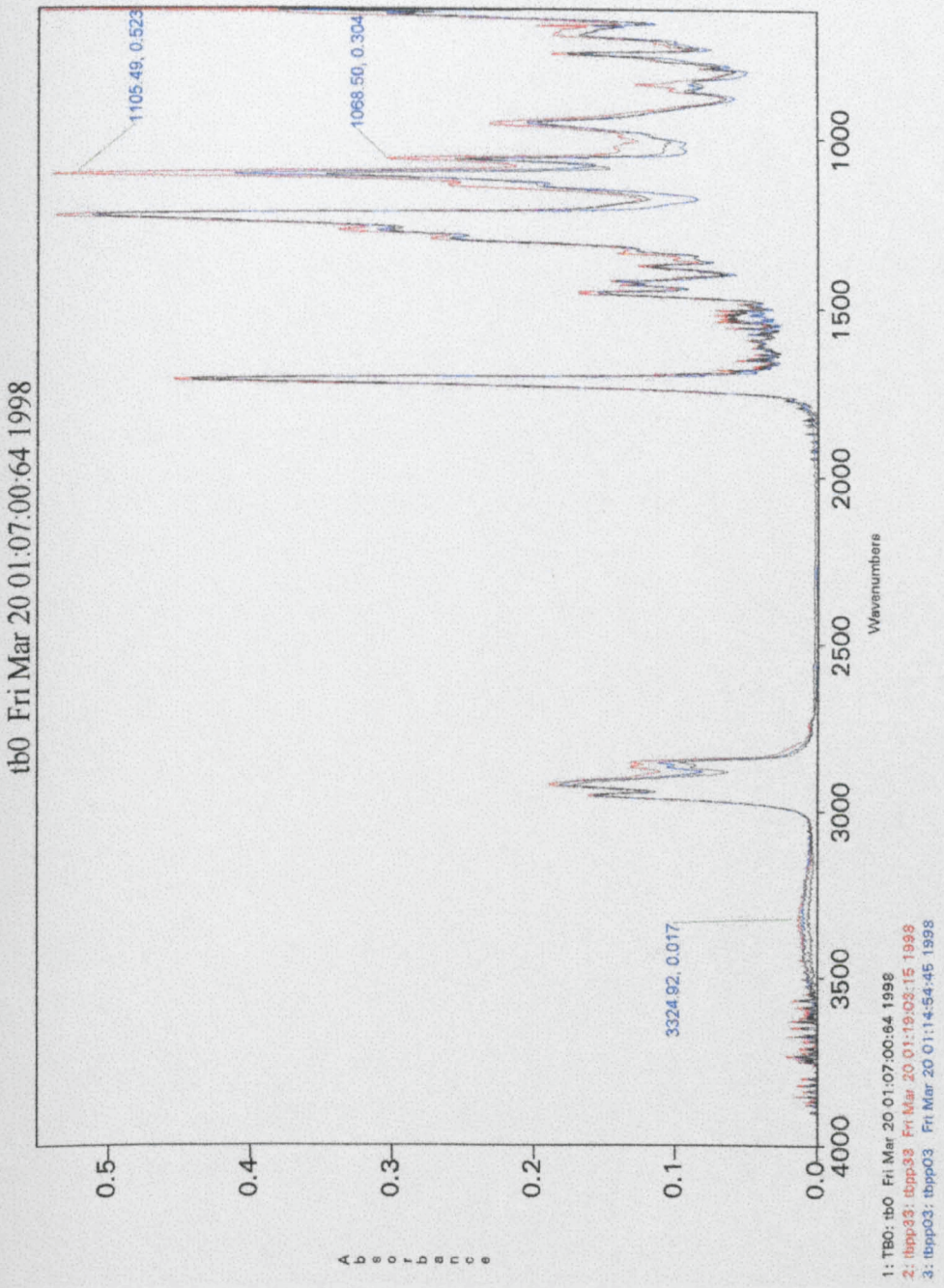


Fig 6.20a ATR-FTIR spectra of B-CD/F68 modified PVC-TEHTM (TBPP03: 3%F68 modified; TBPP33: 3%B-CD/3%F68 modified)

Fig 6.17 gives an example of ATR-FTIR spectra of β-CD/PEO modified PVC-DEHP surface. Clearly, there is a broad band at 3300 cm^{-1} and remarkable absorption around 1000 ~ 1260 cm^{-1}. The appearance of an absorption band around 1600 cm^{-1} also indicates the presence of OH/H_2O bonding vibration as mentioned above for β-CD modified PVC-DEHP. From the spectra, it can be seen that the combination of PEO in a suitable ratio could assist β-CD to the surface. This is evidenced from the more pronounced peaks than those of β-CD alone modified surface at the same wavelength, which are assigned to the great numbers of specific groups of β-CD (21 OH for each β-CD). The β-CD/PEO and PEO alone modified PE serving as control materials were characterised using ATR-FTIR (Fig 6.18). It was found that the incorporation of β-CD and PEO leads to an absorption band at 3350 cm^{-1} and a distinguished absorption band at 1020~1260 cm^{-1}, which is due to C-O-C (ether) stretching vibration. Around 1600 ~ 1730 cm^{-1}, there are many peaks appeared indicating the OH/H_2O bonding vibration. These results indicate that PEO and PEO/B-CD could move to the polymer surface during polymer melting. However, it is difficult to indicate the level of CD/PEO distributed at the surface from the FTIR spectra. This is simply because of the structural similarity between β-CD and PEO.

In the case of the combination of β-CD with Pluronic F68 to modify PVC-DEHP, similarly, the OH specific absorption can be found in the ATR-FTIR spectra as shown in Fig 6.19. The profound absorption at 3345 cm^{-1} for DBPP53 sample indicates the possibility of function of Pluronic F68 for β-CD migration. For the control materials of β-CD/Pluronic F68 modified PE, the absorption at 3338 cm^{-1}, around 1740 ~ 1600 cm^{-1}, and 1020 ~ 1250 cm^{-1} also indicate there is an enriched quantity of β-CD/Pluronic F68 existing at the modified PE surface (Fig 6.20). Similarly, ATR-FTIR spectra of β-CD/Pluronic F68 modified PVC-TEHTM indicate there is am β-CD/F68 enriched surface as well (Fig 6.20a).

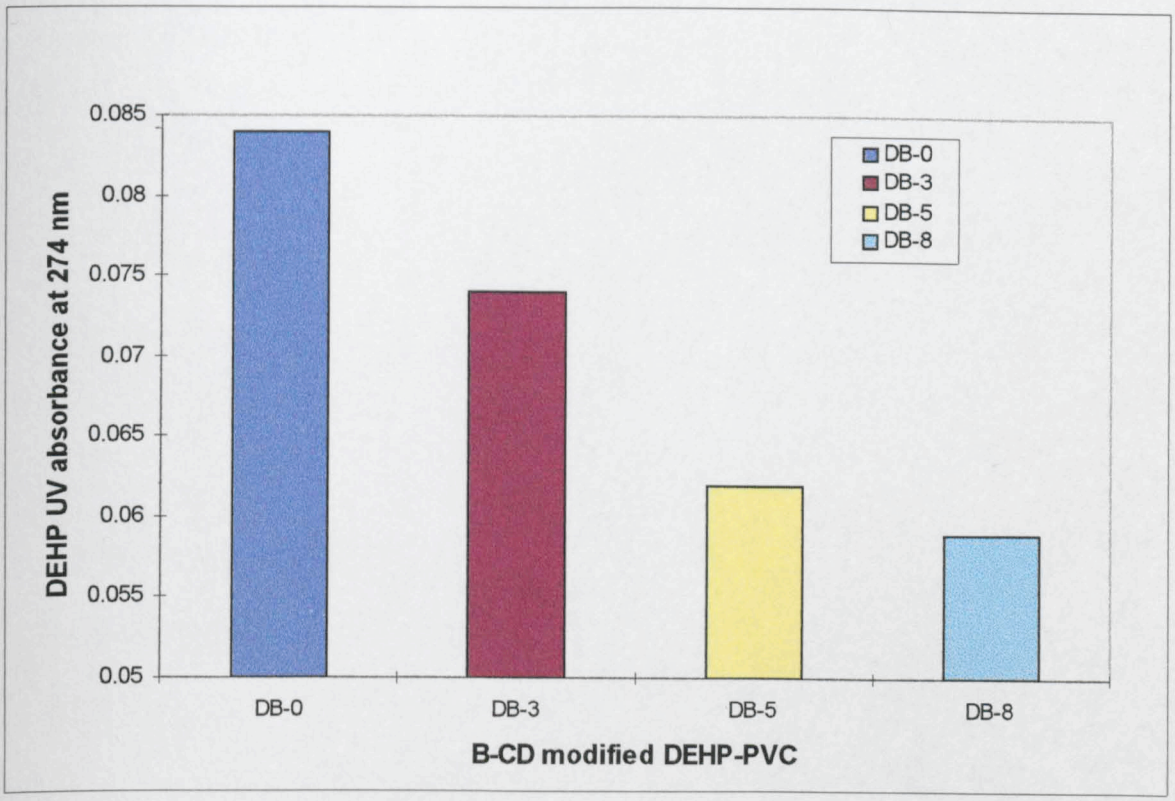


Fig 6.21 Migration test of B-CD modified PVC-DEHP in methanol

(Extraction time: 5 minutes)

Table 6.8 summarises the correlation of absorption peaks with functional groups of β-CD, PEO and PEO-PPO-PEO. From these results, it can be concluded that the incorporation of β-CD or their combination with PEO would alter the surface chemical structures of polymers including PE and PVC-DEHP.

Table 6.8 Assignment of adsorption band of ATR-FTIR spectra to specific functional groups of β-CD, PEO and PEO-PPO-PEO.

Groups	absorption (cm-1)	appearance
O-H stretching vibration	3400~3230	broad
Primary C-OH	1350~1260	
C-O stretching vibration (ether linkage)	1200~1010	s
OH/H_2O bonding vibration	1650~1730	s

6.4 Migration test of CDs modified PVC-DEHP

One of objectives for utilisation of CDs is to retard DEHP migration during contact with media solution such as blood components or other extractants. In this section, two types of extractants were selected for migration assessment. One was methanol and the other was cottonseed oil. The selection was based on the solubility of DEHP in these media. Methanol is an excellent solvent for the dissolution of DEHP, while cottonseed oil is less. The migration test of CDs modified PVC-DEHP in methanol was monitored by UV-spectrophotometry while in cottonseed oil the change in weight loss during extraction period was checked for evaluating the DEHP migration behaviour. Fig 6.21 gives the DEHP migration results of β-CD modified PVC-DEHP using methanol as extractant. It was shown that the extractability of modified PVC-DEHP by methanol was restricted due

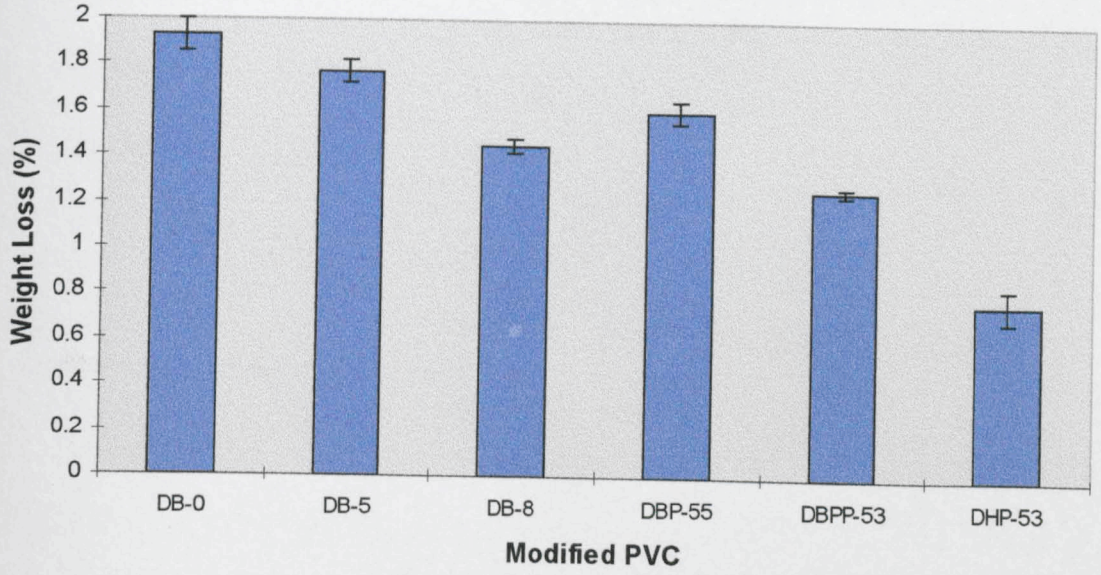


Fig 6.22 Migration test of CDs modified PVC-DEHP using cotton seed oil as an extractant (extraction time: 24 hours)

to the presence of β-CD at the surface, with the higher incorporated amount of β-CD, the more migration resistant. Methanol is such a powerful solvent for DEHP that a longer extraction time than 10 minutes would cause no difference in the DEHP migration property of CDs modified PVC-DEHP. Therefore, cottonseed oil, which is milder than methanol, was selected as an extraction medium for investigation of the effectiveness of CDs for retardation of DEHP migration. Table 6.9 and Fig 6.22 give the migration test results. It was shown that in the first 24 hours contact with cottonseed oil, the modified PVC-DEHP shows a significant migration resistant effect compared to non-modified PVC-DEHP. The surface with improved hydrophilicity due to the incorporation of PEO and hydroxylpropyl-β-CD (HP-β-CD) gave more promising results. However, the longer extraction will lead to a similar final weight loss percentage at 120 hours. In some cases, for examples, DBP-55 and DBPP-53, the results were even higher than that of non-modified PVC-DEHP.

Table 6.9 Migration test of CDs modified PVC-DEHP in cottonseed oil *

SAMPLE		DB-0	DB-5	DB-8	DBP-55	DBPP-53	DHP-53
	24h	1.93±0.07	1.77±0.04	1.45±0.03	1.60±0.02	1.26±0.04	0.77±0.07
Weight loss (%)	48h	4.63±0.05	4.60±0.03	4.58±0.05	4.68±0.06	4.64±0.02	3.18±0.04
	120h	7.98±0.04	7.84±0.04	7.56±0.02	8.11±0.07	8.31±0.05	7.12±0.04

** Migration Test Condition: 4 cm^2 (two-side) flat sheet was placed in a 6-well cell and 10 ml of cottonseed oil was added for extraction at 37℃ from 24h to 120h.*

6.5 Discussion

6.5.1 Effects of CDs, PEO /CDs or Pluronic F68 /CDs combination on protein dsorption

Modification of biomaterials for improved blood compatibility can be achieved by many approaches, which have been critically reviewed in Chapter 3. The work presented in this chapter is an attempt to develop a novel approach to provide biomaterials including PVC-P and polyethylene (PE) control material with improved blood compatibility. The objective of utilisation of CDs is to try to reconstruct a surface with a balance of hydrophilicity and hydrophobicity through CDs' unique chemical structures, which might be beneficial for improving blood compatibility. Therefore, the key point is to make sure that CDs will migrate to the polymer surface during polymer melting processing.

PE without any major additives was selected as a control material. The incorporation of β-CD and its combination with PEO and Pluronic F68 into PE blending system have been found to be effective in reducing protein adsorption. The surface characterisation of these modified surfaces using ATR-FTIR shows that β-CD would appear at the PE surface due to the IR absorption at 1020cm^{-1} ~ 1260 cm^{-1} assigned to C-O-C linkage of β-CD. In addition, the specific OH vibration at 3350 cm^{-1} also confirms the β-CD presents at the PE surface. The β-CD/PEO and β-CD/Pluronic F68 would lead to these additives more enriched surface, which is evidenced by the remarkable IR absorption at 3200~3500 cm^{-1}, 1020 ~ 1260cm^{-1} and 1600 ~ 1730 cm^{-1}. From these results, it could be concluded that the CDs or their combination with PEO or F68 would alter the polymer surface structure, which determining the blood compatibility in terms of protein adsorption.

In the case of PVC-P modification, either CDs or their combination with PEO or F68 influenced the protein adsorption. In some cases with a suitable feeding ratio of β-CD/PEO or β-CD/F68, there seems to have a synergistic effect on reduction in protein adsorption. It was believed that the alteration of the protein adsorption properties of

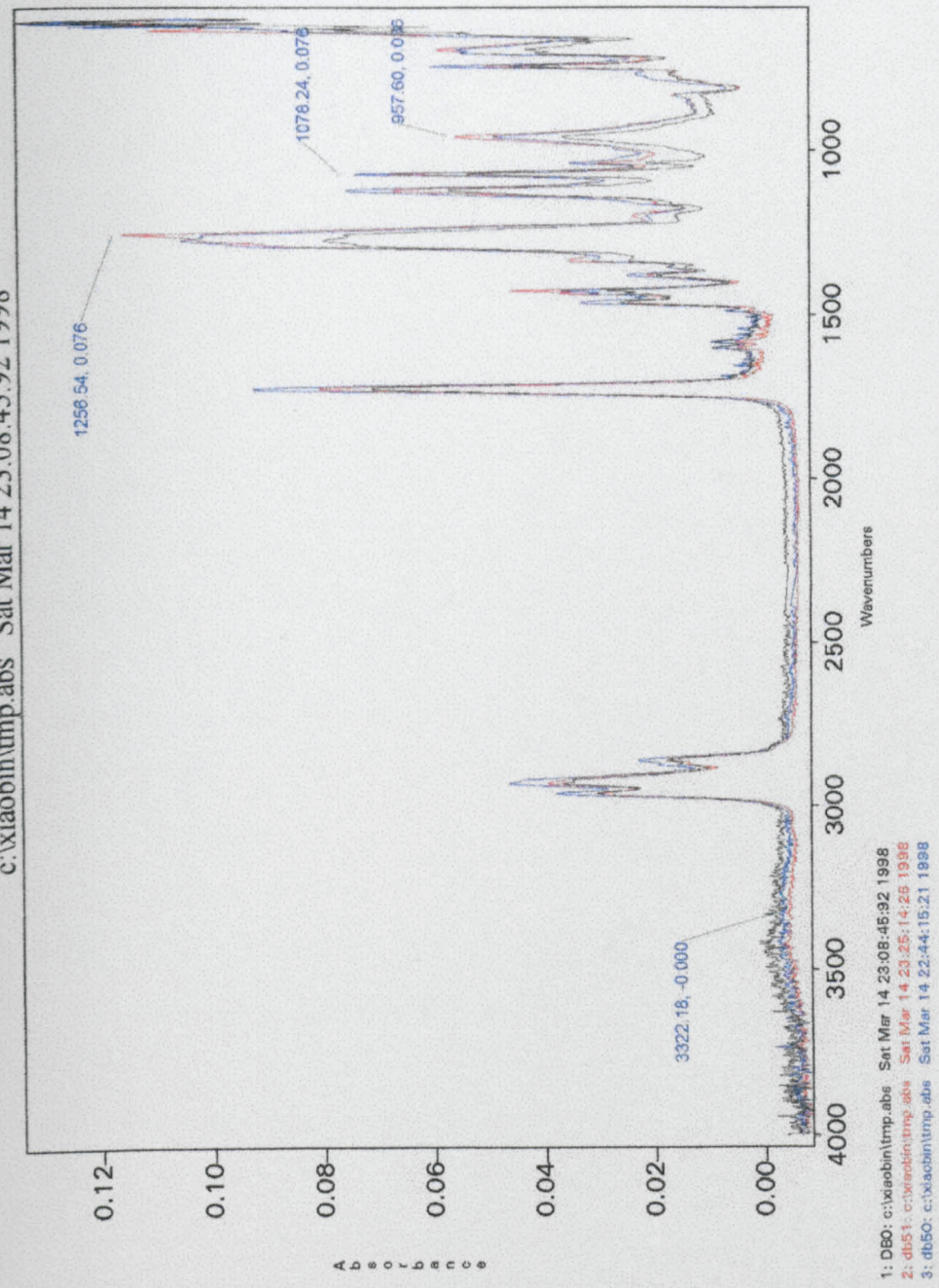


Fig 6.23 ATR-FTIR spectra of methanol surface treated B-CD modified PVC-DEHP (DB5o: original 5% B-CD modified ; DB51: 30 minutes washed)

modified PVC-P must be related to the surface structures. By using ATR-FTIR, the surface of modified PVC-DEHP was characterised. Similarly, the appearance of an absorption band at 3200 ~ 3500 cm^{-1}, 1600 ~ 1730 cm^{-1} and the remarkable increase in intensity at 1020 ~ 1320 cm^{-1} indicate the surface is enriched with β-CD, β-CD/DEHP inclusion complex, β-CD/PEO or β-CD/F68 combination. Unlike PE, the PVC-P system is so complicated and many additives are present at the surface. From the ATR-FTIR spectra, although it is very difficult to predict the proportion of CDs at the surface, the new peaks or increased absorption intensity do suggest that CDs or their combined substances are present at the surface.

For confirming the effects of CDs on surface protein adsorption properties, the methanol surface extraction technique was again employed. It was believed that there are many components presented at the PVC-DEHP surface, including DEHP, β-CD, β-CD/DEHP inclusion complex, β-CD/other additives aggregates, and PVC. The methanol surface treatment would remove surface DEHP more easily than other components due to its relatively lower molecular weight and high solubility in methanol. It is still possible to get β-CD/DEHP inclusion complex removed from the surface depending on the extraction time. The ATR-FTIR spectra of washed DB-5 (DB-5-1) (30 minutes surface treatment) and original DB-5-0 showed that there was a great increase in intensity at 1240 cm^{-1}, which perhaps indicates the increase of β-CD proportion at the surface (Fig 6.23). The appearance of a pronounced absorption band at 3300 cm^{-1} for both washed and unwashed samples indicates the presence of OH groups at the surface. Unlike the ATR-FTIR spectra of surface treated un-modified PVC-DEHP, there is no reduction in intensity around 1726 cm^{-1} after surface treating. This might imply that the contribution of IR absorption enhancement due to the β-CD/DEHP inclusion complex formation would compensate the DEHP intensity loss due to its reduced surface level.

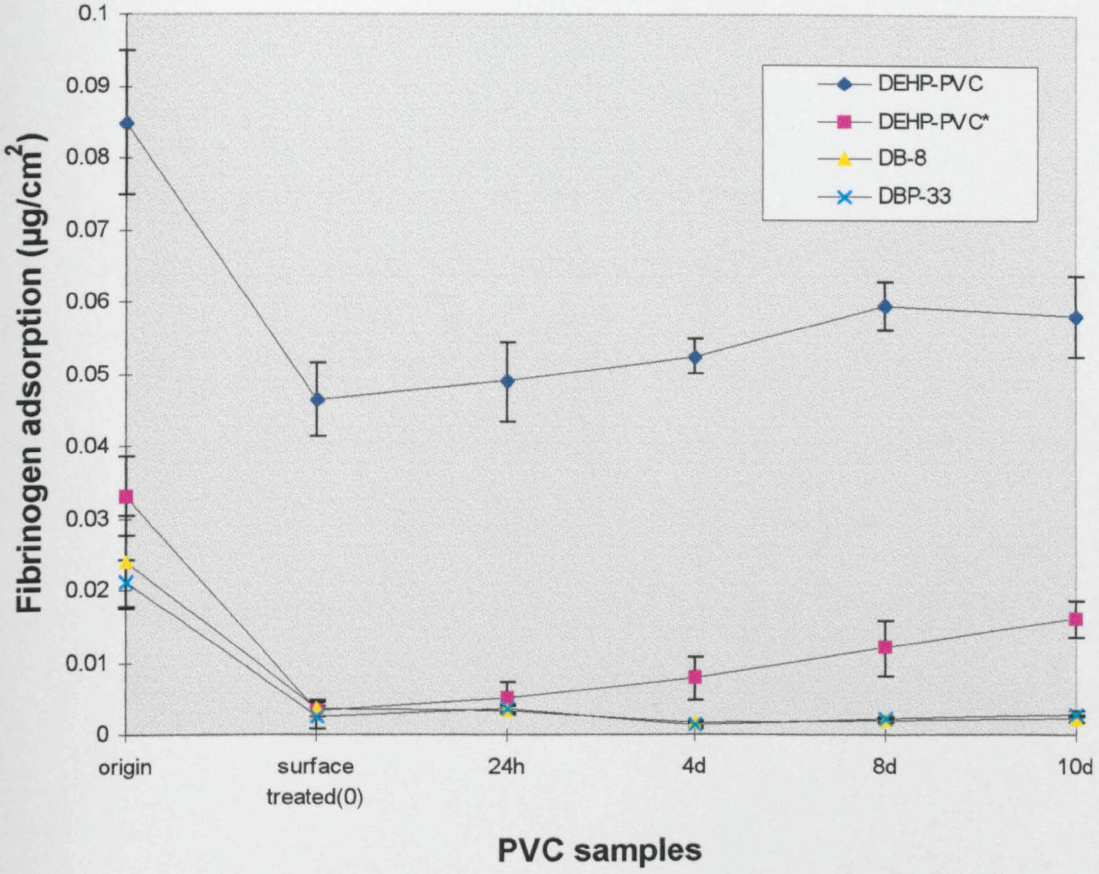


Fig 6.24 Influence of surface treatment and storage on fibrinogen adsorption

PVC-DEHP*, DB-8, DBP-33: Fibrinogen bulk concentration: 1.3microgram/ml

PVC-DEHP: 2.56 microgram/ml

The fibrinogen adsorption on the washed surfaces showed very interesting results (Table 6.10, Fig 6.24). The surface cleaning treatment leads to a dramatic reduction in fibrinogen adsorption, which is more significantly than that of non-modified PVC-DEHP. It indicates that the β-CD and β-CD/PEO would dominate the surface after removal of the surface plasticiser. The β-CD and β-CD/PEO enriched surface must play a more important role than PVC. More interestingly, when these surface cleaned samples were continuously monitored using the fibrinogen adsorption test, it was found that the fibrinogen adsorption capacity of unmodified PVC-DEHP recovered for certain degrees while the CD modified PVC-DEHP could maintain the low fibrinogen adsorption capacity for a rather longer period than ten days. These results strongly indicate the β-CD enriched at the PVC-DEHP surface could possibly restrict the DEHP migration towards the surface.

Table 6.10 Influence of methanol surface treated PVC-P samples and storage afterwards on fibrinogen adsorption (n=9)

FIBRINOGEN ADSORPTION (μg /cm^2) (MEAN ± SD)						
Samples	origin	surface treated(0)	24h	4d	8d	10d
PVC-DEHP *	0.0849±0.01	0.0465±0.005	0.0489±0.0056	0.0525±0.0024	0.0595±0.0034	0.058±0.0056
PVC-DEHP	0.033±0.006	0.0035±0.001	0.0052±0.002	0.0078±0.003	0.012±0.0037	0.016±0.0025
DB-8	0.024±0.003	0.0036±0.0015	0.0035±0.0004	0.0016±0.0005	0.0021±0.0004	0.0023±0.0004
DBP-33	0.021±0.006	0.0024±0.0012	0.0036±0.0008	0.0013±0.0005	0.0022±0.0003	0.0029±0.0006

* Fibrinogen adsorption bulk concentration: 2.56 μg/ml; others: 1.3 μg/ml

6.5.2 Migration behavior of CDs modified PVC-P

DEHP migration problems have received great concern since PVC-DEHP remains as a widely applied blood-contacting biomaterial. The β-CD modified PVC-DEHP was able to retard DEHP migration but not significantly in methanol solution after long-time extraction. The incorporation of other additives such as Pluronic F68, in some cases, even promotes the DEHP migration as a surfactant. However, the increased hydrophilicity at the PVC-DEHP surface due to the presence of PEO, β-CD and HP-β-CD effectively retards DEHP migration. This could be found from the migration test in cottonseed oil. HP-β-CD shows a great solubility in water (>60% w/v) which might be effective to improve surface hydrophilicity more effectively than β-CD. In addition, HP-β-CD is more blood compatible in terms of haemolytic effects than β-CD, as shown in Table 6.11. This suggests that CD derivatives modified surface merit future studies.

Table 6.11 Haemolytic effects of CDs (0.4ml suspension of human erythrocytes + 4ml CD solution in 10 mM isotonic phosphate buffer, pH 7.4, 37°C, 30 min)(Szejetli, 1994)

CYCLODEXTRIN	CDS CONCENTRATION (mg/ml)	
	No haemolysis	50% haemolysis
α-CD	5.7	11.7
β-CD	1.8	7.8
γ-CD	11.0	32.0
HP-βCD(hydroxypropyl-β-CD)	9.0	75
CDPs(Mw=5000)	7.0	37

6.5.3 Novel processing of PVC-P with improved blood compatibility

From the experimental results, it was found that surface enriched CDs, CD/PEO or CD/Pluronic F68 are effective in reducing protein adsorption. The enriched surface can be achieved through a novel polymer process, which can be divided into two steps: step one: polymer blending with CDs or their combination with PEO, or Pluronic F68; step two: surface removal of DEHP plasticiser. However, the possible surface damage due to excess washing should be avoided, since this could lead to a change in surface topography.

6.6 Summary

In this chapter, protein adsorption on CDs modified PVC-P was investigated. The modification technology involved is polymer blending, which is industry relevant. CDs are found to be able to accumulate at the PVC surface, which was evidenced from surface characterisation. The combination of PEO and Pluronic F68 with CDs would aid CDs move to the surface but the distribution ratio is hard to predict because of their structural similarity. These modified PVC-Ps show a remarkable protein resistant surface. The combination of B-CD/PEO and B-CD/F68 in certain feeding ratio seems to be synergistic for such an effect. Since the PVC-P blending system is so complicated, the possible inclusion complex formation between additives and B-CD might cause such an effect. Experience gained in the investigation led to the belief that a more detailed study might provide the basis for a novel form of surface modification. This more detailed study is described in Chapter 7.

CHAPTER 7

CYCLODEXTRIN INCLUSION COMPLEX(CIC) MODIFIED PLASTICISED POLY(VINYL CHLORIDE)

7.1 Introduction

In Chapter 6, cyclodextrins such as α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) , γ-cyclodextrin(γ-CD) and hydroxylpropyl-β-cyclodextrin (HP-β-CD) or their physical mixtures with poly(ethylene oxide) (PEO) and poly(ethylene oxide) (PEO)-poly(propylene oxide) (PPO) nonionic surfactant have been investigated for their influences on protein adsorption of their modified PVC-P surfaces. Polymer solution casting and polymer blending via melting achieved the incorporation of these components.

Cyclodextrins are considered to be such unique natural bioproducts being able to form inclusion complexes with many low molecular weight substances varying from ions, inorganic and organic chemicals to high molecular weight polymers depending on the fitness of their molecular sizes with the cavity of cyclodextrins. The force to form such an inclusion complex is by *van der Waal*'s interaction force, hydrogen bonding and hydrophobic interaction. In 1990, Harada et al (1990; 1990a) first reported that some linear polymers such as polyethylene glycol (PEG) and poly(propylene glycol) (PPG) can form inclusion complexes with cyclodextrins. Later, some other polymers including PPO (Harada,1997) and PEO-PPO-PEO Pluronic surfactants (Topchieva et al, 1993; 1994) have been reported to form inclusion complexes with cyclodextrins. It was generally accepted that only α-CD can form solid complexes with PEG (MW>400) while PPG can form crystalline complexes with both β-CD and γ-CD. For the PEO-PPO-PEO tri-block copolymer, depending on the each block length, the formed inclusion complexes might be water insoluble or water-soluble. In the case of solid complexes, the PPO blocks are included by β-CD or γ-CD to form polyrotaxanes, while in the latter case, β-CD or γ-CD only are strung along the polymer chain depending on the size of the hydrophilic block. The formed solid inclusion complexes are stable thermally by heating. For example, there is no decomposition found even at 320 °C, i.e. at a temperature higher than that of

nonincluded β-CD, which melts and decomposes below 310 °C, indicating that complexing with PPG stabilises β-CD (Harada & Kamachi, 1990).

Recently, Lemos-Senna et al (1998) found there is an interaction between an amphiphilic γ-CD and Pluronic F68 which is able to form mixed micelles. Unfortunately, they could not separate these micelles by centrifugation due to their monophasic behaviour and low density. Therefore, it was impossible for them to prove the interaction between Pluronic F68 and γ-CD is due to inclusion complexation. We employed the methodology according to Gustavo et al (1997) to prepare the inclusion complex of Pluronic F68 with β-CD. In simple addition of 1.36% of β-CD aqueous solution to a dilute solution of Pluronic F68 (less than 3%), we could not find any precipitation, ie. solid inclusion complex. However, it is still possible for β-CD to react with F68 via hydrophobic interaction between the PPO block and the hydrophobic cavity of β-CD, forming a water soluble inclusion complex. The reason is that there are 76 EO block unit, which is too hydrophilic to allow β-CD to form water insoluble inclusion complexes at PPO block sites. The possible formed inclusion complex structure is shown in Fig 7.1a. It was one of our objectives to use this advantage in order to aid cyclodextrins to move to the surface during the polymer blending process. In addition, it was another objective for us to clarify if there was any influence on surface properties due to the inclusion complex formation between cyclodextrins and PEO, PPO or their copolymers when they are used for surface modification of PVC-P.

In this chapter, two types of β-cyclodextrin inclusion complexes (CICs), β-CD-Pluronic L81(CIC1) and β-CD-PPG-PEG-PPG (CIC2) were prepared and incorporated in PVC-DEHP and PVC-TEHTM by polymer blending. Pluronic L81 and PPG-PEG-PPG were reported to be able to form solid inclusion complexes with cyclodextrins, which being able to produced in lab. In addition, some β-CD or its combination with F68 modified

PVC-P were produced for comparison reason. The comparison between the CIC modified surface and the physical mixtures modified surface were investigated using protein adsorption studies. From these results, a possible mechanism by utilisation of the combination of cyclodextrins and other polymers could be postulated.

7.2 Materials

Pluronic L81 (*(PEO)6-(PPO)39-(PEO)6*, MW 2750) was purchased from BASF Wyandotte Corp., Parsippany, NJ, USA. (PPG)8-(PEG)23-(PPG)8 (MW2000) was from Aldrich Chemicals Ltd. β-CD formed inclusion complexes with L81 and PPG-PEG-PPG were named as CIC1 and CIC2 respectively. Their modified PVC-P samples were prepared by polymer blending in Royalite Plastics.Ltd, Edinburgh, according to the formulation in Table 7.1, in which, M represents the mixture of β-CD and other additives; D represents DEHP-PVC; T represents TEHTM-PVC.

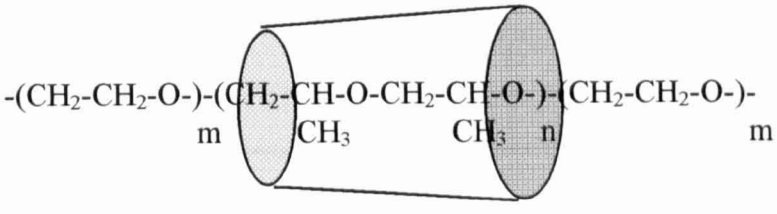


Fig 7.1a β-CD inclusion complexing with PEO-PPO-PEO copolymers

Table 7.1 Formulation of modified PVC-P using β-CD (B-CD)

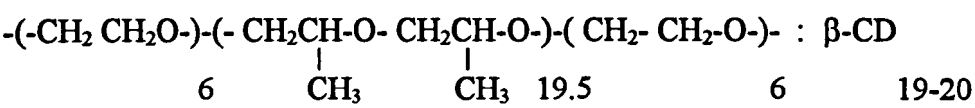
SAMPLES	PVC-DEHP	PVC-TEHTM	CICS (PHR)	MIXTURES(PHR)
DB-0	100	/		
DB-5	100	/		5 (B-CD)
DBPP-35	100	/		3/5 (B-CD/F68)
D-CIC1	100	/	5 (CIC1)	
D-M1	100	/		4.42/0.58 (B-CD/L81)
D-CIC2	100	/	5 (CIC2)	
D-M2	100	/		4.1/0.9 (B-CD/PPG-PEG-PPG)
D-M3	100	/		5 (L81)
D-M4	100	/		4/1 (L81/B-CD)
D-M5	100	/		3/2 (L81/B-CD)
D-M6	100	/		2/3 (L81/B-CD)
D-M7	100	/		5 (PPG-PEG-PPG)
TB-0	/	100		
TB-5	/	100		5 (B-CD)
TBPP-35	/	100		3/5 (B-CD/F68)
T-CIC1	/	100	5 (CIC1)	
T-M1	/	100		4.42/0.58 (B-CD/L81)

The fibrinogen adsorption was carried out by the same method as described in Chapters 5 and 6. However, the fibrinogen bulk concentration was increased to 2.0 μg/ml instead of 1.3 μg/ml.

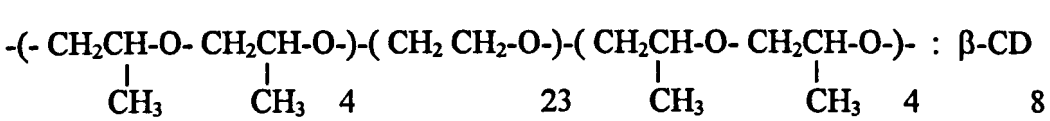
7.3 Results

7.3.1 Preparation of modified PVC-P

When β-CD solution was added to L-81 and PPG-PEG-PPG aqueous solution, during stirring, a large quantity of precipitation forms. This is due to the inclusion complex formation. It is confirmed that only PPG or PPO segment could interact with β-CD and two unit of propylene glycol will bind one β-CD molecule. Therefore, the formed solid CIC1 and CIC2 will have the possible structures as follows (Fig7.1b):



(CIC1)



(CIC2)

Fig 7.1b Structures of β-CD inclusion complexes with L-81 and PPG-PEG-PPG

From the above CIC structures, the modification of PVC-P with β-CD and L81 or PPG-PEG-PPG physical mixtures comparable to the inclusion complexes can be calculated. In addition, PVC-DEHP with different composition of physical mixtures of β-CD and L81 was also formulated in order to evaluate the processibility.

Table 7.2 gives the processing results in terms of plasticisation degree. Results indicate that the addition of β-CD, L-81, PPG-PEG-PPG and CICs would affect the compatibility between plasticiser and PVC in the blending system. Also, the migration of these additives to the surface would be expected due to the incompatibility among these additives, PVC and plasticiser. Only certain feeding compositions of β-CD with L-81 produced excellent blends in sheet form while achieving modified surfaces.

Table 7.2 Processibility of PVC-P blending in the presence of β-CD, CIC and its mixtures

SAMPLES	PROCESSIBILITY	APPEARANCE
DCIC1	+++	transparent
DCIC2	+++	transparent with little un-molten white powder
DM1	+++	transparent
DM2	+++	transparent
DM3, DM4, DM5 DM7	---	pellets, not being able to press into sheet
DM6	++	transparent
TCIC1	+++	transparent
TCICM1	+++	transparent
DBPP-35	++	opaque
TB5	+++	transparent
TBPP-35	+++	opaque

+++: Excellent ; ++: good; ---: poor

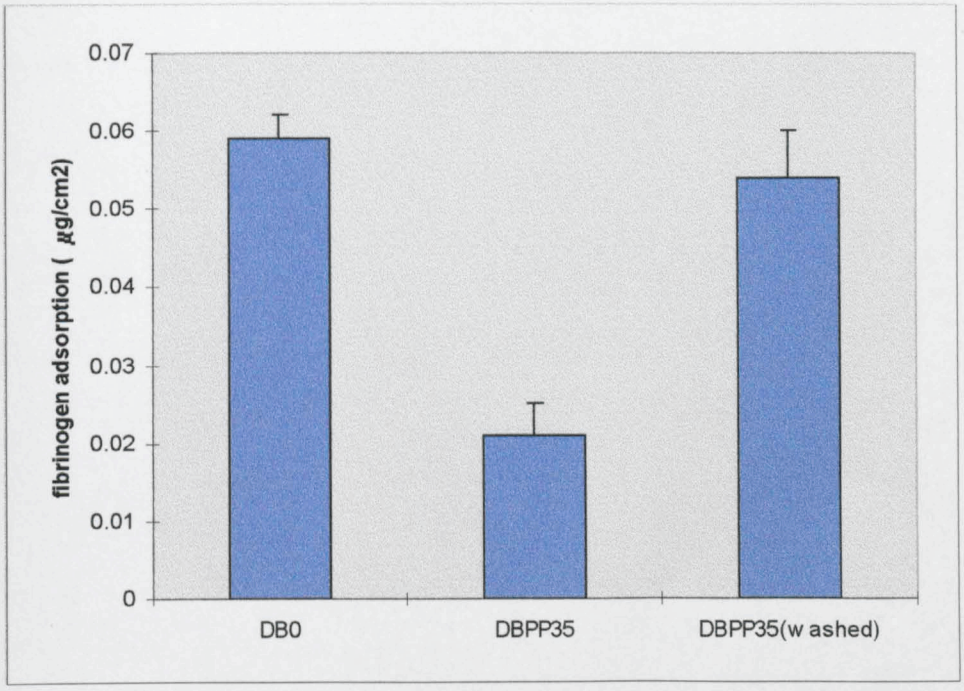


Fig 7.1 Fibrinogen adsorption on B-CD/Pluronic F68 physical mixture modified PVC-DEHP (DBPP-35), compared to PVC-DEHP without B-CD addition and surface methanol washed DBPP-35. (DBPP35: 3phrB-CD and 5phrF68 modified PVC-DEHP).

The processibility of modification of PVC-P changes with different composition. In particular, those with a higher content of surfactant will reduce the plasticisation degree, causing the difficulty of calendering PVC-P. Therefore, a suitable feeding composition for β-CD with Pluronic L81 is β-CD >=3 and L81<=2.0 in total 5 phr. The utilisation of higher molecule weight Pluronic F68 reaches the limit of 5 phr for F68 and 3 phr for β-CD without affecting too much the processibility, while the transparency is reduced. Similar results were also obtained for PVC-TEHTM.

7.3.2 Fibrinogen adsorption studies

Firstly, to confirm the effectiveness of β-CD modified PVC-P in reducing fibrinogen adsorption, β-CD or its combination with Pluronic F68 at a different composition as that in Chapter 6 were repeated for PVC-P modification by polymer blending. Again, it could be clearly seen that the combination of β-CD with F68 with 3/5 feeding ratio (by phr) significantly reduced fibrinogen adsorption, which is in agreement to the previous studies. This is shown in Fig 7.1. In this chapter, a higher fibrinogen bulk concentration was taken to increase the counting number for statistical purposes. It has already been shown in Chapter 5 and Chapter 6 (Fig 6.24) that fibrinogen adsorption on PVC-P is fibrinogen bulk concentration dependent. The increase in fibrinogen bulk concentration leads to an increase in fibrinogen adsorption capacity of plasticised PVC. Comparing the fibrinogen adsorption of DB-0 and DB-5 as measured in Chapter 6, the fibrinogen adsorption capacity increased from 0.032 to 0.059 $\mu g/cm^2$ and 0.028 to 0.042 $\mu g/cm^2$. This does not mean that there is any change in the fibrinogen adsorption pattern with respect to the reactivity towards blood.

Surface methanol treatment was used to clean the β-CD/Pluronic F68 modified PVC-DEHP with the same treatment condition as before. Unlike the results of β-CD alone or the combination of β-CD and PEO modified PVC-DEHP as shown in Chapter 6, the

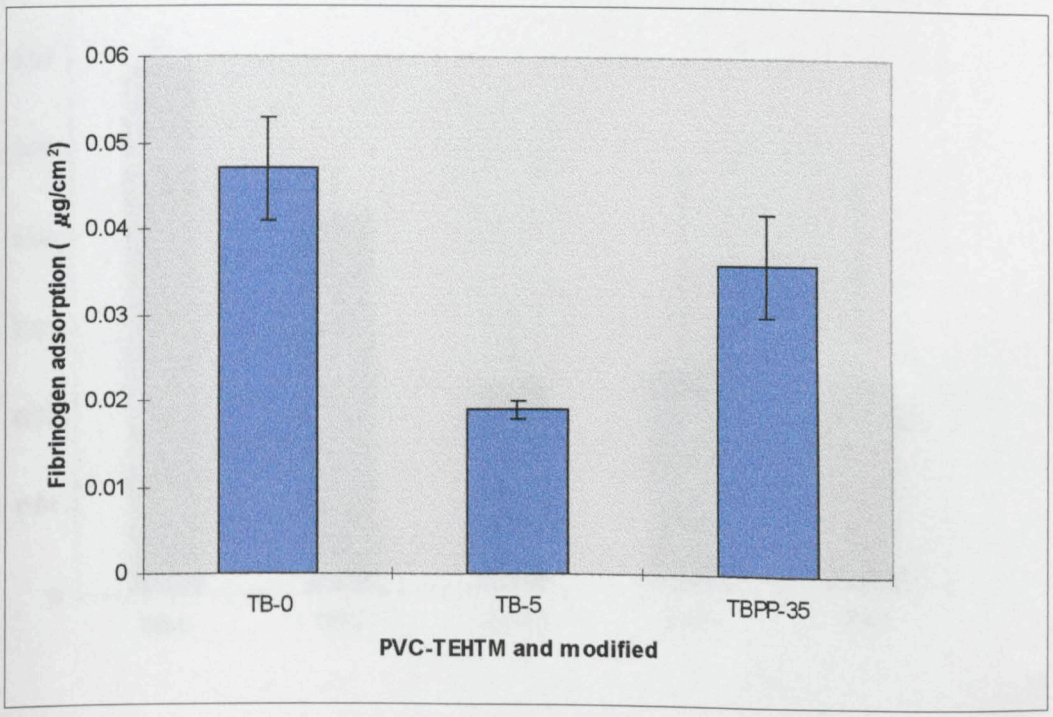


Fig 7.2 Fibrinogen adsorption on PVC-TEHTM without B-CD (TB-0), with B-CD (5phr) and B-CD/Pluronic(3phr B-CD/5phrF68) modification.

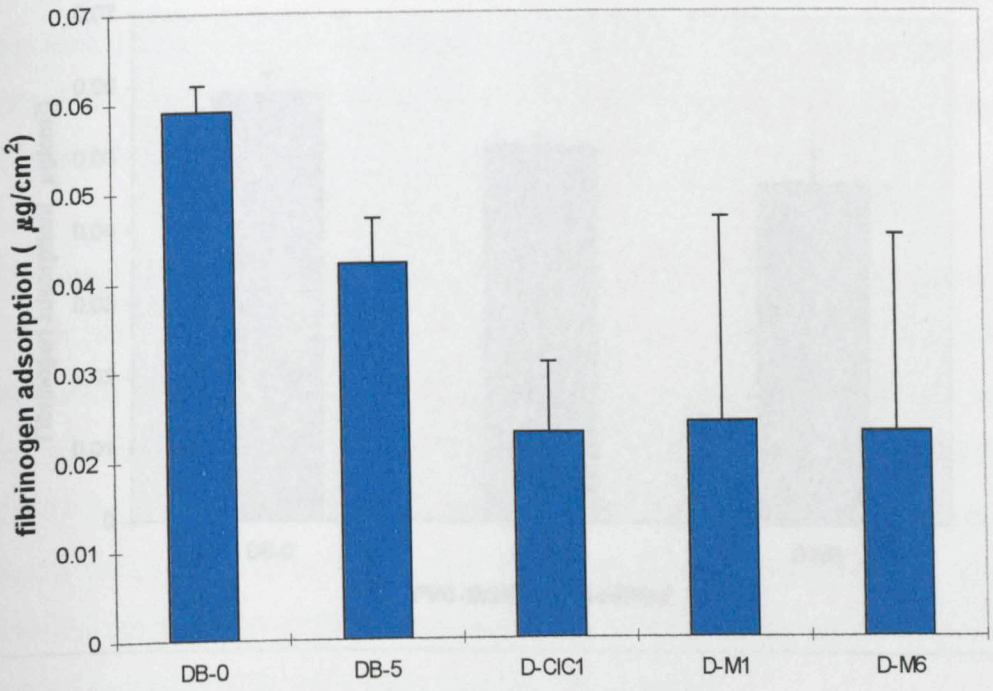


Fig 7.3 Fibrinogen adsorption on B-CD/Pluronic L81 inclusion complex (CIC1) modified PVC-DEHP (D-CIC1), compared to PVC-DEHP without B-CD(DB-0), with B-CD alone, PVC-DEHP modified with physical mixture of B-CD and L81 in different composition (D-M1 and D-M6)(as shown in formulation Table 7.1)

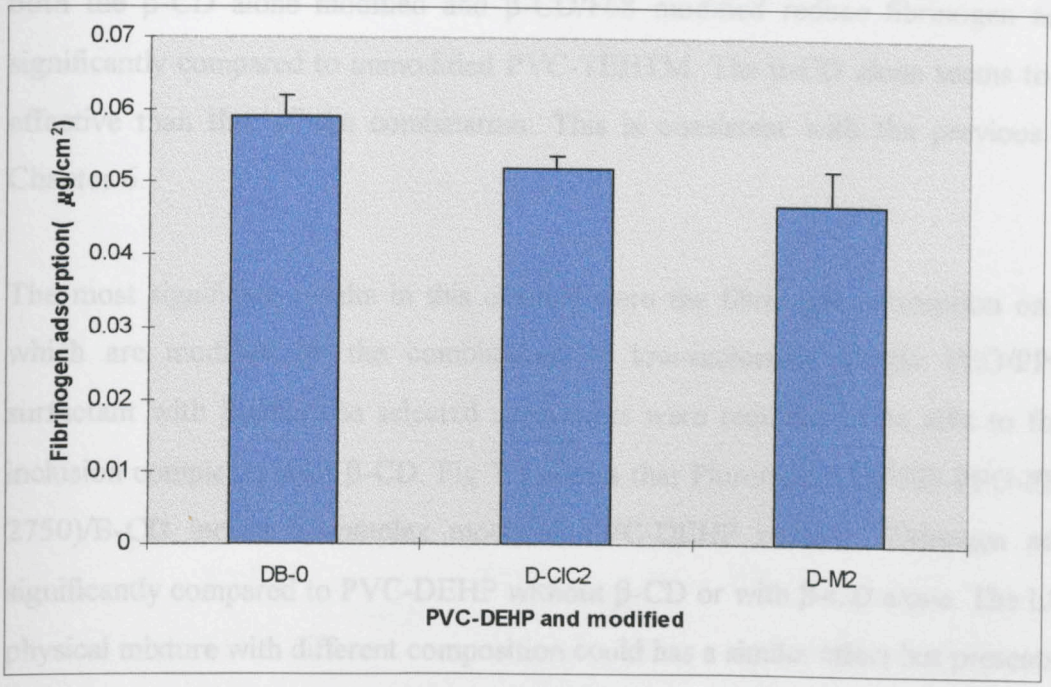


Fig 7.4 Fibrinogen adsorption on B-CD/PPG-PEG-PPG inclusion complex (D-CIC2) modified PVC-DEHP, compared to PVC-DEHP without B-CD modification (DB-0), and PVC-DEHP modified with physical mixture of B-CD and PPG-PEG-PPG (D-M2).

methanol surface treatment of β-CD/Pluronic F68 caused an increase in fibrinogen adsorption as shown in Fig 7.1. The significance of this result is discussed in section 7.3 from the surface characterisation results.

Fig 7.2 shows the fibrinogen adsorption of modified PVC-TEHTM. It can be seen that both the β-CD alone modified and β-CD/F68 modified reduce fibrinogen adsorption significantly compared to unmodified PVC-TEHTM. The β-CD alone seems to be more effective than that of the combination. This is consistent with the previous study in Chapter 6.

The most significant results in this chapter were the fibrinogen adsorption on PVC-Ps which are modified by the combination of low-molecular weight PEO/PPO block surfactant with β-CD. The selected surfactants were required to be able to form solid inclusion complexes with β-CD. Fig 7.3 shows that Pluronic L81 (PEO-PPO-PEO, MW 2750)/B-CD inclusion complex modified PVC-DEHP reduces fibrinogen adsorption significantly compared to PVC-DEHP without β-CD or with β-CD alone. The L81/β-CD physical mixture with different composition could has a similar effect but presents a wider range of measurement statistically than the CIC modified.

In Fig 7.4, when the sequence of block copolymer of PEO/PPO was changed to PPO-PEO-PPO (PPG-PEG-PPG) (MW=2000), the structure of formed inclusion complex is different from the Pluronic L81/β-CD inclusion complex, which results in a different pattern of fibrinogen adsorption. The results indicate that the degree of reduction in fibrinogen adsorption is not so significant as that of the PEO-PPO-PEO sequence. Even in a very low percentage of PPG-PEG-PPG combined with β-CD (D-M2), the presence of this surfactant could restrict the function of β-CD in its reduction of fibrinogen adsorption. This indicates that the sequence of PPO/PEO copolymer is important in its

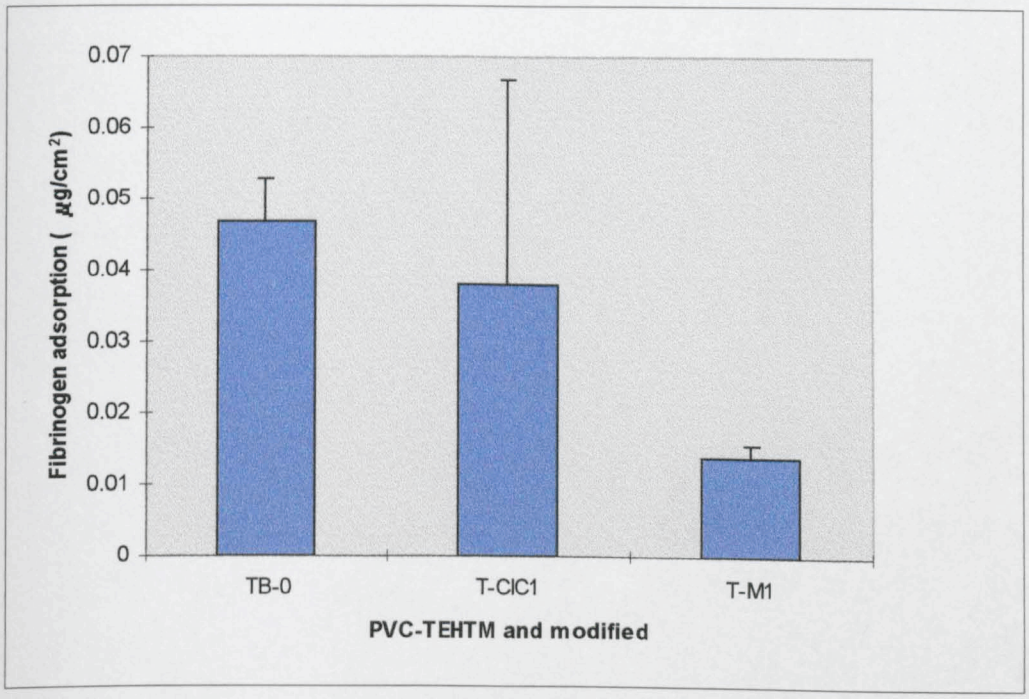


Fig 7.5 Fibrinogen adsorption on CIC1 modified PVC-TEHTM (T-CIC1), compared to PVC-TEHTM without B-CD and with B-CD/L-81 physical mixture (T-M1)

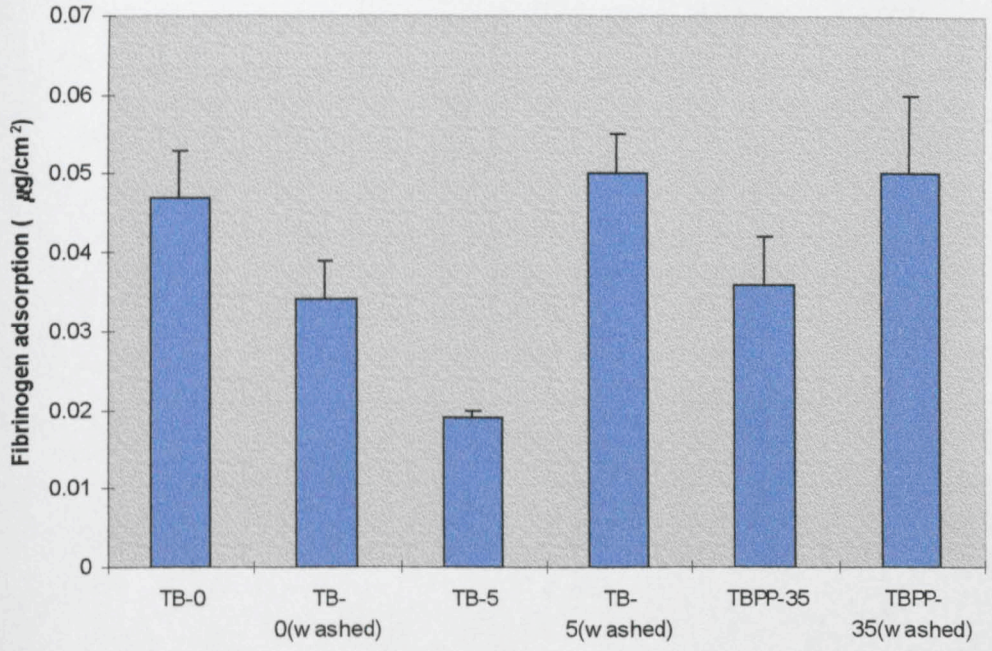


Fig 7.6 Fibrinogen adsorption of PVC-TEHTM, B-CD modified PVC-TEHTM, B-CD/F68 mixture modified PVC-TEHTM, compared to a further surface methanol treatment. (treatment time: 30 minutes; room temperature; volume (ml)/surface area (cm^2) = 2:1)

reactivity towards fibrinogen adsorption. This could be the reason that surfactants with the sequence of PEO-PPO-PEO are the most commonly utilised for biomaterial modification.

With respect to modification of PVC-TEHTM, which has a higher plasticiser level than PVC-DEHP, the fibrinogen adsorption results in Fig 7.5 show that the inclusion complex modified PVC-TEHTM has a wider range of measurement statistically (SD) than the physical mixture modified. The physical mixture modified PVC-TEHTM shows the lowest fibrinogen adsorption capacity.

Fig 7.6 gives the results of the influence of surface methanol treatment on fibrinogen adsorption. Surprisingly, the modified PVC-TEHTM either with β-CD alone or its combination with Pluronic F68 displays an increase in fibrinogen adsorption after surface methanol treatment, regardless of the reduced plasticiser level at the surface. This significance is discussed in section 7.3.

In summary, the study on fibrinogen adsorption of β-CD/Pluronic F68 modified PVC-P shows again the effectiveness of this combination in terms of reduction of fibrinogen adsorption. There is no significant difference between CIC1 and M1 modified PVC-DEHP regarding to reduction in fibrinogen adsorption, however, CIC1 modified PVC-DEHP seems to be dispensed more homogeneously than the simple physical mixture, according to the statistical error analysis. With respect to plasticiser selection, there is a different pattern for PVC-TEHTM. The physical mixture modification is more effective in reducing fibrinogen adsorption than the inclusion complex. It is also found that the selected surfactant with different sequence of block can change the fibrinogen adsorption pattern. PPG-PEG-PPG is not as effective in the reduction of fibrinogen adsorption.

For the further surface treatment using methanol, although the surface plasticiser level can be reduced to achieve a plasticiser-free surface, the fibrinogen adsorption surprisingly goes up. This implicates that there are other factors other than plasticiser surface level, which can influence fibrinogen adsorption.

Table 7.1 Fibrinogen adsorption on PVC-DEHP and modified PVC-DEHP (n is the number of measurement)

FIBRINOGEN ADSORPTION ($\mu G/CM^2$)	DB-0 (N=6)	DBPP35 (N=6)	DBPP35 (WASHED) (N=9)	TB-0 (N=9)	TB-5 (N=6)	TBPP35 (N=6)
Mean	0.059	0.021	0.054	0.047	0.019	0.036
SD	0.003	0.004	0.006	0.006	0.001	0.006

Note: Fibrinogen adsorption of PVC CONTROL: 0.018+/-0.002($\mu G/CM^2$); CUPROPHAN CONTROL: 0.015+/-0.003($\mu G/CM^2$)

Table 7.2 Fibrinogen adsorption on PVC-TEHTM and modified PVC-TEHTM using methanol surface treatment for 30minutes (2ml methanol/cm^2 contacted area)

FIBRINOGEN ADSORPTION	TB-0 (N=9)	TB-0 (WASHED) (N=6)	TB-5 (N=6)	TB-5 (WASHED) (N=9)	TBPP-35 (N=6)	TBPP-35 (WASHED) (N=9)
Mean	0.047	0.034	0.019	0.05	0.036	0.05
SD	0.006	0.005	0.001	0.005	0.006	0.01

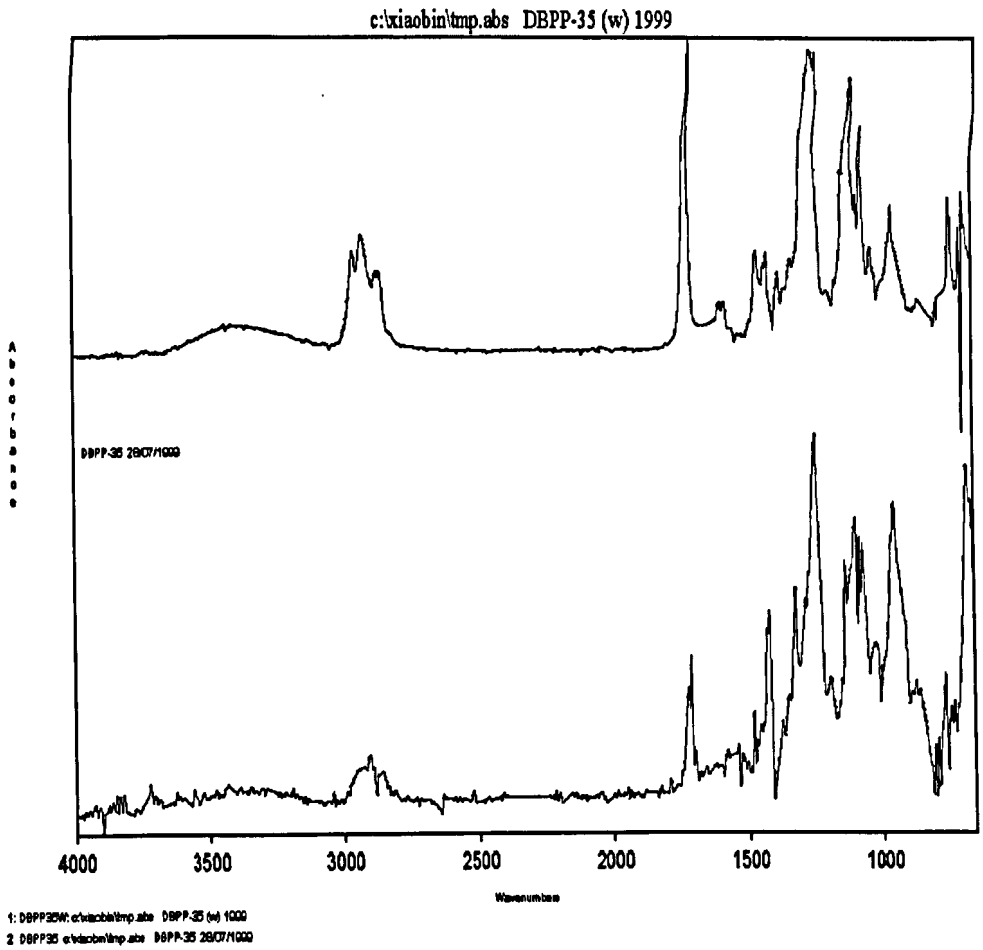


Fig 7.7 ATR-FTIR surface characterisation of DBPP-35 and surface cleaned DBPP-35

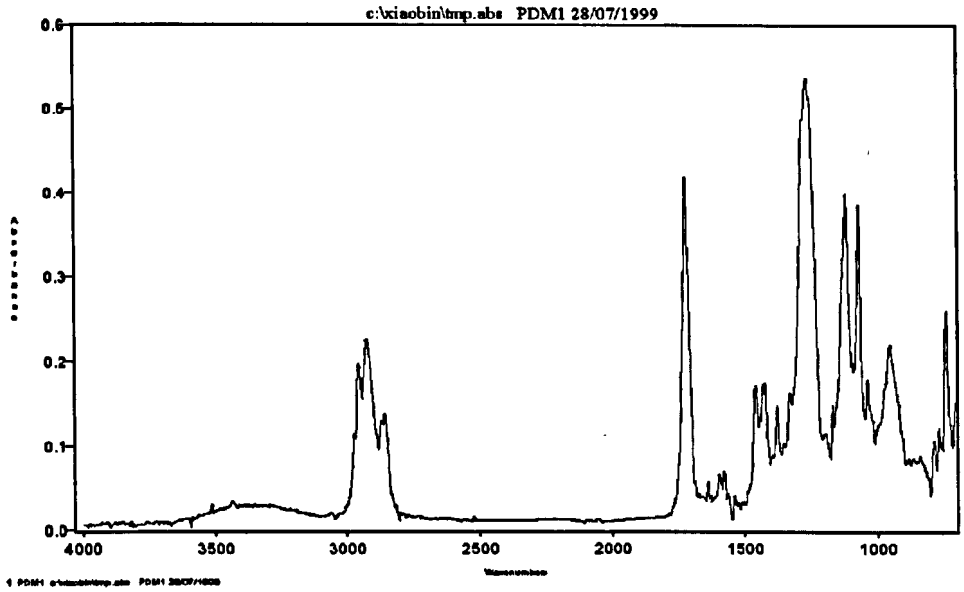
Table 7.3 Fibrinogen adsorption on modified PVC-P by CICs or physical mixtures of β-CD and surfactants

FIBRINOGEN ADSORPTION	DB-0 (N=6)	DB-5 (N=9)	D-CIC1 (N=9)	D-M1 (N=9)	D-M6 (N=9)	D-CIC2 (N=9)	DM2 (N=9)	T-CIC1 (N=9)	T-M1 (N=9)
Mean	0.059	0.042	0.023	0.024	0.023	0.052	0.047	0.038	0.014
SD	0.003	0.005	0.008	0.023	0.022	0.0018	0.0047	0.029	0.016

7.3.3 Surface characterisation using ATR-FTIR

ATR-FTIR surface characterisation technique was employed again in this chapter to clarify the surface chemical characteristics after surface modification using cyclodextrin inclusion complexes (CICs) or simple physical mixtures of β-CD and surfactants and surface methanol treatment.

In Fig 7.7, it is clearly shown that the surface treatment of DBPP-35 using methanol can get a DEHP-cleansed surface. The IR absorption at 2940-2900 cm^{-1} and 1726 cm^{-1} referring to DEHP plasticiser is very weak compared to the unwashed sample However, the IR adsorption at 1425 cm^{-1} and 960 cm^{-1} is increased, which indicates the PVC structure. In addition, the wide band around 3400 cm^{-1} indicates the presence of β-CD and Pluronic F68 at the surface before and after washing. It was also found that after washing, the surface gets rigid which could change the surface roughness. This was very clear from the recorded spectrum of the washed sample. Due to the increased roughness, the attachment between PVC sample and the crystal in the ATR accessory is not as tight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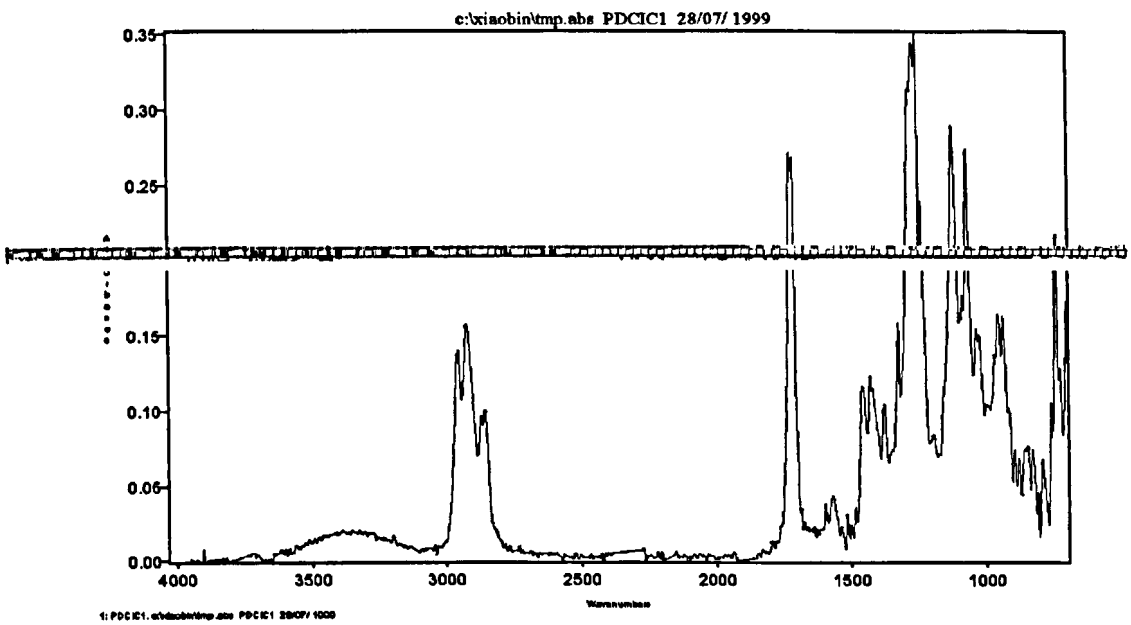
c:\xiaobin\tmp.abs PDCIC1 28/07/1999
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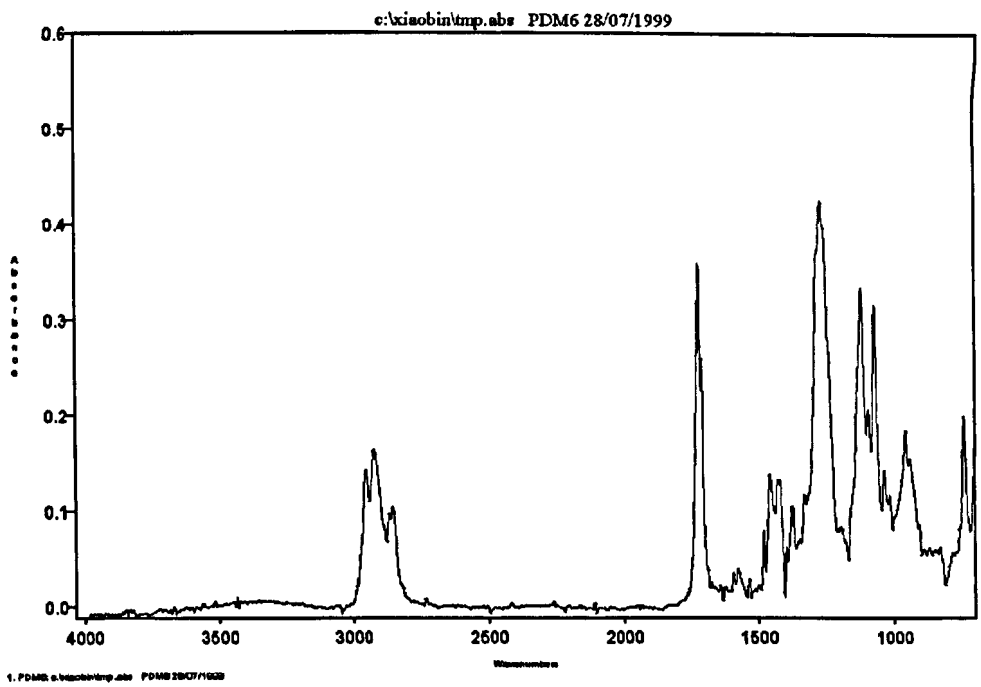

Fig 7.8 ATR-FTIR surface characterisation of CIC-1 modified PVC-DEHP (PDCIC1 or DCIC1), B-CD/Pluronic L81 physical mixture modified PVC-DEHP (PDM1or DM1) both with the same B-CD/L81 composition, while PDM6 or DM6 is the sample modified with B-CD/Pluronic L81 physical mixture with higher Pluronic L81 concentration

as with the unwashed sample. Thus, an increase in noise signal is induced. This is not the case with washed PVC-DEHP modified with β-CD alone or PEO combination as shown in Chapter 6. Ideal spectra can be recorded with low noise signal with their washed samples. All these experimental observation indicates that a surface topographical change would be significant in the case of PVC modification using the combination of β-CD with Pluronic F68. Another possible reason could be due to the occurrence of a reduced density of β-CD/Pluronic F68 molecular layer caused by methanol washing, which leads to an increase in fibrinogen adsorption. It has been postulated that high surface density and long chain length of PEO-type surfactant are desirable for protein resistance (Jeon & Andrade, 1991).

From Fig 7.8, the IR absorption at 3400 cm^{-1} assigned to the νOH vibration indicates the accumulation of β-CD and Pluronic L81 at the surface. CIC1 modified PVC-DEHP seems to have the most pronounced absorption at this wavelength compared to M1 and M6 modified PVC-DEHP. It implies the inclusion complexation between L81 and β-CD could aid β-CD move to the surface. In addition, the strong absorption at 1050-1260 cm^{-1} is partly attributed to νC-O-C of the ether linkage of β-CD and Pluronic surfactant. Therefore, ATR-FTIR surface characterisation gives the indication of its presence of CIC1 or β-CD/L81 physical mixture at the surface. However, it is hard to evaluate the surface level of presented β-CD/Pluronic L81 only using ATR-FTIR. This could be achieved using ESCA (Electron Spectroscopy of Chemical Analysis).

In contrast to Fig 7.7 and 7.8, Fig 7.9 gives the ATR-FTIR results of β-CD/PPG-PEG-PPG modified PVC-DEHP. It is found that the IR absorption at 3300cm^{-1} is not distinguished and the absorption is very weak, which indicates the low level of the presence of β-CD/PPG-PEG-PPG at the surface. This is the reason why there was no

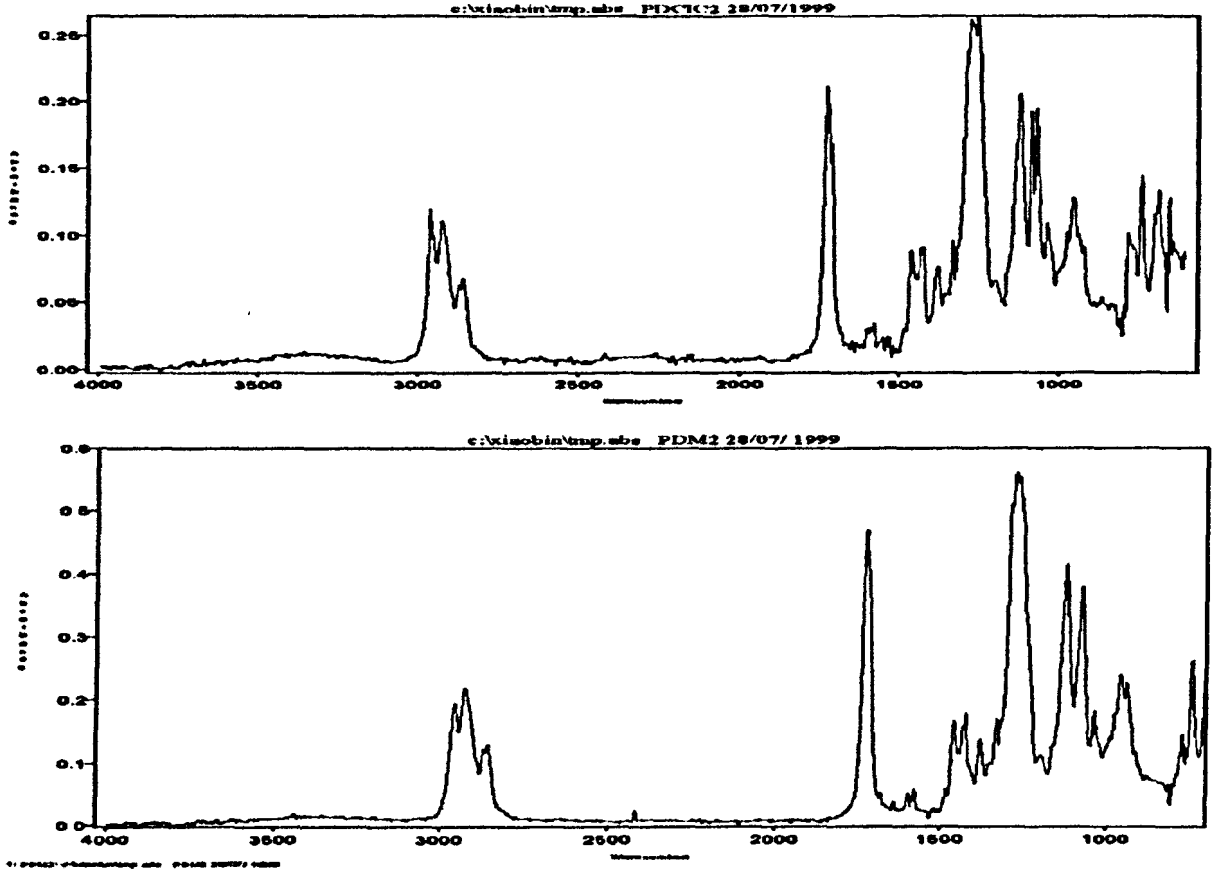

Fig 7.9 ATR-FTIR spectra of D-CIC2(β-CD/PPO-PEO-PPO inclusion complex modified PVC-DEHP) and DM2(mixture modified PVC-DEHP)

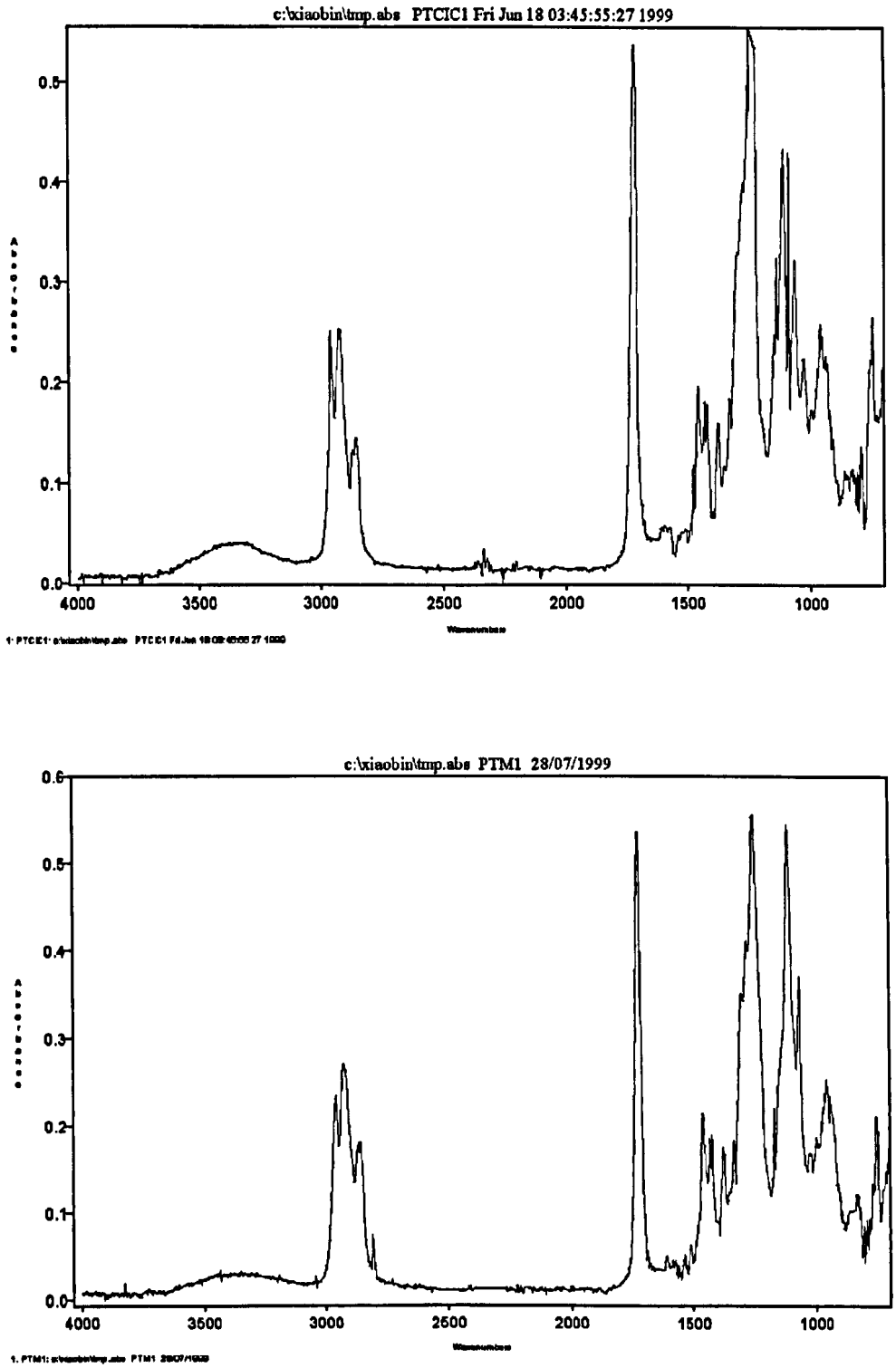

Fig 7.10 ATR-FTIR surface characterisation of CIC1 and M1 modified PVC-TEHTM

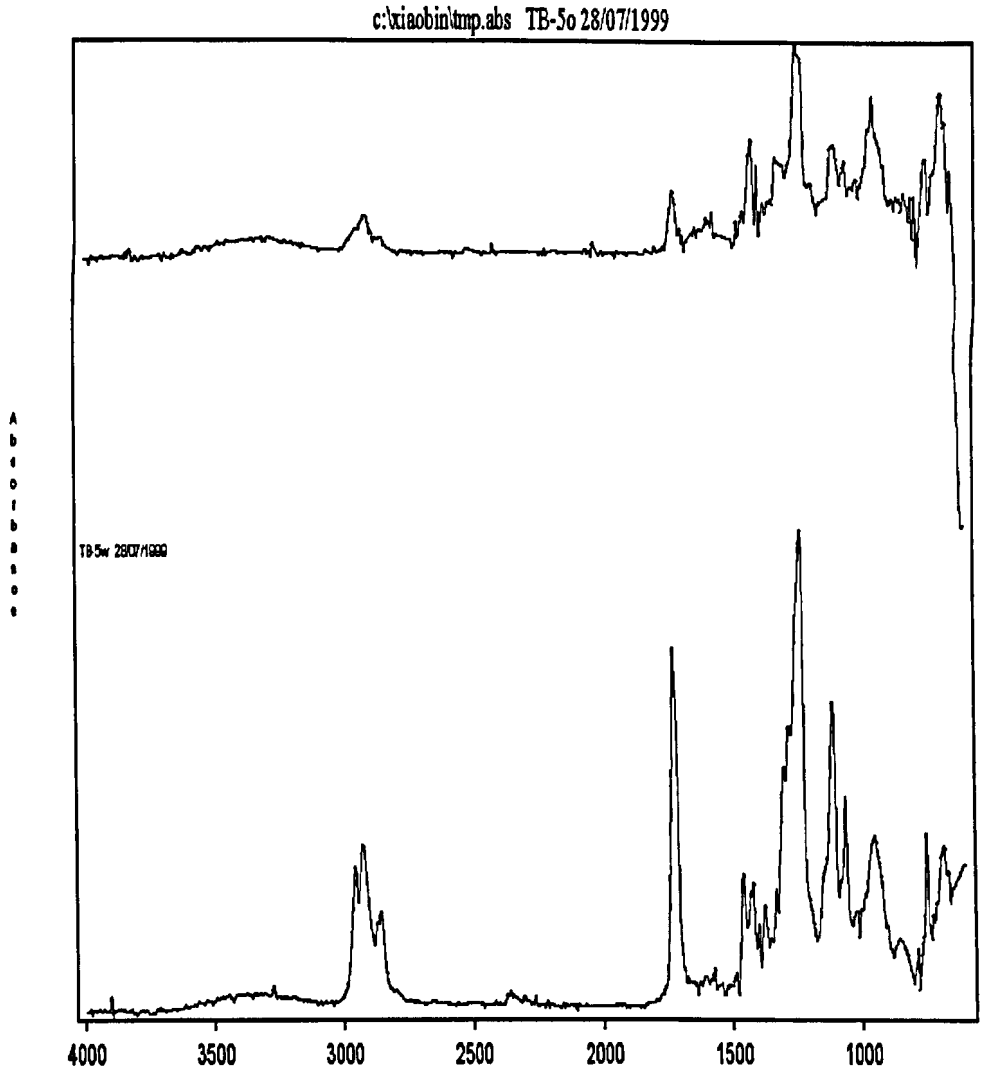

Fig 7.11 ATR-FTIR surface characterisation of original B-CD modified PVC-TEHTM (TB-5 o) and its surface washed sample (TB-5w)

significant reduction in fibrinogen adsorption found using this combination to modify PVC-DEHP.

When the plasticiser is changed from DEHP to TEHTM with a larger molecular weight, the absorption band at 3300 cm^{-1} provides the evidence that CIC1 or the β-CD/L81 mixture are present at the surface. The adsorption band seems to be more pronounced than some of the modified PVC-DEHP samples, as shown in Fig 7.10. The lack of interaction between β-CD and TEHTM could possibly increase the diffusion coefficiency of β-CD to the surface during the blending processing, while the inclusion complex formation between β-CD and DEHP could restrict the diffusion of free β-CD to the surface. This can be seen from Fig 7.10, in which β-CD and L-81 mixture modified PVC-DEHP have less distinguished absorption band at 3300cm^{-1} than CIC1 modified. The reason is that once the hydrophobic cavity of β-CD is occupied by Pluronic L81, there is no free space for β-CD to interact with DEHP. The lack of interaction between CIC1 and DEHP acts to aid CIC1 to the surface. However, there is no significant difference in resistance to fibrinogen adsorption between CIC1 and β-CD/L81 mixture modified PVC-DEHP from the fibrinogen adsorption test as shown in Fig7.4 even though there is a CIC1 enriched surface. This is because of the function of surfactant, which cannot be excluded.

In addition, the surface characterisation of β-CD modified PVC-TEHTM compared to its further methanol surface treated sample using ATR-FTIR shows again the increase of surface roughness, which causes a increase of noise signal as shown in Fig 7.11 due to surface washing. Obviously, the TEHTM level is reduced because of the decrease of intensity at 1726 cm^{-1} and 2900 cm^{-1}, which are characteristic absorption band for phthalate ester plasticiser. Meanwhile, the band at 3300 cm^{-1} seems to be weakening slightly due to washing. This might imply a slight leach of β-CD due to washing.

In summary, ATR-FTIR surface characterisation indicates the possible accumulation of β-CD/L81 either in CIC form or as a physical mixture at the surface. The surface composition could be β-CD/DEHP complex, free β-CD, free Pluronic L81, PVC and free DEHP in the case of physical mixture modified PVC-DEHP. For CIC1 modified PVC-DEHP, the possible surface composition consists of CIC1, PVC and free plasticiser, which would be more straightforward and less complex to understand the surface composition. The β-CD combination with low molecular weight Pluronic surfactant can create a fibrinogen resistant surface, which is more effective than that of β-CD alone. This is a synergistic effect. Even though the low molecular weight Pluronic L81 is less effective than Pluronic F68 in producing a fibrinogen resistant surface by itself, the combination of such a LMW surfactant with β-CD has achieved the protein-resistant surface property, probably without any mechanical property compromise. In this case, the main function of L81 is to help β-CD move more easily to the surface. In addition, the formed inclusion complex could create an ideal balance between hydrophobicity and hydrophilicity at the surface, which is of biomimetic towards the blood environment. This could be beneficial for improving blood compatibility.

For PVC-TEHTM, no interaction occurs due to inclusion complex formation between TEHTM and β-CD. Therefore, it would be advantageous for β-CD to move to surface. This is the reason why the β-CD/L81 mixture modified PVC-TEHTM exhibits a good fibrinogen resistant surface. However, since the miscibility between TEHTM and PVC is not so ideal as DEHP with PVC, the extra addition of B-CD/Pluronic L81 inclusion complex (MW is about 25,750) will definitely increase the process difficulty and alter the surface microphase structure. It is the factor causing higher statistical error in the fibrinogen adsorption study.

The methanol surface treatment leads to a reduced plasticiser surface level. However, it will change the surface topographically. Therefore, it is not necessary to carry out a further surface treatment when using β-CD/Pluronic F68 combination to modify PVC-P.

7.4 Discussion

In this chapter, a novel approach to provide a PVC-P surface with improved blood compatibility in terms of fibrinogen adsorption resistance was achieved. Unlike Chapter 6, two low molecular weight surfactants were selected to combine with β-CD to modify PVC-P. One was Pluronic L81 and another was different sequenced PPG/PEG copolymer, PPG-PEG-PPG. In particular, the β-CD inclusion complex (CIC) was applied for modification of PVC-P. The advantages of using CIC can be summarised as the following points:

1. More easily to move CIC to PVC-P surface to create a fibrinogen resistant surface.
2. More straightforward and easier to evaluate the surface chemical composition.
3. More consistent in evaluation of fibrinogen adsorption of CIC modified PVC-DEHP statistically.
4. Avoiding the leaching of free β-CD and L81 due to a higher MW of CIC.
5. Easier to understand the synergistic effect than to understand that of β-CD/Pluronic F68.
6. More transparent than Pluronic F68 modified material at the same incorporation percentage.
7. CIC1 could be employed to modify polymeric biomaterials other than PVC

According to the fibrinogen adsorption and the ATR-FTIR surface characterisation results, CIC1 was found to be able to accumulate at the surface to achieve a significantly reduced fibrinogen adsorption compared to either PVC-DEHP or PVC-DEHP modified by β-CD alone. The result was more consistent than the physical mixture modified samples. The chemical composition at the surface could be CIC1, DEHP, PVC and other

formulation additives. For the physical mixture (M1) modified PVC-DEHP, the surface chemical composition could be free DEHP, β-CD/DEHP inclusion complex, free L81, L81/β-CD inclusion complex, PVC and other additives. However, it is very difficult to tell the actual composition at this stage using ATR-FTIR.

Unlike Pluronic F68 (MW 8600) modified PVC-P, L81 (low molecule weight) and β-CD is possible to leach to the contacted hydrophilic media. However, the CIC1 with MW25, 750 could limit its leachability due to the entanglement in the surface, as with Pluronic F68 or it's combination with β-CD. This could be assessed by measuring the concentration in the extractant such as PBS buffer solution. However, the possible leaching of plasticisers and other additives leads to a difficulty to assess the content of β-CD and L81. This could be simply done if radiolabelled β-CD was utilised.

Since Pluronic F68 cannot form solid inclusion complex with β-CD by commonly used preparation methods, it was not possible to use β-CD/F68 complex to modify PVC-P. Therefore, it is difficult to understand the possible mechanism of the synergistic effects on fibrinogen adsorption resistance by the F68/β-CD combination. However, in the case of combination of β-CD with L81, where L81 is not so effective in retarding fibrinogen adsorption as F68 does because of its lack of long PEO chain, the CIC1 modification was clearly shown to be a synergistic effect, which is better than either β-CD alone or L81 alone. For the physical mixture modified PVC-DEHP, the poor consistency in measurement indicates its poor processibility, in addition to the possibility of leaching of low molecular weight substances into contacted media. This could rule out its practical application.

In addition, CIC1 could possibly be used for modification of other polymers, such as polyurethane and polyethylene containing less additives than PVC. This provides another opportunity to modify polymeric biomaterials for improved blood compatibility.

The results also showed that surface methanol treatment was not effective in reducing fibrinogen adsorption as Chapter 6 reported for β-CD/F68 modified PVC-P. This is due to the surface topographical change. In addition, the decrease in density of the Pluronic layer could be another reason for increased fibrinogen adsorption. Therefore, it is not recommended to add another surface treatment in the modification process for β-CD/F68 modified PVC-P unless there is no influence on the surface topography during the cleaning process. The results also indicate the importance of processing, which could change the microphased surface structure leading to an alteration of fibrinogen adsorption. In consideration of the possible influence on the surface morphology or topographical change by methanol surface treatment, it would be interested to see how the correlation could be between surface treatment and surface roughness using Atomic Force Microphotography (AFM). This is discussed in chapter 8.

7.5 Hypothesis of β-CD/Pluronic combination for improving blood compatibility

Based on the data of fibrinogen adsorption and ATR-FTIR surface characterisation of B-CD/Pluronic modified PVC-P, coupled with the ATR-FTIR surface characterisation of β-CD/Pluronic modified polyethylene control material, it is clear that the reduced fibrinogen adsorption is due to the altered surface chemical composition of PVC-P. The presence of β-CD, Pluronic surfactant, the formed inclusion complex between them, and formed β-CD/DEHP inclusion complex in the case of PVC-DEHP modification at the polymer surface could be the key reason for achieving low fibrinogen adsorption. In addition, the reduced plasticiser surface level due to β-CD/Pluronic incorporation could be another reason.

Why could a synergistic effect on reduction of fibrinogen adsorption be achieved when polymer surface was modified by β-CD/Pluronic combination?

Pluronic F68 and other higher molecular weight Pluronic surfactants such as F88 and F108, which consist long PEO chain length have been found to be capable of building a protein resistant surface, with the longer chain the better (Li et al, 1991). The key mechanism is that the flexible long PEO chain can create an ideal hydrophilic surface, which is also very dynamic. However, it was also reported that a stable surface configuration was essential for improving blood compatibility, in which Pluronic surfactant at the surface exhibited a dense layer instead of a loose structure (Jeon & Andrade, 1991). It was also found that it was not necessary to have a very long PEO chain length to achieve such a protein resistant property (Lopez et al, 1992).

In the case of β-CD and Pluronic F68 combination, the inclusion complex formation between β-CD and F68 was expected to develop a stable configured surface, while maintaining the flexibility of PEO. The complex block of β-CD and PPO or PPG is much bulky, which could restrict the configuration change. Therefore, CD could be acting as an "ANCHOR" to fix Pluronic surfactant at the surface. This "anchoring hypothesis" could be the mechanism to explain the synergistic effects on fibrinogen adsorption. Already, there is evidence that the loss of this configuration due to surface washing can change the fibrinogen adsorption pattern. Furthermore, when β-CD/L81 inclusion complex (CIC1) was applied for modification of PVC-P, where L81 has a shorter PEO chain than F68, it is almost impossible to get long flexible PEO-chains at the surface, in contrast to Pluronic F68, F88 and F108. However, the extremely stable configuration at the surface with an increased hydrophilicity due to β-CD polysaccharide could also achieve the synergistic effect successfully. Fig 7.12 shows the possible structures and configuration of β-CD/Pluronic complex modified surface, which has the effect of reducing fibrinogen adsorption.

In the case of modification of PVC-P, because of the blend system containing large quantity of additives including plasticiser, heating stabiliser, lubricant, and others, the possible inclusion complexation of β-CD with these additives will also influence the fibrinogen adsorption through the balance of hydrophilicity and hydrophobicity. In addition, the interaction between β-CD/Pluronic surfactant and PVC blends will result in different chemical surface composition and surface microphased structure. All of these factors have to be considered when assessing blood compatibility of modified PVC-P.

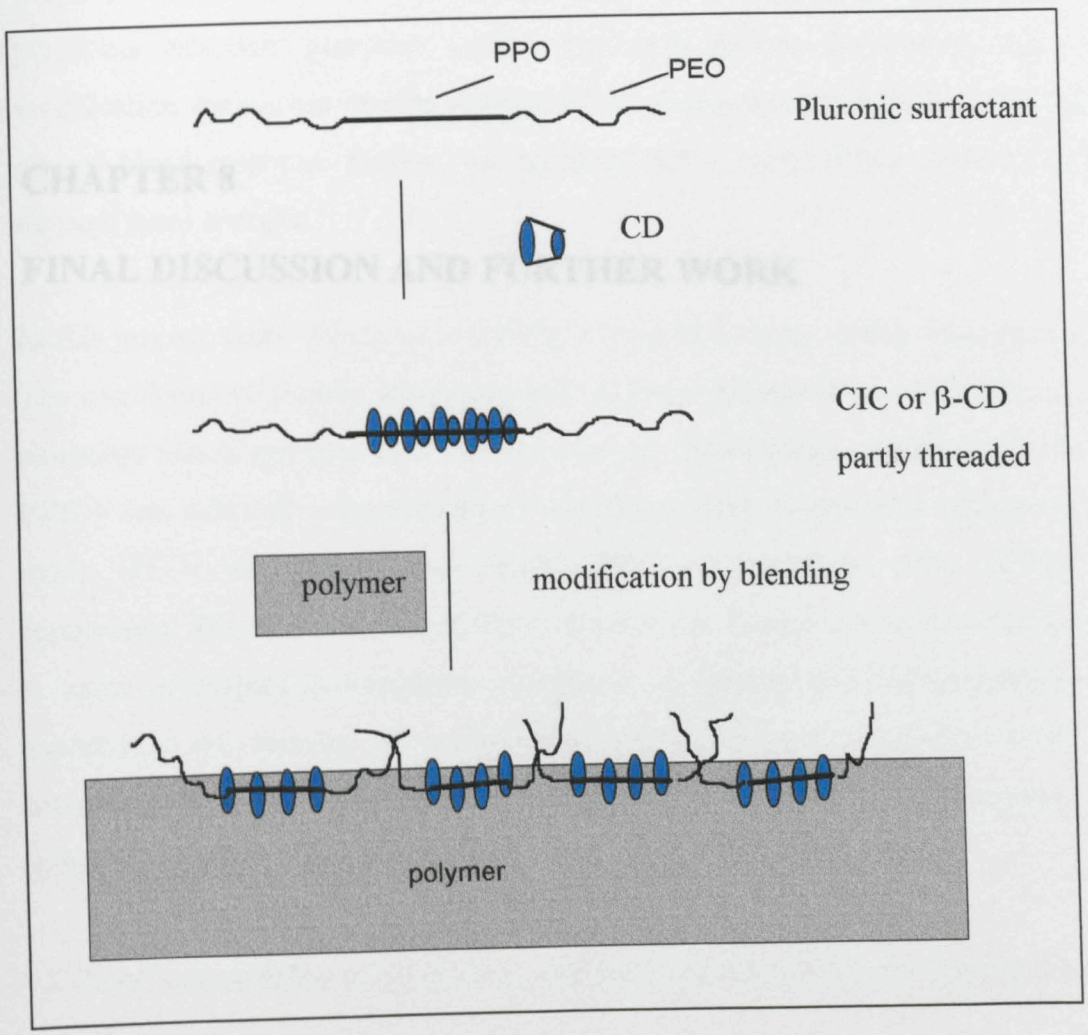

Fig 7.14 "ANCHOR" hypothetical mechanism for improving blood compatibility using the combination of B-CD and Pluronic surfactant

CHAPTER 8

FINAL DISCUSSION AND FURTHER WORK

8.1 Introduction

The high percentage of plasticiser present in a PVC-P formulation provided PVC-P with many characteristics either in bulk properties or surface properties, which are different from PVC. In the case of PVC-P as a blood contacting biomaterial, the surface composition of a PVC-P plays a very important role in blood response. In fact, when a PVC-P contacts blood, the contact surface consists mainly of plasticiser rather than PVC polymer. Therefore, the blood compatibility of PVC-P is believed to be dependent on plasticiser selection, plasticiser surface level and surface distribution. Any surface modification approaches causing changes of the above-mentioned factors can lead to an altered blood response. Further, an improved blood compatibility could be achieved through these changes.

In this project, these effects were closely investigated using protein adsorption studies. The correlation of protein adsorption with surface characteristics of PVC-P, including plasticiser nature and plasticiser surface level was investigated. Surface modification of PVC-P was achieved using cyclodextrins (CDs) or their combination with polyethylene oxide (PEO) and polyethylene oxide (PEO)-polypropylene oxide (PPO) block copolymers. The incorporation of these additives can change the surface characteristics in terms of surface hydrophilicity or balance of hydrophilicity/hydrophobicity, most importantly the stability of configuration of accumulated molecular layer, which determines the blood response. In the end, a novel process for improving blood compatibility of PVC-P was obtained.

8.2 Blood compatibility of plasticised poly(vinyl chloride) (PVC-P): protein adsorption studies

8.2.1 Dependence on plasticiser selection

In this project, three types of medical grade PVC-P in sheet form were selected for protein adsorption studies. The selected plasticisers include two phthalates (DEHP and TEHTM) and one citrate (BTHC). 125-I-fibrinogen and albumin were selected as the blood components for interaction with PVC-P. The material/protein contact was

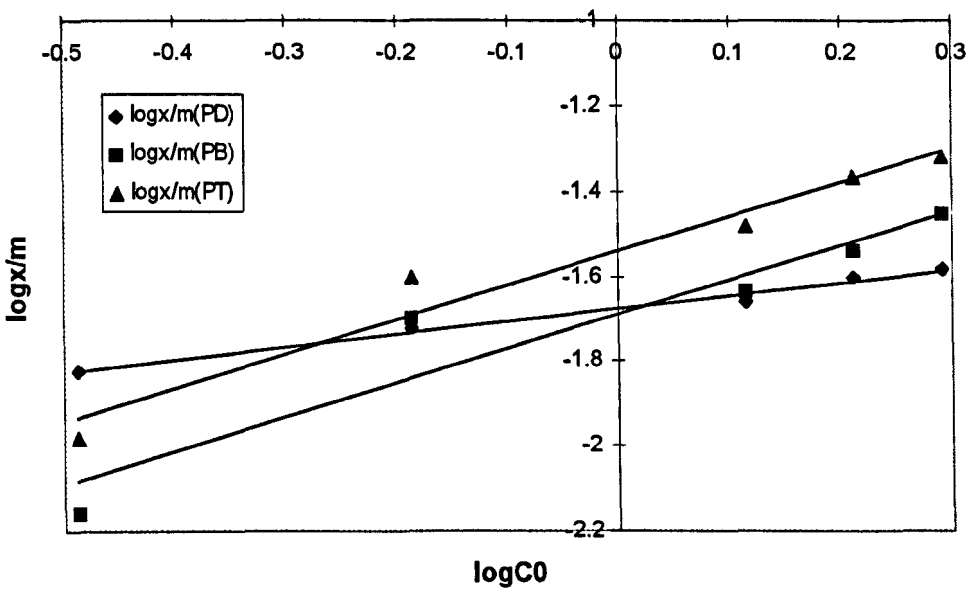

Fig 8.1 Freundlich modelling of fibrinogen adsorption of PVC-P

achieved with a modified 24-well incubation test cell. Protein adsorption studies indicate that at very dilute fibrinogen bulk concentration, PVC-BTHC shows the lowest fibrinogen adsorption, while PVC-TEHTM is the most reactive to fibrinogen among the three types of PVC-P. The PVC-U was found to be unreactive to fibrinogen and albumin, with the same pattern as Cuprophan dialysis membrane. The Freundlich and Langmuir isotherms were employed for modelling protein adsorption. For fibrinogen adsorption, results indicate there is a strong relation between fibrinogen adsorption capacity and fibrinogen bulk concentration. All three types of PVC-P fit the Freundlich equilibrium isotherm indicating a monolayer adsorption in the dilute bulk concentration. PVC-DEHP has the highest n value indicating a weakest adsorptivity. PVC-TEHTM possesses a highest K value and a low n value, which indicates the highest adsorption capacity. For PVC-BTHC, it has the lowest K value indicating the lowest adsorption capacity and a very similar n value (slope of curves) to that of PVC-TEHTM, which indicates a different plasticiser nature, but with similar adsorptivity as PVC-TEHTM. This can be seen from the Freundlich modeling isotherms summarised in Fig 8.1. In addition, it emphasises the importance of plasticiser at the surface as opposed to the adsorption pattern of poly(vinyl chloride) (PVC) polymer itself (Chuang etal, 1978).

For albumin adsorption, the three types of PVC-P fit Langmuir adsorption isotherm while PVC-BTHC fits Freundlich model as well. PVC-BTHC presents the lowest albumin adsorption when the albumin bulk concentration was lower than 0.5211 μg/ml. These data indicate that albumin adsorption is not strongly dependent on albumin bulk concentration.

In conjunction with the surface characterisation results, the lower fibrinogen reactivity of PVC-DEHP is due to its lower DEHP surface level and higher PVC composition (0.252 mg/cm^2; $A_{PVC\ 980cm-1}/ A_{DEHP\ 1726cm-1}$ = 1.04; DEHP%=49) than those of PVC-TEHTM (0.355 mg/cm^2, $A_{PVC\ 980cm-1}/ A_{TEHTM\ 1726cm-1}$ = 0.30; TEHTM%=77%). For PVC-BTHC, the value of $A_{PVC\ 980cm-1}/ A_{BTHC\ 1726cm-1}$ is the lowest about 0.045 (BTHC%=95.7) which means it has the highest BTHC level at the surface. However, it exhibits the lowest

reactivity to fibrinogen within a range of fibrinogen bulk concentration. It implies that protein adsorption is also influenced by the plasticiser nature.

8.2.2 Dependence on plasticiser surface level

The methanol surface treatment leads to a gradually reduced plasticiser surface level. The protein adsorption studies on these treated surfaces made it possible to reveal the correlation between blood compatibility and plasticiser surface level. Generally, the higher the plasticiser surface level, the higher the fibrinogen adsorption, and the lower the albumin adsorption. However, the surface texture or morphology will affect this phenomenon. The washed samples might also lead to a strong reactivity to fibrinogen due to the increase in surface roughness. This is the case particularly in the surface treatment of PVC-TEHTM and PVC-BTHC. The methanol surface treatment after 60 minutes caused an increased fibrinogen adsorption.

From this correlation study, it is concluded that fibrinogen adsorption on PVC-DEHP and PVC-TEHTM is strongly dependent on plasticiser surface level, which implies DEHP and TEHTM have the same chemical nature. If plotting fibrinogen adsorption capacity against plasticiser surface level and crossing Y-axis further, it is found that they have a very similar slope and a very similar Y intercept value, which is the fibrinogen adsorption on PVC with a clean surface. This also indicates that PVC is non-reactive to fibrinogen (fibrinogen adsorption on PVC is around 0.010 $\mu g/cm^2$) (Fig 8.2 and 8.3). PVC-BTHC also showed a very similar trend but for the first 5 minutes, the fibrinogen adsorption increased and then fell gradually with a reduced BTHC surface level. This might also imply that BTHC is of different chemical nature from phthalates. The certain high surface level could be beneficial for improving blood compatibility at the surface treatment before 5minutes, but a further cleansed surface (after 5 minutes) will be more advantageous for reducing fibrinogen adsorption.

8.2.3 Conclusions

From the above discussions, several conclusions can be drawn:

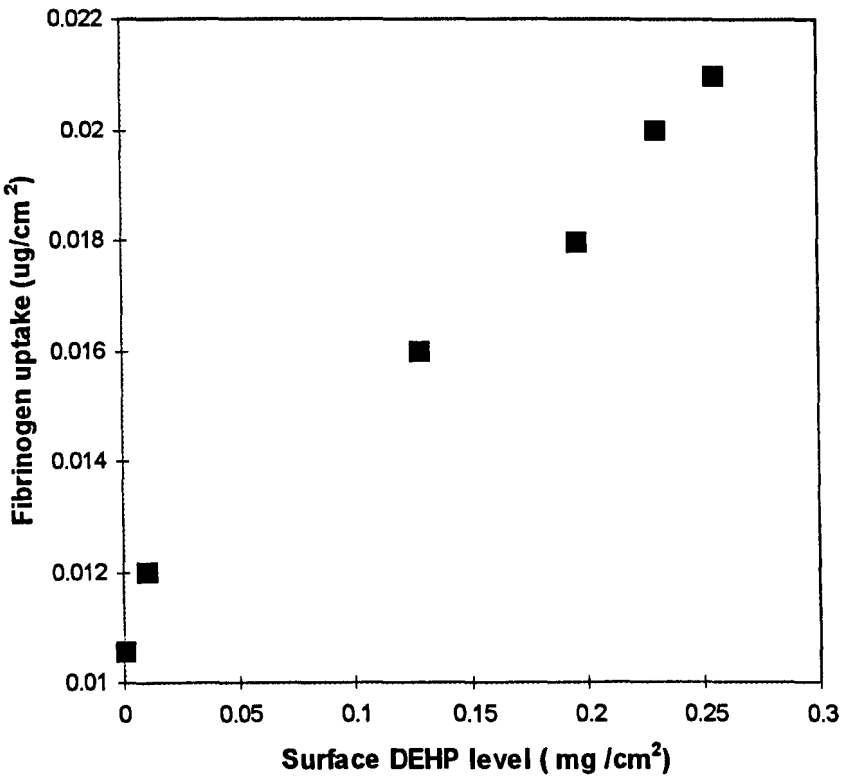

Fig 8.2 Linear correlation of surface DEHP level with fibrinogen adsorption

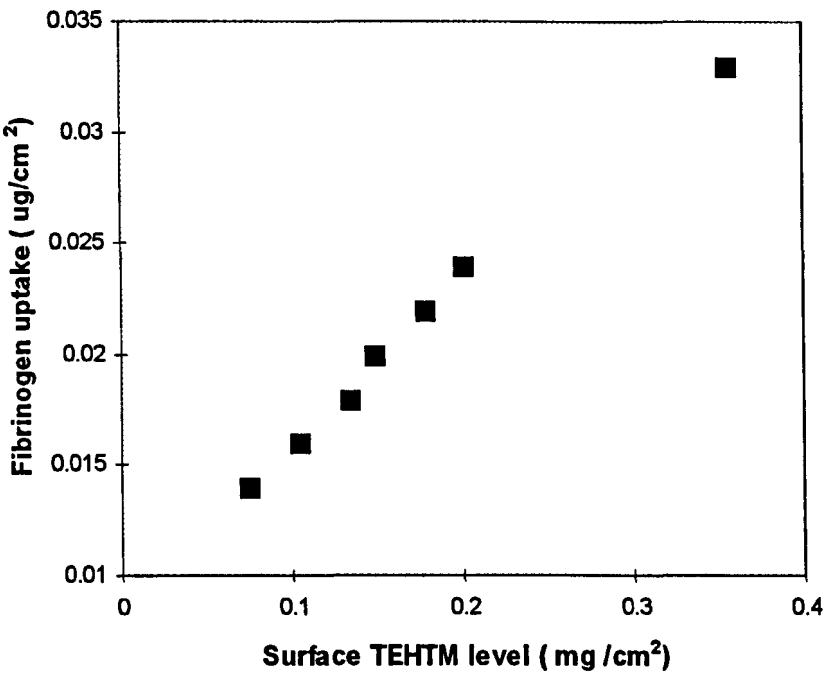

Fig 8.3 Linear correlation of surface TEHTM level with fibrinogen adsorption

(1) Blood compatibility assessment of PVC-P in terms of protein adsorption indicates there is a strong dependence on plasticiser selection (nature) and plasticiser surface level. Higher phthalate plasticiser surface level leads to higher fibrinogen reactivity.

(2) For surface treatment using methanol washing, a possible change in surface texture or morphology might be able to affect protein adsorption pattern, particularly in the case of PVC-TEHTM.

(3) PVC-BTHC has a similar adsorption pattern to PVC-TEHTM from Freundlich equilibrium isotherms with a very similar n value. This indicates both of them have a very similar surface hydrophobicity. However, a higher K value of PVC-TEHTM indicates a stronger reactivity to fibrinogen. Meanwhile, BTHC-PVC adsorbs the least proteins within a certain range of protein bulk concentration, which indicates the different nature of BTHC.

8.3 Surface modification of PVC-P

8.3.1 Influence on protein adsorption of cyclodextrins (CDs) modified PVC-P

The initial considerations of the utilisation of CDs for modifications of PVC-P were based on the fact that β-CD could retard DEHP migration due to the formation of an inclusion complex. However, there is a lack of research on the blood response of CDs modified polymers in the literature. In this project, protein adsorption studies of CDs modified PVC-P were performed.

Basically, CDs are types of cyclic polysaccharides containing various units of glucose, forming a hydrophobic cavity surrounding with a hydrophilic shell. By *van der Waal*'s force, hydrogen bonding and hydrophobic interaction, CDs can form inclusion complexes with many guests. The important features for CDs utilised in the biomedical field are non-toxicity, biocompatibility and the capability of inclusion complex formation. The incorporation of CDs (α-CD, β-CD, γ-CD and HP-β-CD) into PVC-DEHP was achieved using casting technology. Fibrinogen adsorption studies indicated that a reduced fibrinogen adsorption could be achieved due to the presence of CDs at the PVC-DEHP surface. The mechanism could be related to an increase in surface hydrophilicity or building a balance between hydrophilicity and hydrophobicity. More industry relevant,

PVC-P modification using CDs was carried out by polymer melting or blending in a two-roll mill. In this way, CDs or β-CD/DEHP inclusion complex can migrate to PVC-DEHP or PVC-TEHTM surface by the strong sheer force during polymer blending. The increase in CDs incorporation concentration leads to a gradually reduced protein adsorption.

For three reasons, polyethylene oxide (PEO) and Pluronic surfactants (PEO-PPO-PEO) were selected as combinations with CDs for modification of PVC-P. Firstly, to reduce the cost of polymer process by parcial replace of CDs. Secondly, the bulk properties could be improved by this combination instead of using CDs or Pluronic alone. Thirdly, it could possibly aid CDs to migrate to the surface and achieve a highly reduced protein adsorption.

Protein adsorption results show there is a remarkable reduction in protein adsorption both for fibrinogen and albumin. The combination of β-CD and Pluronic F68 seems to have a synergistic effect on fibrinogen adsorption in a certain composition. Similar results were also found for CDs modified PVC-TEHTM. However, the combination of β-CD with F68 was no more effective than β-CD alone in PVC-TEHTM modification. This could be due to a richer β-CD surface concentration at PVC-TEHTM surface than that in the case of PVC-DEHP. The lack of inclusion complex formation between TEHTM and β-CD could be beneficial for β-CD's getting to the surface.

More interestingly, a further surface washing treatment of CDs modified PVC-DEHP can lead to a further reduced fibrinogen adsorption, which is believed to be due to a clean surface with low DEHP surface level. This surface can maintain its low fibrinogen adsorption as long as 10 days, which is quite different from that of PVC-DEHP. The reason is that β-CD's presence at the surface can form a barrier to mobilise the diffusion of DEHP from the bulk to the surface, which could create a clean surface for a relatively long time. Therefore, a novel process for modification of PVC-P could be summarised in Fig 8.4

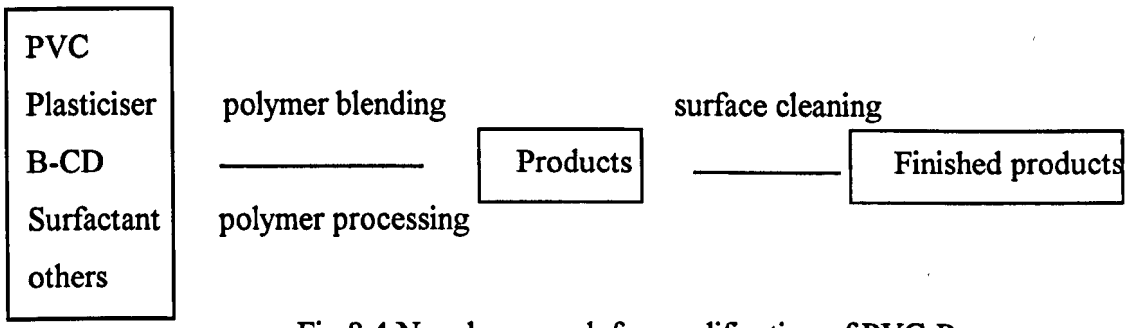

Fig 8.4 Novel approach for modification of PVC-P

8.3.2 Influence on protein adsorption of cyclodextrin inclusion complexes (CICs) modified PVC-P

Supramolecular chemistry is a new division in modern chemistry. It is now more involved in designing new materials according to some sorts of specific recognition via non-covalent bond. In this thesis, cyclodextrins (CDs), the most widely applied natural host molecules, were employed to form inclusion complexes with PEO-PPO-PEO type surfactants. Then, these formed cyclodextrin inclusion complexes (CICs) were incorporated into PVC-P blend system. The objectives of this study firstly were trying to clarify the synergistic effect of the combination of Pluronic surfactant with β-CD on reduction in fibrinogen adsorption. Secondly, to develop a novel approach to modify PVC-P for improving blood compatibility, and finally, to consider a possible mechanism of such reduced fibrinogen adsorption using this CIC supramolecule.

Results in Chapter 7 clearly indicate that the incorporation of an inclusion complex between β-CD and Pluronic L81 (low molecule weight surfactant) (CIC1) into PVC-DEHP blend system can achieve a CIC1 enriched surface, which is effective in reducing fibrinogen adsorption. The other advantages include more consistency in adsorption assessment, less influence on the physical properties such as transparency, and easier processing than the physical mixture of β-CD and Pluronic L81. It is also found that the block sequence of PEO and PPO is very important for creating a protein resistant surface. PPO-PEO-PPO sequenced surfactant is not acceptable for this particular application.

Regarding the further surface treatment using the methanol cleaning technique, the surface washing can change the surface topography, particularly of the B-CD/Pluronic F68 modified PVC-P surface. This leads to an increase in fibrinogen adsorption. Therefore, it is not necessary to follow with such a surface cleaning process in this case. For β-CD/Pluronic L81 inclusion complex modified PVC-P, the influence on fibrinogen adsorption of surface washing was not investigated due to the limit of quantity of materials. However, it is believed that the already CIC-enriched surface must play a more important role rather than the plasticiser present at the surface, since there is no significant difference between CIC1 modified PVC-DEHP and PVC-U, and also Cuprophan.

From these results, it is hypothesised that the CIC1 can help to create a stable PEO-anchored surface, which is beneficial for a reduction in protein adsorption. Compared to other approaches, such as surface coating combined with plasma fixing, chemical immobilisation and other surface modification techniques, this supramolecule modification approach has many advantages, which have been summarised in Chapter 7. In particular, is more industry relevant.

8.3.3 Influence on plasticiser DEHP migration

DEHP migration from the bulk into the contacted media has caused many environmental concerns. However, DEHP is still the most studied plasticiser utilised in medical devices and is widely accepted for plasticisation of medical grade PVC. Therefore, the objective of using β-CD is not simply to develop a DEHP migration resistant type of PVC-DEHP, but to focus on a novel modified surface, which might be effective in retarding DEHP to get to the surface.

The migration of DEHP into contacted extractants depends on many factors, such as the interaction of DEHP with other components in the blend, the processing, the contact area, the selection of media and the extraction conditions such as temperature, pressure

and volume of extractant etc. In this study, methanol and cottonseed oil were selected for migration study, which are two extractants having been reported for DEHP migration studies. In the case of β-CD alone modified PVC-DEHP, the incorporation of β-CD is effective to retard DEHP migration into the methanol medium. However, this effect is not significant in the case of β-CD/PEO and β-CD/Pluronic F68. When the medium was changed to a milder extractant, cotton seed oil, the DEHP migration behavior of the most of modified PVC-DEHP is similar and HP-β-CD/PEO modified sample shows a more significant reduction in DEHP leaching, which indicates a hydrophilic surface is also effective in retarding DEHP migration. More interestingly, the surface washed modified PVC-DEHP, particularly, β-CD alone and β-CD/PEO modified PVC-DEHP can restrict the migration of DEHP to get the surface resulted from the fibrinogen adsorption test. Therefore, this is one of the advantages for using β-CD to get a surface with improved blood compatibility.

8.4 Surface characterisation

8.4.1 Measurement of plasticiser surface level using UV-visible spectrophotometer

Electron Spectroscopy for Chemical Analysis (ESCA) and ATR-FT-IR conjunction with other surface characterisation technique can evaluate the level of plasticiser at the PVC-P surface. In this thesis, a simple method using UV-visible spectrophotometer was achieved for evaluation of plasticiser surface level. The obtained results are somehow consistent with the ATR-FTIR surface characterisation results. However, it is difficult to tell the accuracy of the results since the definition of the surface itself is rather difficult. In addition, for this study, it is more important for us to try to correlate the plasticiser surface level with protein adsorption rather than the plasticiser surface level measurement.

8.4.2 ATR-FTIR surface characterisation of PVC-P and modified PVC-P

ATR-FTIR surface characterisation was employed to tell how clean the surface was after different surface methanol treatment period. This could be observed from the decrease in the absorption intensity at the bands, which attributed to DEHP, TEHTM and BTHC

and the increase in the absorption intensity at the bands, which attributed to PVC. Clearly, the methanol surface treatment can achieve a reduced plasticiser surface level. The longer time is needed for surface treatment of PVC-TEHTM than PVC-DEHP and PVC-BTHC. In addition, there is the highest percentage of plasticiser at the PVC-BTHC surface among these three types of PVC-P.

For β-CD or its combination with Pluronic surfactants, PEO modified PVC-P in Chapter 6, or CICs modified PVC-P in Chapter 7, ATR-FTIR surface characterisation effectively shows some of the distinguished changes due to the surface modification. Since the plasticiser overlaps some IR absorption range with β-CD, PEO and PEO-PPO-PEO around 1600-1700 cm^{-1}, 1000-1200 cm^{-1}, it is difficult to evaluate the β-CD and PEO level at the surface. However, when the modification was carried out using a material without large quantity of plasticiser, such as LDPE, which served as a control material, their ATR-FTIR surface characterisation results show a very clear image that β-CD, PEO and PEO-PPO-PEO are enriched at the surface. This might tell us the general rule, that the hydrophilic modifiers or additives in the hydrophobic blend like to move to surface by high shearing, is also met in the case of PVC-P.

8.5 Further work

8.5.1 Optimisation of modification process

This project was involved in finding the possible application of cyclodextrins or their combination with other additives such as PEO and PEO-PPO-PEO surfactant to achieve a protein adsorption resistant surface, which could lead to a reduced blood response, rather than optimisation of the modification process. Therefore, in the future, the blend system should be optimised with respect to the polymer formulation and the polymer processing. This is particularly important in knowing the actual concentration of each ingredient rather than using pre-mixed PVC-P blend as this project involved.

8.5.2 Characterisation of modified PVC-P

The characterisation of modified PVC-P can be divided into bulk properties and surface property characterisation. The first catalogue is mainly involved in the mechanical

property measurement and the latter is mainly on surface chemical composition, surface topographical or microphase structure, and their influence towards blood response. In addition, the DEHP migration test could be considered.

8.5.3 Blood response of modified PVC-P

Once an optimised PVC-P biomaterial is available, which exhibits comparable properties either in mechanical properties or other physical properties such as gas transmission properties to the most commonly used medical grade PVC-P, a blood compatibility study has to be considered.

8.5.4 Mechanism studies

The mechanism of the influence of cyclodextrins or their combination with other additives or their inclusion complexes on blood compatibility of modified polymers has to be considered in the future. This could be done by measuring the interaction force between protein and CDs or their combination, or their inclusion complexes with PEO or PEO-PPO-PEO , or simply using adsorption technique. In addition, the modification could be extended to polymers other than PVC. This could help us to understand the possible mechanism more easily than that in the PVC system.

Therefore, there is much work to be done, particularly, if considering the huge number of CD derivatives instead of only β-CD. This could open an even wider area, involving the use of supramolecules to modify polymeric biomaterials.

8.6 Future objective

This thesis demonstrates the possibility of a novel form of surface modification, where an increased hydrophilicity or other property is achieved by "anchoring" a section of a nonionic surfactant or other copolymer to a cyclodextrin. This "anchor modification" has important implication for PVC-P and other polymeric biomaterials.

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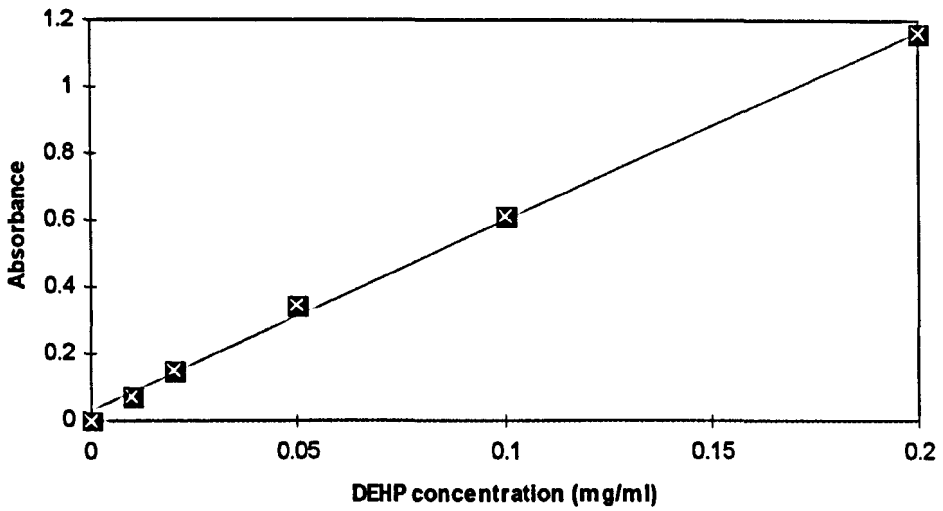
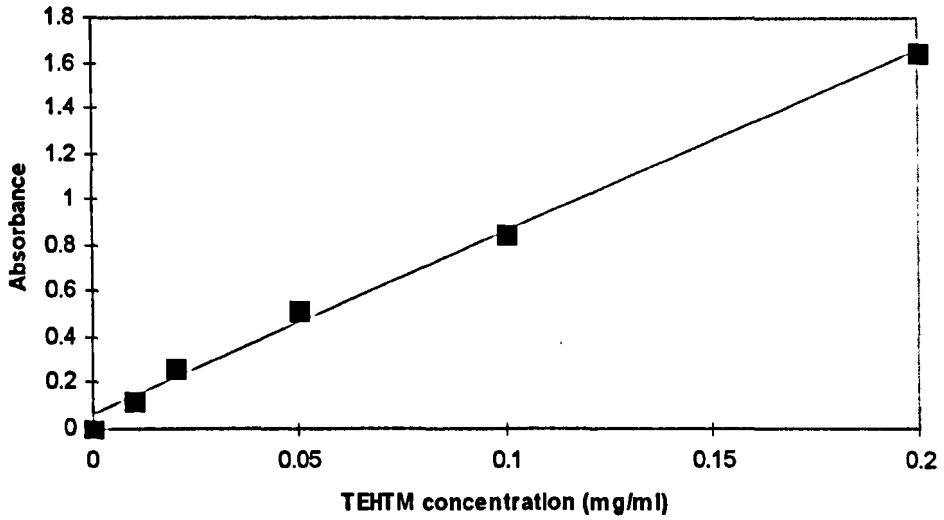


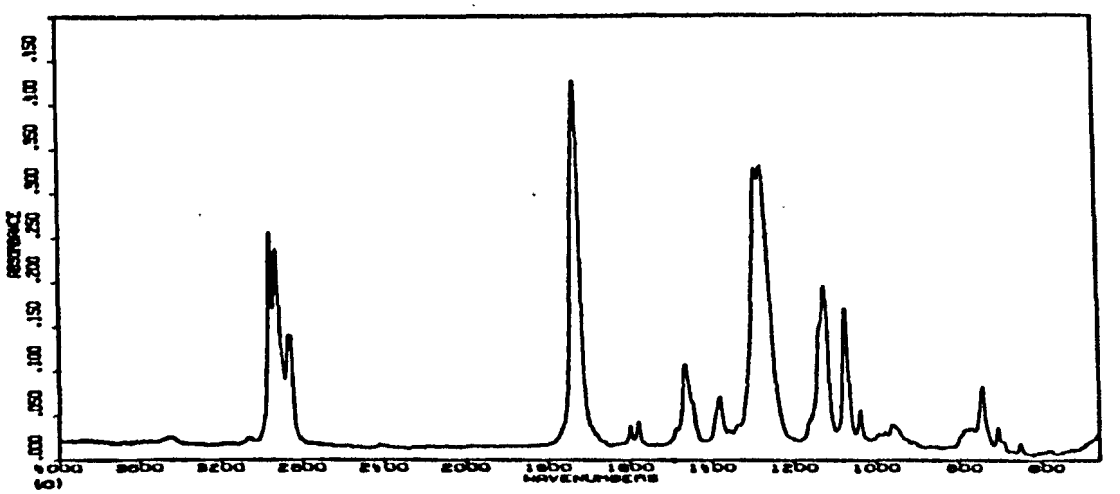
SUMMARY OUTPUT		RESIDUAL OUTPUT	REGRESSION FORMULA: Y=5.89X	
Regression Statistics		Observation	Predicted Y	Residuals
Multiple R	0.996453	1	0	-0.04272
R Square	0.992918	2	0.098558	-0.01256
Adjusted R Square	0.991147	3	0.154391	0.057609
Standard Error	0.040104	4	0.321889	0.021111
Observations	6	5	0.601054	-0.02605
		6	1.159384	0.002616

Appendix Fig 4.1 Calibration plot of DEHP concentration vs UV absorbance at 274 nm

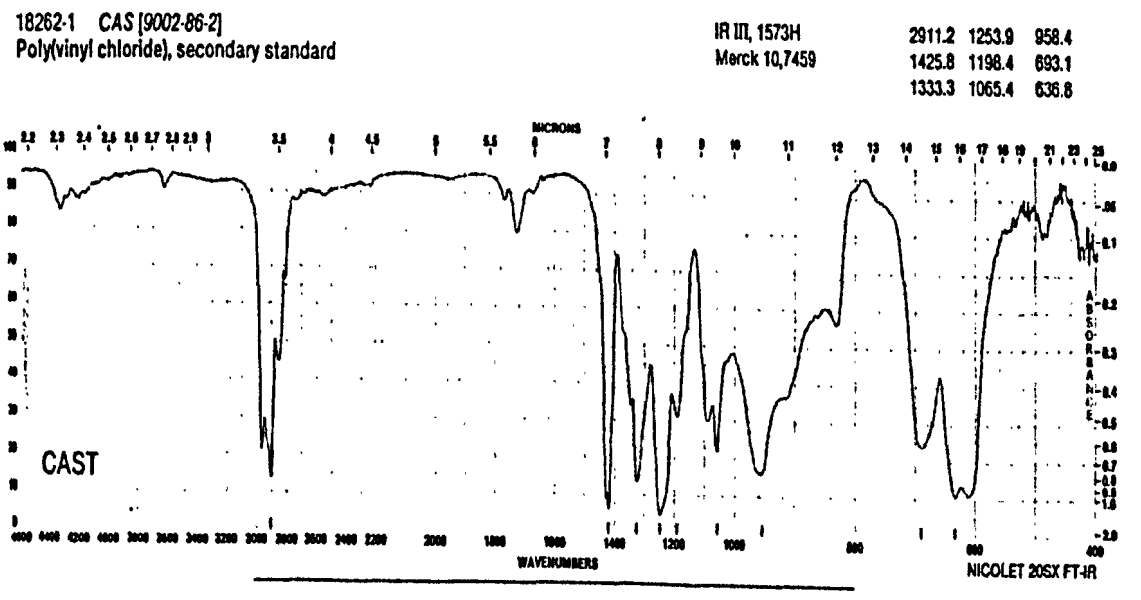


SUMMARY OUTPUT		RESIDUAL OUTPUT	Y=8.45X	
Regression Statistics		Observation	Predicted Y	Residuals
Multiple R	0.99623	1	0	-0.06976
R Square	0.992474	2	0.149247	-0.02725
Adjusted R Square	0.990593	3	0.229	0.074268
Standard Error	0.058866	4	0.467187	0.048813
Observations	6	5	0.864612	-0.01561
		6	1.659462	-0.01046

Appendix Fig 4.2 Calibration plot of TEHTM concentration vs UV absorbance at 290nm



Appendix Fig 5.1a IR-spectrum of DEHP(Siesler&Holland-Moritz,1980)



Appendix Fig 5.1b IR-Spectrum of PVC(Aldrich FT-IR handbook)