

# **Chapter 1                      Background Review**

## **1.1 Introduction**

A biosensor can be defined as a compact analytical device containing a biological recognition element and a transducer. The biological element in the biosensor is a critical component and confers specificity on the sensor. The biological element can be antibodies, proteins, DNA, microorganisms, animal and plant tissues.

The biological element is located on or in close proximity to the transducer and converts the analyte into a physical or chemical response. The transducer converts response of the biological element into an analytically useful signal. The type of transducer in a biosensor can compromise the selectivity of the biological component to some extent. Hence, the type of transducer used in a biosensor is selected, carefully and appropriately, to match the physical or chemical response that is produced by the biological recognition element. There are various types of transducers such as piezoelectric, thermal and electrochemical types of transducers.

Biosensors are part of our everyday life and are used in almost all fields including the food industry, environmental control, biotechnology and clinical diagnostics. A biosensor is unique in the sense that it combines the multidisciplinary skills of the physicists, biologists, engineers and chemists to provide innovative solutions to analytical problems. Moreover, biosensors provide cost effective, simplified, reagent-less analysis of the intermediary metabolites with increased speed and reduced skill requirement.

## **1.2 Amperometric Biosensors**

The amperometric biosensors are devices that utilize electrochemical transducers. The most commonly used biological recognition element in amperometric biosensors is a group of enzymes known as the oxidases.

The oxidase enzyme catalyzes the substrate in presence of oxygen, which leads to the production of an electro active species. The general reaction for an oxidase enzyme is shown (equation 1.1) as follows:



The measurement of increase in hydrogen peroxide concentration or the decrease in oxygen concentration is possible by means of the electrochemical reaction at the working electrode. The glucose sensor is a typical example of such enzyme based amperometric type of electrochemical biosensor.

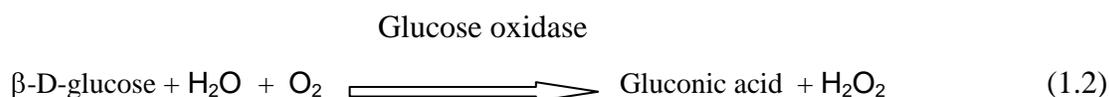
In glucose sensors, the platinum electrode is most commonly used as the “transducer” and the biological recognition element is the enzyme glucose oxidase (GOD), which is immobilised onto the electrode surface. In the earliest model of enzyme electrodes proposed by Clark and Lyon for determination of glucose (1962), glucose oxidase was retained between layers of dialysis membrane. The enzyme was used in high concentration to compensate for any loss due to leakage. The enzyme and polymer membranes sandwich was held over a pH electrode or an oxygen electrode for the measurement of gluconic acid (product) or the depletion of oxygen respectively. However it was proposed in a later model that the hydrogen peroxide formed during the reaction could be exploited as an alternative to the detection of changes in oxygen concentrations at the cathodic potentials (Clark, 1979).

The electrochemical oxidation of the hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) at a platinum electrode allows accurate measurement of the glucose concentration and thus the correction required for the variations in background oxygen tension associated with the amperometric oxygen sensors is avoided.

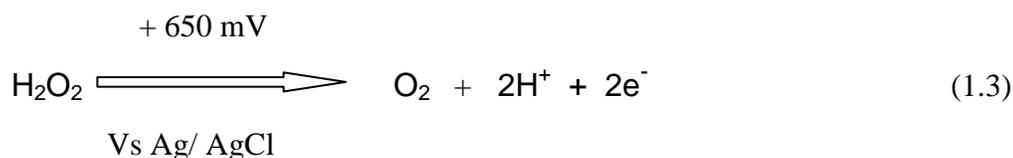
The system utilises the turnover of glucose oxidase (when the glucose is oxidised) to produce or generate an electrical current. The magnitude of this current is proportional to the glucose concentration (Ishikawa et. al. 1998).

The process can be divided into two parts namely glucose oxidation and current production. The two-step process is as follows:

- Firstly, the glucose oxidase converts  $\beta$ -D-glucose (substrate) in the presence of oxygen and water into gluconolactone (product) along with hydrogen peroxide. The reactions can be presented (equation 1.2) as follows:



- Secondly the hydrogen peroxide produced is oxidised at the platinum electrode at high potentials. The hydrogen peroxide concentration at the surface of the sensor is proportional to the current generated. The reaction that takes place can be expressed (equation 1.3) as follows:



The optimum potential for the dissociation of hydrogen peroxide, (at Pt. i.e. the working electrode), is + 650 mV vs Ag/AgCl (reference electrode).

The current generated is directly proportional to the surface area of the working electrode, hence the bigger the surface area of the working electrode, the higher the current generation. At steady state and diffusion limited situations the following equation (1.4) can be used to measure the current generated.

$$i = nFAf \quad (1.4)$$

Where

$i$  = current

$n$  = number of electrons transferred per molecule

$F$  = Faraday constant

$A$  = surface area of the electrode

$f$  = flux of the electroactive species.

The equation (1.4) allows for the determination of current with respect to the electrode surface area. It is assumed that at the surface of the electrode all electroactive species are converted immediately and that the concentration at the surface is effectively zero. Hence, the measured current can be directly related to the concentration of the analyte. However, in real time and practical situations there are many factors that affect the rate of a reaction and the generation of current.

### **1.2.1 Factors that affect current and the rate of a reaction**

The rate of a reaction and the current generated are affected by many factors and reactions that take place at the surface of the electrode, mass transport and electron transfer. These are shown schematically in Fig. 1.1.

The following processes and factors control the current and the electrode reaction:

- The rate of electron transfer at the electrode surface.
- Surface reactions.
- Chemical reactions before and after the electron transfer, including processes such as decomposition on the surface of the electrode.

- Mass transfer of the electroactive species, i.e. the oxidised or reduced species to and from the bulk solution respectively.

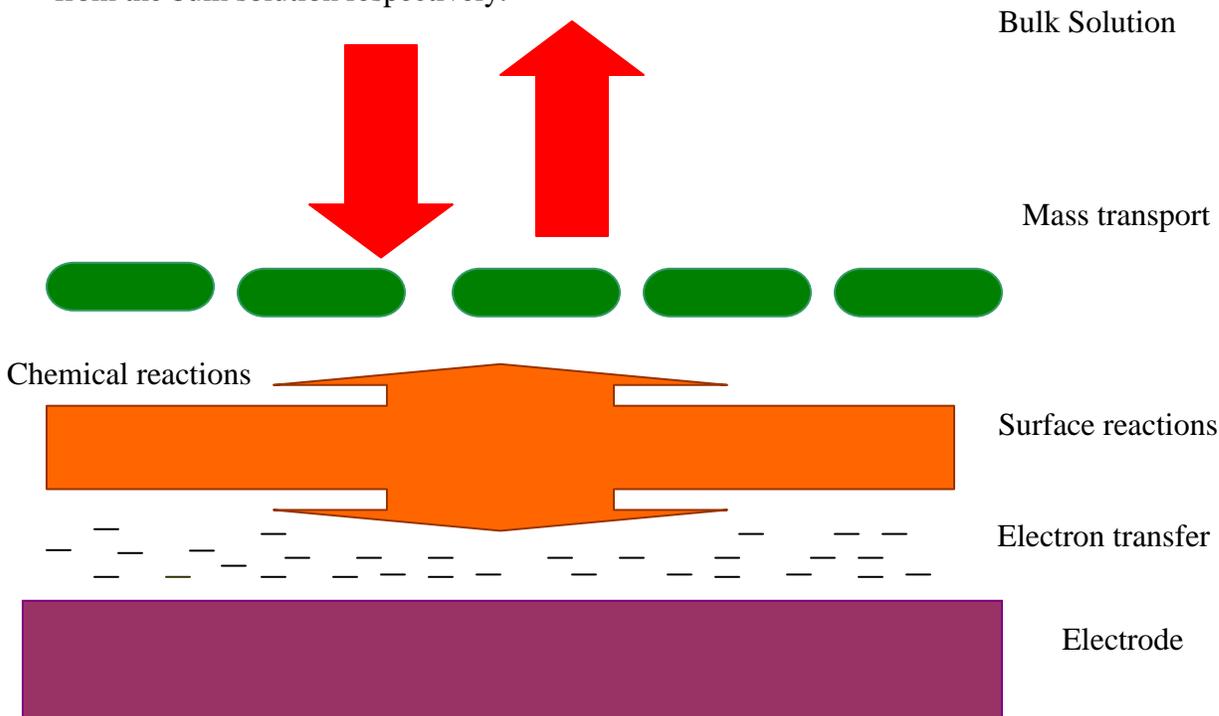


Fig.1.1: Schematic diagram of the reactions taking place at and in near vicinity of a general electrode.

### 1.2.1.1 Electron Transfer

Reactions involving electron transfer at an electrode surface are radiationless electronic rearrangements of a reacting complex. The electrons move during the course of a reaction, from an initial state (e.g. a reductant or an electrode) to a receiving state (e.g. another electrode or a solvated species). The same level of energy between the two states makes the transfer of the electrons possible, a process known as isoenergetic electron transfer. If the density of the possible receiving state is high, then activation overpotential is required for reduction in order to adjust the electrode potential, so that the energy on the initial state would match the energy of the possible receiving state and the electron transfer is made possible.

### **1.2.1.2 Surface reactions**

The reactions taking place at or in the near vicinity of an electrode affect the rate of a reaction. These include chemical reactions that take place before or after the electron transfer, e.g. oxidation of a species at the surface of an electrode, which may result in the formation of a free radical that undergoes free radical polymerization resulting in the formation of a product that gets deposited at the electrode surface. Other reactions taking place at the surface of an electrode include adsorption and electrodeposition, e.g. if the products or reactants of an electrochemical reaction are not very soluble in the solution, they get attached to the surface of the electrode. When this happens they affect the rate of a reaction by obstructing the transport pathway of the reactants and products to and from the surface of the electrode respectively. However, in some cases these surface reactions can be advantageous, e.g. in the case of modified electrodes where an electrochemical species is used to impart specificity for a particular analyte and to facilitate the transfer of electrons. This approach has made a significant contribution to the electrochemical sensors.

### **1.2.1.3 Chemical Reactions**

These are chemical reactions that affect the rate of overall electrode reaction and involve processes that take place before or after the electron transfer; electrochemical polymerisation is one such process. The chemical reaction involves oxidation of a species, at an electrode surface, which results in the formation of a free radical that then initiates free radical polymerisation and results in a product that becomes deposited at the surface of the electrode.

### **1.2.1.4 Mass Transport**

Most species that are detected at the electrode, including hydrogen peroxide, are detected under conditions of mass transport control (Higson et. al. 1994). Hence, it can be assumed that the solute mass transport is the main factor that controls the current and rate

of reaction at the electrode. The three main modes by which the solute and the electroactive species interact with the electrode, which constitute mass transfer processes are as follows:

- Convection
- Diffusion
- Migration

All of these processes take place during a reaction at or in close vicinity of the surface of electrode and affect the rate of electrode reaction.

#### **1.2.1.4.1 Convection**

The fluid conditions that determine convection can be divided into stagnation, turbulent flow and the laminar flow. When the flow is unsteady and involves chaotic motion, it is known as the turbulent flow, which results from the fluid passing sharp angles or obstructions in the flow stream. However, when the flow is steady and smooth, then it is known as laminar flow. In stagnant zones, flow velocity will be effectively equal to zero.

Solute convection, or generally, forced convection can be effected by stirring or hydrodynamic transport in the bulk solution. The process of convection takes place commonly in conjunction with the linear diffusion.

#### **1.2.1.4.2 Diffusion**

At the surface of an electrode, the concentration of species constantly changes due to the diffusion of species, to and from the bulk solution and also because of the oxidation and reduction of species at the electrode surface. This results in a concentration difference between the species at the electrode surface and the bulk solution. The process of diffusion can be defined as movement of species under the influence of a gradient of

chemical potential. Fick's laws of diffusion can be used to explain the rate of diffusion to a planar surface.

Fick's laws are differential equations, which describe the concentration and flux of a substrate as functions of position and time. In the case of linear diffusion, Fick's First Law can be used to describe the steady-state diffusion in a single direction, and states that flux is proportional to the concentration gradient. The general form of Fick's First Law is expressed (equation 1.5) as follows:

$$J = -D \frac{\delta C}{\delta x} \quad (1.5)$$

Where

- J = Solute flux to the electrode
- D = Diffusion coefficient
- C = Concentration of the diffusing solute
- x = Linear distance co-ordinate

The negative sign in the expression indicates that the direction of the diffusion is down the concentration gradient, from a high to low concentration.

Sometimes the term "driving force" is used in the context of what compels a reaction to take place. For diffusion processes several such forces are possible. However, in the context of Fick's First Law, the concentration gradient is the driving force (Callister, 1997). This concentration gradient facilitates transfer of species from the higher concentration gradient to the lower concentration gradient.

As Fick's First Law assumes steady transport of the diffusing substance, it does not allow for variations in flux, from point to point or with time. Most practical diffusion states are non steady-state ones, i.e. the diffusion flux and the concentration gradient may vary with time at some particular point. However with diffusion, concentration changes take place

within small volume elements due to solute flux. Hence, diffusion can also be defined by a partial differential equation (1.6) as follows:

$$\frac{\delta C}{\delta t} = \frac{\delta(D \frac{\delta C}{\delta x})}{\delta x} \quad (1.6)$$

This is known as the Fick's Second Law of diffusion, and defines change of concentration with time. These equations are generic and can be used to describe diffusion not only in solution but also in polymers.

Fick's Second Law can be solved for a planar active electrode surface to obtain a current response on the application of a potential step, from a potential where the electrode process is negligible to one where it is diffusion controlled. The expression thus obtained (equation 1.7) is as follows:

$$j_D = \frac{D^{1/2}}{\pi^{1/2} t^{1/2}} C \quad (1.7)$$

Where  $j_D$  is the diffusion controlled flux per unit area. The expression (1.7) above is known as the Cottrell equation and is applicable to diffusion limited conditions.

### 1.2.1.4.3 Migration

Migration can be defined as the movement of a charged body or particle as a result of forces exerted on it by an electric field, i.e. a gradient of electrical potential. For a planar electrode configuration, which has potential difference  $V$ , applied between electrodes in an isotropic medium, the motion for the charged particle can be shown by the equation (1.8) as follows:

$$\frac{d^2x}{dt^2} = \frac{ZV}{MS} \quad (1.8)$$

Where

- x = Direction of the field
- M = mean of the charged particle
- S = Separation between the electrodes
- V = potential difference between the electrodes.
- Z = Size of the particle

It represents the mechanism by which charge flows through a solution between two electrodes, under the influence of an applied potential gradient.

Migration does not play a major role in the overall rate of mass transport. Diffusion and convection are typically the only significant mechanisms of mass transport in electrochemical reactions (Eddowes, 1990). Analyte and electroactive species reach the surface of the electrode mainly by means of diffusion.

Biosensors in general, glucose sensors in particular, encounter many electroactive species, which dissociate at the electrode surface and lead to signal modulation. In blood there are many other species that also passivate the surface of electrode, which results in many problems such as altered selectivity along with the loss of sensitivity. Hence, the overall performance of biosensor is affected. Even after four decades of research, problems associated with biosensors have not been totally eliminated.

### 1.3 Problems related to *in vivo* biosensors

*In-vivo* biosensors face various problems, which can lead to their failure. There are many potential sources of malfunction for an *in-vivo* electrochemical biosensor such as the glucose biosensor.

Glucose sensors are commonly used to monitor glucose levels in patients with diabetes. Diabetes affects 100-120 million people world-wide (Rigby et. al. 1999). Insulin dependent diabetes mellitus may result in many problems such as long-term micro-vascular, neurological and macro-vascular complications, which can lead to retinopathy, neuropathy and cardiovascular diseases. The diabetes control and complications trial research group (1993) reported, after studies on 1441 patients with insulin dependent diabetes mellitus, that intensive therapy effectively delays the onset and progression of neuropathy and diabetic retinopathy in such patients. Thus, close monitoring and tight control of the glucose levels in such patients is required to reduce the risk of long-term complications and disease. Hence, the most important requirement for glucose sensors or any other sensor that is used *in vivo* is its durability and effective working life.

The malfunction of a biosensor may be caused by two major categories of problems namely mechanical stability and biocompatibility. The problems related to mechanical stability may be due to the component failure such as electrical short in a lead or detachment, membrane delamination and enzyme degradation. The problems related to biocompatibility and other physiologically related causes include electrode passivation, fibrous encapsulation, membrane degradation and biofouling. These problems affect the stability and working life of the biosensor (Wisniewski et. al. 2000).

The needle-type of enzyme electrode is used to monitor the glucose levels in patients that are under observation in hospitals and has been the subject of much ongoing research in recent years. These sensors are implanted subcutaneously *in vivo*. The analyte, i.e. the glucose molecule in this case, has to diffuse out of a capillary and then through the surrounding tissue without being consumed by the surrounding cells to the surface of the sensor. As the sensor is implanted, two types of reactions have to be considered in terms of biocompatibility, firstly the sensor's reaction to the body also known as sensocompatibility (Wisniewski et. al., 2000) and secondly the body's reaction to the sensor.

The body responds to the sensor as a foreign material. Moreover, the cut that is made to implant the sensor goes through wound healing processes. The body's immune system is activated around the implanted biosensor. This causes many problems including biofouling, where the cells and proteins adsorb and adhere onto the surface of the biosensor and also avascular and fibrous scar like tissue develops. Thus, diffusion of glucose towards the sensor is reduced which in turn may lead to a decreased response of the sensor along with loss of sensitivity.

Processes taking place in close vicinity to the implant at post implantation can thus be summarised as follows:

- Proteins are deposited or adsorbed onto the interfacing surface of the sensor from the surrounding body liquid within the first few seconds; this protein layer controls further reactions with the surrounding tissue and is dependent on characteristics of the material of the implant.
- Initiation of wound healing processes and the onset of infection in the tissue, which borders the implant, results in the formation of a granular type of tissue consisting of inflammatory cells, collagen fibres etc. around the implant.
- The biomaterial's components may react with the body. The aggressive body medium may cause degradation processes, leading to release of the degradation by-products.

These factors together determine the biocompatibility of an implant (Klee et. al. 2000).

Rebrin et. al. (1992) investigated the amperometric glucose oxidase/ hydrogen peroxide based sensor, implanted subcutaneously into the neck of normal and diabetic dogs, to elucidate the conditions for long term stable functioning of the sensor *in vivo*. Their studies showed that the sensor lost sensitivity after 14-96 hours, evident post implantation and this appeared mainly due to inflammatory tissue reactions, regardless of the biomaterial (polyurethane and cellulose acetate) used. The inflammatory reactions also

alter the glucose concentration within the measuring compartment of the electrode, as movement of glucose to the surface of the electrode is restricted, thus causing unreliable measurements. In addition to these problems, the underlying electrode may suffer from fouling (also known as the electrode passivation), where molecules penetrate the surface of the sensor and come in contact with the electrode or transducer. These small molecules are present in the body (e.g. ascorbate, urate, etc) and are known as interfering electroactive species. Electroactive species oxidise and generate current at the surface of the electrode at potentials similar to those required for the dissociation of hydrogen peroxide. The increase in current signals due to the oxidation of interfering substances at the electrode may lead to an impression of a higher than true value of analyte concentration, hence giving rise to unreliable and incorrect responses.

The factors and problems affecting biosensors, especially those experienced by a needle-type glucose amperometric biosensor in in-vivo are illustrated schematically in the figure 1.2.

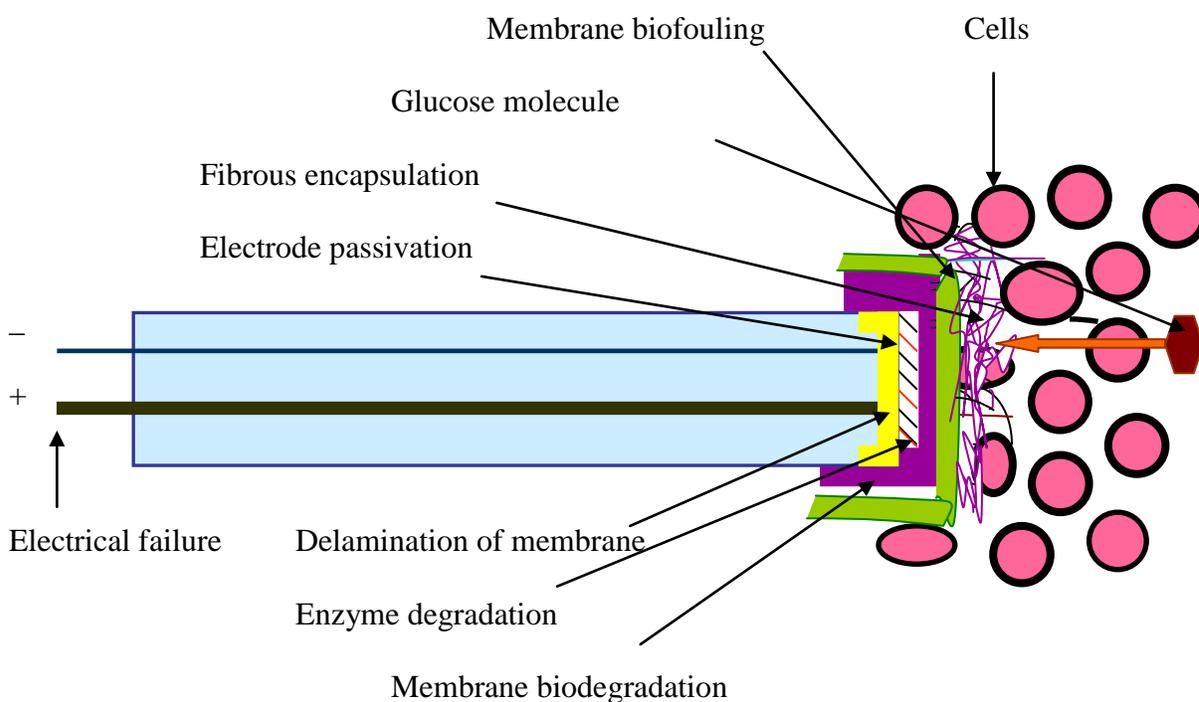


Fig. 1.2: The schematic representation of various problems experienced by a subcutaneously implanted needle-type glucose biosensor (adapted from Wisniewski et. al., 2000).

The surface of a biosensor is exposed to various organic materials, colloids and cellular components present in biological fluids, during operation. Hence regardless of the type of transducer being used, signal modulation may occur. The *in vivo* environment gives rise to the twin problem of biofouling and interferents.

Biofouling affects the long term stability of a biosensor. It has deleterious effects on the working life of a sensor as it involves accumulation of cells, proteins and other biological materials on surface of the sensor. Thus, affecting the performance and consequently may lead to failure of the sensor *in vivo*.

The biosensor requires good stability to other operating conditions such as temperature and pH along with retention of the biological activity in the immobilised biolayer.

There are various techniques that are used for immobilization of biological components such as enzymes, proteins or other macromolecules on the surface of the electrode.

## **1.4 Immobilisation of biological components in a biosensor**

There are various methods for immobilising a biological component of a biosensor such as an enzyme onto the surface of an electrode or a transducer. These include physical entrapment techniques and chemical attachments of the biological component.

### **1.4.1 Physical immobilisation techniques**

This approach involves deposition of the biological component, usually enzyme, on the electrode surface using physical techniques excluding the use of chemicals. The simplest physical immobilisation method involves retention of enzyme by using inert membranes. The membranes are used in the form of a sandwich where enzyme is held physically in between two layers of membranes. However, this method is not very effective, as the

enzyme can easily leach out. Adsorption of enzyme at the electrode surface is another form of physical immobilisation technique. Thus, the use of additional reagents is avoided.

Micro-encapsulation is another type of physical immobilisation. As the name suggests the process involves encapsulation of enzyme, usually in a small porous or semi-permeable polymeric sphere of about 100 microns in diameter. A semi-permeable polymer at the interface of a stable emulsion forms these spheres. Polymers used for the process of micro-encapsulation include nylon and cellulose.

Entrapment of enzyme in polymer gels and matrices is also a method of physical immobilisation. In this method, enzyme is present in solution from which the gel is formed and is entrapped in a gel lattice. Permeability and flexibility of the gel can be controlled by controlling the gel forming conditions, such as the concentration of the monomer and enzyme. Commonly used gel materials include silicone rubber, poly(vinyl alcohol) and gelatin. The thickness of the membrane thus formed covers the surface of the electrode and affects the response time.

Electropolymerised films have been used to physically entrap enzymes (Yasuzawa et al, 2000, Quinto et. al. 2000, Wang et. al. 2000, Adelouju et. al. 2001). This method allows retention of enzyme activity. Moreover, ultra-thin polymer-enzyme films can be produced. Controlling the polarising potentials along with the duration of the process can provide control over conditions.

Electropolymerisation is a relatively simple process that requires the use of electrochemical oxidation of an appropriate monomer from a solution containing the enzyme. This approach allows easy control of film thickness (Adelouju et. al. 2001) and enables deposition of enzyme at the electrode surface without using chemical cross-linking.

### **1.4.2 Chemical immobilisation**

This type of immobilization technique involves chemical agents that alter the structure of the biological component. In biosensors where enzymes are used, the enzyme is crosslinked using a crosslinking reagent such as glutaraldehyde, which leads to the formation of covalent bonds. It can also involve chemical attachment of the enzyme to other surfaces or solid supports such as surface of the sensor.

Covalent bonding between the support matrix and enzyme is achieved through functional groups of the enzyme that are not essential for its catalytic activity. It has the advantage that the enzyme is unlikely to be released during use from the optimum support chosen and the diversity of methods allows active sites to be avoided during the process of linking (Barker, 1987).

The enzyme can be crosslinked on its own but this may cause some problems as the active sites on the enzyme may bind and be compromised, thus distorting the structure of the enzyme. However, if the enzyme is cross-linked with an inert protein such as albumin, then enough active sites are made available for the substrate to be catalysed. Chemical immobilisation guarantees that the enzyme will be retained and is fixed in its place on the transducer. However, less robust enzyme may lose its activity if its three-dimensional structure is altered during the reaction. One important factor while working with enzyme electrodes is the effect of temperature on the activity of the enzyme.

### **1.5 Temperature effects**

The rate of reaction for most enzymes increases with temperature, until an optimum temperature is reached, where the enzyme works best. After this point more increases in temperature cause a reduction in the rate of reaction, until a point is reached where the three dimensional structure of the enzyme destabilises and the enzyme denatures. At this temperature, there is a drop in activity of the enzyme, which is usually irreversible.

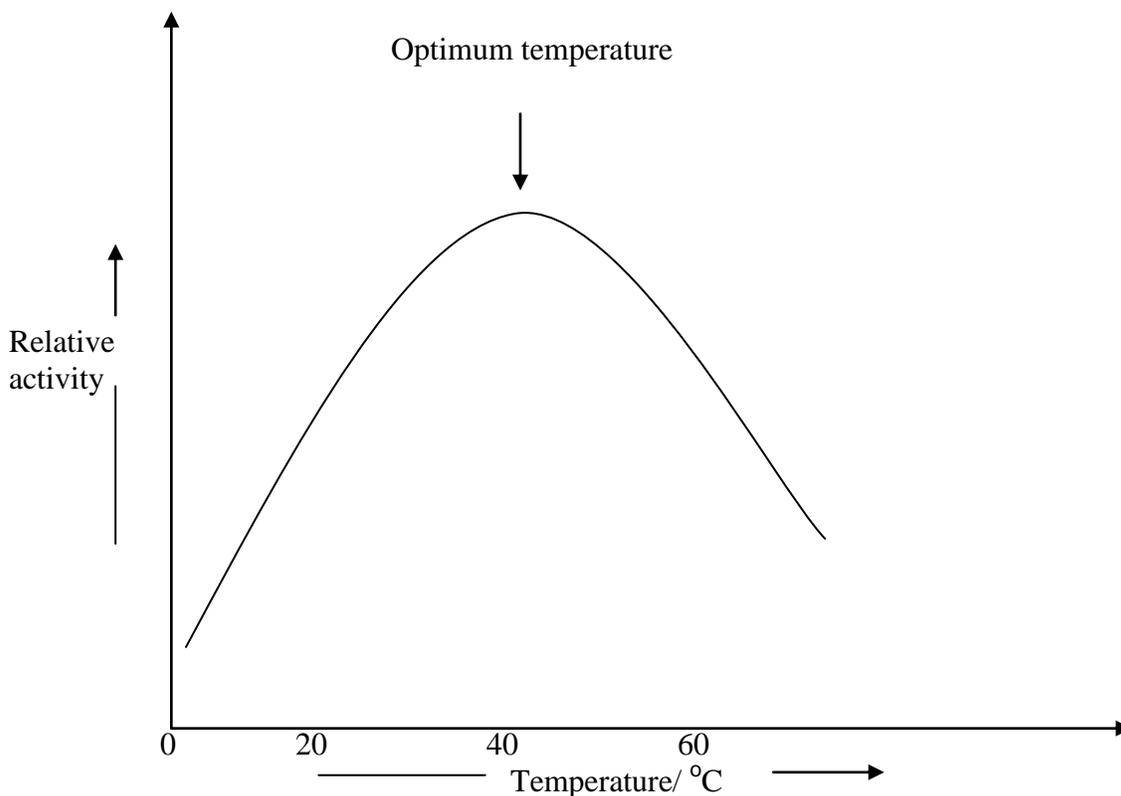


Fig. 1.3: The temperature dependence of enzyme activity.

The rate of an enzyme-controlled reaction is doubled for every rise of ten degrees Centigrade until the optimum temperature is reached (Green et. al. 1990). The increased rate of reaction is a consequence of increased molecular kinetic energy. At a basic level this can be envisaged as an acceleration of the velocity of molecular movement, which leads to an increase in the number of molecular collisions. The number of effective collisions also increases and therefore the rate of reaction also increases. However, if the temperature is increased above the optimum level then a decrease in the rate of reaction occurs despite the frequency in collisions. Hence, temperature control is essential for enzyme electrodes in order to obtain reliable and accurate results.

The rate of reaction and the rate of diffusion or flux of analyte to the electrode in a sensor can be controlled by applying barrier membranes at surface of the electrode. Membranes play an important role in a sensor. The amperometric biosensors, developed for analyzing

biological activity *in vivo*, make use of polymers as outer biointerfacing membranes to enhance biocompatibility of the sensor and as inner membranes to control the diffusion of reaction products to and from the surface of the electrode. Biointerfacing membranes have a profound effect on the life and operation of a sensor *in vivo*.

## 1.6 Membranes and their role in biosensors

The initial contact between blood and the surface of an implant, results in protein deposition within the first few seconds. Subsequent protein deposition is dependent on the surface properties of the outer membrane and also on protein type (Klee et. al. 2000).

Proteins interact with the surface of the sensor, they adsorb and also denature at the interface. When this happens it results in the formation of a persistent layer, then blood platelets aggregate at surface of the sensor, which results in the release of clotting factors. This leads to thrombus formation, which results in further biofouling of the surface of a biosensor. As a consequence of this, a diffusion barrier is created at surface of the sensor, therefore the response time of the biosensor is increased and sensitivity reduced.

The response or analytical signal of a biosensor (i.e. the current signal in the case of glucose sensor) is increased by oxidation of the electroactive species, other than the analyte at surface of the electrode. These include small molecules such as ascorbic acid, urate, small peptides and amino acids, all of which are present in the blood. These electroactive species cause interference problems and produce higher current signals, thus leading to greater than the true value of concentration.

By careful design it is possible to remove the interfering components from biological samples instead of using traditional sample cleanup methods such as chromatography and solvent extraction. One approach has been the application of membrane technology to biosensors (Treolar et. al., 1995).

Membranes are used to modify solute access to the electrode tip and to reduce the twin problems of biofouling and interferents. Biofouling can be minimised by the application of an appropriate outer membrane. Electrode passivation and interference can be resolved to a large extent by the application of a suitable inner membrane.

In amperometric enzyme electrodes the enzyme is generally immobilised between two membrane layers, the outer membrane is interfaced with the test solution and the inner membrane with the surface of the electrode.

The membrane material to be used in biosensors must have the following properties:

- It must not have deleterious effects on the activity of the biological component.
- The material must be in good contact with or adhere to the outer material of the sensor, e.g. surface of the electrode.
- The material must allow the transport of the analyte or substrate and products.
- Undesired biological response by the material must not be produced i.e. the material must elicit the desired biological response in the host.

In short the membrane material has to be biocompatible, perm-selective and stable. In addition it should have good wear properties along with mechanical durability. Properties of the membrane can affect response of the electrode. Hence, by changing the type of membrane, the response of the sensor can be optimised (Bartlett, 1990). Maines et. al. (1996) showed that by using specially designed and developed membranes, which are diffusion limiting, it is possible to measure aqueous glucose concentration to values which are higher than the enzyme's  $K_M$ . The membrane material can be selected and applied so that only the desirable analyte is selected and the interfering species are excluded.

## A study of biointerfacing polymeric barrier membranes for sensors

A membrane can exclude electroactive species or interferents by two processes, as follows:

- Charge exclusion
- Size exclusion.

The schematic mechanisms on which these processes operate are shown in Fig. 1.4.

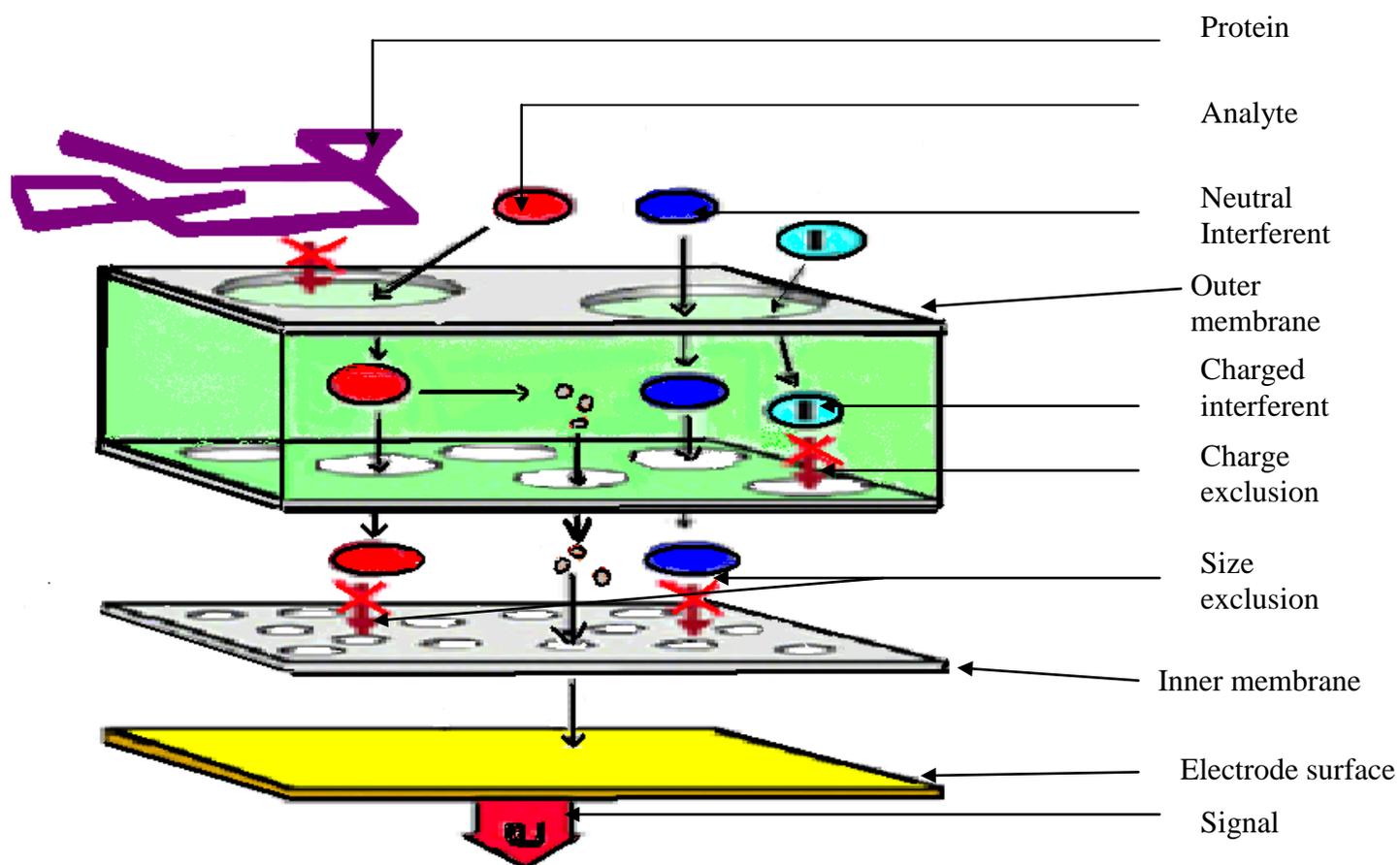


Fig. 1.4: Schematic representation of the processes by which membranes operate in order to exclude interfering species. -

Most of the interference or electroactive species present in the body are charged molecules. For example ascorbate and urate are negatively charged. If the inner

membrane is made of a negatively charged polymer then it will repel a negatively charged electroactive chemical.

Charge exclusion may take place due to ionogenic groups that are present either fixed to or adsorbed on the membrane. The membrane excludes co-ions (ions of the same sign, either negative or positive charge) by electrostatic repulsion. The degree of exclusion is governed by the concentration of the external electrolyte (Lakshminarayanaiah, 1969).

Some interfering chemicals are not charged. They may not be selected if size of the molecule is larger than the pore size of the membrane. Size exclusion takes place on the basis of the nominal pore size of the membrane. Thus, the presence of high charge density of the ionogenic groups and pores in membranes are desirable for enhancing the response of a biosensor.

Complications associated with biosensors such as thrombosis, tissue inflammatory responses, infection, calcification to some extent and the most undesirable i.e. the problem of interfering species can thus be eliminated or minimised and response of the biosensor can also be enhanced by using the appropriate membrane material.

A variety of polymeric materials have been used to modify surface of the electrode and to improve the response of the biosensor.

### **1.6.1 Inner membranes**

The operational stability of a biosensor can be enhanced by using internal membranes, especially those interposed between the working electrode and the enzyme layer (Treolar et. al., 1995). The inner membrane provides a supporting surface for immobilisation of enzyme close to the surface of the electrode, allowing the substrate to reach the electrode and at the same time may also act as a molecular sieve.

Several polymers have been used as inner membranes to overcome the problem of interfering species. These polymers include cellulose acetate, electropolymerised films such as poly(phenol) and poly(pyrrole) films.

### **1.6.1.1 Electropolymerised films**

Ultra-thin electropolymerised films can be used to modify and protect the surface of an electrode. These films are directly electropolymerised electrochemically on the surface of the electrode. Electropolymerised films can be formed by oxidation of a phenolic monomer (e.g. poly(phenol), poly(pyrrole)) at the surface of the electrode, which results in the formation of an insoluble conducting or insulating, polymer film that coats the surface of the electrode. These films are self-limiting and show characteristics of molecular self-assembly, having uniform thickness and completely cover the surface of the electrode regardless of size or shape.

An increase in the concentration of the monomer generally increases the rate of electropolymerisation. If the correct potential and polymer is selected, then electropolymerised membranes can provide enhanced analytical results by lowering the response due to interference while still keeping the response of interest, e.g. hydrogen peroxide, relatively high (Daly et. al. 1999). Moreover, enzymes can be entrapped in these electropolymerised films.

The electrodeposition of enzymes or proteins in a conducting polymer has a number of distinct advantages. For example, it allows controlled and reproducible deposition of enzymes irrespective of their size or overall charge, the deposition of high activities from lower concentrations of enzyme in the polymerisation mixture and the prospect of covalent incorporation of a variety of other mediators, reagents and coenzymes, concurrent with the enzyme deposition, while at the same time retaining the main advantage of a one-single step deposition procedure (Wolowacz et. al. 1992).

### 1.6.1.1.1 Poly(pyrrole)

Pyrrole has been used in the electropolymerisation process, to modify the surface of an electrode, to form ultra-thin electropolymerised films. The Fig. 1.5 shows the schematic diagram of the electropolymerisation process for poly(pyrrole) from its monomer.

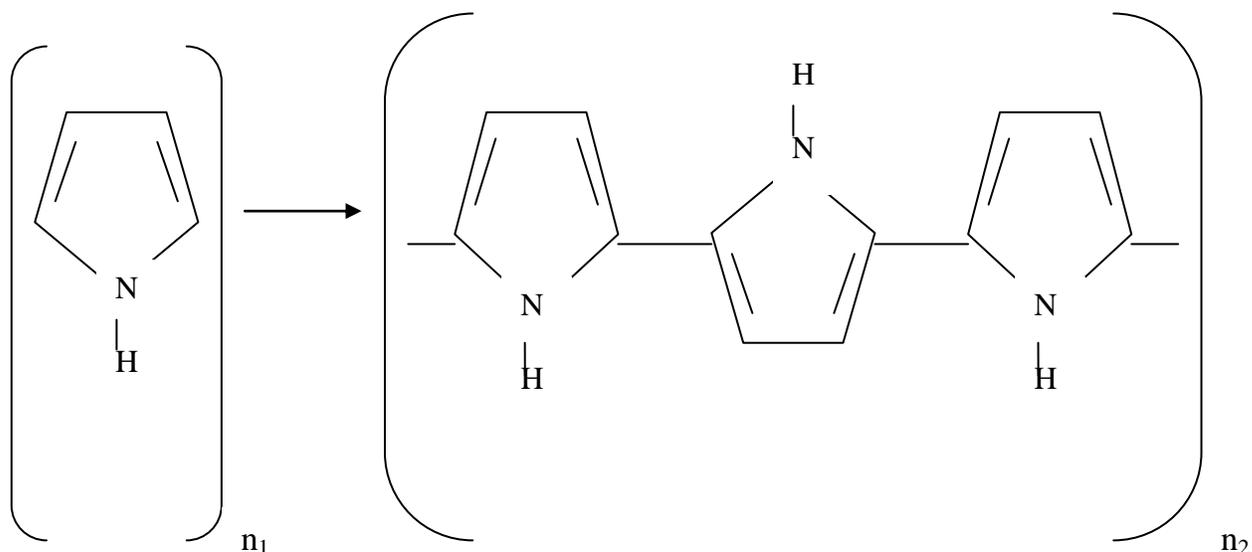


Fig. 1.5: Schematic diagram of the electrochemical polymerisation of poly(pyrrole) from its monomer.

Electropolymerised poly(pyrrole) is an electronically conducting organic polymer. Glucose oxidase has been immobilised in poly(pyrrole) films (Vidal et. al. 1998). Hence, the effect is to enfold the enzyme in a conducting layer on the surface of the electrode. Moreover, products of the enzyme reaction with glucose are produced in the immediate proximity of this conducting material (Umana et. al. 1986). These ultra-thin poly(pyrrole)-glucose oxidase electrodes can be reused for more than two months without major loss in sensitivity (Adelaju et. al. 2001).

The selectivity of poly(pyrrole) films improves the determination of glucose in the presence of electroactive species, such as ascorbic acid and uric acid. Moreover, by controlling the charge passed during polymer electrosynthesis, it is possible to control the

thickness of the polymer film formed on the platinum electrode surface, along with the amount of enzyme entrapped. Thus the analytical performance of the sensor can be controlled.

In short, the one step process of electrodeposition of enzyme and the polymer gives rise to many advantages such as ease of production, direct use of the working electrode for electrochemical detection and a long working life of the biosensor.

There are some disadvantages associated with the entrapment of enzyme in a polymer layer. Sensitivity of the sensor does not vary appreciably during storage. However, there may be problems associated with long-term stability of the enzyme, and the toxicity problems associated with leaching of the enzyme, *in vivo*, which are not covalently bound to the electrode surface (Foulds et. al. 1988).

One approach to solve these problems can be covalent immobilisation of the enzyme onto such films. The polymer can be directly electropolymerised onto the surface of the electrode and then the enzyme can be covalently immobilised onto the electropolymerised ultrathin film. Alternatively a further film or membrane can be applied on top of the enzyme-polymer layer.

#### **1.6.1.1.2 Poly(phenol)**

Poly(phenol) is a non-conducting polymer. Non-conducting polymer films are self-regulating with uniform thickness; they cover the surface of the electrode completely regardless of the morphology of the electrode. The schematic diagram of the monomer of poly(phenol) is shown in Fig (1.6).

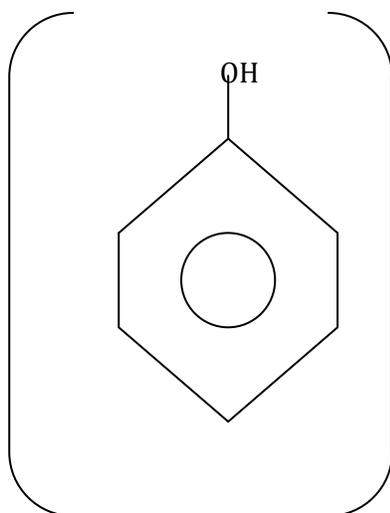


Fig. 1.6: Schematic diagram of the structure of the monomer of poly(phenol).

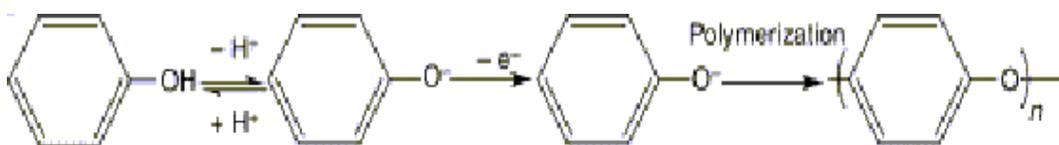


Fig. 1.6.1: The electrooxidation (electropolymerisation) processes of phenol (Yuqing et. al. 2004).

Electropolymerised films of phenol are produced by *ortho*- or *para*-coupling of phenolate radicals that are generated from oxidation of the phenolate anion at surface of the electrode. Subsequent reactions produce oligomers, thus the poly(phenylene oxide) films are polymerised and deposited directly at surface of the electrode (Nakabayashi et. al. 1998; Yuqing et. al. 2004).

These films only grow thick enough to achieve uniformity and a self-limiting depth, to become an insulator. The polymerisation continues until the surface of the electrode is covered, then the process stops. This is because the monomer is unable to penetrate the film any further. The modified electrode displays a high degree of selectivity to hydrogen

peroxide. Response to other electroactive chemicals such as urate, ascorbate, serum and paracetamol are greatly reduced. The problem of interference is thus alleviated.

Glucose oxidase can be incorporated into electropolymerised poly(phenol)films. These films are continuous and largely free of pinholes. When the enzyme is incorporated into these films, their appearance changes and clumps or islands of immobilised enzyme can be seen on the surface of the electrode.

The thickness of these ultrathin films has been measured; they are 1 – 2 enzyme molecules thick. Amperometric measurements in the presence of glucose show that a significant proportion of these immobilised enzyme molecules remain active (Arrigan et. al. 1998). Hence, these non-conducting phenol films provide selectivity and robustness and an improved analytical response to the glucose sensor.

Phenol films have been used in conjunction with phenol derivatives such as poly(phenol red) films. The proposed structure and redox reaction is shown schematically in Fig. 1.6.2 below.

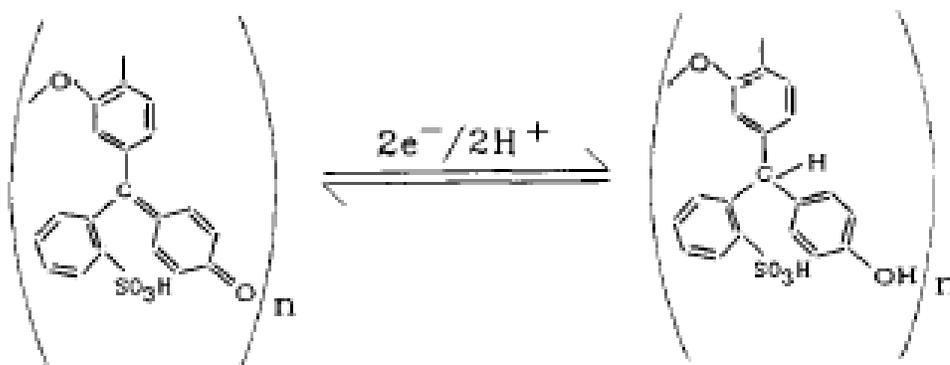


Fig. 1.6.2: The schematic representation of the proposed structure and redox reaction of poly(phenol red), (Warriner et. al. 1996).

Selective properties of such modified electrodes depend on the final polymeric structure of the membrane. Although phenol films are essentially non-conducting, dual phenolic film deposition is still possible. Poly(phenol red) forms an initial porous polymer film on the surface of an electrode, followed by a more diffusion limiting film of oxidised phenol. The resulting composite film combines the characteristics of both component polymers. Response of these films is selective but exceptionally fast and of a magnitude similar to that of a bare electrode. Thus, modified electrodes are stir-independent (Eddy et. al. 1995).

These non conducting defect free electropolymerised films provide an alternative to standard polymer membranes such as cellulose acetate, poly(carbonate), for use as the inner membrane in a biosensor. The role of the outer membrane is also very important for the functioning of biosensors.

### **1.6.1.2 Cellulose acetate films**

Cellulose acetate membranes have been used as inner membranes as well as outer membranes in classical amperometric enzyme electrodes. Mullen et. al. (1986) used cellulose acetate as inner membrane in conjunction with the outer poly(carbonate) membrane to make enzyme electrodes for the measurement of lactate in undiluted blood. Their studies showed that the presence of a cellulose acetate membrane between the electrode surface and the enzymatically active poly(carbonate) membrane significantly reduced the interference caused by electroactive species.

Cellulose acetate has also been used as a composite material with nafion, as inner membrane, in implantable amperometric glucose sensors. This composite has been found to eliminate acetaminophen and other electrochemical interferences while at the same time maintaining reasonable diffusivity for hydrogen peroxide. These sensors have exhibited significantly reduced steady state sensitivity to acetaminophen in vivo (Zhang et. al. 1994).

Cellulose acetate is commercially available as a precast membrane. It can also be applied on the surface of the sensor by dip coating method.

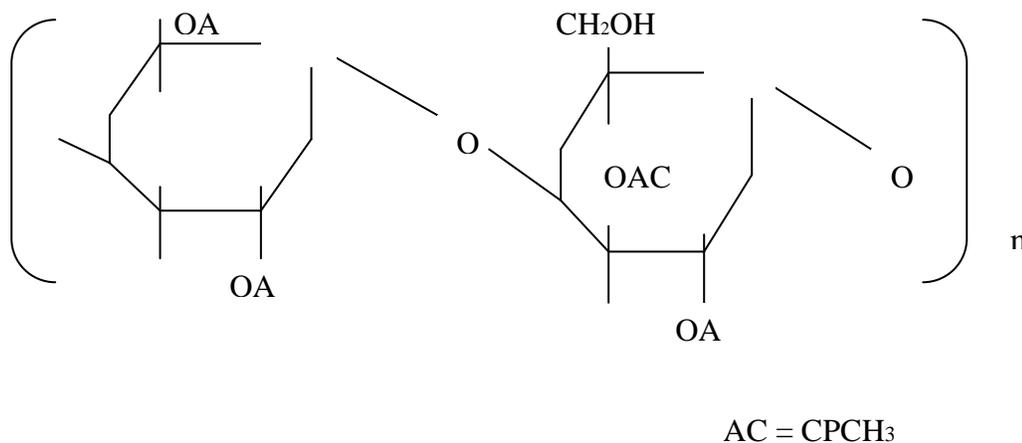


Fig. 1.7: Structure of Cellulose acetate.

The polymer structure contains some negative charge, which is derived from the presence of residual carboxyl groups. At physiological pH conditions these groups are ionised. Hence, these consequently enable the cellulose acetate membrane, not only to exclude the electroactive or interfering species such as ascorbate and urate but also proteins. However, the actual selectivity depends on membrane thickness, pore size, preparation conditions such as the composition of the solvent, the rate of evaporation, humidity and the environmental temperature (Wilson et. al. 1990). Therefore, by controlling the preparation conditions it is possible to reproduce membranes with similar selectivity. As the cellulose acetate membrane not only excludes interfering species but also proteins, it has been widely employed as an inner as well as outer protective membrane (Abdel-Hamid et. al. 1995) in amperometric enzyme electrodes. The role of the outer membrane is very important for the functioning of biosensors.

## 1.6.2 Outer membranes

The outer membrane provides protection to the biosensor from the medium surrounding it as this membrane prevents large molecules that are present in physiological solutions and blood e.g. proteins, from entering and interfering with the biological layer. Moreover, it reduces the chances of enzyme leakage *in vivo*. This function of the outer membrane is important. For example, in glucose sensors the enzyme glucose oxidase is of non human origin and its leakage *in vivo* may cause immunological reactions (Thevenot et. al. 1999, 2001).

The outer protective membrane, in general, must be biocompatible and stable *in vivo* and at the same must allow the passage of substrate to the surface of the electrode. For sensors with essentially planar active surfaces, precast membranes can be used. Generally, these have more uniform properties than membranes that are directly deposited onto the surface of the electrode from solution. However, precast commercially available membranes can be disadvantageous if the geometry of the surface of the electrode is not planar, e.g. in case of needle type of electrodes. This is because it may be difficult to place the membrane in uniform contact with the surface of the electrode. In this case, response characteristics change with time. For needle electrodes, the deposition of polymer layers from solution, e.g. by dip coating, produces a more adherent layer which can have a spherical geometry. Moreover, if the precast membranes are less than 10 – 15 microns thick, they cannot be handled and manipulated without tearing (Wilson et. al. 1990). Hence, if thinner membranes are required (< 10 microns) then direct deposition of the polymer layer onto the surface of the electrode or sensor from solution is employed.

Factors that determine the selectivity of a polymer membrane include its thickness, pore size, preparation conditions such as the composition of the solvent, the rate of evaporation, humidity and environmental temperature (Wilson et. al. 1990).

Many polymer materials have been employed as outer membranes in sensors including cellulose acetate (Rebrin et. al. 1992), silicone rubber (Baker et. al. 1995), polyvinyl chloride (Abdel-Hamid et. al. 1995, Kyrolainen et. al. 1997), polycarbonate (Mullen et. al. 1986) and polyurethanes (Churchouse et. al. 1986, Shichiri et. al. 1987, Abdel-Hamid et. al. 1995, Labat-Allietta et. al. 1998).

### 1.6.2.1 Poly(vinyl chloride)

The wide use of Poly(vinyl chloride) or PVC in medical devices has been due to its good biocompatibility properties and its ability to undergo structural changes through plasticisation. It retains mechanical integrity upon plasticisation. PVC membranes (Abdel-Hamid et. al. 1995) and modified PVC membranes (Reddy et. al. 1997, Kyrolainen et. al. 1997) have been used as outer protective membranes in biosensors. The general reaction scheme for the polymerisation of PVC from its monomer (vinyl chloride) is shown in Fig. 1.8.



Fig. 1.8: General reaction scheme for the synthesis of PVC.

PVC undergoes degradation process at high temperatures. On exposure to ultraviolet light, hydrochloric acid is released and the material acquires a brown black colour. PVC has a glass transition temperature of 78 –80 degrees centigrade. PVC on its own is a hard material. Plasticisers can be added to increase the flexibility of the polymer.

Plasticisers are compounds that have very low glass transition temperatures. They are blended with polymers of higher glass transition temperature and in miscible systems

thus plasticise, i.e. reduce the glass transition temperature of the other component. The process of plasticisation is often achieved by mechanical mixing of the required components.

Plasticisers are technologically important compounds as they impart flexibility to hard plastics. However, plasticisers with very low molecular weight, which have high free volume content and are soluble materials with low glass transition temperature, often have the disadvantage of being volatile and can slowly evaporate and thus can be lost from the matrix polymer (Eastmond, 2000). In implantable materials such as PVC the plasticisers should be moderately large, non-volatile molecules, so that they do not leach out of the polymer matrix in vivo.

The choice of plasticiser requires consideration not only of its compatibility with the polymer matrix, but also the chemical nature of the end product, because the resultant membrane properties, such as selectivity, depend on the functional groups of the plasticiser used. Selectivity is conferred by the relaxation of the ion impermeability properties of the membrane, which is specified by the incorporation of the plasticiser, ion exchanger or neutral carrier molecule. PVC membranes have been modified with various plasticising agents including surfactants. So far, electrodes thus constructed have enabled measurements of species such as  $H^+$ ,  $K^+$ ,  $Ca^+$ ,  $Cl^-$  and organic anions such as formate, salicylate and oxalate (Lakshminarayanan, 1969). Cationic surfactants are used in the transport of anionic species.

Surfactants are also known as surface active agents. These are molecules, which possess distinct hydrophobic and hydrophilic characteristics. Surfactants are classified as anionic (e.g. sodium dodecyl sulphate and potassium laurate), cationic (e.g. quaternary alkylammonium salts), zwitterionic (e.g. dodecyl-N,N-dimethyl betaine) and nonionic (e.g. sorbitan trioleate). This classification is dependent on their size and substituents.

PVC membranes modified by incorporation of nonionic surfactants such as Tween 80 and Triton X – 100 and anionic bis(2-ethylhexyl) hydrogen phosphate (BEP), exhibit

superior biocompatibility over the commercially available porous poly(carbonate) and cellulose acetate membrane (Reddy et. al. 1997). This is because these membranes mimic the high permeability and selectivity properties of conventional dialysis membranes to a range of low molecular weight solutes. BEP contains extensive side chain branching. When PVC is plasticised with BEP, the loss of surfactant (through physical leaching) is minimised from the membrane matrix (Reddy and Vadgama, 1997).

Kyrolainen et. al. (1997) used PVC plasticised with Triton X – 100, as outer membrane material, in their studies on lactate enzyme electrodes. Their results show that the incorporation of this nonionic surfactant into outer poly(vinyl chloride) membrane acts as a hemocompatible interface and also as a diffusion barrier, extending the sensor linear range.

For glucose sensors, linearity with unplasticised PVC as outer membrane is observed to be up to 300 mM glucose. However, higher linear range can be achieved by using mixed PVC/polycarbonate membrane, up to 2000 mM glucose (Maines et. al. 1996).

### **1.6.2.2 Poly(carbonate)**

Poly(carbonate) or PC membranes have been used in biosensors as outer protective membranes (Mullen et. al. 1986). Poly(carbonate) is a linear thermoplastic based on bis-phenol, a dihydroxy compound. The poly(carbonate) resin is characterised by unusual toughness and clarity. It has a high melting point (225 – 250 degrees centigrade) a high glass transition temperature (150 degrees centigrade) and is degraded by mineral acids and alkalis.

The polymer does not show any crystallinity (Teoh et. al. 1998). Glass transition temperature,  $T_g$ , is the temperature at which the polymer undergoes a change from a glassy brittle material to a more elastic and rubbery material.

The general reaction scheme for the synthesis of poly(carbonate) is shown in Fig. 1.9.

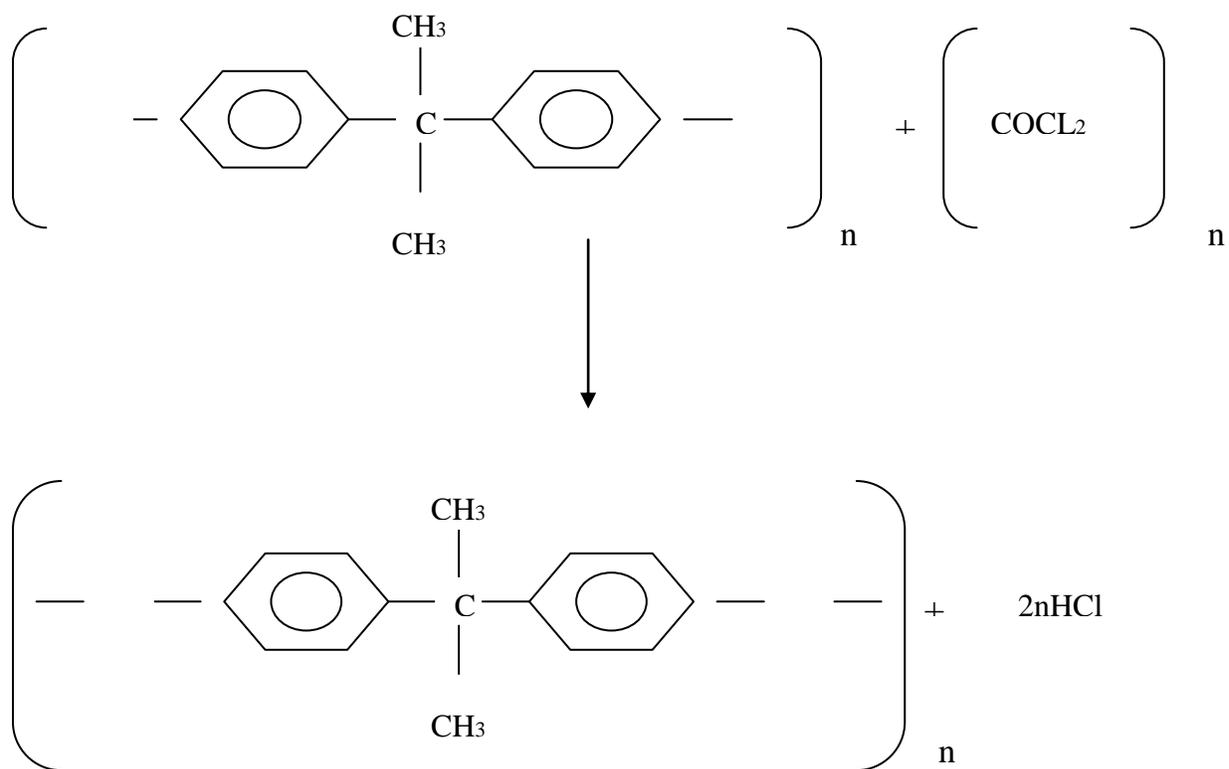


Fig. 1.9: General reaction mechanism for the synthesis of poly(carbonate).

Poly(carbonate) membranes used for biosensor applications have pores in order to control the flux of the solute to the enzyme layer. The analytical linear range increases with decreasing the pore size (Maines et. al. 1996).

Glucose sensors with nonporous poly(carbonate) as an outer membrane were investigated in vivo by Wisniewski et. al. (2001), using subcutaneous implants in rats over eight days. The probes showed a buildup of cellular and fibrous debris on the surface and a reduced permeability to glucose after ex-plantation. Electrodes were more stable between 0 and 2 days. The probes retrieved on day zero were covered with loose connective tissue, with blood vessels adjacent to the probe surface. However, on day eight the tissue surrounding the probe was found to be denser with a high density of white cells. It was thought that the tissue on day eight, on the surface of the probe, had less interstitial space, reduced

vascular proximity and an increased cellular metabolic consumption. All of these effects led to an increase in resistance to transport of glucose to the membrane surface.

The search continues for more biocompatible materials to be used in biosensors. Silicone membranes have also been used as membrane materials in biosensors.

### 1.6.2.3 Silicone membranes

The elastomers most extensively used for the biomedical applications are the medical grade silicone rubbers. This elastomer is of heat vulcanising type and it consists of methyl and vinyl monomer copolymers. The formula of this copolymer is in Fig. 1.10 as follows:

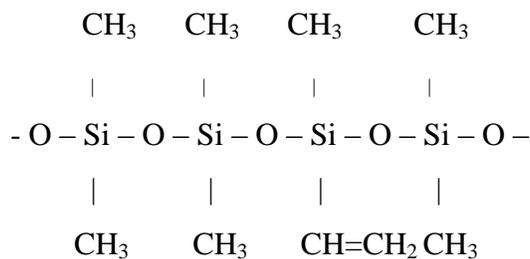


Fig. 1.10: Chemical formula of the silicone rubber.

The polymer chains have molecular weight of up to 500,000. In silicone gel the polymer is cross-linked, the more cross-linking, the more solid is the gel. Silicones first became commercially available in the early 1940s. Silicones have since been developed for a variety of medical applications. These elastomers have many desirable attributes such as high flexibility, good resistance to body fluids and non-toxicity. They have thrombo resistance properties better than many other elastomers. Baker et. al. (1995) used silicone rubber in the manufacture of lactate sensors and reported in their studies that the sensor showed no indication of clot formation at ex-plantation and no signs of signal modifications ascribable to clotting during the experiment. This relative non-

thrombogenicity of silicone rubbers has been reported before (Van Noort et. al. 1981). These membranes exhibit selective permeability and low biofouling.

The properties of medical grade silicone rubbers vary with curing time. Van Noort et. al. (1979) showed in their studies that silicone rubber must be cured under controlled conditions to achieve optimum properties. Their studies showed degradation of these rubbers in “*in vivo*” experiments in guinea pigs. A calcific surface deposit on the prosthesis was also observed, which caused substantial alterations in surface characteristics.

Silicone rubber implants may cause inflammatory reactions as well as silicone hypersensitivity (Kossovsky, 1986). Gradual degradation of these elastomers can also take place. Silicone rubbers are prone to swelling, which leads to an increase in the weight of the polymer, which in turn may affect its physical and chemical properties. Hence, the search continues for better biocompatible materials, to be used in biosensor.

Desai et. al. (2000) used nanoporous silicone membranes in their investigations to find biocompatible membranes for biosensors applications. Their results show that such membranes can be fabricated with uniform pore sizes and are capable of simultaneous diffusion of glucose and exclusion of albumin. Moreover, these silicone membranes exhibited no morphological change or degradation in the presence of biological proteins and fluids at 37 degrees Centigrade. Hence, they offer advantages in their stability and reproducibility. Other polymers such as polyurethanes have also been used as membrane materials in biosensors.

#### **1.6.2.4 Polyurethanes**

Polyurethanes have been used in glucose sensors because of their good mechanical properties and blood biocompatibility. The most common technique used for applying the outer polyurethane membrane is from solution by dip coating method.

Churchouse et. al. (1986), Shichiri et. al. (1987), Abdel-Hamid et. al. (1995) and Yang et. al. (2000) used dip coating techniques to apply the outer polyurethane layer on needle type of glucose electrodes, which were implanted subcutaneously in vivo. The outer polyurethane membrane extended the linear range of glucose concentration to 70 mM and the response time of 60 seconds, of the sensor (Churchouse et. al. 1986). However, the linearity of the sensor response may be affected by varying the concentration of polyurethane in the outer membrane (Vadgama et. al. 1988, Abdel-Hamid et. al. 1995).

If the desired linear range is not achieved after the first coat, it is possible to increase the thickness of the membrane by applying multiple coats. Hence, by varying the thickness of the polyurethane membrane, the required linear range may be achieved (Bindra et. al. 1991).

The general reaction scheme for synthesis of polyurethane from its monomer is shown in Fig. 1.11 as follows:

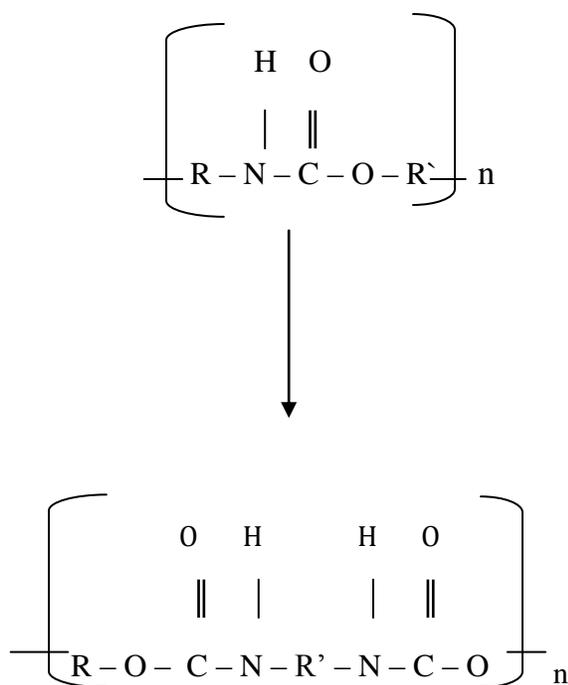


Fig. 1.11: General reaction scheme for polyurethane synthesis.

The polyurethane structure consists of alternating hard segments, which may be glassy or crystalline, and soft segments i.e. elastomeric chain segments. The micro phase separation of these two chemically distinct components gives rise to some very useful physical and mechanical properties of polyurethane. The hard segments are composed of urethane segments and form glassy or semi crystalline domains. They act as multifunctional cross linking sites, resulting in materials, which have high modulus and exhibit elastomeric behaviour. The soft segments form an amorphous or semi crystalline matrix in which the hard segments are dispersed. Segmented polyurethane appears to be the best compromise material available in terms of biocompatibility and mechanical flexibility. However, in terms of durability it may not be ideal.

Material properties and the surface finish may promote calcification. The defects in surface and porosities provide sites for the deposition of minerals and other biological components. Golomb et. al. (1991) reported calcification of plain polyurethane films in their studies. Calcification on the surface of plain polyurethane films indicates that the material properties of a biomedical material play a vital role in determining the durability and life of an implant in the body. Calcified deposits on the surface of an implant may provide further sites for the deposition of proteins and thrombus formation.

The interaction between calcification and thrombosis is not yet clear. It is likely that calcific alterations cause severe hemodynamic and surface changes, which may be the prime reason for thrombus formation, which in turn leads to the problem of biofouling. Hence, polyurethane may not be the best material as an outer membrane for biosensors on its own and may need some surface modification.

Yang et. al. (2000) used copolymers to modify polyurethane membranes in their investigations to improve the hemocompatibility of glucose sensors. Their results show that thus modified polyurethanes extend linear response, up to 100 mM glucose, due to the enhancement of diffusion. Moreover, non coated polyurethane was covered with large amorphous deposits, containing numerous red cells and fibrin strands, these were not

found on the coated surface, which indicates that the modified (coated) polyurethane is protein resistant.

The surface of a sensor can be modified by various techniques to reduce biofouling effects, extend linear range of the sensor and therefore enhance the overall response of the biosensor.

## **1.7 Membrane modifications**

Ideally, the surface of the sensor should be able to select specifically required molecules, similar to a cell membrane, while resisting undesirable and non specific interactions. One approach to improve biocompatibility and to enhance selectivity of a biosensor is incorporation of surfactants.

Surfactants, also known as surface active agents, are molecules, which possess distinct hydrophobic and hydrophilic characteristics. Surfactants are classified as anionic (e.g. sodium dodecyl sulphate and potassium laurate), cationic (e.g. quaternary alkylammonium salts), zwitterionic (e.g. dodecyl-N,N-dimethyl betaine) and nonionic (e.g. sorbitan trioleate). This classification is dependent on their size and substituents. Cationic surfactants are used in the transport of anionic species.

PVC membranes have been modified with various surfactants (Lakshminarayanan 1969). Cellulose acetate membranes have been modified by means of high molecular weight surfactant. By simple entrapment of nonionic Tween or Triton X-100 it is possible to improve the mechanical properties of cellulose acetate and to reduce the surface fouling (Maines et. al. 1996). Hence, incorporation of surfactants into membranes is a viable solution for modification of membranes in order to achieve better response.

### 1.7.1 Naturally derived materials

Many materials of biological origin such as silk fibroin and cellulose have been used in biosensors applications. However, the classical amperometric glucose sensors that use cellulose acetate membrane of wood origin have limited clinical use as they are not stable in blood for long. Cellulose acetate membranes of bacterial origin have been used instead of cuprophan membranes, to improve the long term stability of glucose sensors in vivo. In diluted blood the bacterial cellulose acetate covered sensors exhibit long term stability of more than 200 hours. In undiluted blood they are stable for 24 hours, which is 6 – 7 times longer than the stability of the classical cuprophan membrane covered sensor (Ammon et. al. 1995).

Naturally derived albumin and heparin are used to improve the response and biocompatibility of biosensors. Albumin is used with glutaraldehyde to immobilise glucose oxidase and to improve the response of the biolayer. Thus, it is used to enhance the response of the biosensor. Heparin is used on the outside to modify the outer membrane of the biosensor to reduce the biofouling.

#### 1.7.1.1 Albumin

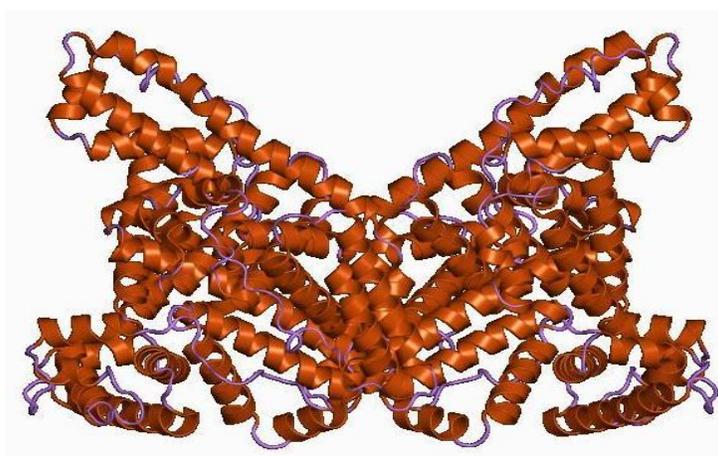


Fig.1.12: Crystalline structure of albumin (Sugio et. al. (1999)).

Albumin is the most abundantly present protein in plasma in the body is. It is a globular protein. It binds to water, cations (such as  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$ ), hormones, fatty acids and drugs. It plays a vital role in the body as its main function is to regulate the colloidal osmotic pressure of the blood. Albumin consists of three homologous domains that assemble together to form a heart shape molecule. Serum albumin is produced in the liver. It forms a large proportion of all plasma proteins and it normally constitutes 60% of the human plasma protein. Albumin is widely used in the cross linking of the enzyme in the biological layer of amperometric biosensors.

### 1.7.1.2 Fibrinogen

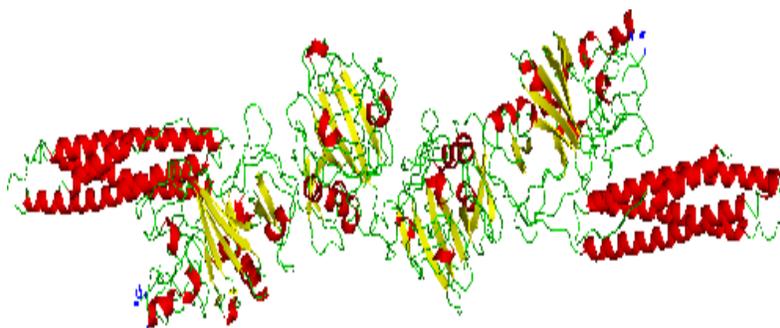


Fig.1.13: Crystal structure of human fibrin (Everse et. al. 1998)

Fibrinogen is synthesised in the liver, it is a soluble plasma glycoprotein that is converted by thrombin into fibrin during blood coagulation. It is possible to measure the levels of fibrinogen in venous blood. Normal levels are 1.5-2.77 g/L, higher levels of fibrinogen, amongst other factors, are associated with cardiovascular disease ( $>3.43$  g/L). In its natural form, fibrinogen can form bridges between platelets, by binding to their surface membrane proteins. However, its major function is as the precursor to fibrin. Fibrinogen is the main protein in clotting of the blood. It is essentially a hexamer, containing two sets of three different chains ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) that are linked to each other by disulfide bonds. The conversion of fibrinogen into fibrin fibers is associated with the formation of the fibrin gel. Size of the fibrinogen molecule in its hydrated form is larger than its dry form and is cylindrical in shape with round edges (Bachman et. al. 1975).

### 1.7.1.3 Lysozyme

Lysozyme is an enzyme that is abundantly found in body secretions and fluids such as saliva, tears, mucus and human milk. It contains all of the 20 common amino acids. Lysozyme can damage bacterial cell walls by catalyzing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins.

Lysozyme plays a vital part in the immune system as it is a natural form of protection from gram positive pathogens like Bacillus, Staphylococcus and Streptococcus. The skin is a protective barrier due to its dryness and acidity, however, the conjunctiva (membrane covering the eye) is instead protected by secreted enzymes, mainly defensins and lysozyme.

### 1.7.1.4 Hyaluronan

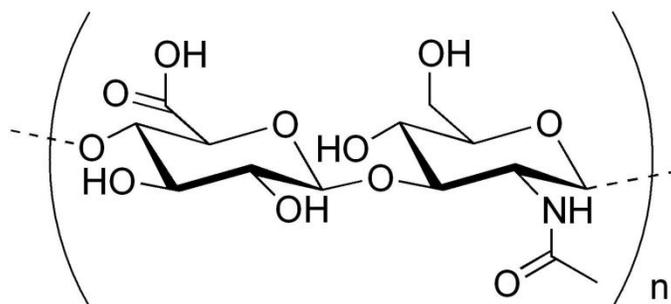


Fig. 1.14: Schematic structure of hyaluronan.

Hyaluronan is also known as hyaluronic acid. It is an anionic, non sulfated glycosaminoglycan, which is widely distributed throughout the body and is found abundantly in the connective, epithelial, and neural tissues. It is unique among glycosaminoglycans in that it is non-sulfated and is produced in the plasma membrane instead of the Golgi. It can be a very large molecule, with its molecular weight often reaching the millions. Hyaluronan is one of the major components of the extracellular

matrix and it contributes significantly to cell proliferation and migration. The average 70 kg (154 lbs) person has roughly 15 grams of hyaluronan in the body, one third of which is turned over (degraded and synthesized) every day.

Hyaluronan is an important component of articular cartilage, where it is present as a lubricating coat around each cell. When aggrecan monomers bind to hyaluronan in the presence of a link protein such as a glycoprotein, large highly negatively charged aggregates form. These aggregates imbibe water and are responsible for the resilience of cartilage (its resistance to compression). Hyaluronan can be 25,000 disaccharide repeats in length. Polymers of hyaluronan can range in size from 5,000 to 20,000,000 Da *in vivo*. The average molecular weight in human synovial fluid is 3–4 million Da and hyaluronan purified from human umbilical cord is 3,140,000 Da.

Hyaluronan is synthesised by a class of integral membrane proteins called hyaluronan synthases. These enzymes lengthen hyaluronan by repeatedly adding glucuronic acid and N-acetylglucosamine to the nascent polysaccharide as it is extruded via ABC-transporter through the cell membrane into the extracellular space. Hyaluronan is degraded by a family of enzymes called hyaluronidases. In humans, there are at least seven types of hyaluronidase like enzymes, several of which are tumor suppressors. Hyaluronan plays a key role in the repairing of skin wounds.

Hyaluronan is found in many tissues of the body, such as skin, cartilage and vitreous humour. Therefore, it is well suited to biomedical applications targeting these tissues. Native hyaluronan has a relatively short half life so various manufacturing techniques have been deployed to extend the length of the chain and stabilise the molecule for its use in medical applications. Hyaluronan is medically applied in eye injury and cataract surgery, dry skin ointments for dermatitis and many more applications.

In the present study the naturally derived macromolecules namely the albumin, fibrinogen, lysozyme and hyaluronan were investigated as potential candidates as coating materials to modify the polymer membranes for use in sensors.

## 1.8 Aims and Objectives

The aim of this study is to produce a needle type of electrode that would give optimum response and resolve the problem of interference by utilising polymeric biointerfacing barrier membranes derived from naturally occurring macromolecules and would be biocompatible. By utilising naturally derived macromolecule in the form of coatings on the commercially available membranes and electropolymerised films that are selective of the analyte and reduce interference from electroactive substances, it is possible to obtain a biocompatible biointerfacing membrane that acts as a barrier for the interferents.

Naturally derived macromolecules such as hyaluronan and albumin have been employed in order to impart selectivity to the sensor and to improve the biocompatibility and durability of the biosensor.

The objective of the study is to select a membrane that would reject the interfering species and thus maximize the response of the biosensor. Various membranes have been investigated in order to achieve this goal. Commercially available membranes such as poly(carbonate) and poly(vinylidene fluoride) membranes and electropolymerised films have been studied.

Electropolymerised phenol films were investigated as these provide ultrathin defect free films, which enhance the response of the biosensor along with sensor linearity and biocompatibility. These films provide selectivity and thus solve the problem of interference. Different types of phenol films were investigated as potential candidate material for inner membranes on needle type of electrodes. As outer membranes, different types of PVC, mixed cellulose acetate and PVDF were investigated.

Membrane modifications and adsorption studies with various naturally derived macromolecules i.e. hyaluronic acid, albumin, fibrinogen and lysozyme have been done on the precast membranes and electropolymerised films. The macromolecules used in the study i.e. hyaluronan, albumin and fibrinogen are negatively charged at physiological

conditions. Hence, the objective was to produce a biointerfacing layer that rejects electroactive species on the basis of charge selection. However, initial results showed that all these membranes give reduced response on exposure to the macromolecules. Therefore, investigations were carried out on these membranes and films in order to find out the impact of the thickness of the deposited macromolecular film on the selectivity of the modified films. These studies involved SEM, AFM and QCM investigations to investigate the adsorption pattern of macromolecules on the membranes after various exposure times.

The scanning electron microscope was used to characterise the surface of the membranes before and after modification post deposition with macromolecules. The membranes used in the SEM studies were commercially available precast membranes including poly(carbonate), poly(vinylidene fluoride) and mixed cellulose acetate membranes. These investigations provided an insight into the adsorption mechanisms of the macromolecules on the surface of the membranes. However, it was difficult to investigate the exact nature of deposition and thickness of the deposited layer.

The atomic force microscope was used to study the surface roughness and the thickness of the deposited macromolecular films after a given period of time. QCM was used to investigate the macromolecule deposition in real time and aqueous conditions on the poly(carbonate) films for various time periods.

The design of needle type of electrode was investigated in order to acquire a microelectrode that gives optimum response. Gold and platinum were investigated as suitable candidate materials for utilization in the needle type of electrodes. The platinum disk type of microelectrode was used in selectivity studies involving various electroactive substances.

## **Chapter 2      Experimental**

### **2.1    Materials**

#### **2.1.1   Membranes**

The commercially available isoporous polyvinyl pyrrolidone coated poly(carbonate) membranes of 0.05, 0.22 and 5.0  $\mu\text{m}$  pore diameter were obtained from Millipore, U.K. Polycarbonate resin was obtained from Aldrich. Poly(carbonate)membranes of 0.22 and 5.0  $\mu\text{m}$  pore diameter were obtained from Fieder Filter Systems, U.K. Poly(vinylidene fluoride) and mixed Cellulose acetate membranes of 0.22  $\mu\text{m}$  pore diameter were obtained from Millipore, U.K. The dialysis membrane used was a Cuprophan dialysis membrane, removed from a hemodialysis cartridge (Gambro, Lund, Sweden).

#### **2.1.2   Buffer Solutions**

Phosphate buffer saline solution of pH 7.4 was prepared using 10.8 g of sodium dihydrogen orthophosphate dihydrate (BDH Chemicals), 37.47 g of disodium hydrogen orthophosphate (BDH Chemicals) and 15 g of sodium chloride (Sigma) dissolved in 1 L of ultra pure distilled water. The buffer solution of pH 3 was made by mixing solutions 20.55 ml of 0.2 M di-sodium hydrogen orthophosphate anhydrous (BDH chemicals) and 79.45 ml of 0.1 M citric acid (Sigma).

#### **2.1.3   Macromolecules**

Hyaluronic acid (from rooster comb), bovine serum albumin and newborn calf serum were obtained from Sigma. Lysozyme (from hen egg white) and fibrinogen (from bovine plasma) were obtained from Fluka Biochemica.

## 2.1.4 Chemicals

Electrochemically active chemicals including catechol, acetaminophen and hydrogen peroxide (30% v/v) were obtained from BDH. Ascorbic acid, uric acid and phenol (Analar R grade) were obtained from Sigma. Phenol red, potassium ferrocyanide (II) trihydrate, hydrochloric acid and ethanol were obtained from Aldrich. Dichloromethane and ammonia (25% v/v) were also obtained BDH.

Solutions of all the chemicals and macromolecules were made fresh prior to the experiments in buffer solution as required.

## 2.2 Methods

The following techniques were used in this study

- 1 Field emission scanning electron microscopy (SEM).
- 2 Atomic force microscopy (AFM) non-contact, tapping mode in air.
- 3 Quartz crystal microbalance (QCM).
- 4 Amperometry
- 5 Cyclic Voltametry

### 2.2.1 Electron Microscopy

A field emission scanning electron microscope JEOL 6300F (JEOL UK Ltd) with the secondary detectors was used to examine the membranes used in the biofouling studies. The field emission source in the SEM is for reduction of surface damage by using lower voltages. Moreover, a higher resolution as high as 1.5 nm can be achieved.

The samples used in the study were prepared beforehand. Membranes were immersed in required protein solutions for 1 hour or 24 hours and rinsed with ultra pure water by

immersion for 5 minutes to get rid of any labile proteins or macromolecules present on the membranes. Membranes were then dried in Petri dishes and mounted on stubs. Because of their nonconductive nature the samples were coated with a conductive gold layer (10-20  $\mu\text{m}$  thick), to avoid burning and charge effects of the samples, using a sputter coater (Balzer) for 30 - 60 seconds at 30 mA. Thus prepared samples were investigated on the same day or the following day of coating. The samples were investigated under accelerating voltages of 10 KV. The images taken with the SEM were of high resolution at various magnifications.

### **2.2.2 Atomic Force Microscopy**

An atomic force microscope (AFM) was used in tapping mode in air to investigate the surface properties of membranes before and after exposure to macromolecules. The membranes were soaked in a solution of macromolecules in buffer for given periods of time, 1 hour or 24 hours; the fouled membranes were then immersed in ultrapure distilled water for 5 minutes then put into covered Petri dishes to dry for two days.

The membranes were put onto 15 mm diameter flat disk AFM mounts (Agar Scientific Ltd., Essex, U.K) using a double sided tape. The samples thus prepared were investigated by using tapping mode constant force scanning microscopy. The cantilevers used in these studies were explorer mount Si. Veeco model number 1650-00 (Veeco Instruments, France). The scan areas investigated ranged from large to small surface areas, including 80  $\mu\text{m}$  by 80  $\mu\text{m}$ , 50  $\mu\text{m}$  by 50  $\mu\text{m}$  and 2  $\mu\text{m}$  by 2  $\mu\text{m}$  square areas. The images taken were of high resolution.

### **2.2.3 Quartz Crystal Microbalance (QCM)**

A quartz crystal microbalance (Q-Sense D300 from q-sense, scientific, Cheshire, UK) was used for the biofouling studies to investigate the formation of protein films in hydrated state over given periods of time. The QCM-D enabled real time measurements

of processes including adsorption, adhesion and changes of mass in ultrathin films. The system consists of a measurement chamber that has a sensor crystal, an electronics unit and is connected to a computer, which allows recording and analysis of the data.

All experiments were carried out using standard gold electrodes (Qsx 301 from Q-sense). The electrode typically consisted of a thin (0.3 mm) quartz crystal, which has two electrodes namely the active electrode and the counter electrode.

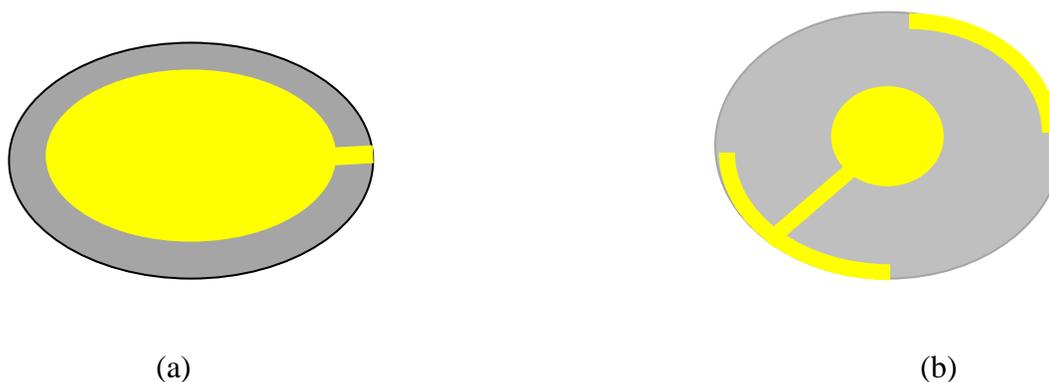


Fig. 2.0: Schematic diagram of a quartz crystal showing (a) active working electrode and (b) the counter electrode (top side (a) lower side (b) of the crystal).

The gold electrodes were used with and without a polycarbonate coating in this study. The polycarbonate membrane was spin coated on the gold electrode using 0.5% W/V (0.05 g / 10 ml) solution of polycarbonate in dichloromethane at 200 rpm, for 20 seconds using a spin coater (Chemat technology, PI KEM LTD, Shropshire, England). The coated crystals were dried for two days in covered Petri dishes.

The gold sensor crystal was placed in the measurement chamber of the QCM-D, which provides a temperature controlled environment for the sensor crystal during the experiment. The chamber with the crystal is connected to the electronics unit and a reservoir inlet. The buffer solution was put into the sample reservoir inlet. Then, 1.5 ml of buffer solution was allowed to flow through the chamber. After acquiring a stable temperature, which was set at 25 degrees Centigrade, 0.5 ml of buffer solution was run

through the sensor side. The data acquisition was then started. The remaining buffer solution was then removed from the reservoir by using a pipette and the reservoir was refilled with the stock solution of the required macromolecule. The buffer solution was used to obtain the baseline reading, after which 2 ml of the stock solution was run through the sensor side and left for the required period of time. Macromolecules adsorption for a given period of time was investigated. The data obtained was used to calculate thickness and viscosity of the films formed over given periods of time.

At the end of the experiment, the electrode was cleaned by immersing the crystals into a mixture of 5 parts water: 1 part ammonia (25%): 1 part hydrogen peroxide (30% V/V) solution, which was heated to 75 degrees Centigrade using a hot plate. For health and safety reasons the cleaning process was carried out in a ventilated fume cupboard. The crystals were removed after 10 minutes and then rinsed with ultra pure distilled water followed by ethanol. The crystal was then blow dried with nitrogen gas.

The polymer coating was removed from the crystal by using a mixture of 50% water: 50% dichloromethane solution in an ultrasonic bath (VWR) at room temperature. The above described method was used to clean the crystal for reuse.

All the experiments were carried out under constant temperature (25 degrees Centigrade).

#### **2.2.4 Amperometry**

The Rank Cell (Rank Bros, Bottisham, Cambs. U.K), which is an oxygen electrode system, was adapted for the electrochemical oxidation of hydrogen peroxide and other electroactive species. The Rank Cell consists of two parts, a lower base plate and an upper section.

The lower base plate holds a 2 mm diameter platinum working electrode (the anode) and a 12 mm diameter annular Ag/AgCl reference electrode. The upper section has a sample chamber where the analyte to be investigated is introduced. The membrane is applied in

between these two sections and is held in place by an O-ring when the Cell is assembled. In figure 2.1(a) picture of the Rank Cell is shown. Figures 2.1(b) and 2.2 illustrate schematic diagrams of The Rank Cell.



Fig 2.1 (a): Photograph of a Rank Cell.

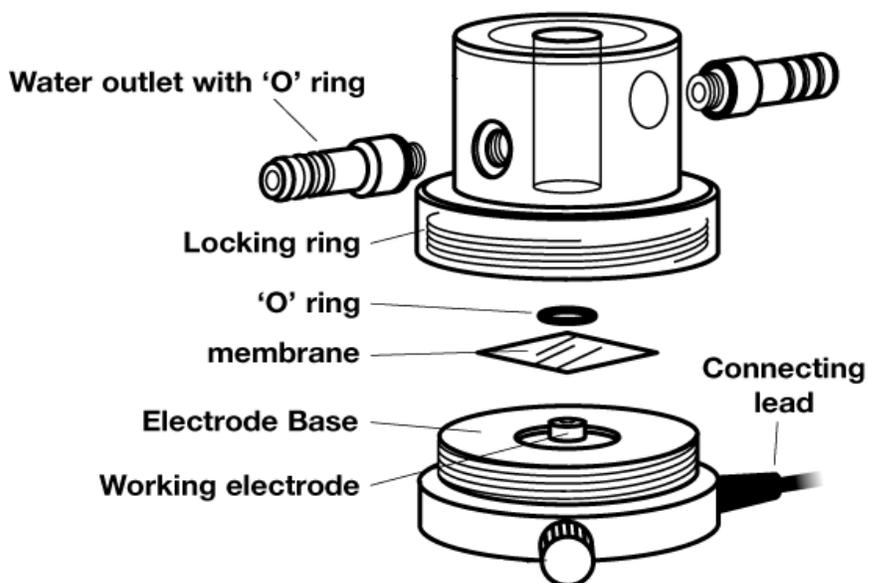


Fig. 2.1 (b): The diagram of the Rank Cell showing the placement of the membrane (modified from <http://www.rankbrothers.co.uk/prod1.htm>).

Figure 2.2 illustrates a schematic diagram of The Rank Cell. The required solution was put into the sample chamber and stirred with a MR100 magnetic stirrer (Heidolph, Kelheim, Germany). The analyte diffuses from the bulk solution through the membrane to the platinum electrode where it is detected.

In order to perform amperometric measurements, the polarisation of the electrode at a constant voltage (+ 0.65V vs Ag/AgCl) was achieved using a potentiostat, Autolab PGSTAT10 (ECO CHEMIE, Utrecht, Netherlands). The output signal was acquired over given period of time.

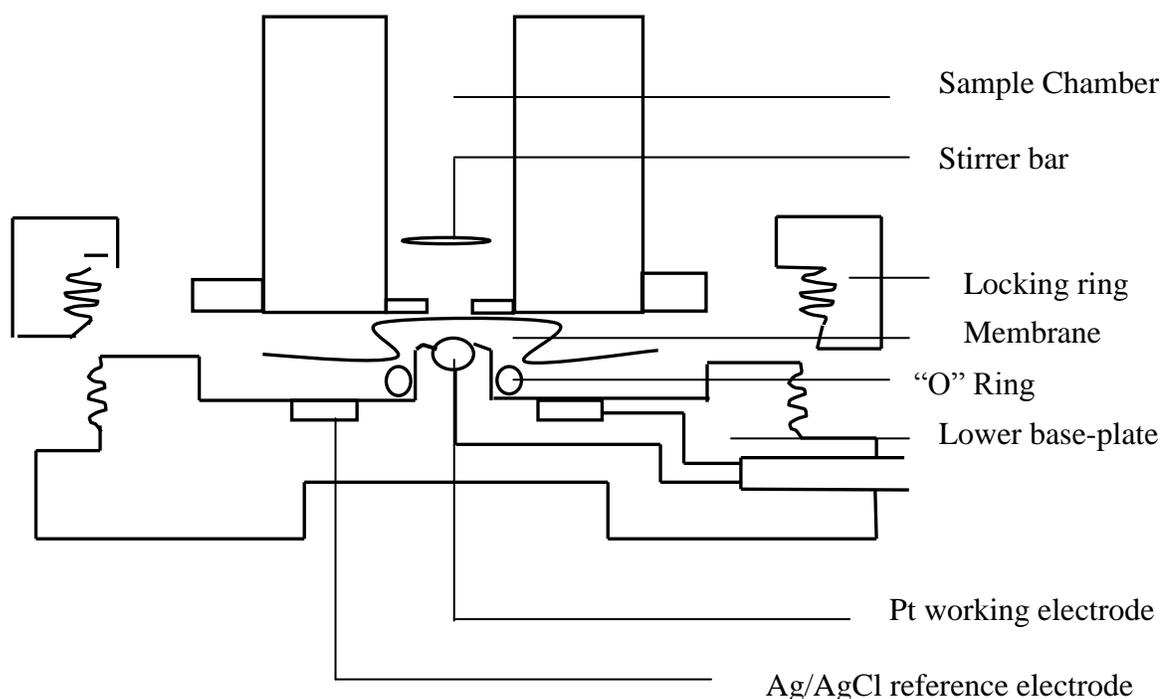


Fig. 2.2: Schematic diagram of a cross-section of the Rank Cell.

The experiments were carried out at room temperature (25 degrees Centigrade). The counter electrode used was MW-1032 platinum wire (Bioanalytical System Inc., Warwickshire, U.K) and the MF-2052 silver/silver chloride electrode (Bioanalytical Systems Inc., Warwickshire, U.K) was used as a reference electrode. The diameter of the counter electrode was 0.5 mm with a length of 75 mm.

### **2.2.4.1 Electrode preparation**

The Rank cell described in section 2.2.4 was utilised as a flat disc electrode in our studies, only the platinum working electrode was used for the experimental purposes. The electrode was polished and cleaned before each use. Electrodes was first rinsed with distilled water and then polished with 0.3  $\mu\text{m}$  alumina powder (BDH) and cotton wool to expose a fresh surface, followed by rinsing with distilled water.

The electrode assembly, in the three electrode system, was calibrated in phosphate buffer for 1 hour at an applied potential of + 650 mV (vs. Ag/AgCl) until a stable baseline current was obtained (0 – 3  $\mu\text{A}$ ).

An overlying high permeability dialysis membrane was usually placed over the electrodes in order to prevent damage to the electrode from the rotating action of the stirrer bar.

### **2.2.4.2 Film formation by electropolymerisation**

Phenolic films, phenol and non conducting phenol red were formed by an electropolymerisation process directly onto the platinum electrode, from a 5 mM buffered solution of the coating species, at an applied potential of + 850 mV (vs. Ag/AgCl). The process was initiated by placing 3 ml of the required solution in the sample chamber, it was oxidised at the applied potential of + 850 mV (vs. Ag/AgCl) to produce the electropolymerised film directly on the working electrode.

The electropolymerisation of poly(phenol red) and poly(phenol) film was found to be self limiting, i.e. once a stable current was obtained after the initial rise the current did not increase. The coating times were between 1/2 - 1 hour. After achieving a stable current for 15 minutes the coating solution was removed from the sample chamber, followed by rinsing with buffer. The cell was further conditioned in buffer for half an hour.

Response to 1 mM hydrogen peroxide was used to assess the integrity of thus formed phenol films. Responses to electroactive species were determined by adding aliquots of stock solutions, of the required chemical, to buffer solution in the sample chamber.

#### **2.2.4.3 Macromolecule exposure for membrane modification**

Fresh macromolecule solutions were prepared in phosphate buffer every time prior to use for the membrane modification investigations. The macromolecules used were hyaluronic acid, albumin, lysozyme and fibrinogen. The solutions of required concentration of each macromolecule i.e. 1% w/v; 4% w/v and 10% w/v were used.

Responses to electroactive species were first measured in the absence of macromolecules. The membranes were then exposed to the required solution of the macromolecule for a given period of time, 1 hr or 24 hrs to determine the effect of protein deposition on the diffusion of electroactive species and membrane fouling. After exposure, the protein solution was removed, the cell was rinsed with buffer and responses to electroactive species were determined. The electrode was then polished, while the membranes coated with macromolecules (fouled) were retained and the response to electroactive chemicals was measured, in order to investigate membrane fouling. This was not possible with the electropolymerised films as the film is directly polymerised on the surface of the electrode hence responses to chemicals in the absence of protein, with the protein present in the solution and after exposure to protein were determined. At the end of the experiment, the cell was cleaned using the method described in section 2.2.4.1.

### **2.3 Procedure for construction of needle electrode**

An eight cm long piece of stainless steel tube, AISI 304, Fe/Cr18/Ni10 (Goodfellow, Cambridge Ltd), with inside diameter of 0.38 mm and 0.5 mm external diameter was cut and edges of the tube were squared on wet and dry Emory paper (RS Components, UK).

## A study of biointerfacing polymeric barrier membranes for sensors

An 11 cm long piece of polyester insulated gold or platinum wire (Goodfellow, Cambridge, Ltd) of 0.125 mm conductor diameter and 0.14 mm insulator thickness was cut and threaded through the stainless steel tube, depending on the type of electrode being constructed. The wire was affixed inside the stainless steel tube with Loctite 3341 UV curing adhesive (Medical grade, Loctite UK Ltd, Herts) and cured in place with a UV gun (3 M Dental Products, Germany) for 10 seconds.

The non sensing end of the platinum and stainless steel tube was attached to 50  $\Omega$  coaxial cable (RS Components, UK). The inner wire of the coaxial cable was attached to the platinum and the outer mesh of the coaxial cable was attached to pseudo stainless steel reference. The other end of the coaxial cable was soldered to a DIN plug (RS components, UK) for connection to the potentiostat (University of Newcastle, UK).

At the working end of the sensor, the polyester insulating material of the platinum wire was carefully removed from the tip to leave a required length of non-insulated platinum. For the flat tip needle type of electrode, the insulated platinum wire was fixed in place with UV curing adhesive and then cut very close to the stainless steel tube making sure that it did not touch the tube, hence a disc type of needle electrode was made.

The stainless steel-Pt needle was affixed inside a 1 ml syringe (Sherwood Medical, Northern Ireland) with Loctite 3321 UV curing adhesive and cured with the UV gun for 120 seconds.

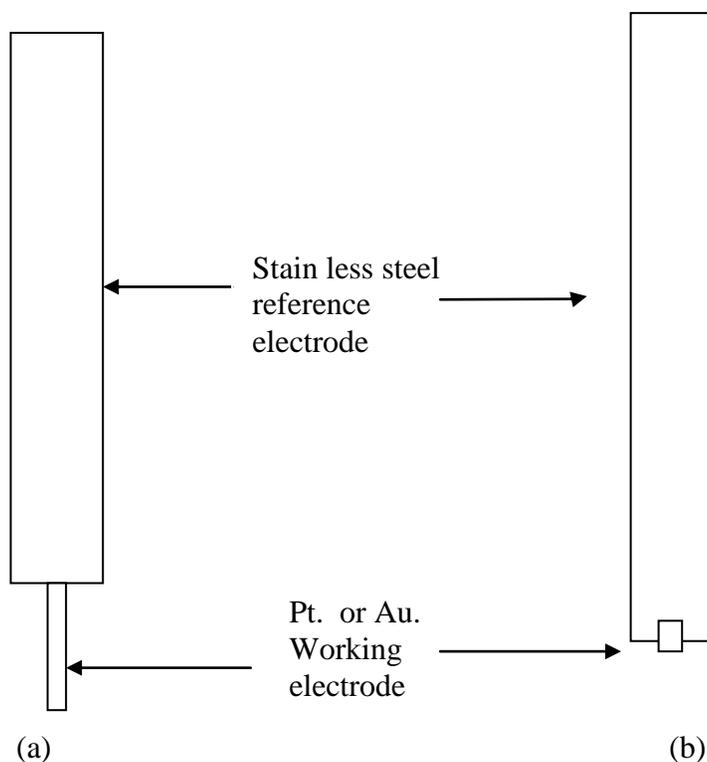


Fig. 2.3: Exploded schematic diagram of the (a) extruded tip (b) flat tip of the needle type of electrode.

The electrodes were tested with a solution of hydrogen peroxide. Sensors were cleaned before using, rinsed with 1 M HCl followed by deionised water to expose a fresh surface before each use. Sensors were conditioned and calibrated in phosphate buffer for 3 hours before each use.

All the experiments were carried out at room temperature under stirred conditions and pH 7.4 unless otherwise stated.

## Chapter 3 Needle Electrodes

### 3.1 Introduction

Needle type electrodes have been used for the detection of various analytes such as glucose, hydrogen peroxide, oxygen etc *in vivo* over the past few decades. There are various designs and types of needle electrodes including those based on the use of working electrodes made from carbon paste, coated wires, platinum, gold and rhodium.

Wilkins (1978) used platinum wire electrodes for electrochemical detection of bacteria. Chen et. al (1992) used platinum electroplated carbon electrodes for the detection of glucose. Gold tip electrode catheters have been used to induce deep lesions using radiofrequency ablations *in vitro* (Lewalter et. al. 2005). Evans et. al. (2004) used needle electrodes for measurement of oxygen in human brains. Needle type electrodes have been used for amperometric detection of electroactive chemicals (Yang et. al. 1998). Commercially available needle type of electrodes have been investigated for *in situ* amperometric detection of hydrogen peroxide and shown to effectively detect the elevated level of hydrogen peroxide *in-vitro* and *in-vivo* (Tamasko et. al. 2007).

In the present studies, needles based on stainless steel with platinum or gold working electrodes were investigated for potential use in electrochemical and biological sensors applications such as hydrogen peroxide or glucose measurements.

### 3.2 Selectivity of needle type of electrodes

Needle electrodes were made according to the procedure described in section 2.3. Two types of needle electrodes were made, namely with gold and platinum working electrodes. These electrodes were used for the selectivity studies as bare electrodes.

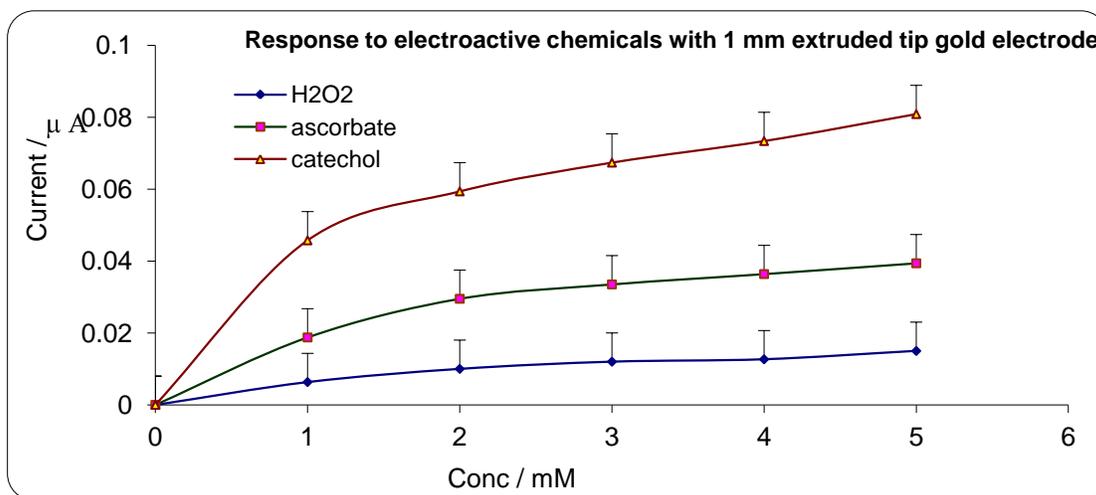


Fig. 3.1: Current response to hydrogen peroxide, catechol and ascorbic acid using 1 mm extruded tip gold electrode (n = 3).

The Fig. 3.1 shows that the gold electrode detects catechol better than ascorbic acid and hydrogen peroxide. The response is linear up to 1 mM concentration of catechol, 2 mM of ascorbic acid and 3 mM concentrated solution of hydrogen peroxide. This can be due to the saturation of the electrode at the above mentioned concentrations of the solutes, so a further increase of the concentration of the solute does not give rise to higher current values.

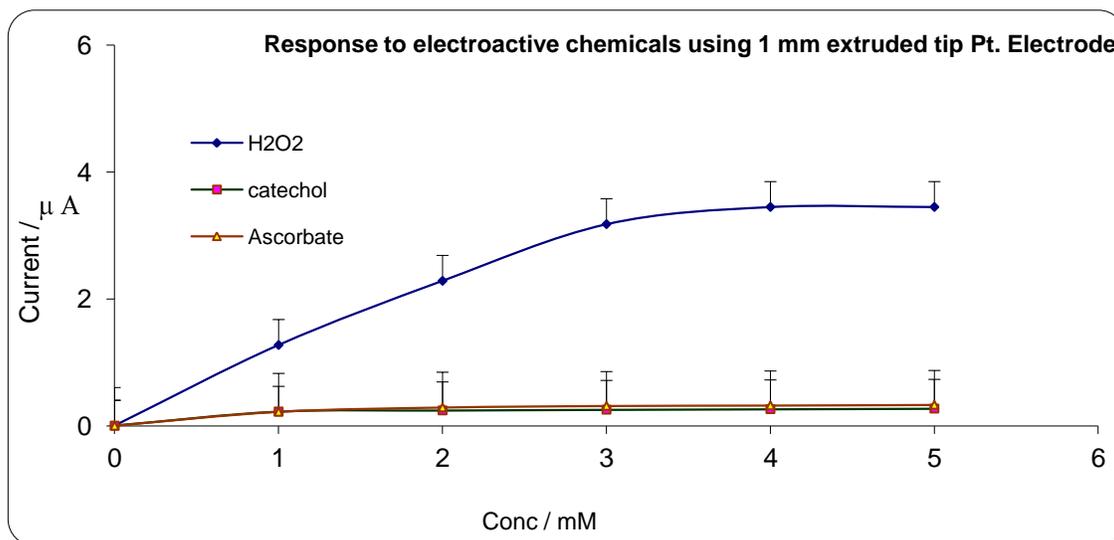


Fig. 3.2: Response to hydrogen peroxide, catechol and ascorbic acid using 1 mm extruded tip platinum electrode (n = 3).

Fig. 3.2 shows that a platinum electrode detects hydrogen peroxide more effectively than catechol and ascorbic acid. The response to hydrogen peroxide is linear up to a concentration of 3 mM of hydrogen peroxide after that the response reaches a plateau. This indicates that the electrochemical reaction at the electrode surface has become rate limiting and the response is no longer dependent on concentration gradients from the bulk solution.

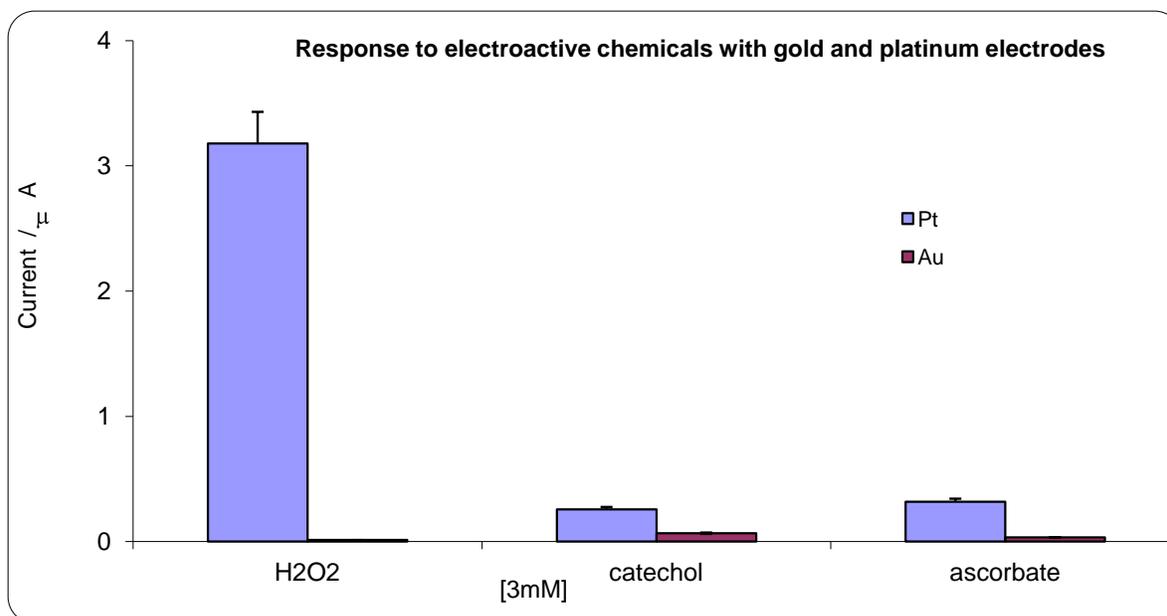


Fig. 3.3: Response to 3 mM hydrogen peroxide, catechol and ascorbic acid using 1 mm extruded tip gold and platinum electrodes (n = 7).

Initial results of the investigation on gold and platinum electrodes showed that platinum electrodes are better than gold electrodes for the detection of hydrogen peroxide. Gold has been in use longer than platinum and has been known for its stability, it appears that when it comes to detecting hydrogen peroxide this noble material may be second best to platinum. This could be due to various factors including the potential used or electrochemical processes taking place on the surface of the electrode.

The electrochemical processes, conductivity, charge transfer and diffusion of ions across the interface and adsorption of reaction products on the electrode surface are dependent to a large extent on the material properties of the electrode.

Table 3.1: Properties of materials:

Material	Atomic number	Expansion Coefficient	Melting Point °C	Thermal conductivity W/cm Kelvin
Platinum	78	8.9	1773	0.716
Gold	79	13.2	1063	3.17

The high atomic number and melting point of gold and platinum makes them a suitable candidate for implanted electrodes as they can be radio graphically visualised (Geddes et. al. 2003). Also, gold and platinum are both inert metals and do not corrode in aqueous solutions at neutral pH.

When an electrode is placed in electrolyte solution, different electrochemical phenomena take place and a potential is generated due to the unequal charge distribution across the electrode-electrolyte interface (McAdams et. al. 1995). The electrochemical oxidation of hydrogen peroxide at the surface of an electrode is affected by pH, temperature and electrode conditioning (Zhang et. al. 1993).

The electrocatalytic properties of gold vary according to the pH of the solution used. Gold has been reported to be a poor electrocatalyst at acidic pH, however, it exhibits good electrocatalytic properties for oxidation of oxygenated saturated compounds at alkaline pH (Beden et. al. 1987). This is perhaps due to the weak interaction of oxygen with the surface of the electrode (Rodriguez et. al. 2014). The oxidation of hydrogen peroxide at gold surface is affected by the composition, cleanliness and redox state of the electrode along with the applied potential. At a clean gold electrode surface, the hydrogen

peroxide oxidation takes place at + 490 mV vs Ag/AgCl and at the oxidation of gold oxide at a higher positive potential (+ 870 mV vs Ag/AgCl) in phosphate buffer at pH 7.4 (Gerlache et. al. 1997, 1998).

The polarisation of the surface of a metal electrode leads to the production of metal oxides. These oxides are adhered at the surface of the electrode in the form of layers, which in turn leads to a reduction in the active surface area of the electrode and lower current detection, since the oxide layer is non-conducting.

The deposition of reaction products on the surface of an electrode, hydrogen in the case of hydrogen peroxide catalysis, or oxide layers can take place in the form of mono-layers (Zuliani et. al. 2010) or multi layers depending on the surface and material of the electrode. The completion of a monolayer of hydrogen adsorption probably takes place in the case of the platinum electrode. In the case of gold electrodes no hydrogen adsorption region is recognisable (Trasatti et. al. 1991). These initial adsorbed elements or layers may facilitate electrochemical reactions at the electrode-electrolyte interface. Vitt et. al. (1990) proposed that adsorption of hydroxyl radicals at the surface of a gold electrode is a prerequisite to the subsequent oxygen and electron transfer steps. The formation and growth of platinum and gold oxide layers occurs at time scales from microseconds to seconds (Zuliani et. al. 2010).

A gold electrode on its own may not be the ideal choice for the electrochemical detection of hydrogen peroxide. However, gold electrodes have been recently modified by using various metal nanoparticles and nanowires. Song et. al. (2010) used a gold electrode modified with silver nanowires for amperometric detection of hydrogen peroxide. They showed that such modified gold electrodes exhibited high sensitivity and good stability.

The present investigations showed that the platinum electrodes offer better sensitivity for the oxidation of hydrogen peroxide compared to gold electrodes which is in agreement with previous studies (Gerlache et. al. 1997). Hence, platinum electrodes were used in further investigations to obtain an electrode that would give optimum response.

### 3.3 Design evaluation of the needle type of electrodes

Platinum electrodes were selected for these studies as the initial results had shown their suitability for the detection of hydrogen peroxide. The designs that were investigated included leveled tip needle electrodes, 1 mm extruded tip needle electrodes with the reference electrode near the working electrode, 1 mm and 2 mm extruded tip electrodes with the working electrodes away from the reference electrode and 1 mm extruded tip electrode with a blocked tip, where the working electrode's tip was coated with a thin film of the non conducting polymer.

The surface area of the working electrode for each design was calculated and is shown in table 3.2.

Table 3.2: Surface area of the tip of the needle type of electrode:

1 mm extruded near ref.	1 mm extruded tip	1 mm extruded block tip	2 mm extruded tip	Flat tip electrode
0.4045 mm <sup>2</sup>	0.405 mm <sup>2</sup>	0.393 mm <sup>2</sup>	0.788 mm <sup>2</sup>	0.012 mm <sup>2</sup>

The calculated surface area is highest for 2 mm extruded tip electrode and lowest for the flat tip needle type of electrode.

Responses to electroactive chemicals were measured and current densities were calculated in order to compare the results. Results of these investigations are shown in Fig. 3.4.

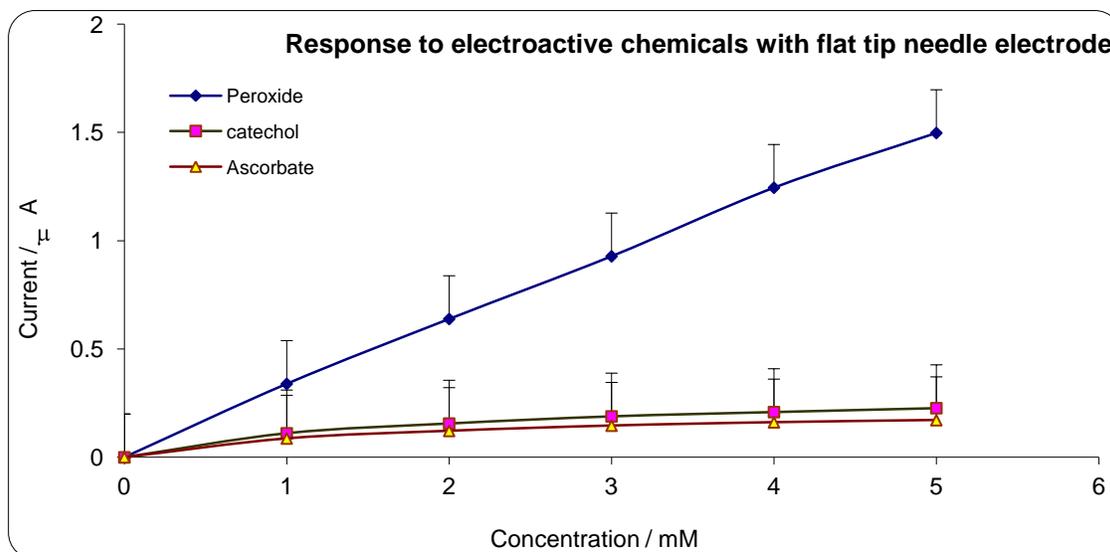


Fig. 3.4: Response of flat tip needle electrode to peroxide, ascorbic acid and catechol.

Fig 3.4 shows that leveled tip platinum needle type of electrodes like other platinum electrodes detect hydrogen peroxide better than catechol and ascorbic acid. The response was observed to increase linearly with the increasing concentration of analyte. This indicates that the saturation point for hydrogen peroxide of the flat tip needle type of electrode is higher than that for extruded type of electrodes. It may be due to the variation in the surface crystallogical structure of the platinum wire as the tip might have different surface structure crystallinity than that of the side of the wire.

The response reaches a plateau after an initial linear increase at lower concentration of analyte in case of the extruded tip needle type of electrodes as seen in Fig. 3.5.

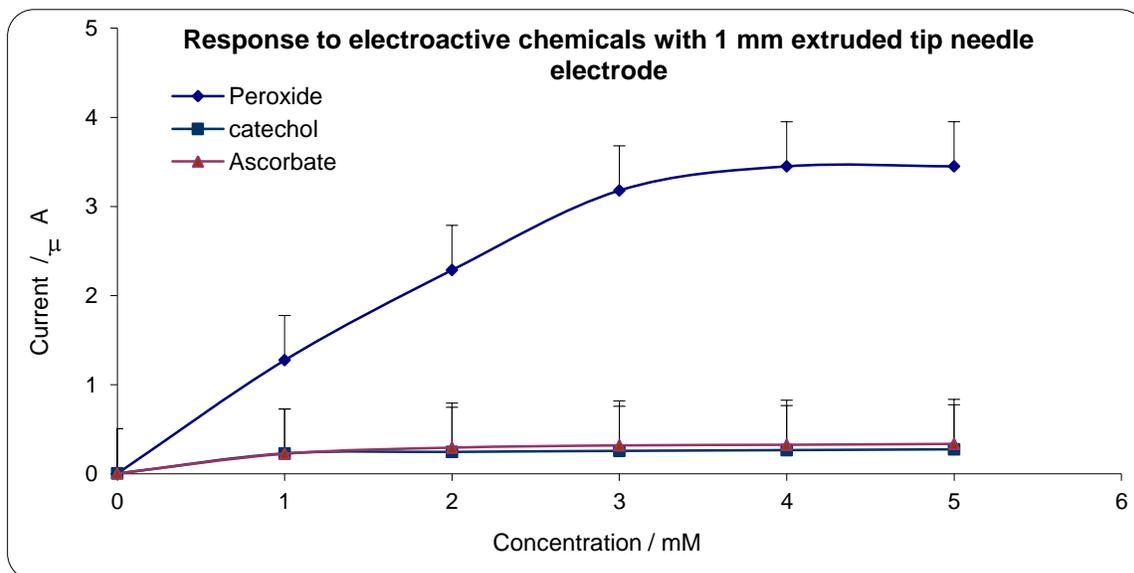


Fig. 3.5: Current response to hydrogen peroxide, catechol and ascorbic acid using 1 mm extruded tip needle electrode.

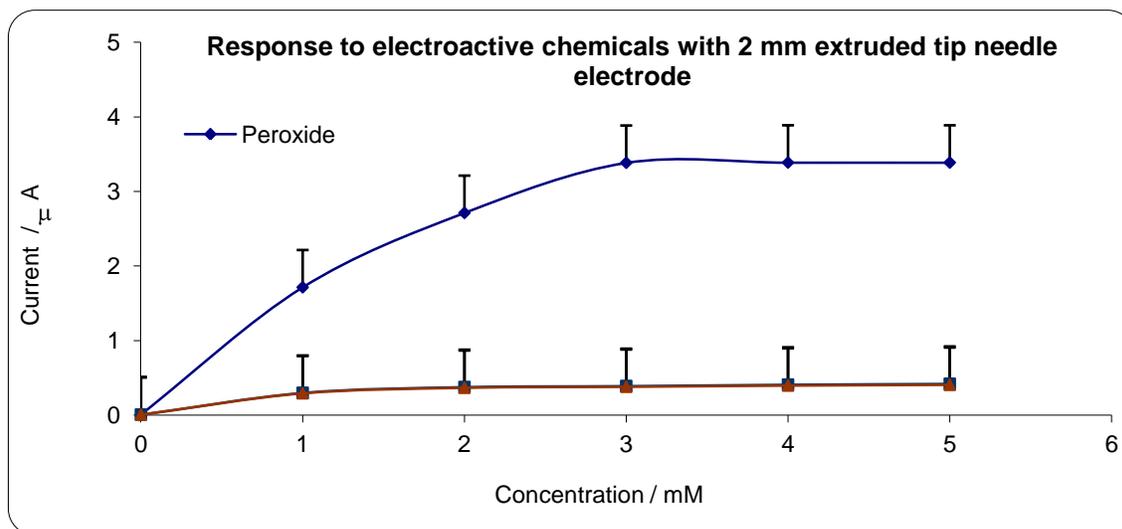


Fig. 3.6: Current response of hydrogen peroxide, catechol and ascorbate to 2 mm extruded tip needle type of electrode.

Response to hydrogen peroxide, catechol and ascorbic acid were observed to be similar with 1mm extruded tip needle type of electrode and 2 mm extruded tip needle type of electrodes. The response was linear up to a 3 mM concentrated solution of hydrogen peroxide with both types of electrodes. This concentration (i.e. 3 mM hydrogen peroxide)

was then selected for further investigations and design evaluation of the needle type of electrodes. The results are shown in Fig. 3.7.

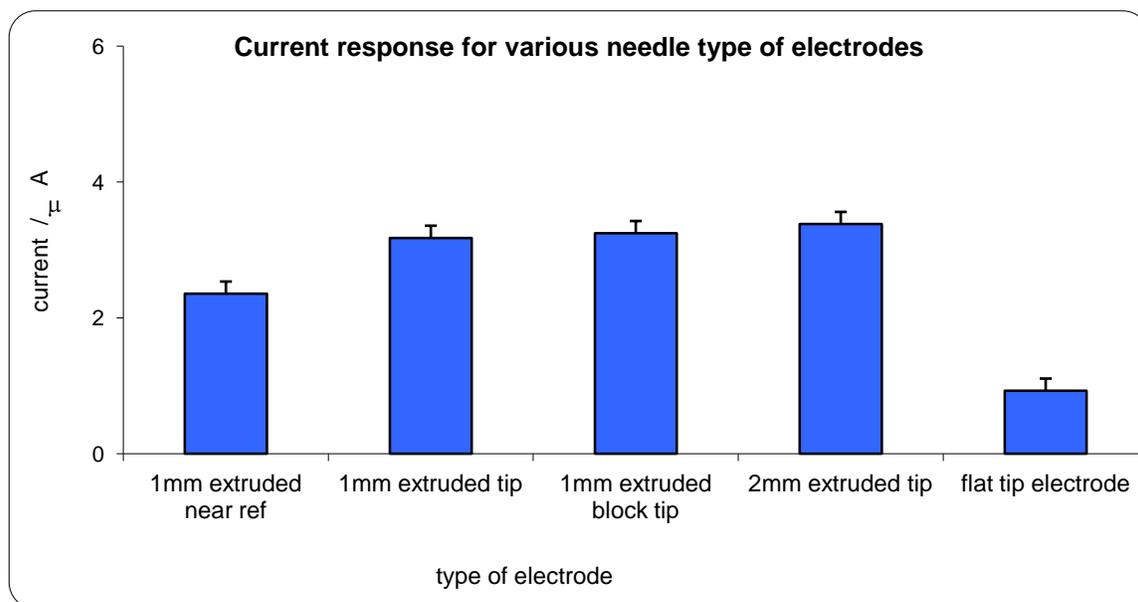


Fig. 3.7: Response of various electrodes to 3 mM concentrated solution of hydrogen peroxide, (n = 7).

The results show that the current response increases in proportion to the increase in surface area, this result is expected as larger surface area will provide more active sites for the molecules to attach and the reaction to take place and hence will result in a higher current response as seen here, in accordance with the Faraday equation.

In order to evaluate the best design, current densities were calculated. The current density is the current response over the active surface area of the electrode and has the units A/m<sup>2</sup>. From the current responses the flat tip type of needle electrode is the best design as it gives a linear response over a larger concentration range of the analyte. The results shown in Fig. 3.8 show the current densities of various needle types of electrodes that were used in present study.

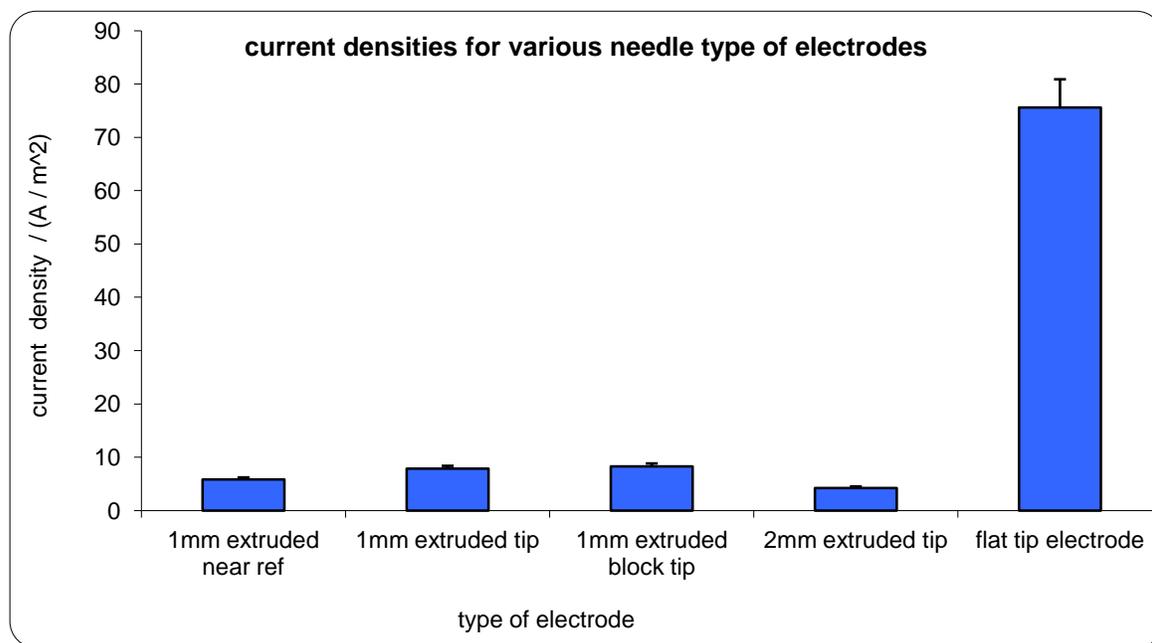


Fig. 3.8: Current densities for various types of electrodes at [3 mM] of hydrogen peroxide.

Results show that as the surface area increases the current density decreases. This may be due to the needle electrode's deviation to the microelectrode type of diffusion as the surface area reduces. The flat tip electrode has higher current density than the 1 mm extruded tip (near reference electrode) needle type of electrode, this is because the diffusion to the flat tip is more like diffusion to the surface for microelectrode and therefore has spherical and not planar diffusion and thus giving an enhanced current. The electrode with 1 mm extruded block tip electrode and the simple 1 mm extruded tip electrode show similar current response and current densities.

The 2 mm extruded tip needle type of electrode gives similar current response as the 1 mm extruded tip needle type of electrode. Theoretically, the current response should be higher for the 2 mm extruded tip electrode than that for the 1 mm extruded tip needle type of electrode as the surface area is greater. The large surface area provides a greater interface between electrode and the electrolyte, compensating for a reduced electrochemical reaction rate as the molecules and chemicals can be catalysed over a greater surface area but the results do not show this. It could be due to the diffusional

differences because of the shape of the electrodes as the current density for the 2 mm extruded tip electrode is half of that for 1 mm extruded type of needle electrode.

The difference in the current densities may be due to the fact that although the 2 mm extruded tip electrode has a larger surface area than the 1 mm extruded tip needle type of electrode, the active unit area per current ratio is not high. The current density per unit area is smaller. This could be due to various factors such as the above including the impedance of the electrode.

The impedance in an electrical circuit, in alternating currents system, is the measure of the resistance that an electrical conductor has to pass the electrical current through the conductor. It quantifies how a conducting material opposes the flow of current through it at the application of a required voltage.

The impedance of an electrode-electrolyte interface depends on the type of metal, electrolyte used, surface area of the electrode and the temperature. The impedance decreases with increasing area and surface roughness and the increasing current density used to make measurements. The impedance of needle electrode with larger surface area is expected to be lower. However, impedance measurements have been reported to be lower for electrodes with lower surface area (Kalvoy et. al. 2010). So, charge differences taking place right at the interface could possibly contribute to the observed differences.

The flat tip disc shape platinum electrode is the best design for a needle type of electrode as it gives linear and reproducible results and is easy to reuse.

The polymer membranes for use on needle electrodes were investigated using the Rank Cell as it provided ease of use. Various types of membranes including electropolymerised films, polycarbonate membranes with cylindrical pores and PVDF and cellulose acetate membranes with interconnecting pores were studied. The selectivity of all these membranes was studied before and after modification with various macromolecules.

## Chapter 4 Membranes without porous structure

### 4.1 Introduction

Membranes are used in close proximity to the surface of the electrode in a biosensor. The membrane not only provides the sensor with protection from fouling but also at the same time acts as a barrier against many interfering or electroactive species and thus enhances selectivity of a biosensor. The electropolymerised polymer films provide membranes without porous structure for use in biosensors. The electropolymerised films including poly(phenol) and poly(phenol red) films have been investigated in these studies.

### 4.2 Bare (uncoated) electrode, effect of stirring

Tests were carried out with hydrogen peroxide and ascorbic acid in order to investigate the effects of stirring conditions. A dialysis membrane was used to prevent electrode damage from the rotating action of the stirrer bar. The results shown are averages of three experiments.

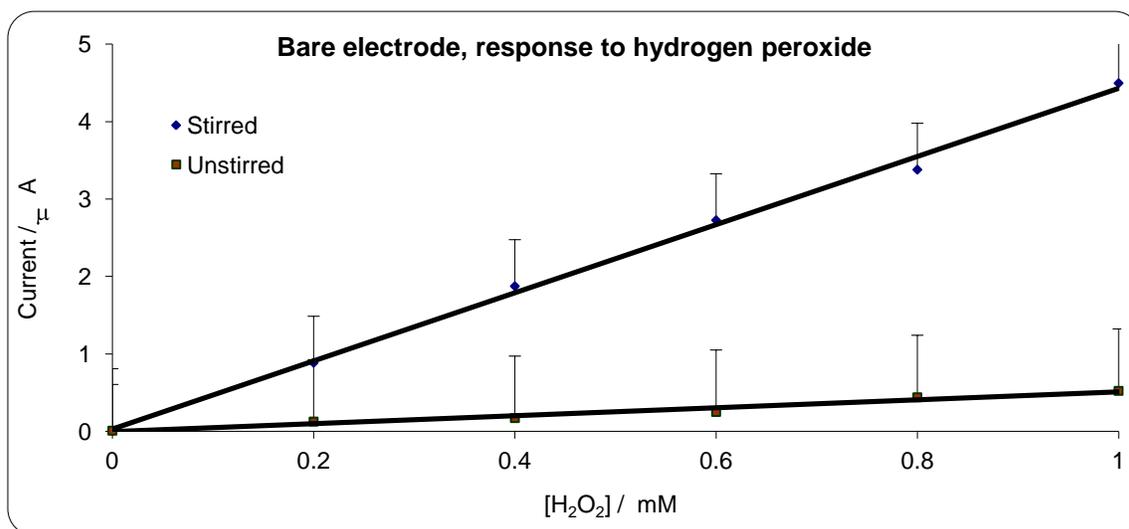


Fig. 4.1: Response to Hydrogen peroxide, effect of stirring conditions, (n = 3).

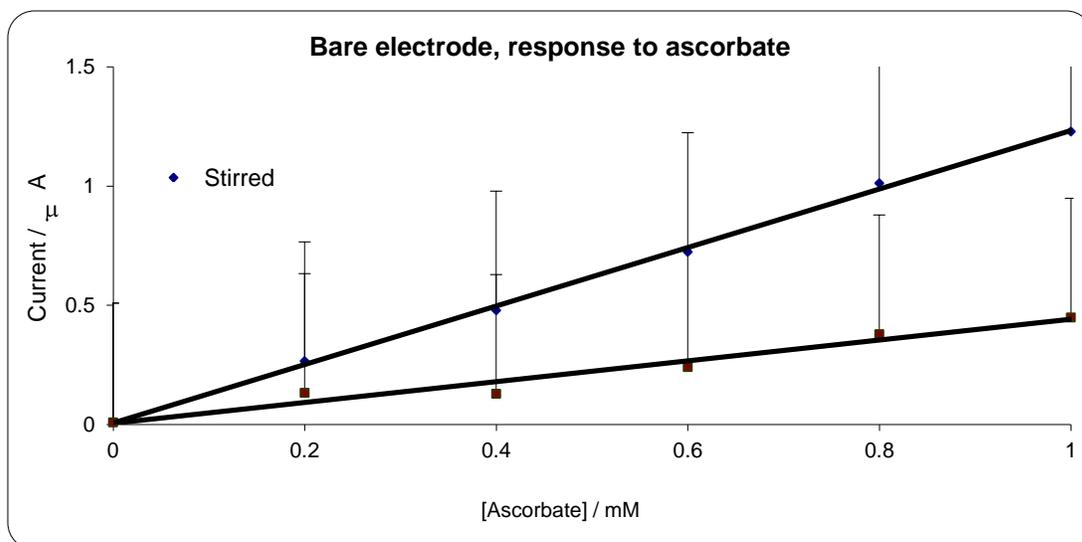


Fig. 4.2: Response to Ascorbic acid, effect of stirring conditions, (n = 3).

The results show that the current increases linearly with increase in the concentration of the hydrogen peroxide as well as of ascorbic acid both under stirred and unstirred conditions. This suggests that current is directly proportional to the concentration of analyte.

The response obtained under unstirred conditions is lower than that obtained under stirred conditions. This may be due to the formation of a diffusion layer (in unstirred conditions), which restricts transport of analyte to the surface of the sensor, which may be the cause of loss of sensitivity. Stirring prevents the buildup of this diffusion restricting barrier layer, allowing a constant flux of the analyte to surface of the sensor, giving rise to higher responses.

### 4.3 Electropolymerised films

Electropolymerised films are used to modify and protect the surface of the electrode. These films are formed by oxidation of a phenolic monomer at the surface of the electrode. The process of electropolymerisation results in an ultra-thin insoluble, conducting or insulating film on the surface of the sensor. The electropolymerised films

are self-regulating, have uniform thickness and completely cover the surface of the electrode.

Two types of phenol films namely poly(phenol) and poly(phenol red) were investigated. The films were tested for the selectivity of various electroactive chemicals.

### 4.3.1 Poly(phenol) films

Phenol films can be directly electropolymerised onto the surface of an electrode. Poly(phenol) films are non-conducting, ultrathin, defect free and have uniform thickness. Phenol films were electropolymerised from 5 mM solution of phenol at an applied potential of + 0.85V (vs Ag/AgCl).

#### 4.3.1.1 Effect of stirring

The effects of stirring were investigated using hydrogen peroxide solutions of 0, 1,2,3,4 and 5 mM concentration.

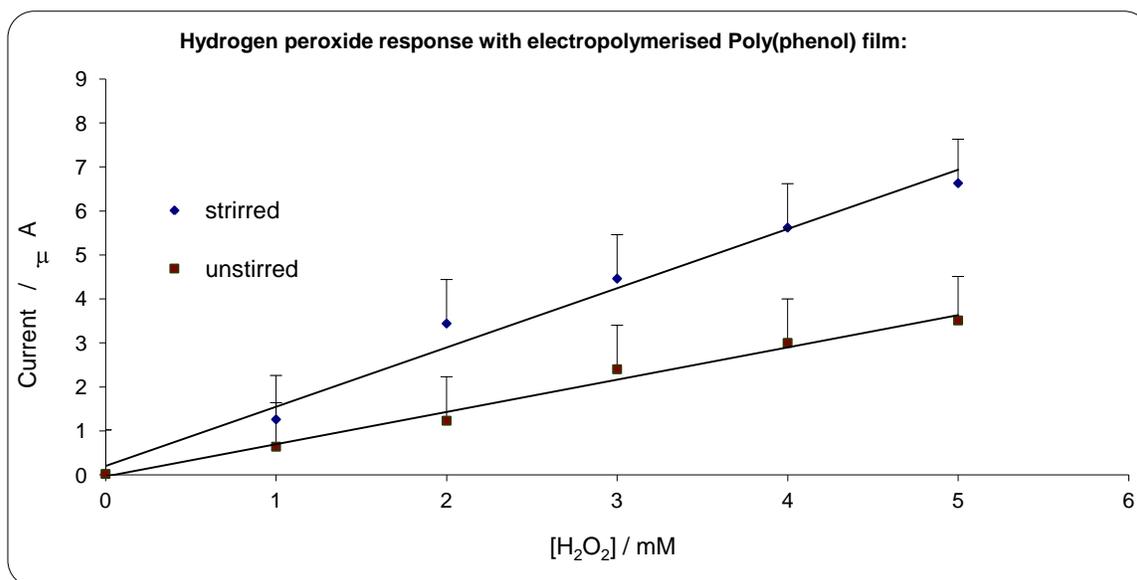


Fig. 4.3: Graph showing effect of stirring, response to hydrogen peroxide under stirred and unstirred conditions, n = 3.

The results show that the response to analyte (hydrogen peroxide) is dependent on the stir conditions. The response increases linearly with the concentration of hydrogen peroxide, both under stirred and unstirred conditions. This indicates that the current response is directly proportional to the concentration of hydrogen peroxide.

Under unstirred conditions, the response to hydrogen peroxide is reduced and under stirred conditions the response obtained is high. This indicates that the stirring of the bulk solution causes more analyte to diffuse or migrate through the membrane to the surface of the electrode and as a result higher current response is obtained. Under unstirred conditions transfer or flux of the analyte to the surface of the electrode is mainly through diffusion. The static or stagnant solution may give rise to a diffusion layer on the surface of the sensor, restricting the diffusion of the analyte to the surface of the electrode.

#### 4.3.1.2 Selectivity of poly(phenol) films

Poly (phenol) film was tested for selectivity to various electroactive species.

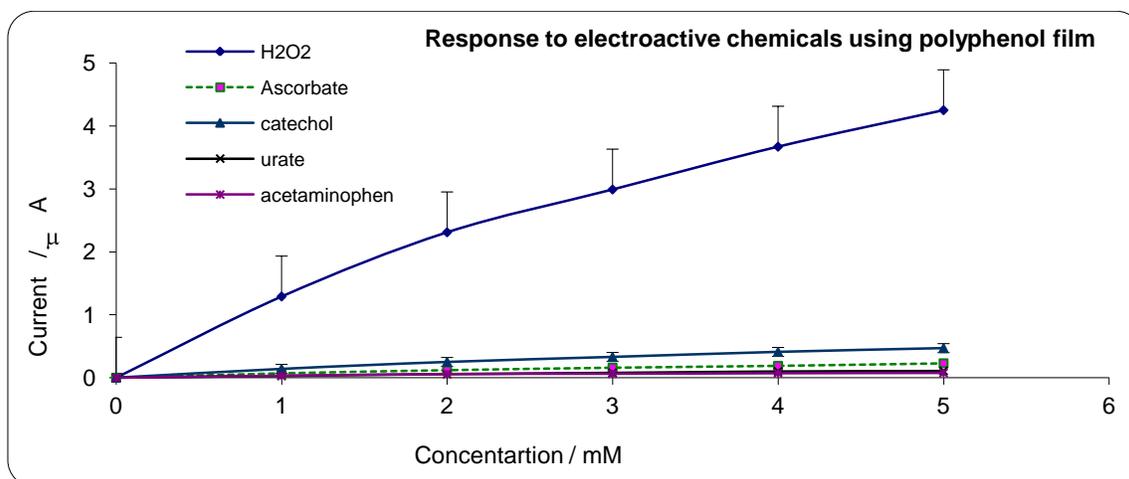


Fig. 4.4: Response to various electroactive chemicals, using poly(phenol) film, (n = 3).

Results show that the poly(phenol) film selects hydrogen peroxide more than any other electroactive chemical. The response to hydrogen peroxide increases linearly with increasing concentration. Response to other electroactive chemicals is minimal compared to the response to hydrogen peroxide. Catechol is selected but the response is very low.

Ascorbic acid, uric acid and acetaminophen do not give a significant response. This indicates that the poly(phenol) film is highly selective of hydrogen peroxide and does not select acetaminophen, uric acid and ascorbic acid. This may be due to the structure of the film.

Poly(phenol) is an insulating and defect free film. Hydrogen peroxide may diffuse through the film due to its small molecular size. Other electroactive chemicals may simply not be selected due to their size. Hence, films are obtained that are selective of hydrogen peroxide and reduce the problem of interference from other electroactive chemicals. These results are in accordance with similar work on phenol films (Christie et. al. 1993, Eddy et. al. 1995).

### **4.3.2 Poly(phenol red) films**

Phenol red is a derivative of phenol. Electropolymerised poly(phenol red) initially forms a relatively porous films as compared to poly(phenol) film (Eddy et. al. 1995). Phenol red films were directly electropolymerised on the surface of the electrode, from 5 mM solution of phenol red, at + 0.85 V (vs. Ag/AgCl). Thus formed films were tested for their selectivity and effect of stir conditions.

#### **4.3.2.1 Effect of stirring**

The effect of stirring was investigated with solutions of hydrogen peroxide of 0, 1, 2, 3, 4 and 5mM concentration.

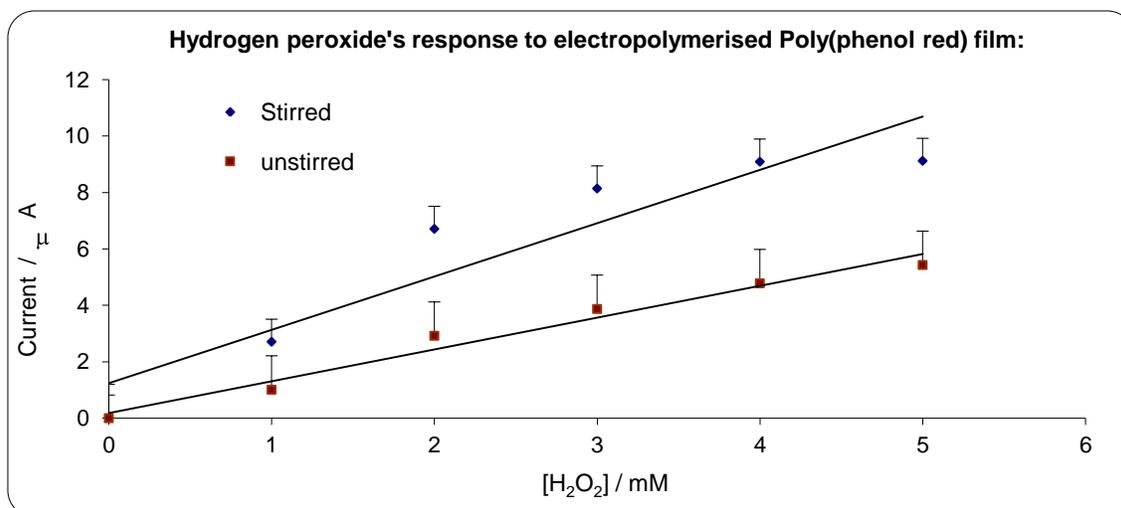


Fig. 4.5: Response to hydrogen peroxide, under stirred and unstirred conditions,  $n = 3$ .

Results show that response to hydrogen peroxide, both under stirred and unstirred conditions, increases linearly initially with increasing concentration.

Response to hydrogen peroxide, using poly(phenol red) film is stir dependent. The response obtained under stirred conditions is higher than the response obtained under unstirred conditions. As stated before this could be due to the formation of a diffusion restricting layer, formed under unstirred conditions. Stirring may prevent the formation of this diffusion restricting layer, allowing better transport of the analyte to the surface of the electrode. Thus giving rise to a higher response under stirred conditions.

#### 4.3.2.2 Selectivity profile of poly(phenol red) film

Poly(phenol red) film was tested with solutions of 0, 1, 2, 3, 4 and 5 mM concentration of various electroactive chemicals to investigate the selectivity of this ultra thin electropolymerised film.

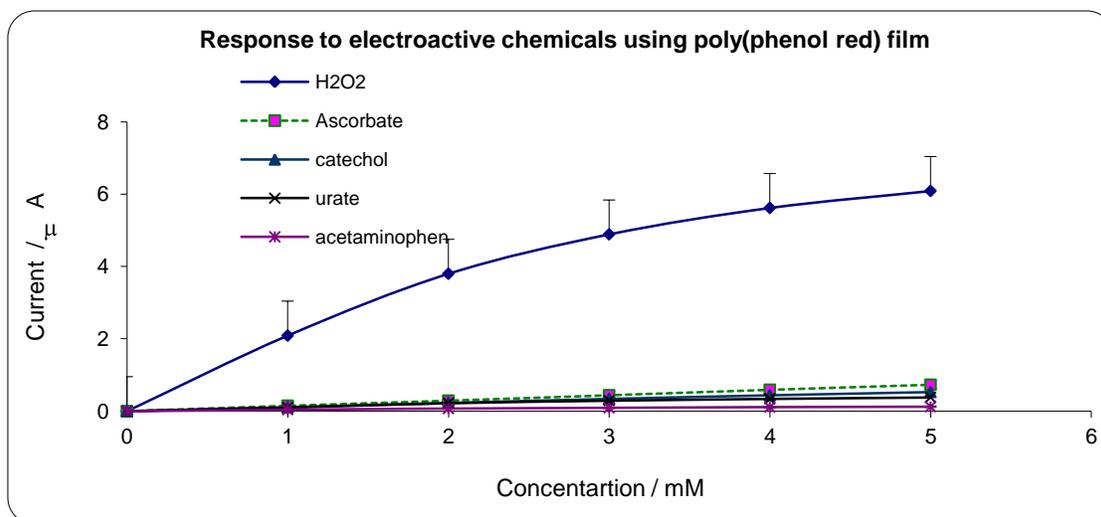


Fig. 4.6: Response to various electroactive chemicals, using poly(phenol red) film,  $n = 3$ .

Results show that hydrogen peroxide gives a higher response compared to other electroactive chemicals. The response to ascorbic acid is 8% of the hydrogen peroxide. Similarly catechol gives a response of 6% of that of hydrogen peroxide. The response to uric acid and acetaminophen is almost insignificant, (less than 5% of the hydrogen peroxide). These results indicate that the poly(phenol red) film selects hydrogen peroxide more than any other electroactive chemical.

The response to hydrogen peroxide is rapid and of a magnitude similar to the bare electrode, whereas the response to catechol, ascorbic acid, uric acid and acetaminophen are either greatly reduced or almost eliminated.

#### 4.4 Biofouling studies with electropolymerised films

Poly(phenol) and poly(phenol red) films were exposed to 4 % w/v fibrinogen for one hour to investigate the extent of fouling of these films.

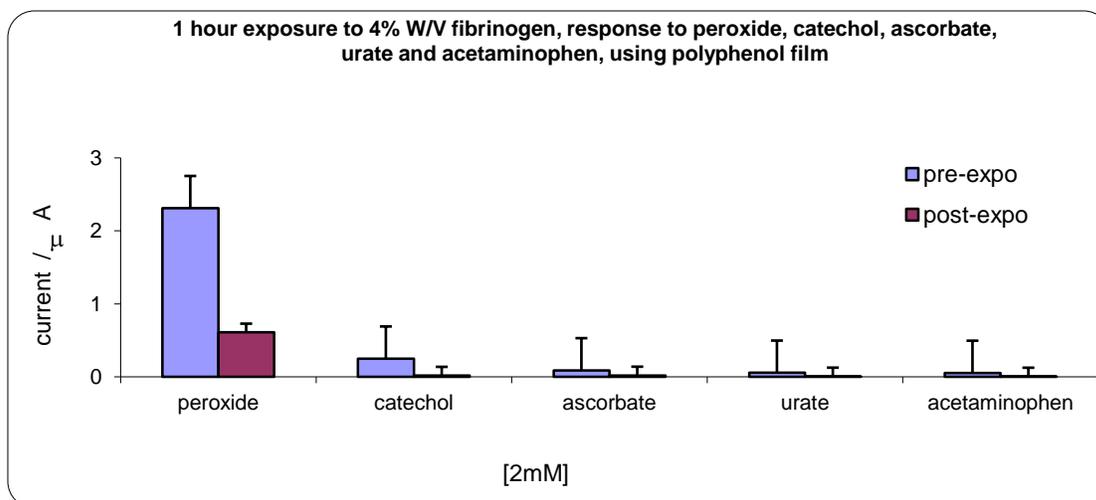


Fig. 4.7: Response to various electroactive chemicals, before and after exposure to 4% w/v fibrinogen using poly(phenol) film.

Results show that poly(phenol) films give reduced response to hydrogen peroxide, 75% loss in sensitivity is observed, after exposure to 4 % w/v fibrinogen solution. Response to other electroactive chemicals was observed to be reduced by 95 % after exposure to the protein.

Exposure to the protein fibrinogen may results in protein adsorption on the surface of the film, which restrict the diffusion of electroactive chemicals or analyte to the surface of the electrode. The film is thus fouled and this is the reason that a reduced response to hydrogen peroxide was observed.

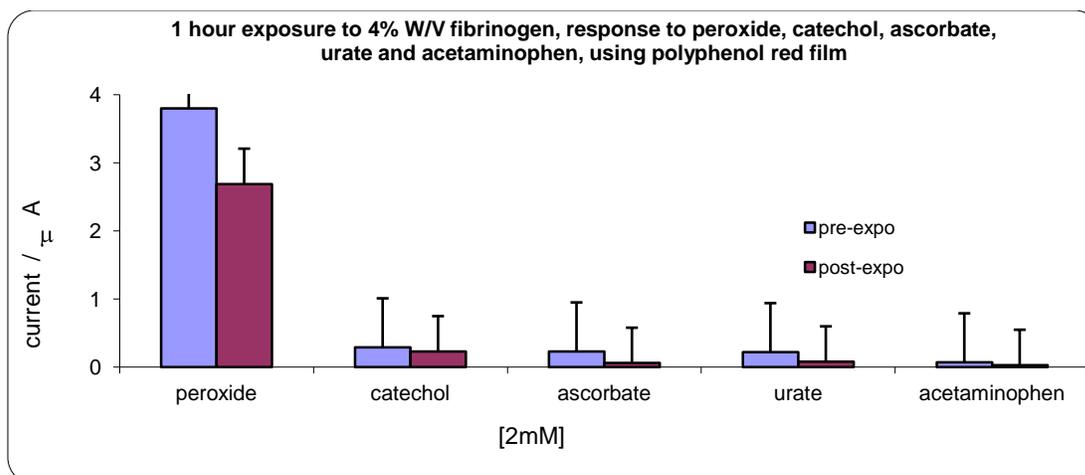


Fig. 4.8: Response to various electroactive chemicals, before and after exposure to 4% w/v fibrinogen solution, using poly(phenol red) film.

Results show that poly(phenol red) films suffer from loss in sensitivity after exposure to 4% w/v fibrinogen solution. The response to hydrogen peroxide was reduced by 30% and 10 % loss in sensitivity was observed with catechol. Acetaminophen and uric acid gave no significant response after exposure to the protein and response to ascorbic acid was also reduced. This may be due to protein adsorption on the surface of the film.

Results indicate that poly(phenol red) selects more hydrogen peroxide than poly(phenol) film, both films reduce the problem of interference. The poly(phenol) films seem to suffer from a loss in sensitivity after exposure to fibrinogen more than poly(phenol red) films. The response to interfering species was reduced significantly with phenol film post exposure to fibrinogen. This effect could be investigated in future work.

A composite of these two films will be a good membrane material for use in needle type of electrodes as an inner membrane. Composite of phenol and phenol red films could be investigated for selectivity to various electroactive chemicals. Fouling studies with albumin and fibrinogen could be done with electropolymerised films and SEMs would be useful to study the morphology of these films.

## Chapter 5 Membranes with 3D interconnected porous structure

The Poly(vinylidene fluoride) (PVDF) and cellulose acetate membranes have 3D interconnected porous structures. These membranes are widely used in micro filtration and ultra filtration processes due to their good chemical resistance, controlled porosity and durability (Wang et. al. 2002, Klee et. al. 2003). The PVDF and Cellulose acetate membranes were investigated in this study for their potential use as biointerfacing barrier membranes in sensors.

### 5.1 Poly(vinylidene fluoride) membrane

PVDF membranes of 0.22 micron pore diameter were used in our study to investigate their possible use as a membrane material in glucose sensors.

#### 5.1.1 Effect of stirring

Effect of stirring was investigated using hydrogen peroxide solutions of various concentrations under stirred and unstirred conditions.

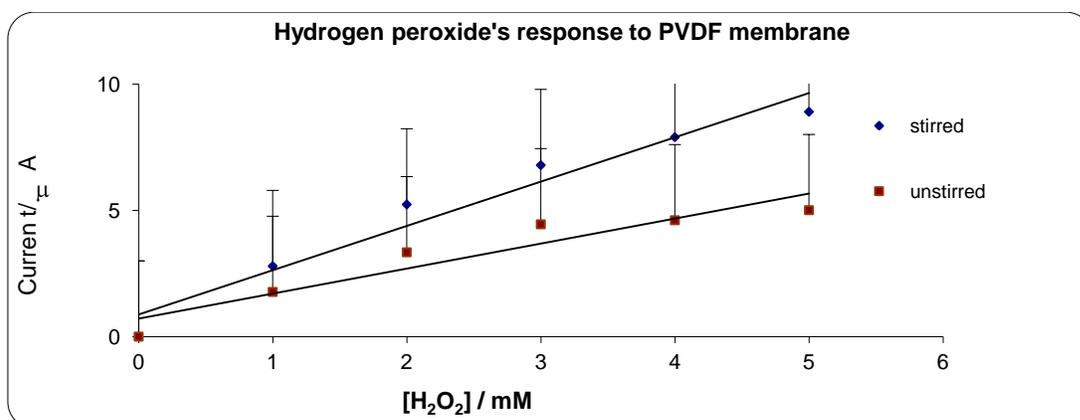


Fig. 5.1: Response to hydrogen peroxide, using 0.22 micron pore diameter PVDF membrane under stirred and unstirred conditions,  $n = 3$ .

Results show that response to hydrogen peroxide increases with increasing concentration. The response obtained under unstirred conditions is linear up to 2 mM concentrated solution of hydrogen peroxide, plateau effect is observed after 3 mM concentration of hydrogen peroxide. The current does not seem to increase significantly with increasing concentration of the hydrogen peroxide, under unstirred conditions, this may be due to the formation of diffusion restricting layer on the surface of the electrode. Under stirred conditions this phenomenon was not observed.

The effect of stirring was investigated using solutions of 0, 1, 2, 3, 4, 6, 7, 8, 9 and 10 mM concentration of hydrogen peroxide. The results shown are only up to 5 mM concentration solutions of hydrogen peroxide. Response to hydrogen peroxide is linear up to 5 mM concentration. The response to hydrogen peroxide after 5 mM concentration was reproducibly irreproducible.

It was hypothesised that the solution of 6 mM and higher concentrations of hydrogen peroxide may cause structural changes in the membrane. So, the membrane was left in concentrated solution of hydrogen peroxide overnight and tested after rinsing with buffer the next day. A control experiment was carried out using buffer. The results of these experiments are shown below.

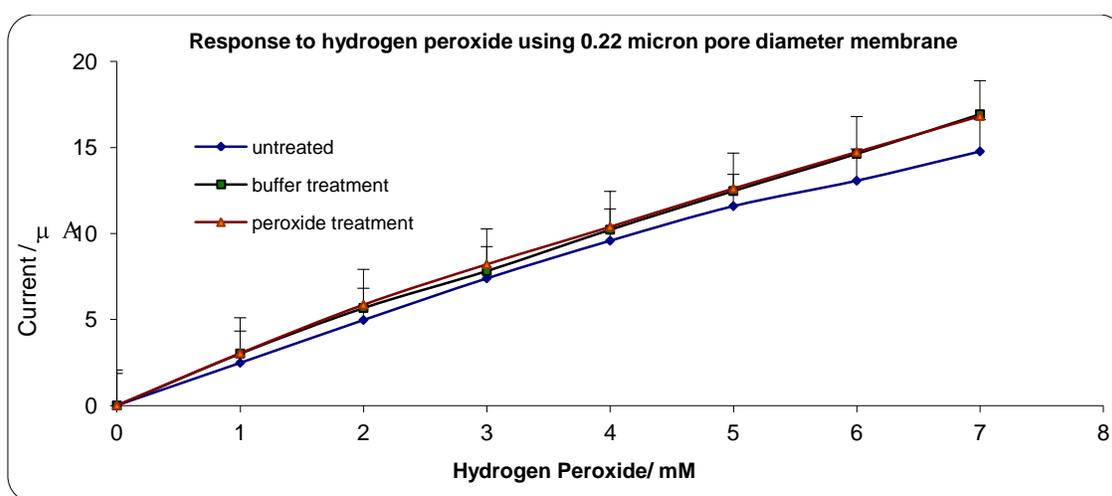


Fig.5.2: Response to hydrogen peroxide, using 0.22 micron PVDF membranes, effect of buffer and concentrated hydrogen peroxide treatment, n = 3.

Results show that the response to hydrogen peroxide after treatment with buffer and concentrated hydrogen peroxide is very similar; however the response is more stable and improved as compared to untreated membrane. Concentrated hydrogen peroxide is a corrosive chemical and when in contact with skin causes burns. The stability of PVDF membranes in this substance indicates that the PVDF membrane has good chemical resistance and durability.

The improved and more stable results after wetting the membrane in either buffer or peroxide solution for long time (24 hours) may be due to some other reasons. PVDF is hydrophobic, after prolonged exposure to solutions it may acquire some surface changes or hydrophilic properties. These results need further investigation. SEMs studies of these membranes showed that the surface of the membranes appeared to be smoother after soaking in buffer. However, the longer exposure times did not show any adverse effects or morphological changes in the structure of these membranes.

PVDF membrane was tested for selectivity to various electroactive chemicals.

### **5.1.2 Selectivity profile of PVDF membranes**

The selectivity of PVDF membranes was investigated to various electroactive chemicals such as catechol, ascorbic acid, hydrogen peroxide, uric acid and acetaminophen. Results show that this membrane selects all of these electroactive chemicals to some extent.

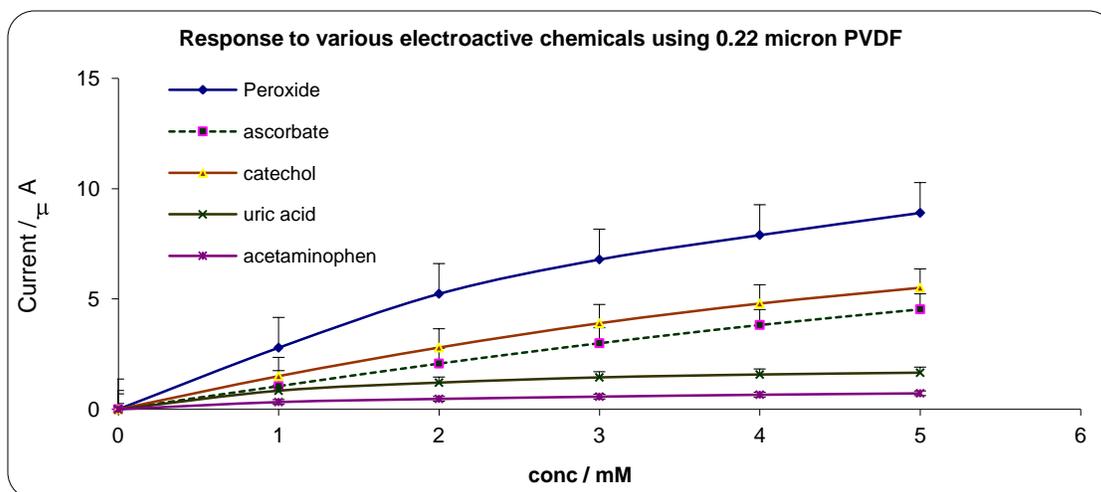


Fig. 5.3: Response to hydrogen peroxide, catechol, ascorbic acid, uric acid and acetaminophen, using 0.22 micron pore diameter PVDF membrane, n = 3.

The results show that with 0.22 micron pore diameter PVDF hydrogen peroxide is selected more than any other electroactive chemical up to a certain concentration as stated before. At higher concentrations the response obtained was not reproducible. Response to hydrogen peroxide increased linearly only at low concentrations, at higher concentration the response increased but not very linearly and at concentrations higher than 5 mM the response to hydrogen peroxide was irreproducible.

Catechol and ascorbic acid gave most stable and linear responses. The results were found to be reproducible. Uric acid and acetaminophen were selected the least. If the response to hydrogen peroxide at 2 mM concentration is taken to be 100 % then response to other electroactive chemicals as compared to hydrogen peroxide is as follows:

Catechol : 50 %

Ascorbic acid : 40%

Uric acid: 30 %

Acetaminophen: 10%

The fastest response time observed was for hydrogen peroxide, but the results were not always reproducible as described earlier. The longest response time observed was for uric

acid and acetaminophen. The response time for the selectivity of catechol and ascorbic acid was very quick.

On the basis of the linearity and reproducibility of the response and the fast response times for catechol and ascorbic acid it is suggested that PVDF membranes may have potential use in sensors where the concentrations of catechol and ascorbic acid are tested or utilised.

### 5.1.3 Fouling studies of PVDF membrane with Albumin

The adsorption of bovine serum albumin at polyvinylidene fluoride microfiltration membranes was studied using 10 % w/v solution of bovine serum albumin. The concentration of albumin solution was increased so that the effect of serum albumin on the fouling of this membrane can be investigated.

Results shown are averages of three experiments and standard deviations are shown.

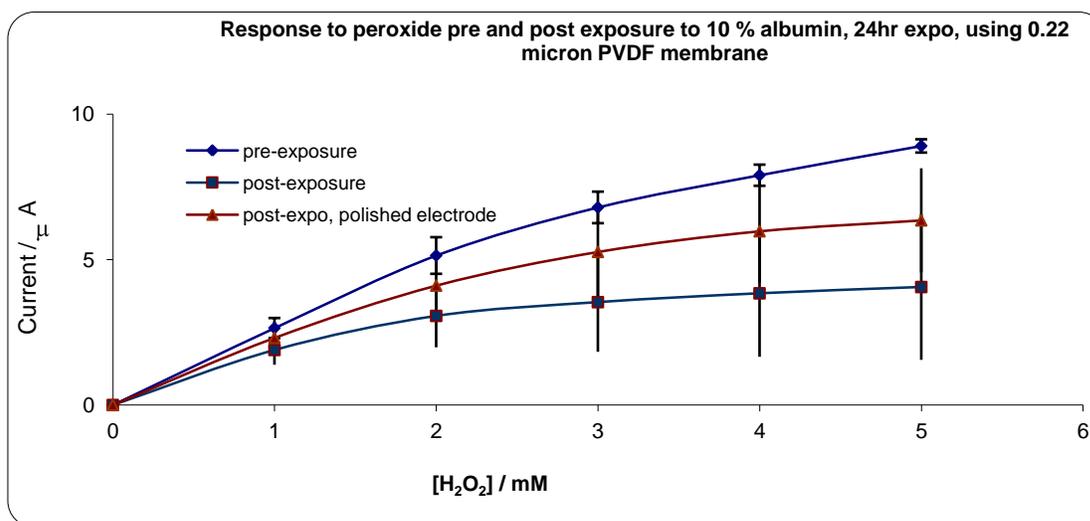


Fig. 5.4: Response to hydrogen peroxide, using 0.22 micron pore diameter poly(vinylidene fluoride) membrane, exposure to 10 % w/v albumin for 24 hour.

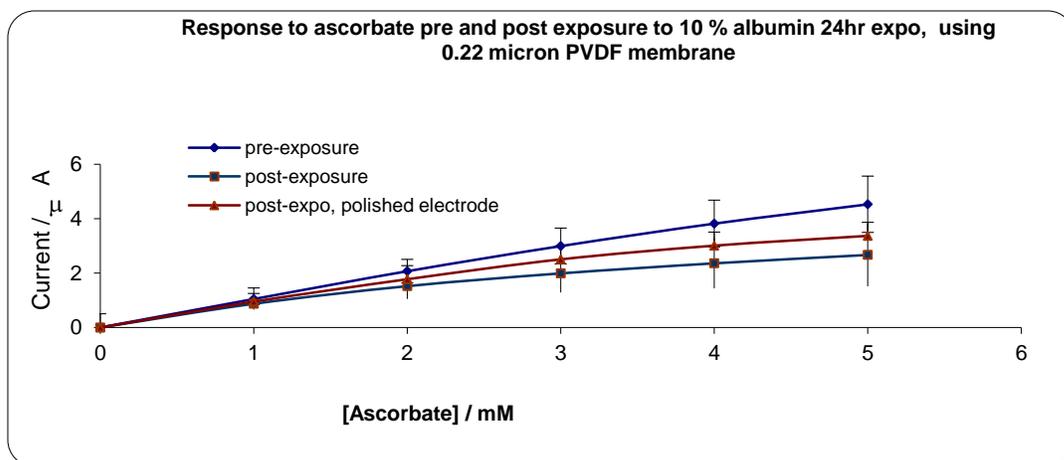


Fig. 5.5: Response to ascorbic acid, using 0.22 micron pore diameter poly(vinylidene fluoride) membrane, exposure to 10 % w/v albumin for 24 hour.

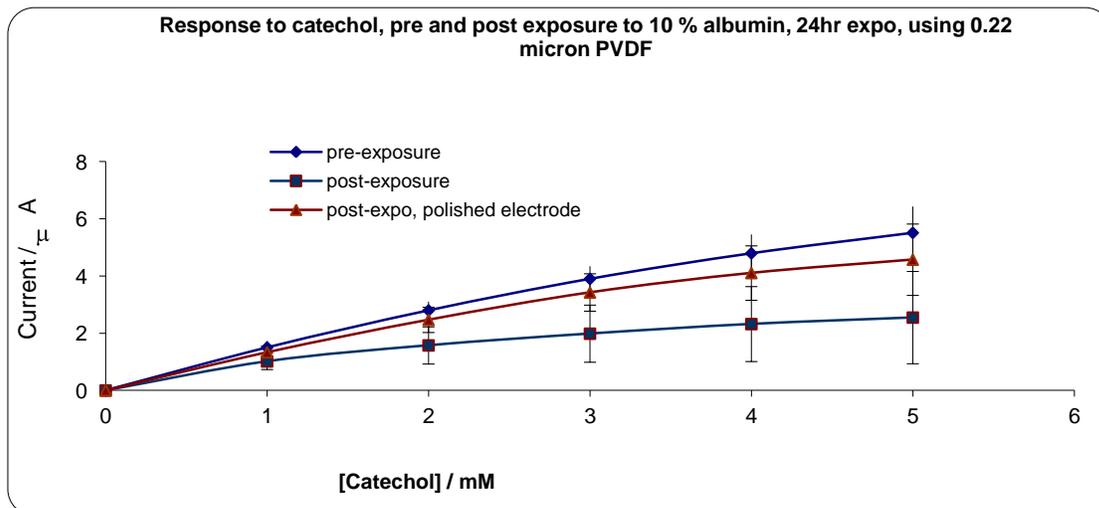


Fig. 5.6: Response to catechol, using 0.22 micron pore diameter poly(vinylidene fluoride) membrane, exposure to 10 % w/v albumin for 24 hour.

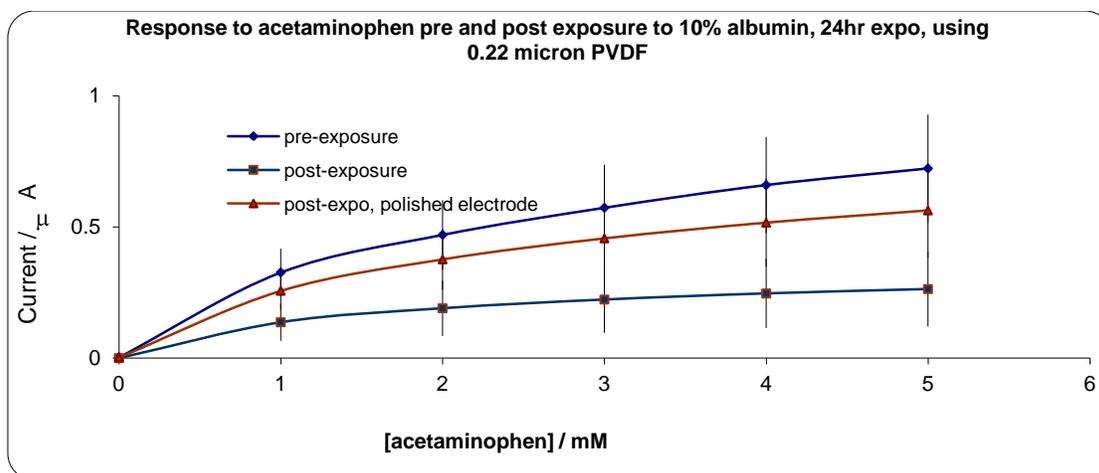


Fig. 5.7: Response to acetaminophen, using 0.22 micron pore diameter poly(vinylidene fluoride) membrane, exposure to 10 % w/v albumin for 24 hour.

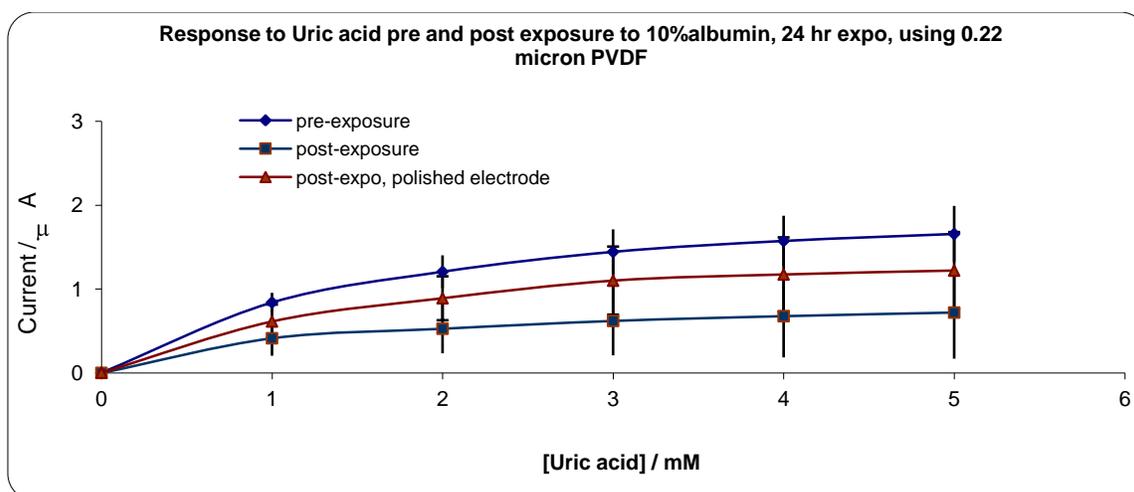


Fig. 5.8: Response to uric acid, using 0.22 micron pore diameter poly(vinylidene fluoride) membrane, exposure to 10 % w/v albumin for 24 hour.

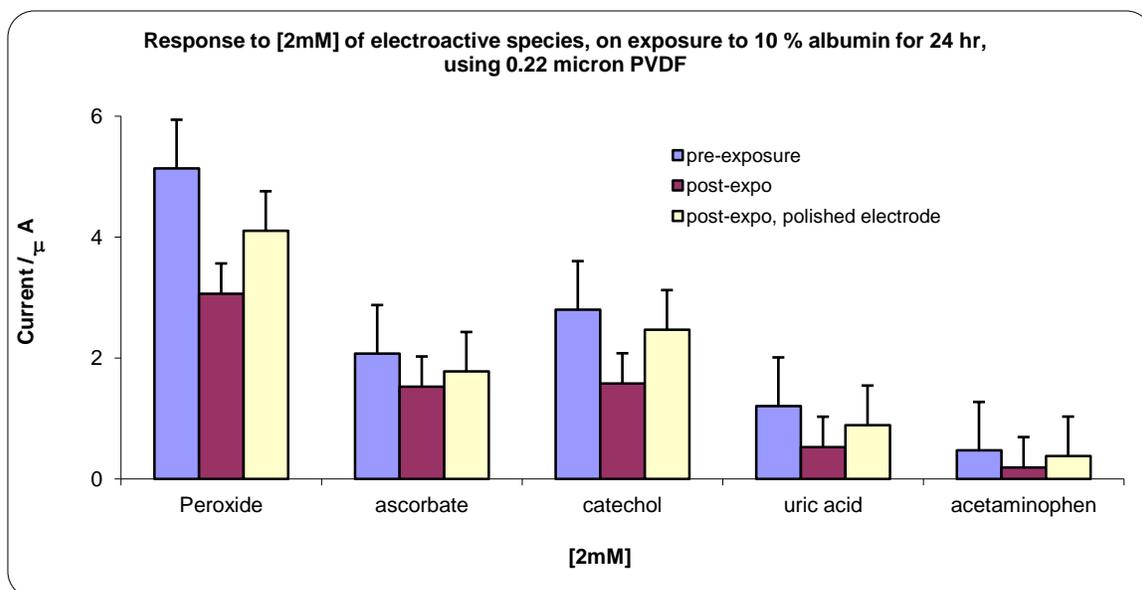


Fig. 5.9: Response to various electroactive chemicals, using 0.22 micron pore diameter poly(vinylidene fluoride) membrane, exposure to 10 % w/v albumin for 24 hour.

The PVDF membranes of 0.22 micron pore diameter were used to investigate the adsorption of bovine serum albumin on this membrane and to investigate the extent of membrane fouling after 24 hour exposure to 10 % w/v solution of bovine serum albumin. The electrode was polished in order to check the extent of membrane fouling. The membranes were tested with various electroactive chemicals to investigate the selectivity before exposure to protein and to see if fouled membranes give rise to changes in selectivity.

Results show that PVDF membranes select catechol, ascorbic acid and uric acid. Acetaminophen is selected the least and hydrogen peroxide is selected if present in low concentrations, as described before. On exposure to protein (10 % w/v solution of bovine serum albumin in buffer), a loss in sensitivity was observed with all the electroactive chemicals. The ratio in selectivity did not change. The response to hydrogen peroxide reduced to 60 % of the original response, 75 % for ascorbic acid, 55 % for catechol, 50 % for uric acid and 50 % for acetaminophen.

It was hypothesised that fouling or adsorption of protein takes place mostly on the surface of the membrane, modifies the membrane. So, the electrode was polished in order to remove the protein from the surface of the electrode and the fouled (protein modified) membrane was then retested. The response to all electroactive chemicals improved. The response improved to 80 % for hydrogen peroxide, 85 % for ascorbic acid, 90 % for catechol, 75 % for uric acid and 70 % for acetaminophen, after polishing of the electrode. This indicates that the protein goes through the pores of the membrane and is adsorbed on the surface of the electrode, resulting in reduced response, when the protein is introduced. However, the polishing and cleaning of the electrode results in removal of the protein layer from the surface of the electrode. When the modified membrane is retested, the response did not go back to the original baseline. This proved the hypothesis to be correct that the protein is adsorbed on the surface of the membrane; it modifies the membrane and confers selectivity to the membrane.

Adsorption of the protein is mostly on the surface of the membrane. This may be due to the formation of aggregates of the protein (bovine serum albumin) on the surface of the membrane, which blocks the pores of the membrane and may cause cake like protein layer formation on the surface of the membrane. According to Richard et. al. (1991) the saturation coverage of the membranes by the protein occurs at substantially less than a monolayer and the amount of protein adsorbed varies with pH.

The protein adsorption on the surface of the membrane may be due to the heterogeneous structure of the PVDF membrane, the surface morphology of the membrane may provide sites for the adsorption of the protein. The initial protein layer may lead to formation of aggregates of the protein or maybe protein cake layer on the surface of the membrane, which restrict the diffusion of the analyte to the surface of the electrode and thus results in a loss of sensitivity.

SEMs of the fouled (protein modified) membranes were taken to further investigate these results.

## **5.2 Topographic investigation of membranes**

Polymer membranes are used widely in filtration and separation processes and also in biosensors. Surface morphology of the membrane can be altered chemically, physically or through bulk polymer modification in order to achieve required surface properties and confer selectivity. The modified membranes can enhance the biostability of polymers and are less susceptible to fouling during ultrafiltration (Khayet et. al. 2003). The morphology of membranes can be studied by image analysis of electron micrographs (Wu et. al. 1995). In this study PVF membranes were investigated using field emission scanning electron microscopy before and after modification with macromolecules.

### **5.2.1 SEM of Poly(vinylidene fluoride) membranes**

The poly(vinylidene fluoride) (PVDF) membranes are tortuous pore, precast commercially available membranes. PVDF membranes are used widely in filtration processes in industry, immunoblotting of proteins and medical research. The poly(vinylidene fluoride) membranes of 0.2 micron pore diameter were used in this study.

#### **5.2.1.1 Poly(vinylidene fluoride) membranes control**

The unmodified PVDF membranes were used for this study.

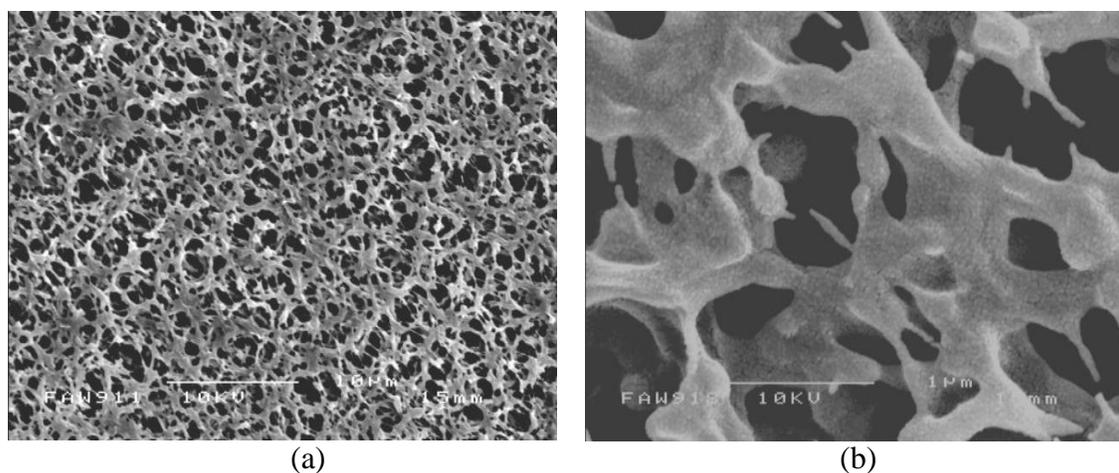


Fig.5.10: Surface of a 0.2 micron pore diameter poly(vinylidene fluoride) membrane. (Magnification: (a) = x 3,000 ; (b) = x 33,000)

The micrographs show that PVDF membranes have a tortuous structure. The pores are interconnected and appear to have branch like features protruding from the surface in all directions. The surface of the membranes appear to have very small cracks, Fig. 5.10 (b), again this could be an artefact due to many factors including charge effects or a result of the vacuum conditions in the scanning electron microscope. These problems were overcome to a large extent by applying a thicker coating of gold on the samples.

### 5.2.1.2 Poly(vinylidene fluoride) membranes – Buffer Exposure

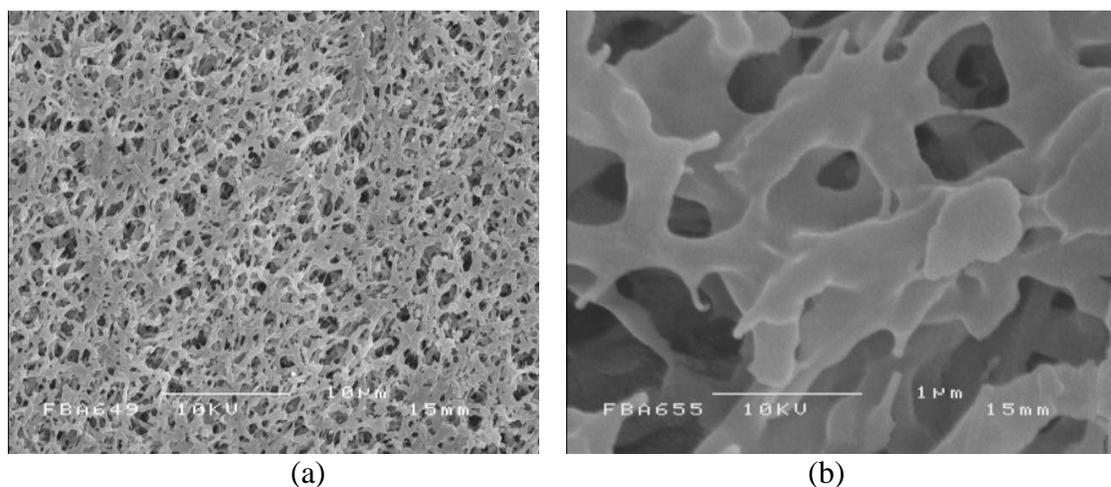


Fig.5.11: Surface of a 0.2 micron pore diameter poly(vinylidene fluoride) membrane post exposure to 1 hour in buffer. (Magnification: (a) = x 3,000; (b) = x 33,000)

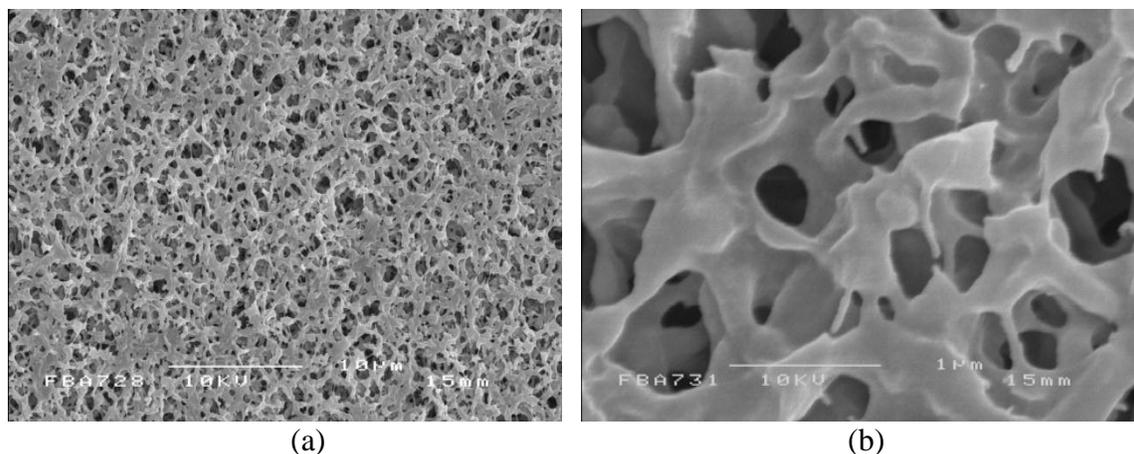


Fig.5.12: Surface of a 0.2 micron pore diameter poly(vinylidene fluoride) membrane post exposure to 24 hours in buffer. (Magnification: (a) = x 3,000; (b) = x 33,000)

The surface of PVDF membranes appear to be smooth after being soaked in the buffer. There is no significant difference in the appearance of the surface of the membrane and morphology of the membrane after longer exposure to phosphate buffer solution. Hence, it can be assumed that these membranes do not suffer adverse effects after exposure to phosphate buffer and are stable at longer exposure times.

### 5.2.1.3 PVDF membranes, post exposure to Albumin

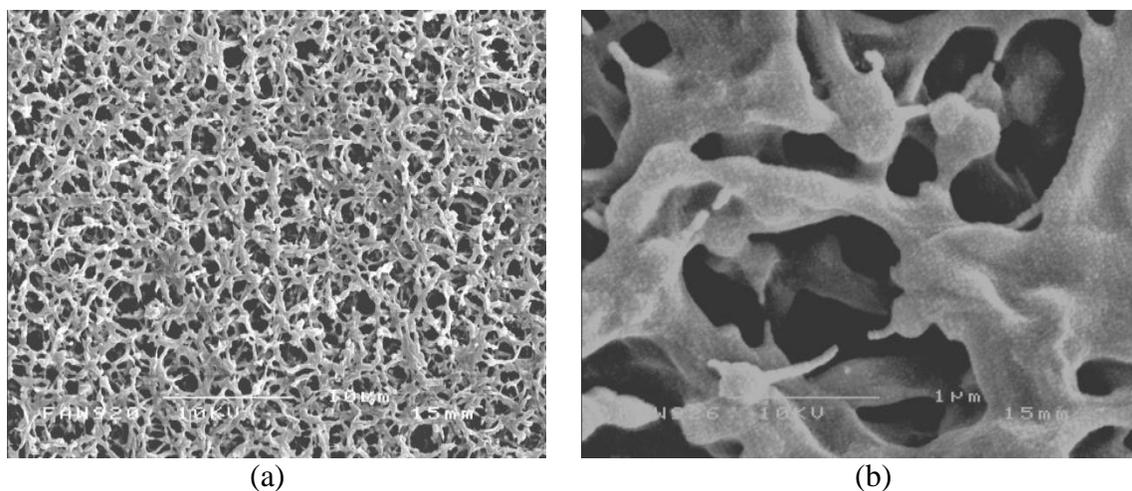


Fig. 5.13: Surface of a 0.2 micron pore diameter PVDF membrane after 1 hour exposure to 4 % w/v albumin solution. (Magnification: (a) = x 3,000; (b) = x 33,000)

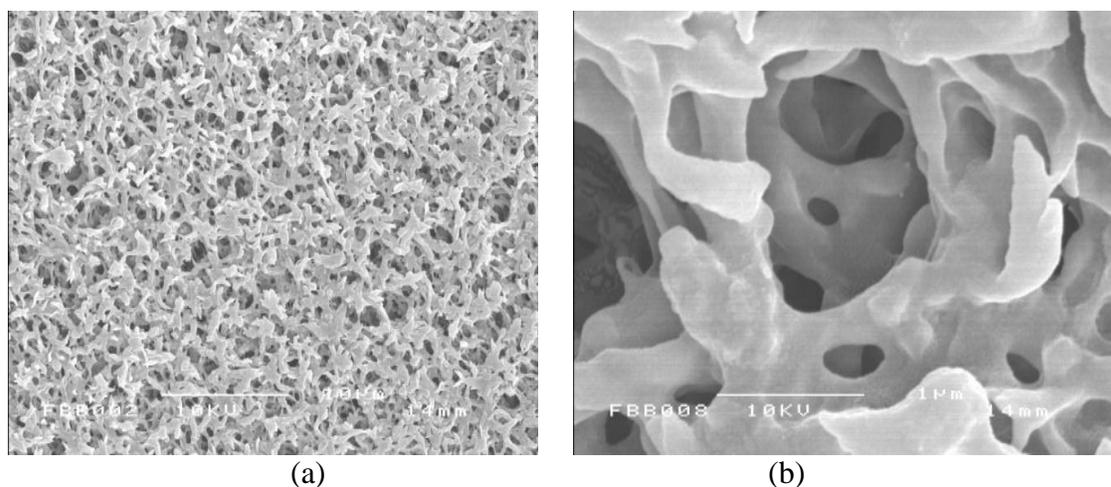


Fig. 5.14: Surface of a 0.2micron pore diameter PVDF membrane after 24 hour exposure to 4 % w/v albumin solution. (Magnification: (a) = x 3,000; (b) = x 33,000)

The exposure to the solution of albumin results in formation of a layer of deposit on the surface of the PVDF membrane in the first hour as can be seen in Fig. 5.13(a). After 24 hours of exposure the protein layer becomes thicker and the surface of the membrane appears to be covered with a continuous deposit of the protein, Fig. 5.14 (b).

#### 5.2.1.4 PVDF membranes, post exposure to Lysozyme

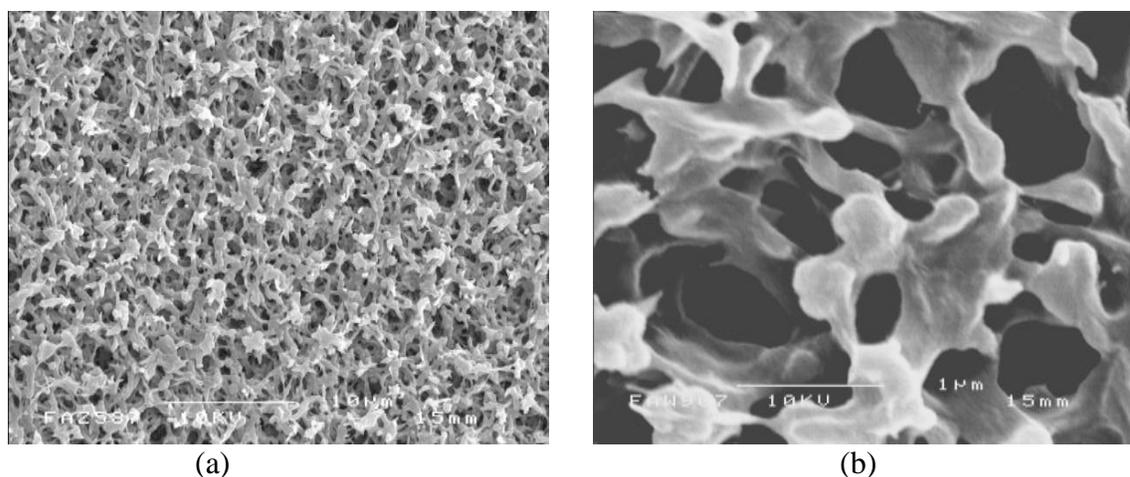


Fig. 5.15: Surface of a 0.2 micron pore diameter PVDF membrane after 1 hour exposure to 4 % w/v Lysozyme solution. (Magnification: (a) = x 3,000; (b) = x 33,000)

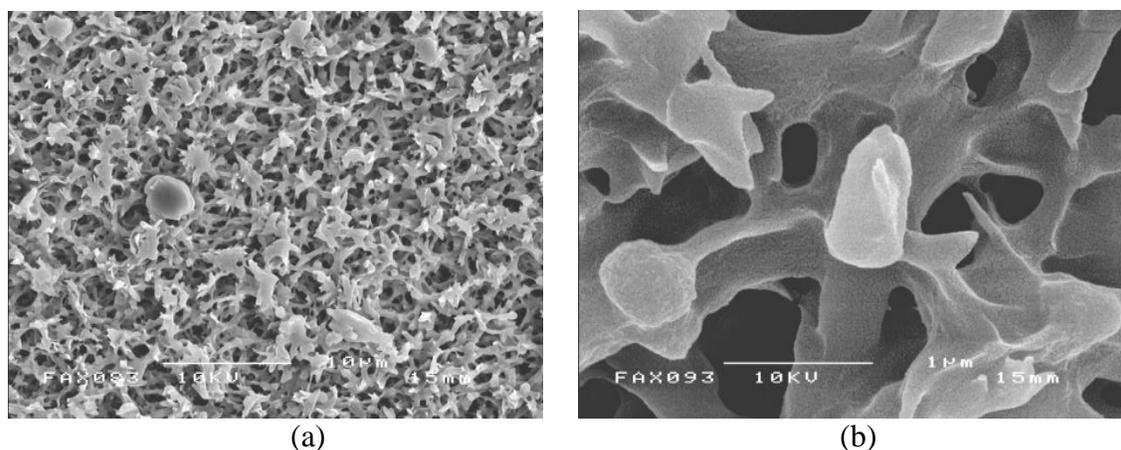


Fig. 5.16: Surface of a 0.2 micron pore diameter PVDF membrane after 24 hours exposure to 4 % w/v Lysozyme solution. (Magnification: (a) = x 3,000; (b) = x 33,000)

The results show that lysozyme adsorbs on the surface of the poly(vinylidene fluoride) membranes. The longer exposure time, twenty four hours, to lysozyme solution results in more adsorption on the surface of the PVDF membranes. The protein seems to be forming a uniform layer on the entire surface of the membrane and also in and around the pores. The aggregates appear to be of similar size after one hour of exposure and twenty four hours of exposure and appear to be uniformly distributed on the surface of the membrane.

### 5.2.1.5 PVDF membranes, post exposure to Fibrinogen

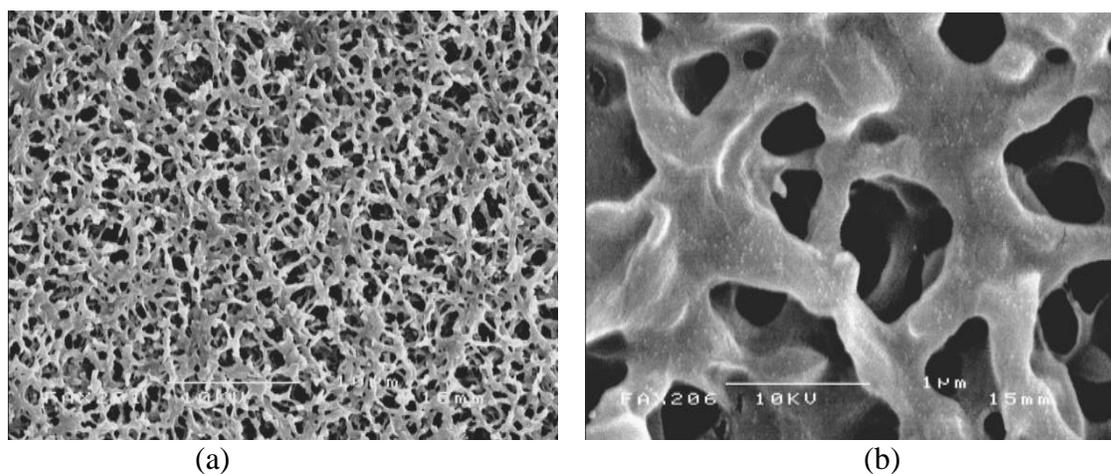


Fig. 5.17: Surface of a 0.2 micron pore diameter PVDF membrane after 1 hour exposure to 4 % w/v fibrinogen solution. (Magnification: (a) = x 3,000; (b) = x 33,000)

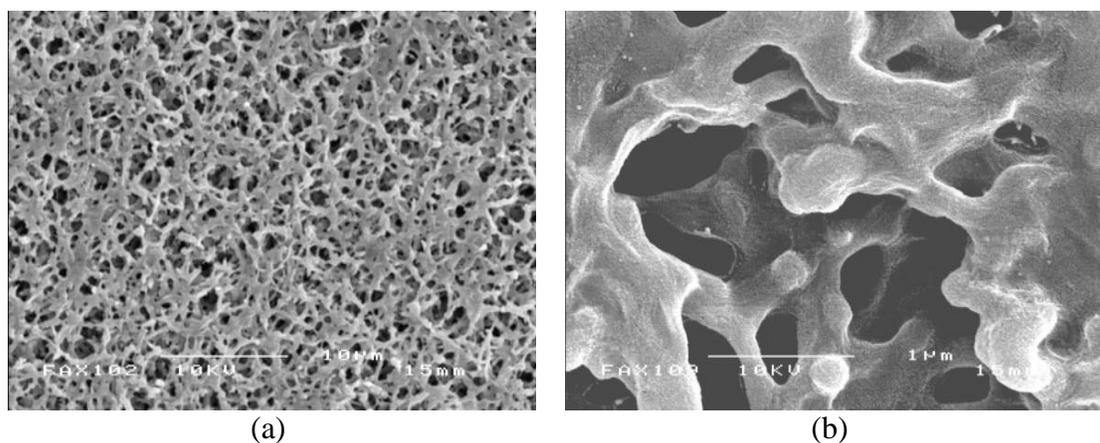


Fig. 5.18: Surface of a 0.2 micron pore diameter PVDF membrane after 24 hours exposure to 4 % w/v fibrinogen solution. (Magnification: (a) = x 3,000; (b) = x 33,000)

Exposure to fibrinogen solution causes rapid fouling of the surface of the PVDF membrane. Aggregates of the protein are observed on the surface of the membrane after one hour exposure (Fig. 5.17 b). A thicker layer of protein forms on the entire surface of the membrane after 24 hours of exposure, as can be seen by observing the Fig. 5.18(b).

### 5.2.1.6 PVDF membranes, post exposure to hyaluronan

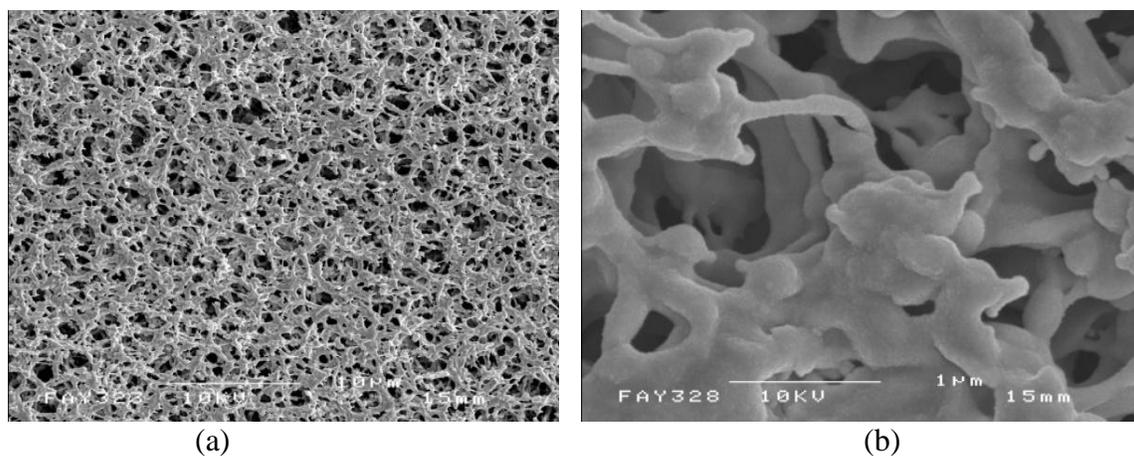


Fig. 5.19: Surface of a 0.2 micron pore diameter PVDF membrane after 1 hour exposure to 1 % w/v hyaluronan solution. (Magnification: (a) = x 3,000; (b) = x 33,000)

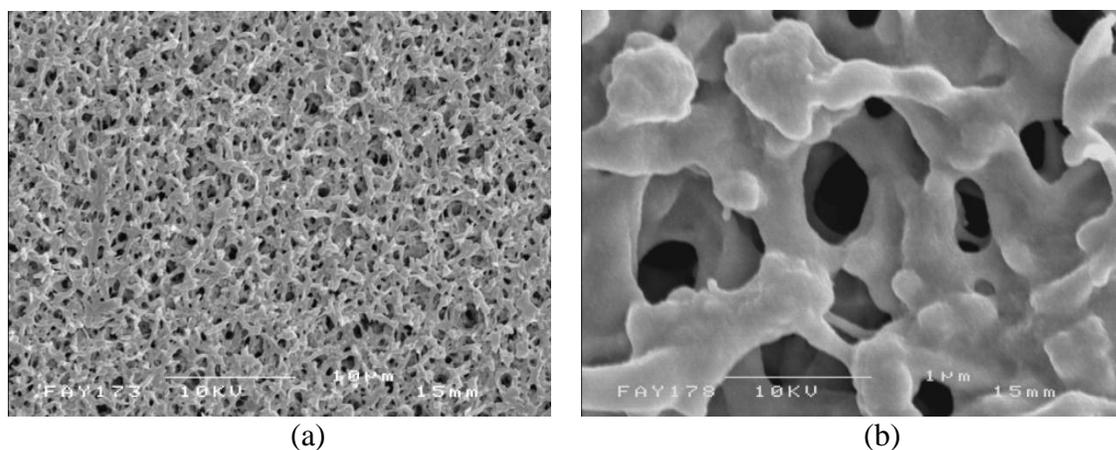


Fig. 5.20: Surface of a 0.2 micron pore diameter PVDF membrane after 24 hours exposure to 1 % w/v hyaluronan solution. (Magnification: (a) = x 3,000; (b) = x 33,000)

The images show that hyaluronan adsorbs on the surface of the PVDF membrane. The deposit, rather like a smooth layer of adsorbate, can be seen after one hour of exposure to solution of hyaluronan (Fig. 5.19 b). The longer exposure time results in an even thicker layer of the deposit being formed in and around the pores and on the surface of the membrane (Fig. 5.20 b). However, after 24 hours of exposure there are no aggregates observed in particular, unlike proteins, this could be due to various factors including the structural differences of the macromolecules.

### 5.2.1.7 PVDF membranes, post exposure to foetal calf serum

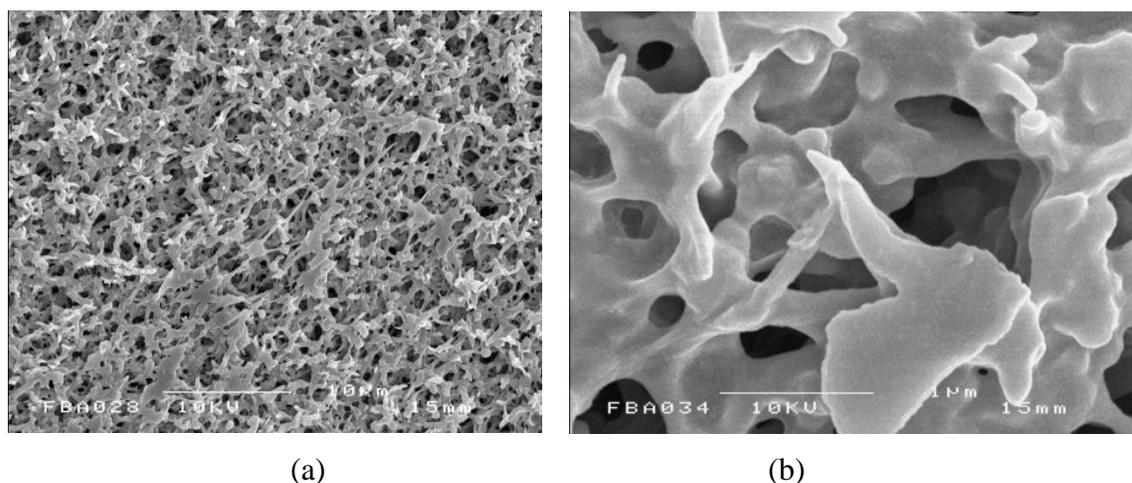


Fig. 5.21: Surface of a 0.2 micron pore diameter PVDF membrane after 1 hour exposure to foetal calf serum. (Magnification: (a) = x 3,000; (b) = x 33,000)

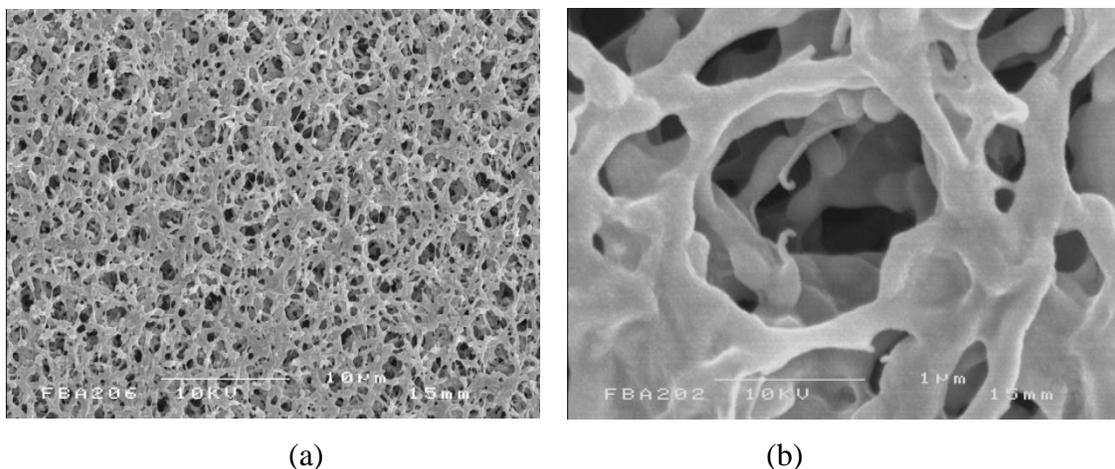


Fig. 5.22: Surface of a 0.2 micron pore diameter PVDF membrane after 24 hours exposure to foetal calf serum. (Magnification: (a) = x 3,000; (b) = x 33,000)

The images show that after one hour exposure to fetal calf serum a thick cake like layer of deposit is formed on the surface of the PVDF membrane (Fig. 5.21). The surface of the poly(vinylidene fluoride) membrane, after 24 hours of exposure to the fetal calf serum, still looks smooth and coated with a thick layer of the deposit (Fig. 5.22). No aggregates as such are visible on the surface of the membrane.

The PVDF membranes were investigated for their stability at the different pH levels.

### 5.2.1.8 PVDF membranes post exposure to phosphate buffer pH 3

For these investigations 0.2 micron pore diameter PVDF membranes and phosphate buffer at pH 3 was selected. The membranes were soaked in the phosphate buffer solution at pH 3 for various time durations including one hour and twenty four hours.

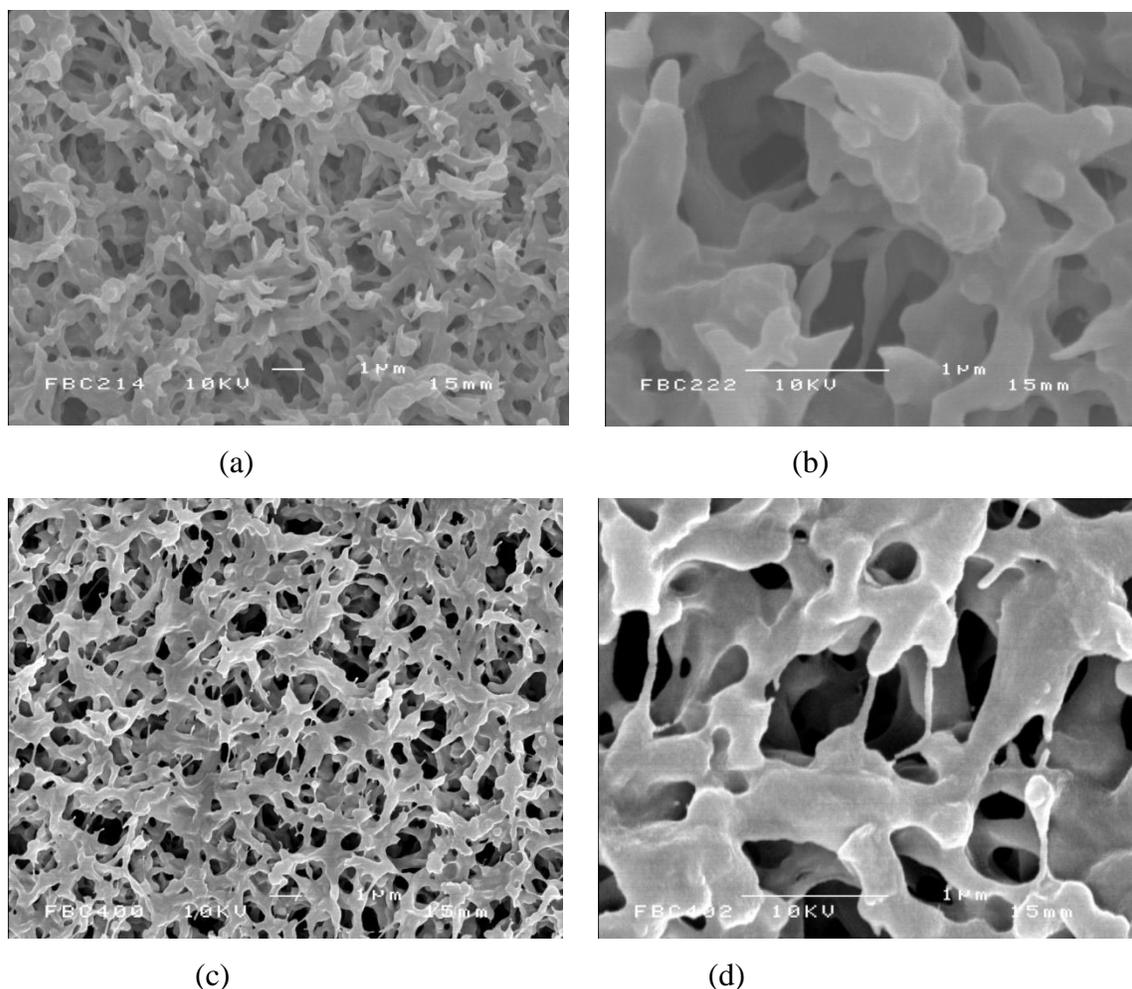


Fig.5.23: Surface of a 0.2 micron pore diameter PVDF membrane after exposure to buffer pH 3 (a),(b) after 1 hour exposure (c),(d) 24 hours post exposure (Magnification: (a), (c) = x 7000; (b), (d) = x 33,000).

The PVDF membranes are stable after exposure to phosphate buffer at pH 3. The results show that longer exposure times do not lead to any changes in the morphology of these membranes (Fig. 5.23 (c, d)).

### 5.2.1.9 Effect of concentration of protein on PVDF membranes

The effect of concentration on the fouling of tortuous pore membranes was also investigated using the 0.2 micron pore diameter poly(vinylidene fluoride) membranes. The protein used for these investigations was albumin at 4 % w/v and 10 % w/v concentration.

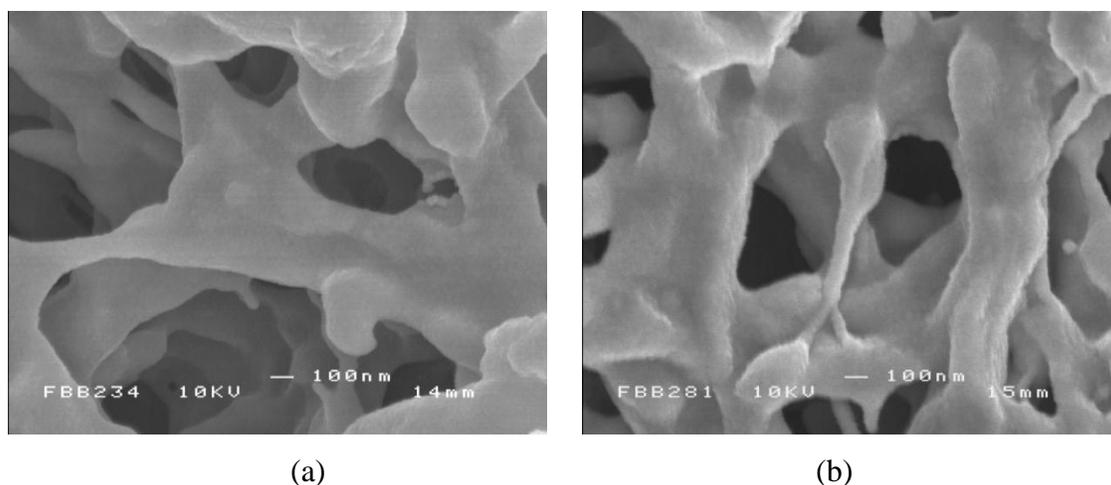


Fig.5.24: Surface of a 0.2 micron pore diameter PVDF membrane, after 1 hour exposure to (a) 4 % w/v (b) 10 % w/v solution of albumin. (Magnification: x 50,000).

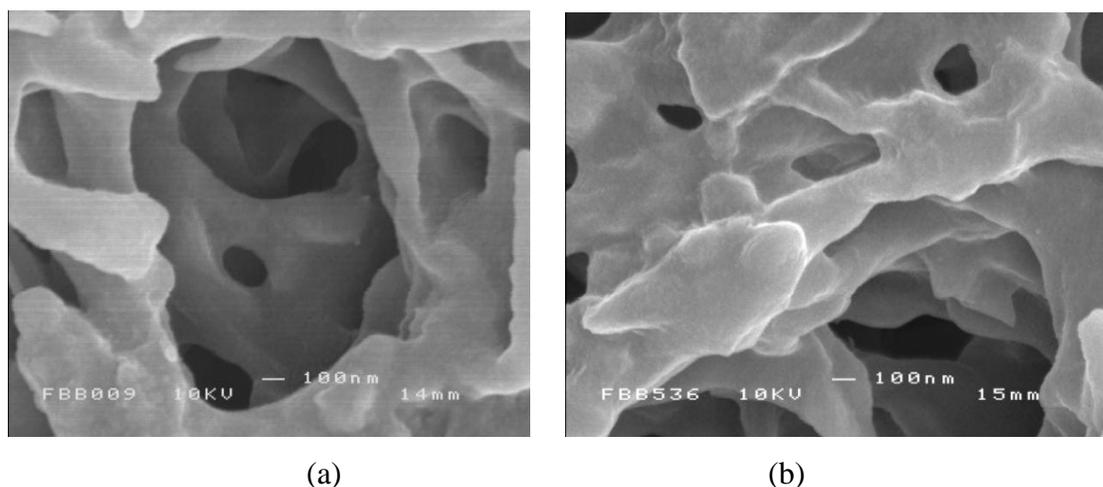


Fig.5.25: Surface of a 0.2 micron pore diameter PVDF membrane, after 24 hours exposure to (a) 4 % w/v (b) 10 % w/v solution of albumin. (Magnification: x 50,000).

The results show that exposure to a higher concentration of albumin solution, i.e. the solution of 10 % w/v of albumin, leads to fouling of the surface of the poly(vinylidene fluoride) membrane, the membrane is observed to be covered with a grainy layer of the protein (Fig. 5.24 b), the surface of the membrane after one hour of exposure looks less fouled (Fig 5.24 a). After twenty four hours of exposure, the same difference is observed in the morphology of the membrane with higher and lower concentration of the protein solution.

### 5.3 SEM of Mixed Cellulose Acetate membranes

The field emission scanning electron microscope was used to characterise and investigate surface morphology of the mixed cellulose acetate membranes. Commercially available 0.2 micron pore diameter cellulose acetate membranes were used in these studies.

#### 5.3.1 Mixed cellulose acetate membranes control

The unmodified mixed cellulose membranes of 0.2 micron pore diameter were used for these studies.

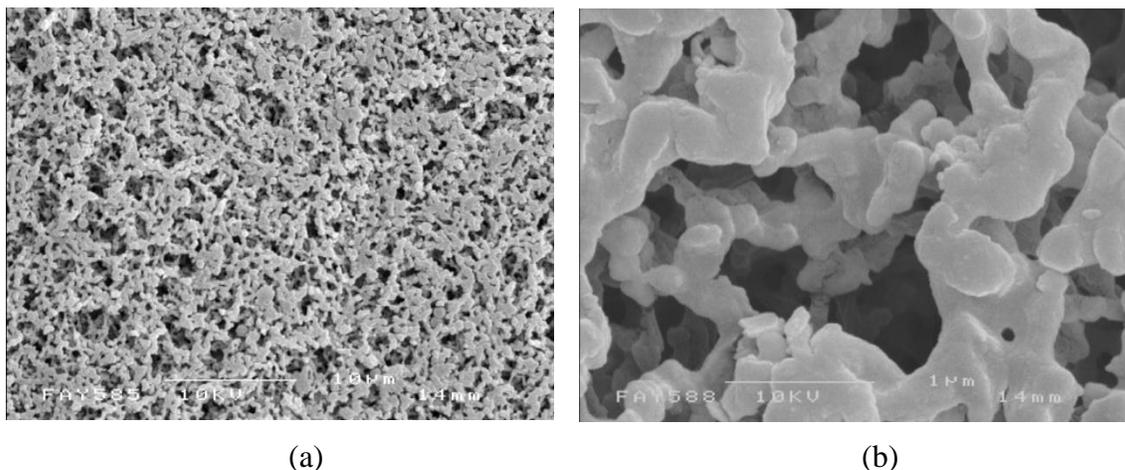


Fig. 5.26: Surface of a 0.2 micron pore diameter mixed cellulose acetate membrane. (Magnification: (a) = x 3,000; (b) = x 33,000)

The structure of the mixed cellulose acetate membrane is heterogeneous. The pores are observed to be interconnected and tortuous in nature. The surface of the membrane is not continuous rather looks like an entangled mesh of the polymer fibres connected together. The actual pore size of these membranes seems to be larger than 0.2 micron pore diameter due to the interconnectivity of the pores.

The mixed cellulose acetate membranes were then soaked in the buffer solution at pH 7 for various time durations including one hour and twenty four hours, in order to investigate the stability of these membranes at physiological pH and conditions.

### 5.3.2 Mixed cellulose acetate membranes – Buffer exposure

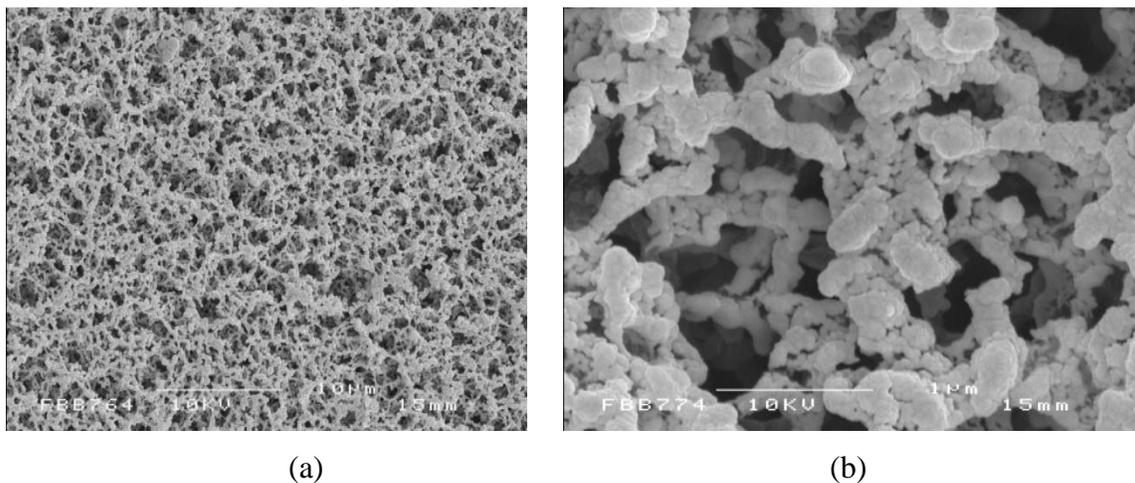


Fig. 5.27: Surface of a 0.2 micron pore diameter mixed cellulose acetate membrane after 1 hour exposure to buffer. (Magnification: (a) = x 3,000; (b) = x 33,000).

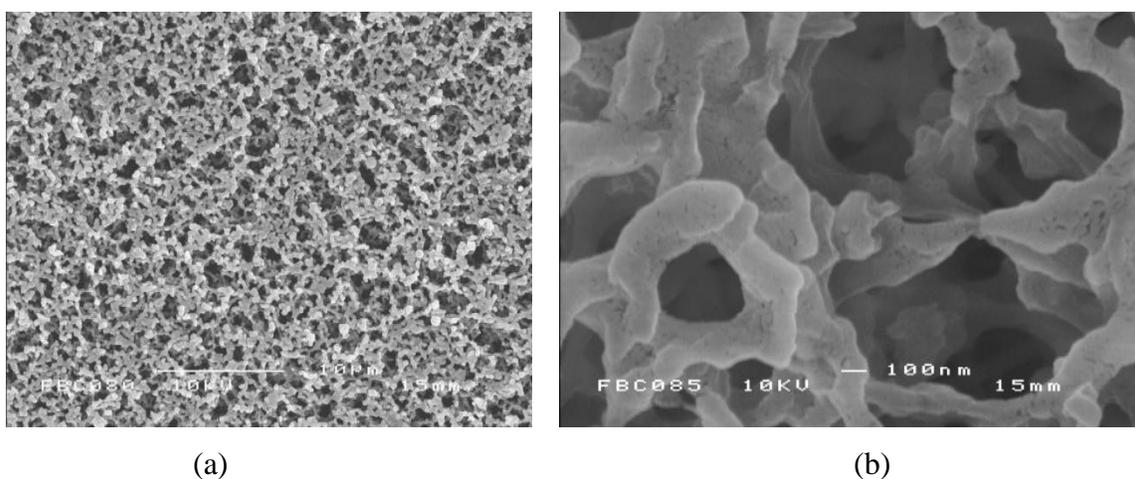


Fig. 5.28: Surface of a 0.2 micron pore diameter mixed cellulose acetate membrane after 24 hours exposure to buffer. (Magnification: (a) = x 3,000; (b) = x 33,000)

The exposure to buffer solution leads to cracking of the surface of the mixed cellulose acetate membrane. After one hour of exposure, the entire surface of the membrane is observed to have clumps of material on it. This could be due to desorption of the material or leaching of substances out of the surface of the membrane. The surface of the mixed cellulose acetate is coated with surfactant Triton, it could also be that the coating

dissolves in the phosphate buffer solution or changes configuration when exposed to phosphate buffer solution (Fig. 5.27). However, at longer exposure times to buffer solution the surface of the membrane is stable. Moreover, the clumped material on the surface appears to be smoother at longer exposure times. The reason could be that all of the coating would either have dissolved and then reabsorbed or just simply leached out from the entire surface or perhaps it stabilises after longer periods of exposure to buffer solution. The mixed cellulose acetate membranes were not used for investigation in the amperometry studies as the SEM studies showed that their structure is not stable in buffer solution at short exposure times. However, exposure to protein was investigated using the cellulose acetate membranes in this study.

### 5.3.3 Mixed cellulose acetate membranes, post-exposure to Albumin

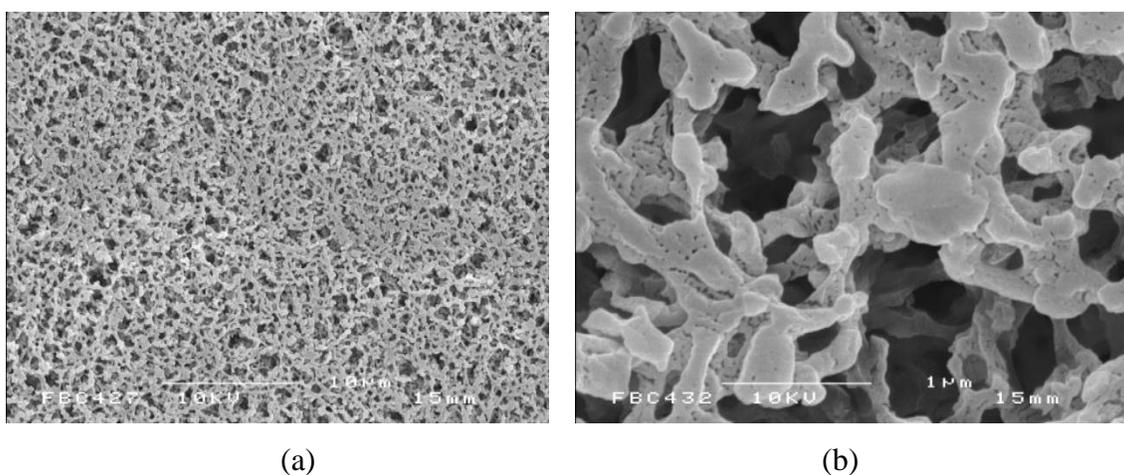


Fig. 5.29: Surface of a 0.2 micron pore diameter mixed cellulose acetate membrane after 1 hour exposure to 4 % w/v albumin solution. (Magnification: (a) = x 3,000; (b) = x 33,000)

The studies were only done with albumin solution and only 1 hour exposure of the membrane was investigated due to the fact that the surface of the membrane is so severely distorted after one hour of exposure to buffer. The exposure to albumin solution also shows the surface to be distorted. However the albumin solution is observed to be coating the surface of the membrane at the same time (Fig. 5.29). The investigation of

A study of biointerfacing polymeric barrier membranes for sensors

protein adsorption on the surface of cellulose acetate was discontinued after these findings, as there are too many factors involved for it to lead to any conclusive evidence. Polycarbonate membranes were selected for investigation of macromolecular adsorption on polymer membranes.

## **Chapter 6 Membranes with vertical cylindrical pores and different pore sizes**

In glucose sensors cast membranes are used extensively. These include poly(carbonate) and PVC. Two types of commercially available pre-cast polycarbonate membranes were used in this study. Isoporous polycarbonate membranes with PVP coatings and polycarbonate membranes without PVP coating, both of these poly(carbonate) membranes have vertical cylindrical pores.

For biosensor applications, poly(carbonate) membranes are used with limited pores in order to control the flux of solute to the enzyme layer. The analytical linear range increases with decreasing pore size. Low pore density poly(carbonate) membrane gives linearity to approximately 200 mM glucose (Maines et. al. 1996).

The polycarbonate membranes of different pores sizes were investigated for use in sensors and for membrane modification with various macromolecules for potential use as barrier membranes for sensors.

### **6.1 Selectivity profile for poly(carbonate) membranes**

Various sizes of commercially available isoporous poly(carbonate) membranes (0.05, 0.2 and 5.0  $\mu\text{m}$  pore diameter) were used for the investigation of selectivity profile of these membranes. Experiments were performed under stirred conditions.

Results shown are averages of three experiments,  $n = 3$ .

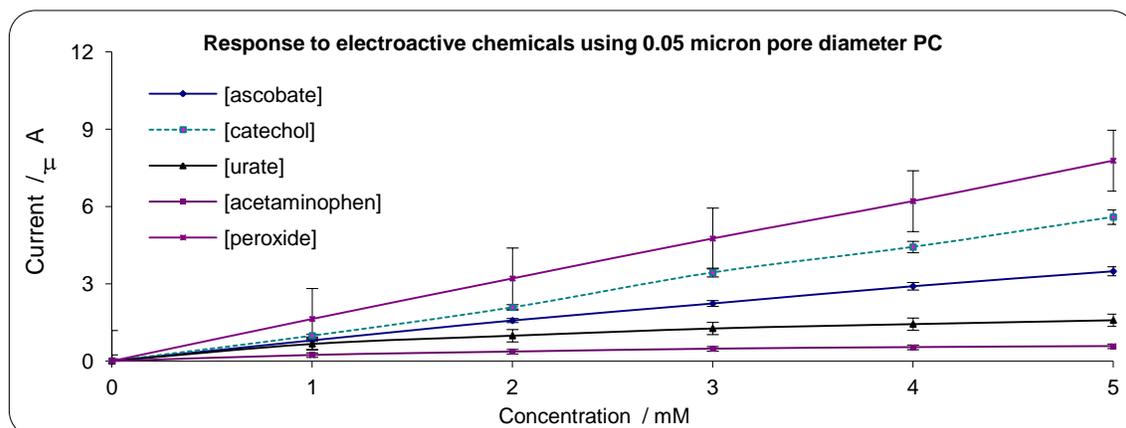


Fig. 6.1: The response to electroactive species using a 0.05 micron pore diameter poly(carbonate) membrane.

Fig. 6.1 shows that as the concentration of the peroxide, similarly other chemicals increases, the current response increases linearly. These results hold true for the large pore size poly(carbonate) membranes.

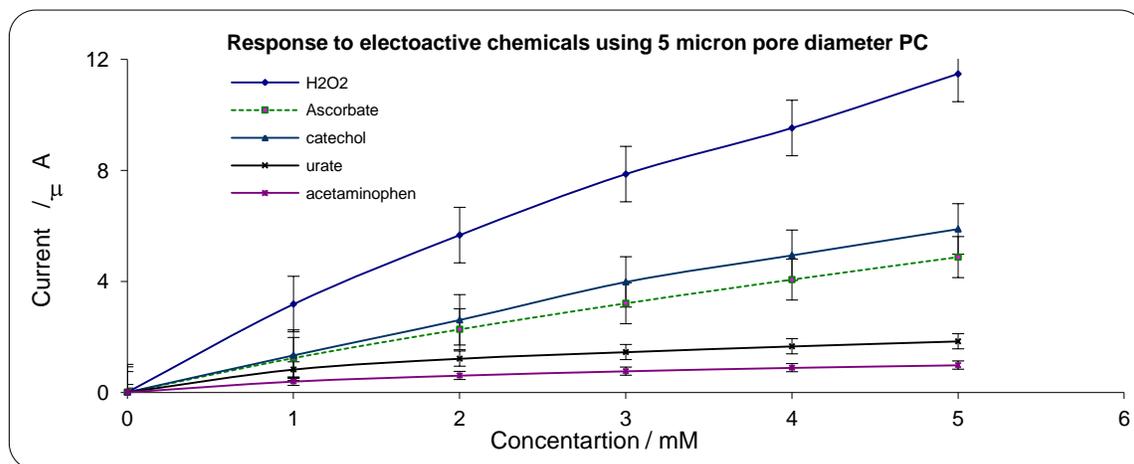


Fig.6.2: The response to electroactive chemicals using a 5.0 micron pore diameter poly(carbonate) membrane.

Results show that the response to all the chemicals is higher with the larger pore size polycarbonate membrane as compared to smaller pore size polycarbonate membranes. This can be due to the fact that the small pore size membrane provides controlled flux and linear diffusion of the analyte to the surface of the electrode.

The graphs show that response to hydrogen peroxide is higher than any other electroactive chemical; this is observed with 0.05 and 5.0 micron pore diameter polycarbonate membranes. Catechol gives the second highest response, followed by ascorbic acid and uric acid respectively. Acetaminophen gives the lowest current response. These results show that poly(carbonate) membranes select hydrogen peroxide the more than any other electroactive chemical. Hence, the poly(carbonate) membranes were used in this study to investigate membrane modification with various macromolecules and to study the selectivity profiles of the modified polymer membranes.

Various macromolecules including fibrinogen (fibrous protein), albumin (globular protein), lysozyme and hyaluronan were used to foul and modify the poly(carbonate) membranes and to investigate the selectivity of these fouled and modified membranes. The membrane as well as electrode fouling was investigated in the initial investigations.

## **6.2 Biofouling studies of poly(carbonate) membranes**

These studies involved assessment of the selectivity profile in the absence of macromolecules and proteins followed by investigation of selectivity after the exposure of a membrane to macromolecule or protein for a specified duration of time in order to investigate the selectivity profile of the macromolecular modified membrane. The membrane was then changed and a new membrane (same batch) was applied in order to investigate the extent of electrode fouling.

### **6.2.1 Fouling studies with Hyaluronic acid**

Hyaluronic acid is a common component of the extracellular matrix, obtained by extraction. It is a linear anionic polysaccharide. The molecular weight of hyaluronic acid is in the range of  $10^5 - 10^7$ . Some of the properties that make it a useful polymer include lubrication and shock absorbance. Viscoelasticity of hyaluronic acid plays an important

role in physical protection and integrity in living tissue such as skin, synovial fluid, vitreous humor and articular cartilage. Hyaluronic acid has been widely tried and used in various medical applications such as eye surgery, pace makers, wound healing and injections for osteoarthritis, due to its biological safety and rheological characteristics (Miyazaki et. al. 2001, Avitabile et. al. 2001).

Hyaluronic acid forms an entangled network in dilute solution. Increased concentration leads to a gel like consistency. This is due to overlapping of the polymer chains, as the network becomes very dense and the flow of the solution is reduced. This property of hyaluronic acid was observed during the experiments, when the concentration of hyaluronan was increased it formed a gel like solution.

## Results and discussion

The results shown are the mean of three experiments ( $n = 3$ ) and standard deviations are indicated.

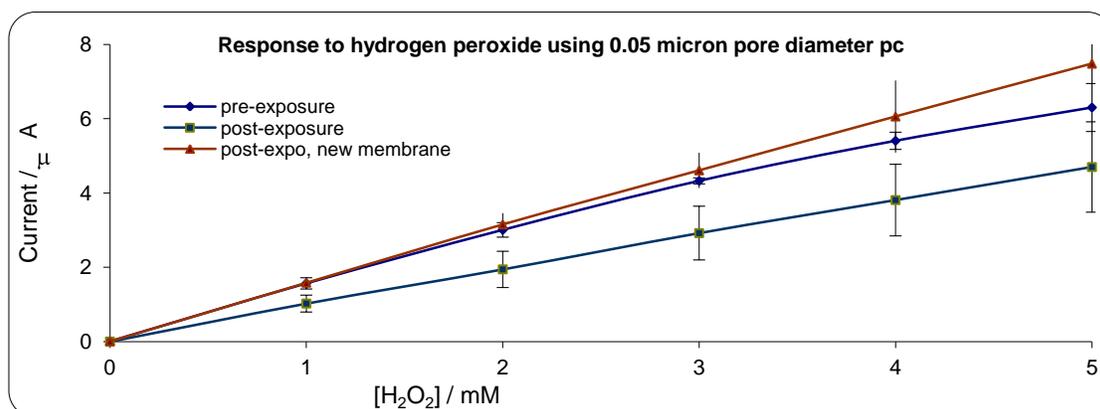


Fig. 6.3: Response to Hydrogen peroxide, using 0.05 micron pore diameter polycarbonate membrane, exposure to 2 % W/V Hyaluronic acid for 1 hour.

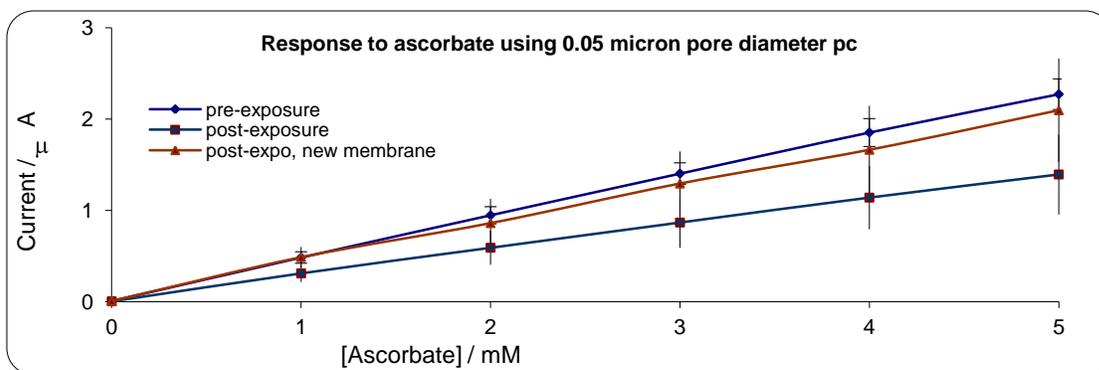


Fig. 6.4: Response to Ascorbic acid, using 0.05 micron pore diameter polycarbonate membrane, exposure to 2 %W/V Hyaluronic acid for 1 hour.

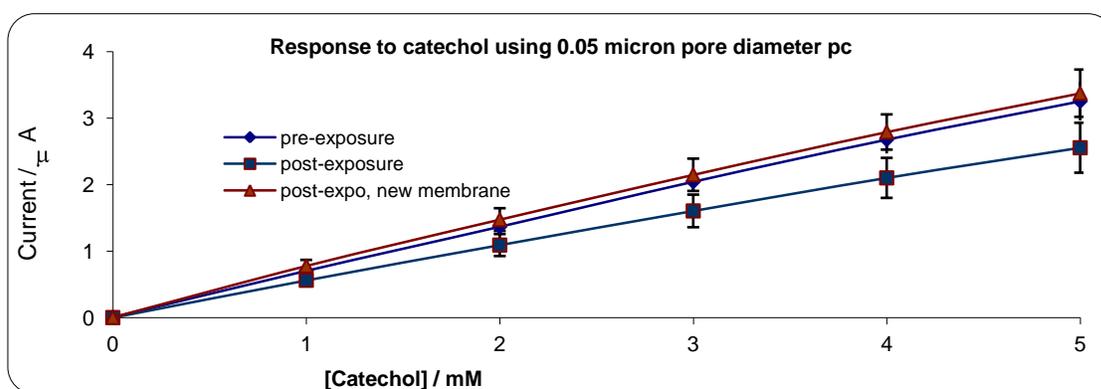


Fig. 6.5: Response to Catechol, using 0.05 micron pore diameter polycarbonate membrane, exposure to 2 %W/V Hyaluronic acid for 1 hour.

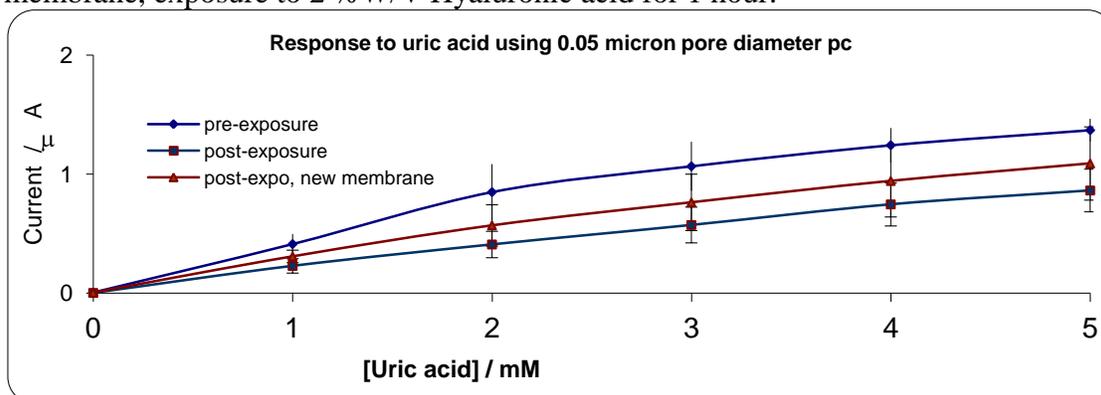


Fig. 6.6: Response to Uric acid, using 0.05 micron pore diameter polycarbonate membrane, exposure to 2 %W/V Hyaluronic acid for 1 hour.

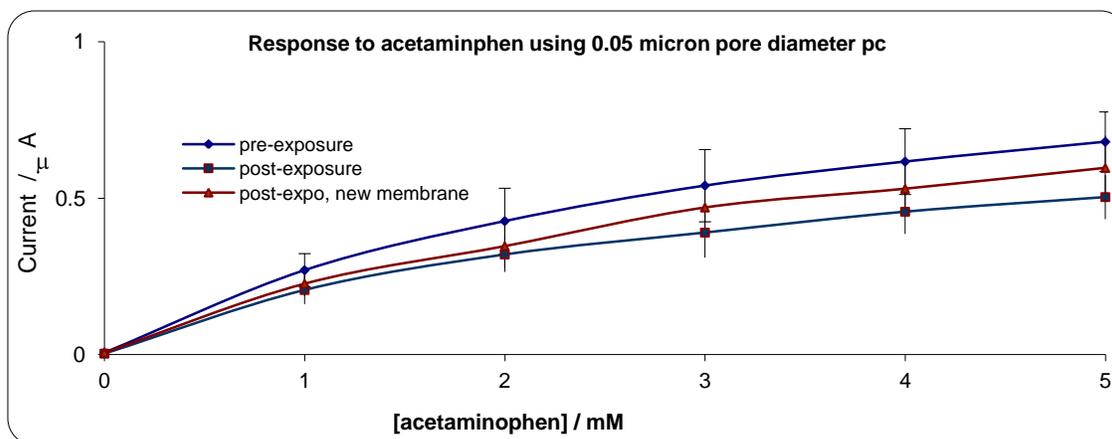


Fig. 6.7: Response to Acetaminophen, using 0.05 micron pore diameter polycarbonate membrane, exposure to 2 %W/V Hyaluronic acid for 1 hour.

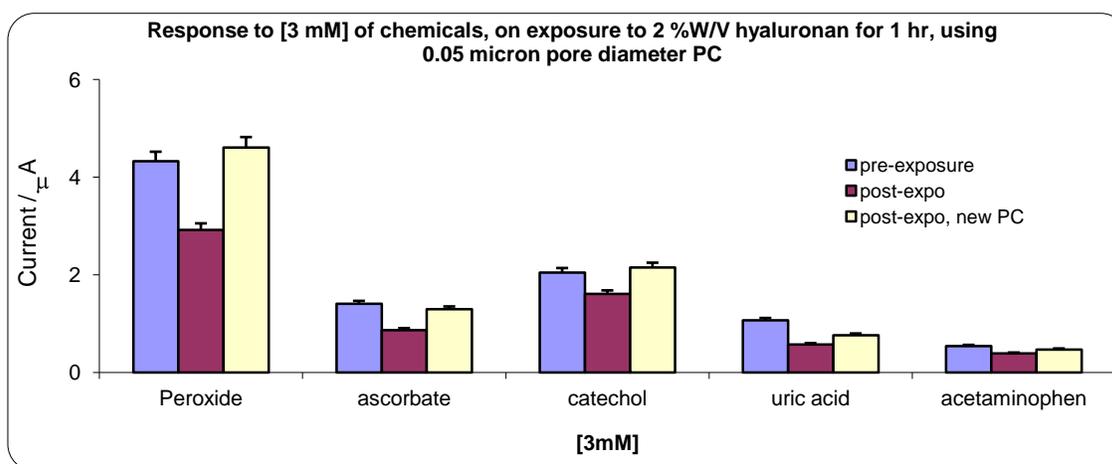


Fig. 6.8: Response to various electroactive chemicals, using 0.05 micron pore diameter polycarbonate membrane, exposure to 2 %W/V Hyaluronic acid for 1 hour, n = 3.

Results show that membrane fouling is higher than the electrode fouling when 0.05 micron pore diameter polycarbonate is exposed to 2% w/v hyaluronic acid, as the response improves after changing the membrane. The response to all electroactive chemicals was observed to be reduced after one hour of exposure. When a new membrane was applied the response obtained was similar to the original response in case of hydrogen peroxide and catechol. In fact the response to hydrogen peroxide increased more than the original response when the membrane was changed. However, the current response did not improve significantly with ascorbic acid and uric acid and acetaminophen. These results show that after exposure to hyaluronan, the membrane is modified, which affects the selectivity profile.

It is hypothesised that when 0.05 micron pore diameter polycarbonate membrane is exposed to 2 % w/v hyaluronic acid, the hyaluronic acid is deposited on the surface of the membrane more than it diffuses through to the surface of the electrode. It may form aggregates on the surface of the membrane, blocking the pores of the membrane, hence restricting further diffusion of hyaluronic acid to the surface of the electrode. This may give rise to the formation of a very thin film of hyaluronic acid on the surface of the electrode, which somehow facilitates diffusion of hydrogen peroxide to the surface of the electrode.

Hydrogen peroxide and catechol are relatively small molecules as compared to other electroactive chemicals, so they may diffuse through the hyaluronic acid film or layer more easily. Other electroactive chemicals may not be selected by the hyaluronic acid film or coating. This may be due to the fact that these electroactive chemicals are large compared to hydrogen peroxide and catechol and so they are excluded on the basis of size exclusion. However, as hyaluronan is negatively charged at pH 7 and ascorbic acid is also negatively charged at physiological pH hence, it may be excluded due to charge effects.

### Response to various chemicals, on exposure to 2 % w/v Hyaluronic acid using 0.2 micron pore diameter PC

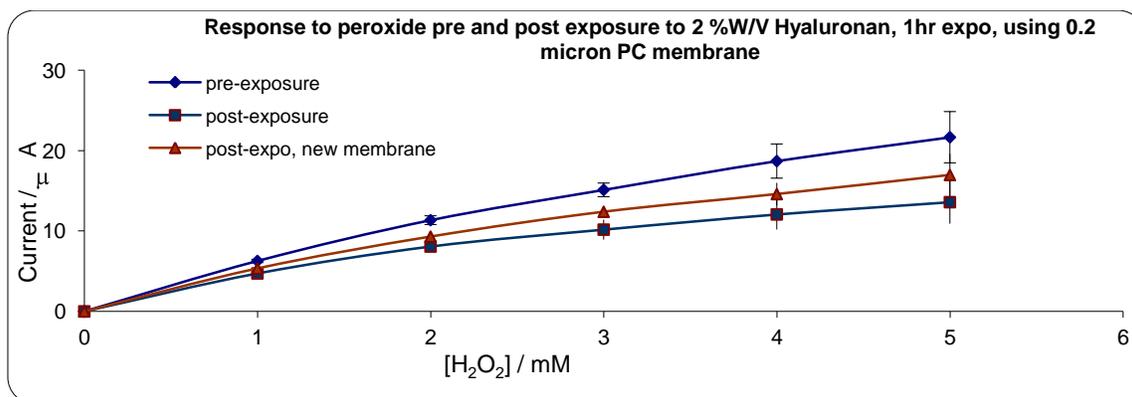


Fig. 6.9: Response to Hydrogen peroxide, using 0.2 micron pore diameter polycarbonate membrane, exposure to 2 % w/v Hyaluronic acid for 1 hour.

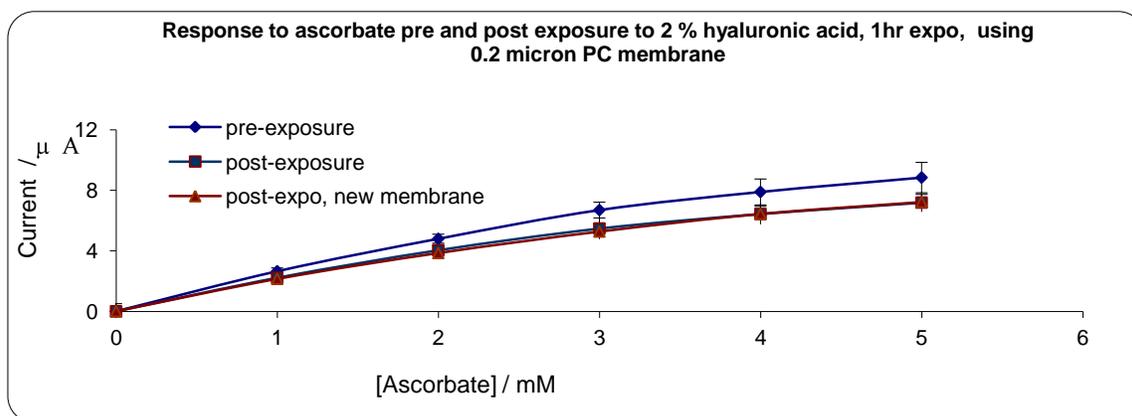


Fig. 6.10: Response to Ascorbic acid, using 0.2 micron pore diameter polycarbonate membrane, exposure to 2 %w/v Hyaluronic acid for 1 hour.

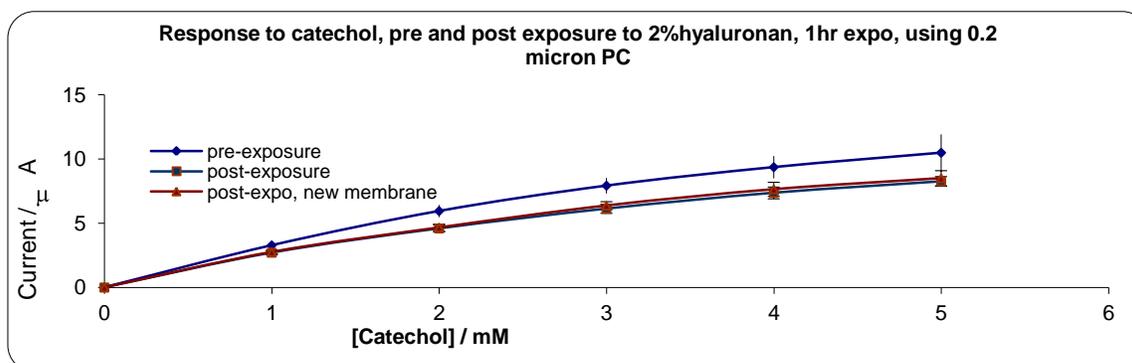


Fig. 6.11: Response to Catechol, using 0.2 micron pore diameter polycarbonate membrane, exposure to 2 %w/v Hyaluronic acid for 1 hour.

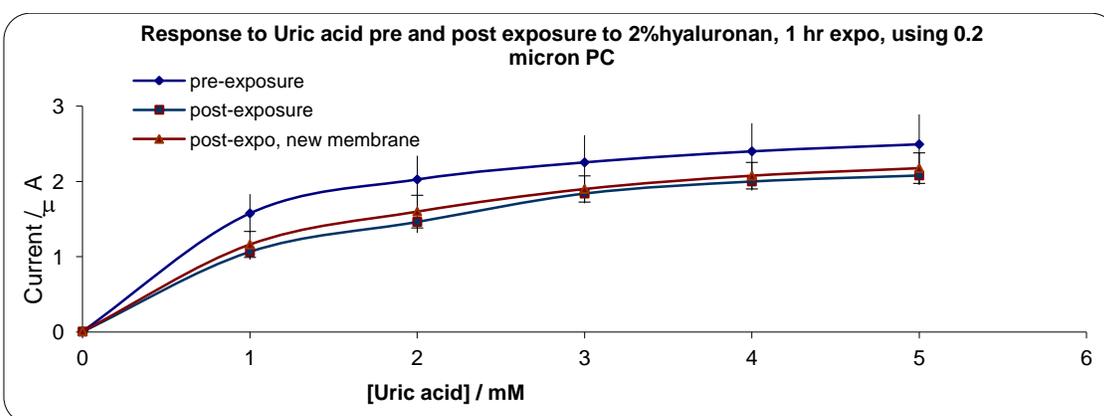


Fig. 6.12: Response to Uric acid, using 0.2 micron pore diameter polycarbonate membrane, exposure to 2 % w/v Hyaluronic acid for 1 hour.

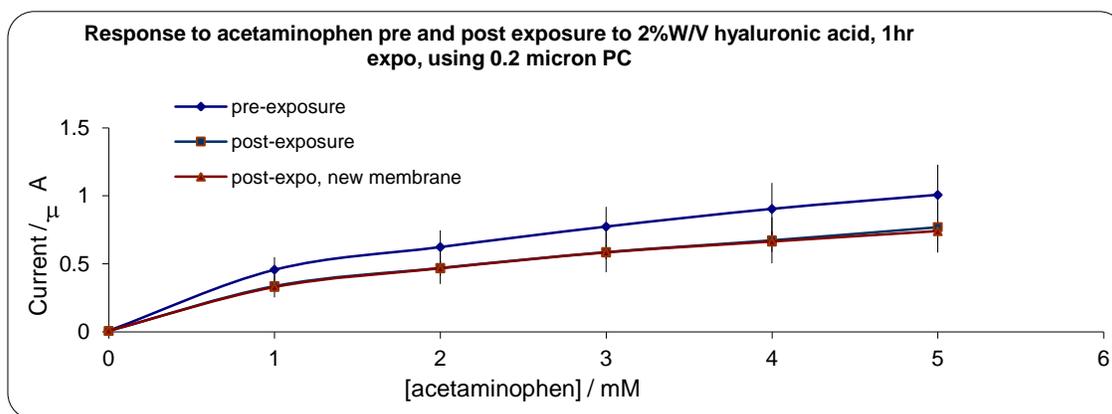


Fig. 6.13: Response to Acetaminophen, using 0.2 micron pore diameter polycarbonate membrane, exposure to 2 % w/v Hyaluronic acid for 1 hour.

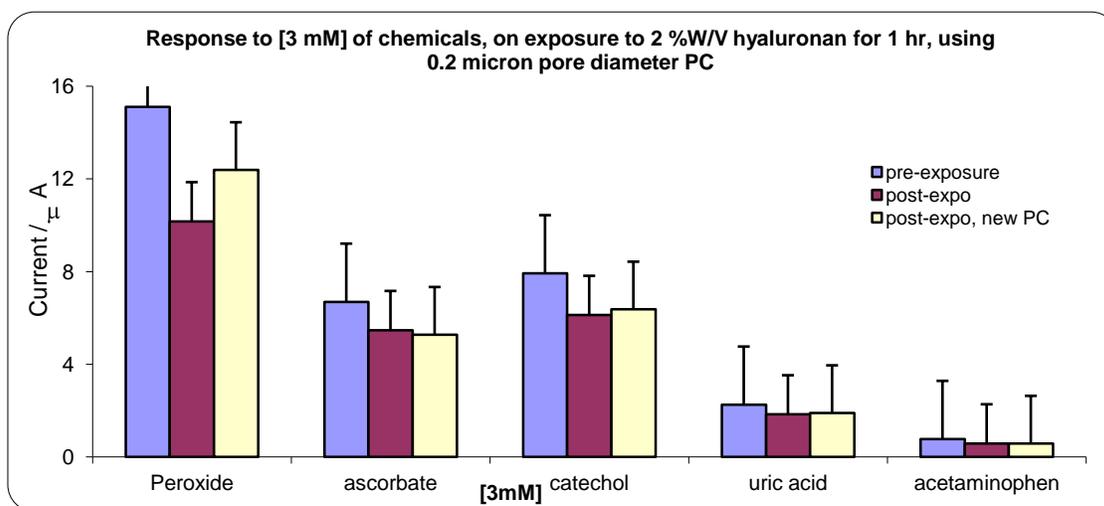


Fig. 6.14: Response to various electroactive chemicals, using 0.2 micron pore diameter polycarbonate membrane, exposure to 2 % W/V Hyaluronic acid for 1 hour.

Results show that the current response is reduced after one hour exposure to hyaluronic acid. This indicates that the exposure to hyaluronic acid results in membrane fouling. The response improves after the membrane is changed and a new 0.2 micron pore diameter polycarbonate membrane is applied.

The current response does not go back to the baseline after the application of new membrane, unlike the 0.05 micron pore diameter polycarbonate membrane. This indicates that the hyaluronic acid goes through the membrane to the surface of the electrode and causes significant electrode fouling, modifying the surface of the electrode,

resulting in loss of sensitivity. However, the response to hydrogen peroxide was not reduced significantly and after application of new membrane the total loss in sensitivity was 5 % of the original response. Loss in sensitivity with other electroactive chemicals was more profound. The current response did not improve significantly in case of acetaminophen, ascorbic acid, uric acid and catechol, even after the application of new membrane. This indicates that hyaluronic acid goes through the pores of the 0.2 micron pore diameter membrane, to the surface of the electrode and coats the surface of the electrode, which results in changes in the selectivity profile.

It is hypothesised that hyaluronic acid forms a film or a protective coating on the surface of the electrode. This film acts as a selective membrane. It selects hydrogen peroxide and prevents selectivity of interfering electroactive chemicals. To test this hypothesis a solution of low concentration of hyaluronan, 0.5 % w/v, was used to coat the surface of the electrode (Rank Cell) for just an hour and then a dialysis membrane was employed to prevent the removal of hyaluronic acid and to avoid effects of stirring during the experiments. Response to hydrogen peroxide was investigated. Results are shown below.

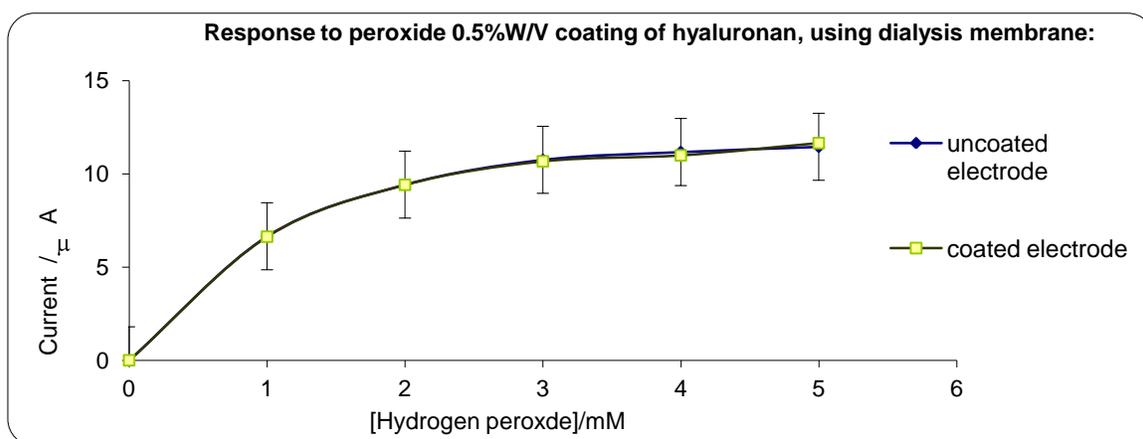


Fig. 6.15: The response to hydrogen peroxide after coating the electrode with 0.5 % w/v solution of hyaluronic acid, n = 3.

The response to hydrogen peroxide is identical in presence of 0.5% w/v coating of hyaluronic acid and uncoated electrode. This indicates that hyaluronic acid in low

concentration does not lead to electrode fouling but acts as a film that is selective for hydrogen peroxide.

### Control experiments

Control experiments were performed in the absence of protein. Results are shown below.

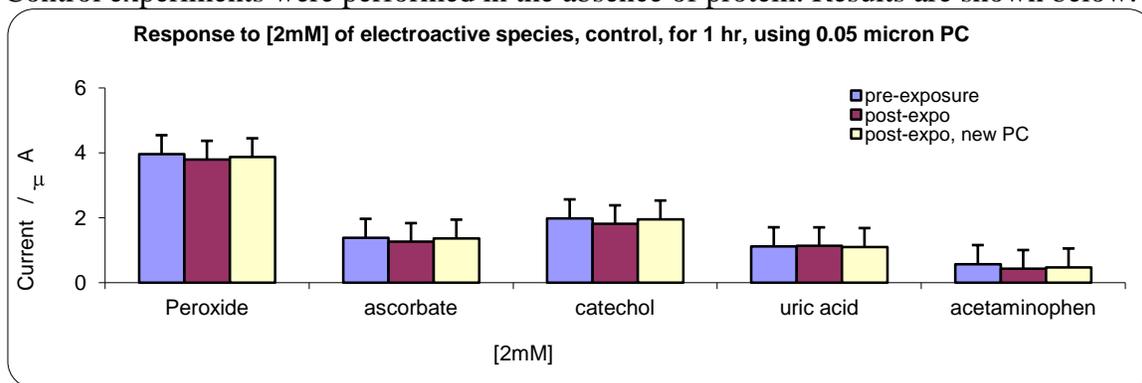


Fig. 6.16: Response to various electroactive chemicals, using 0.05 micron pore diameter polycarbonate membrane, control, in absence of protein exposure for 1 hour.

Results show that with 0.05 micron pore diameter polycarbonate membrane, after one hour exposure to phosphate buffer solution, the response to all the chemicals was slightly reduced, apart from uric acid. This may be due to the formation of an oxide layer on the surface of the electrode or a diffusion restricting barrier layer. When a new membrane was applied the response obtained with all electroactive chemicals was similar to the original response.

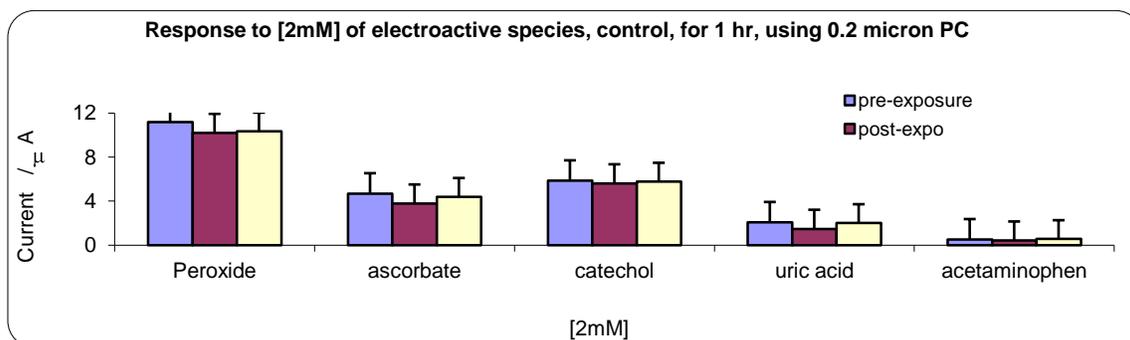


Fig. 6.17: Response to various electroactive chemicals, using 0.2 micron pore diameter polycarbonate membrane, control, exposure in absence of protein for 1 hour.

Fig. 6.17 shows that the sensor suffers loss in sensitivity and reduced response after one hour. The response did not improve for all electroactive chemicals when a new membrane was applied. This indicates that with large pore size polycarbonate membrane a more stable diffusion restricting layer or an oxide layer forms on the surface of the electrode, which is not dislodged on application of a new membrane.

## 6.2.2 Fouling studies with Albumin

Albumin is a globular protein that is present in the body. The 4 % w/v solutions of bovine albumin were used in fouling studies of polycarbonate membranes of 0.05, 0.2 and 5.0 micron pore diameter. Membranes were tested in absence of the proteins and then after exposure to protein with various electroactive chemicals to study the effect of fouling on the selectivity profile of membranes. In these experiments a new membrane was applied in order to investigate the electrode fouling and selectivity profile of the modified electrode after exposure to proteins.

### One hour exposure using 0.2 micron pore diameter polycarbonate membrane

Results shown are averages of three experiments and standard deviations are shown.

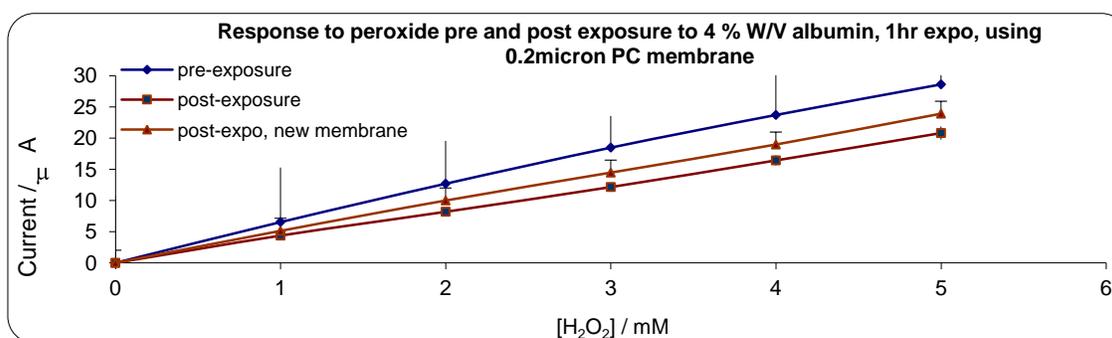


Fig. 6.18: Response to hydrogen peroxide, using 0.2 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

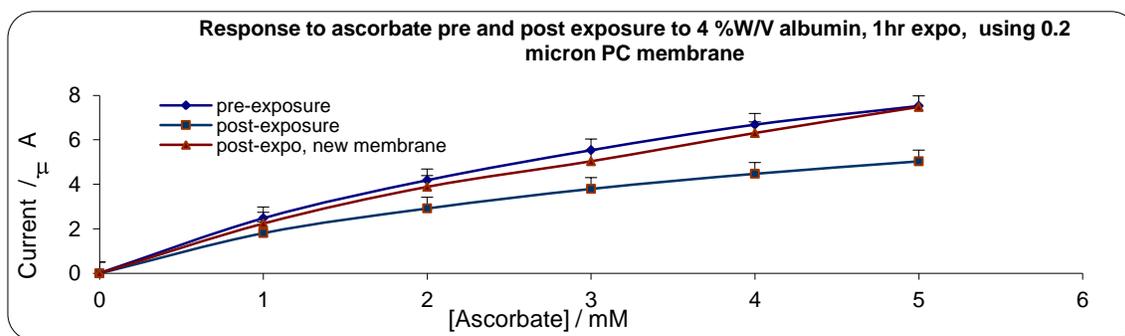


Fig. 6.19: Response to ascorbic acid, using 0.2 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

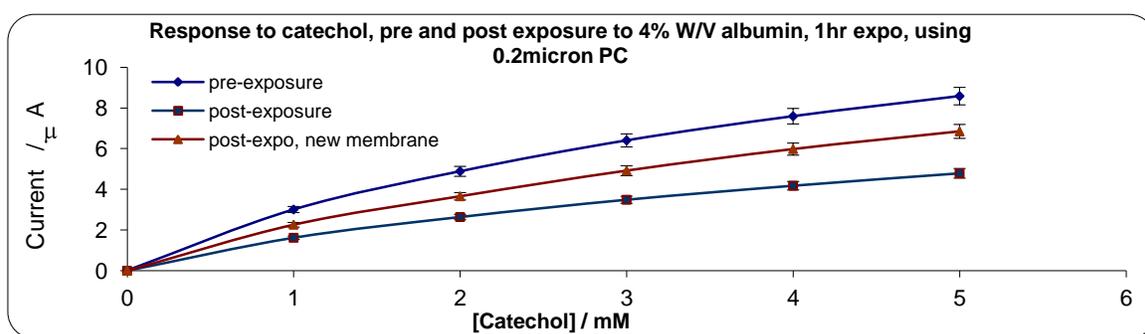


Fig. 6.20: Response to catechol, using 0.2 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

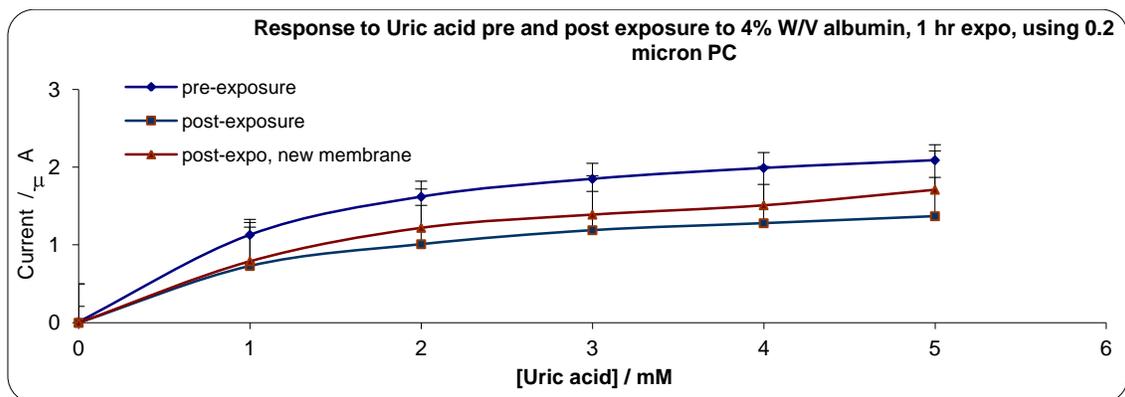


Fig. 6.21: Response to uric acid, using 0.2 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

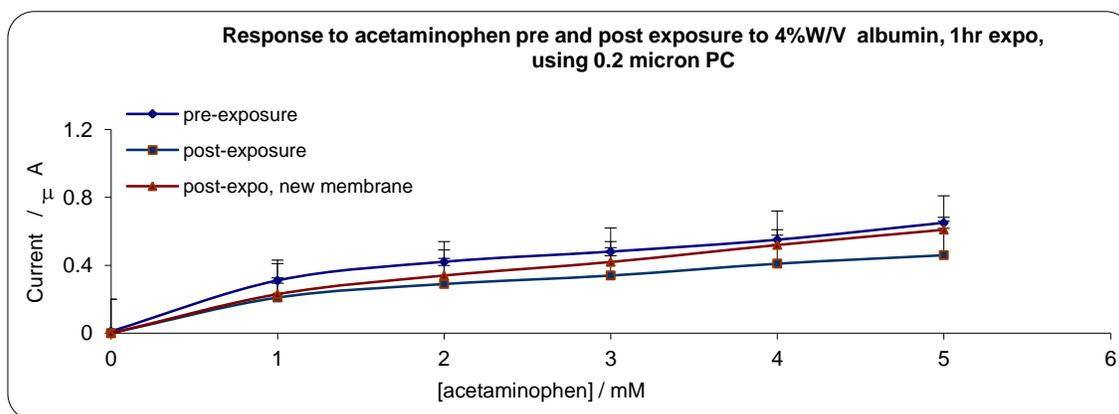


Fig. 6.22: Response to acetaminophen, using 0.2 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

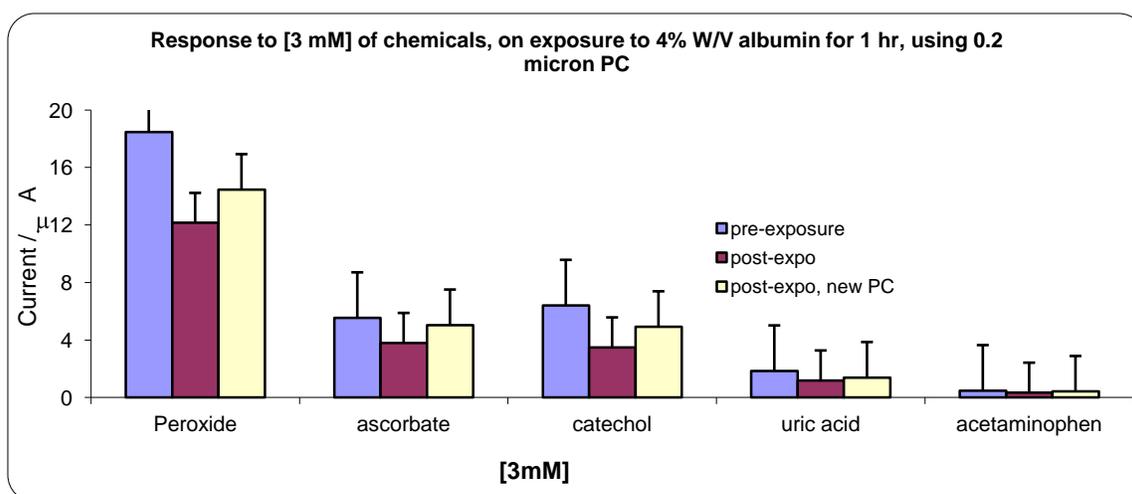


Fig. 6.23: Response to various electroactive chemicals, using 0.2 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

Results show that on exposure to 4 % w/v albumin solution, the sensitivity to acetaminophen was reduced. With uric acid, ascorbic acid and hydrogen peroxide the loss in sensitivity was 40 %, with catechol the loss in sensitivity was 50 %. With the application of a new 0.2 micron pore diameter membrane, the response to all electroactive chemicals improved by 20 %. The response did not go back to the original baseline and on application of a new membrane 100 % response was not obtained. This indicates that membrane fouling as well as electrode fouling takes place when 0.2 micron pore diameter polycarbonate membrane is exposed to 4 % w/v solution of albumin.

The protein may diffuse or migrate to the surface of the electrode through the pores of the membrane. The protein may be adsorbed on the surface of the electrode, leading to the fouling of the electrode. This may cause reduced permeation of the analyte to the surface of the electrode resulting in a loss of sensitivity. The results show that response to all electroactive chemical is reduced after exposure to the albumin solution and application of a new membrane does not result in significant improvement in the response. This indicates that the protein modifies the surface of the electrode.

Polycarbonate membranes have vertical cylindrical pores and the pore radii are more than an order of magnitude larger than the diameters of the bovine serum albumin. According to Tracy et. al. (1994) significant fouling observed under convective conditions is due to the formation of aggregates of the protein occurring in solution or at the mouths of the pores. This may be the reason for the loss in sensitivity after exposure to proteins.

The protein aggregation leads to a multi layer protein deposit on the membrane as well as the surface of the electrode, causing electrode fouling, so the response does not improve significantly after the application of a new 0.2 micron pore diameter polycarbonate membrane.

The experiment was repeated with 0.05 micron pore diameter polycarbonate membrane to investigate the effect of pore size on the extent of membrane and electrode fouling.

### **One hour exposure using 0.05 micron pore diameter polycarbonate membrane**

Results shown are averages of three experiments and standard deviations are shown.

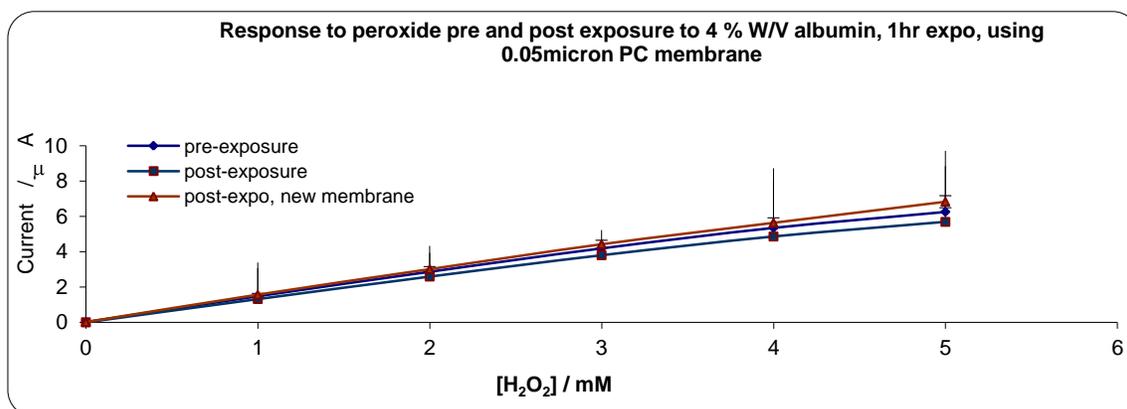


Fig. 6.24: Response to Hydrogen peroxide, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

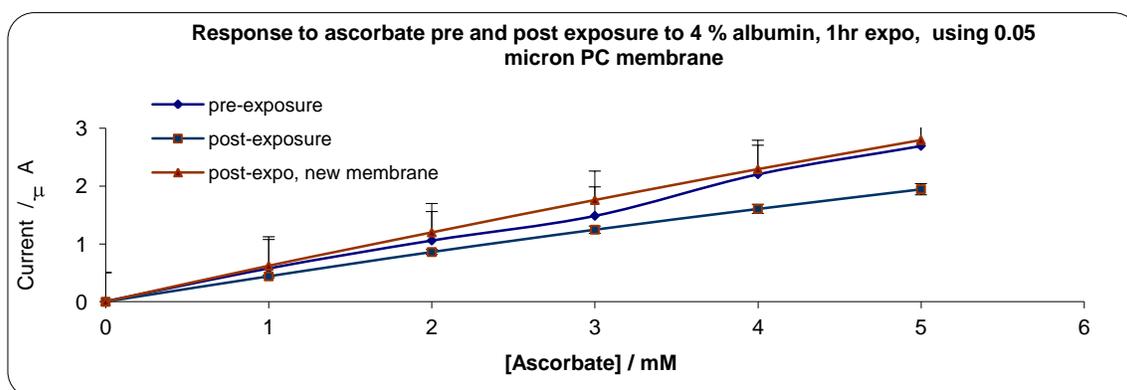


Fig. 6.25: Response to Ascorbic acid, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

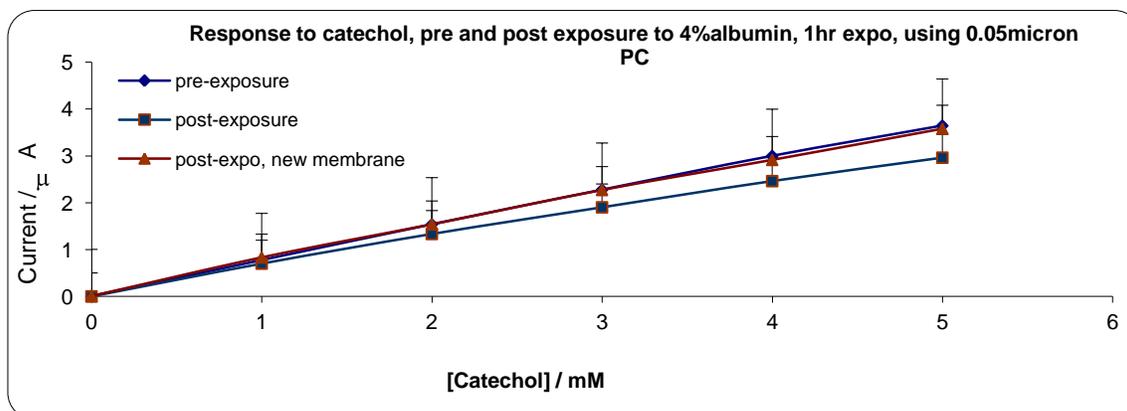


Fig. 6.26: Response to Catechol, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

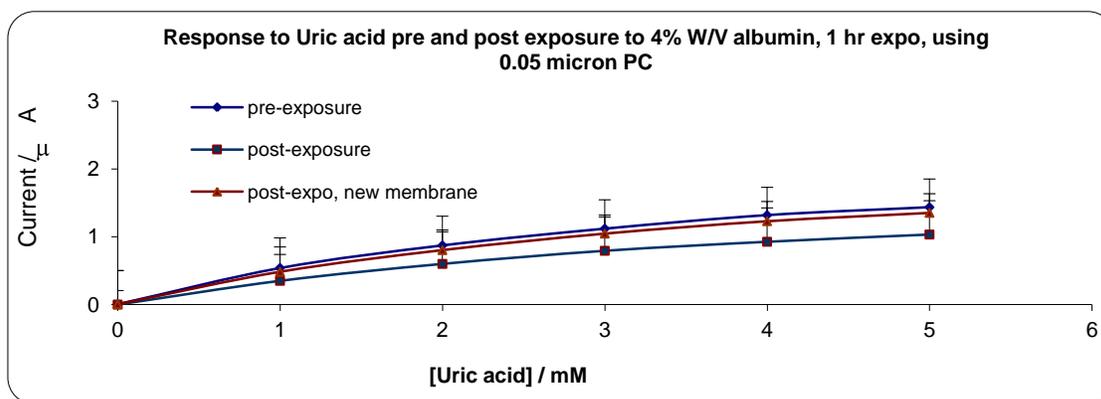


Fig. 6.27: Response to Uric acid, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

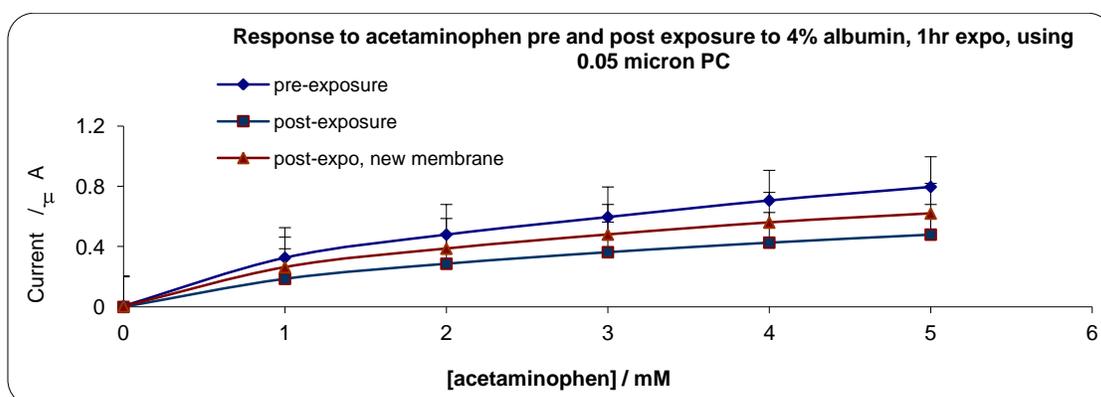


Fig. 6.28: Response to acetaminophen, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

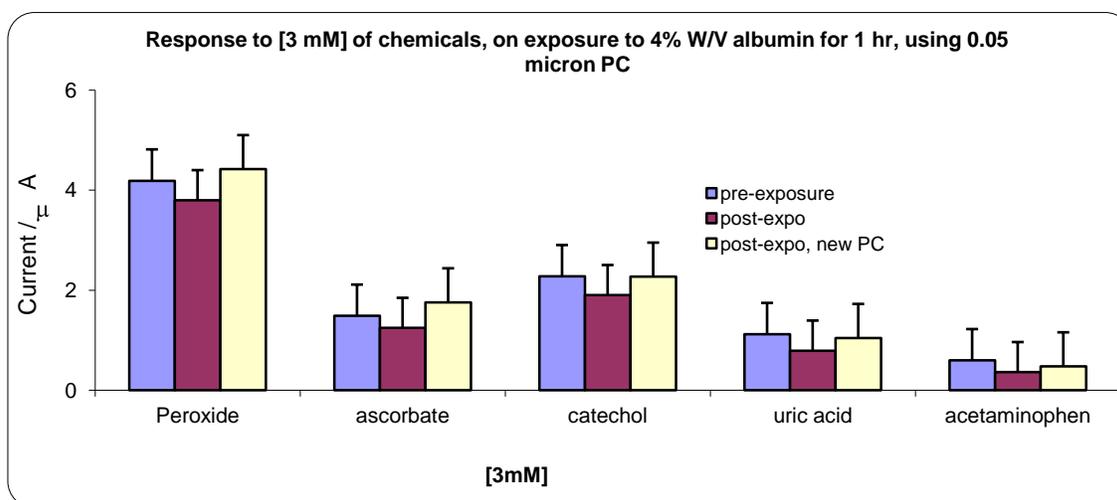


Fig. 6.29: Response to various electroactive chemicals, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 1 hour.

Response to all electroactive chemicals was reduced on exposure to 4 % w/v albumin. The loss in sensitivity was 15 % to the original values with all electroactive chemicals. When a new membrane was applied the response improved, this indicates that the protein does not diffuse through the pores of the membrane. The response was in fact similar to the original response in case of catechol and uric acid. The response to acetaminophen improved but did not go back to original. However, the response to ascorbic acid and hydrogen peroxide was observed to be more than 100%. This result suggests that the protein does diffuse through the pores of the membrane and modifies the surface of the electrode, forming a film on the surface of the electrode that is selective of hydrogen peroxide and ascorbic acid.

These results indicate that with 0.05 micron pore diameter polycarbonate membranes, the protein is adsorbed on the surface of the membrane, it may block the pores of the membrane and cause fouling of the membrane. The protein may form aggregates on the surface of the membrane and on the mouths of pores in the membrane, preventing diffusion of protein to the surface of the electrode. If the protein does diffuse through the pores of the membrane to the surface of the electrode, then sufficient protein is not deposited on the surface of the electrode to cause electrode fouling.

The amount of protein that may diffuse through the pores of the membrane may form a protein film on the surface of the electrode, modifying the surface of the electrode. This protein layer might be the reason for the higher than original response of hydrogen peroxide and ascorbic acid and a reduced response to acetaminophen. This hypothesis could be tested by coating the bare electrode with a dilute solution of albumin and tests carried out with hydrogen peroxide and ascorbic acid to investigate if albumin in low concentration facilitates the diffusion of these electroactive chemicals.

The experiment was repeated with 0.05 micron pore diameter polycarbonate membrane and the exposure time was increased to 24 hours, to investigate if longer exposure time has any effect on the electrode fouling and the selectivity profile.

### Twenty four hour exposure to 4 %w/v albumin using 0.05 micron pore diameter PC membrane

Results shown are averages of three experiments and standard deviation are shown.

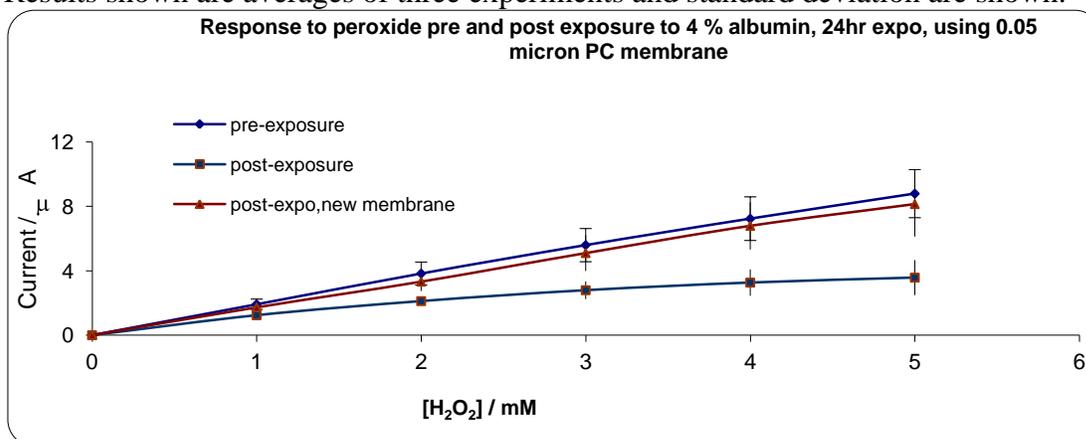


Fig. 6.30: Response to hydrogen peroxide, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

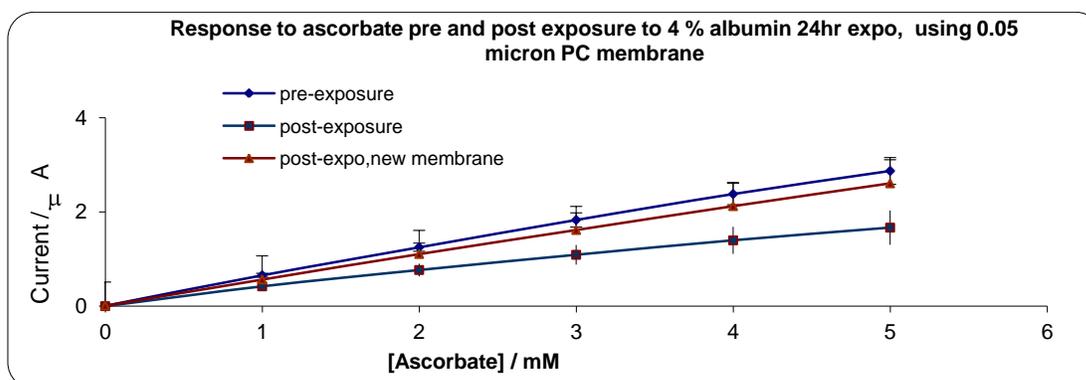


Fig. 6.31: Response to ascorbic acid, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

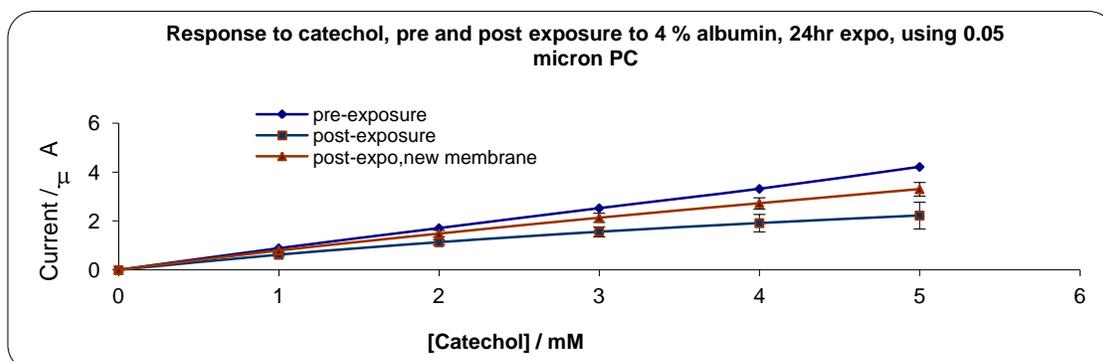


Fig. 6.32: Response to catechol, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

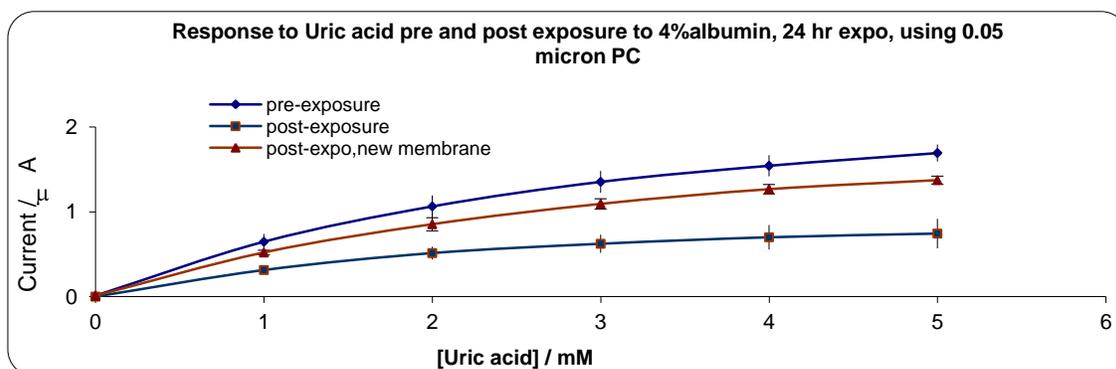


Fig. 6.33: Response to uric acid, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

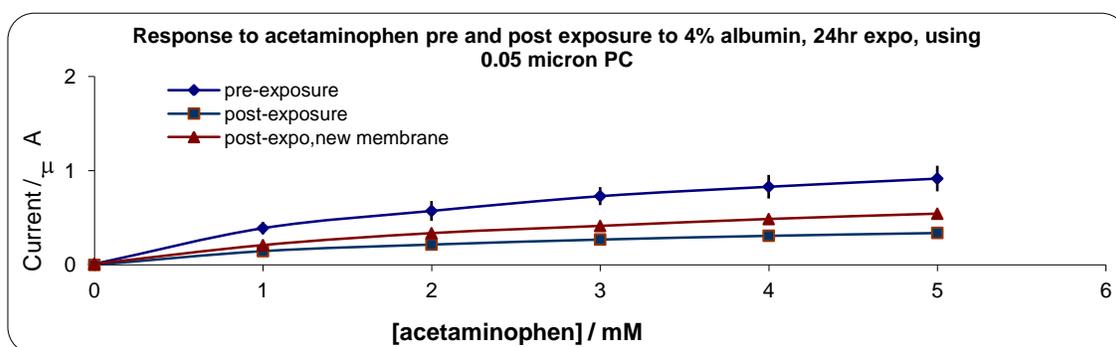


Fig. 6.34: Response to acetaminophen, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

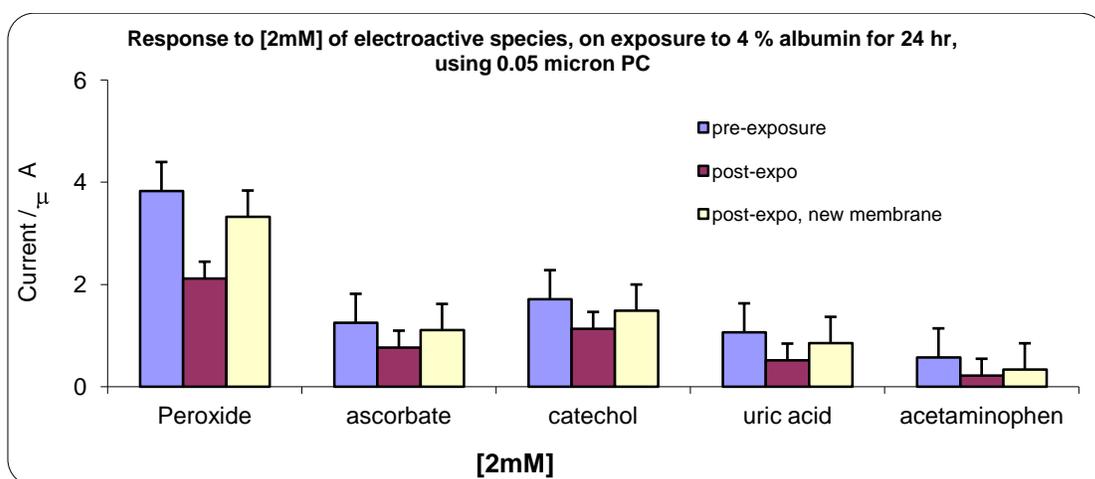


Fig. 6.35: Response to various electroactive chemicals, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

Results show that on exposure to 4 % w/v albumin for 24 hours, using 0.05 micron pore diameter polycarbonate membrane a reduced response was obtained. The loss in

sensitivity was observed to be 50 % to the original response for all electroactive chemicals. When a new membrane was applied the response improved to 90 % but did not go back to the original 100 %. This indicates that longer exposure to protein results in electrode fouling as well as membrane fouling. The protein diffuses through the pores of the membrane and modifies the surface of the electrode, as the response did not go back to the original baseline response after application of new polycarbonate membrane. However, the protein film that modifies the surface of the electrode does not confer selectivity to the electrode for any particular electroactive chemical as response to all electroactive chemicals was reduced. Membrane fouling appeared to be greater than the electrode fouling. The loss in sensitivity maybe due to the formation of protein aggregates which, block the pores of the membrane and restrict the permeation of the analyte to the surface of the electrode.

The experiment was repeated with a very large pore size (5.0 micron pore diameter) polycarbonate membrane to investigate the extent of membrane and electrode fouling after exposure to 4 % w/v albumin for twenty four hours.

### Twenty four hour exposure to 4% w/v Albumin, using 5.0 micron pore diameter polycarbonate membrane

The results are averages of three experiments and standard deviations are shown.

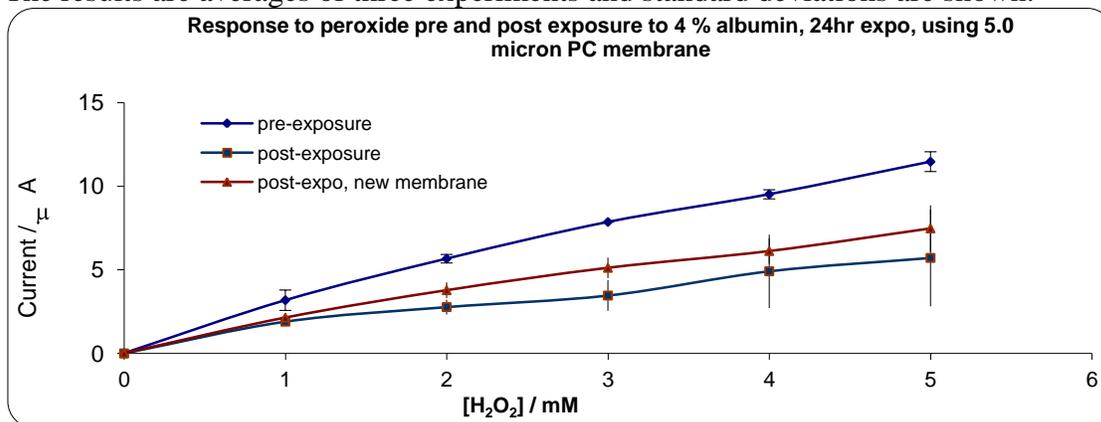


Fig. 6.36: Response to hydrogen peroxide, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

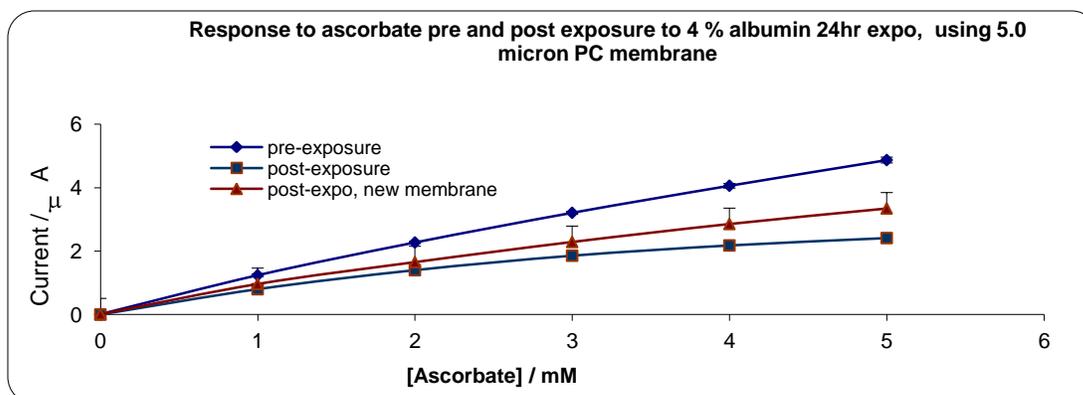


Fig. 6.37: Response to ascorbic acid, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

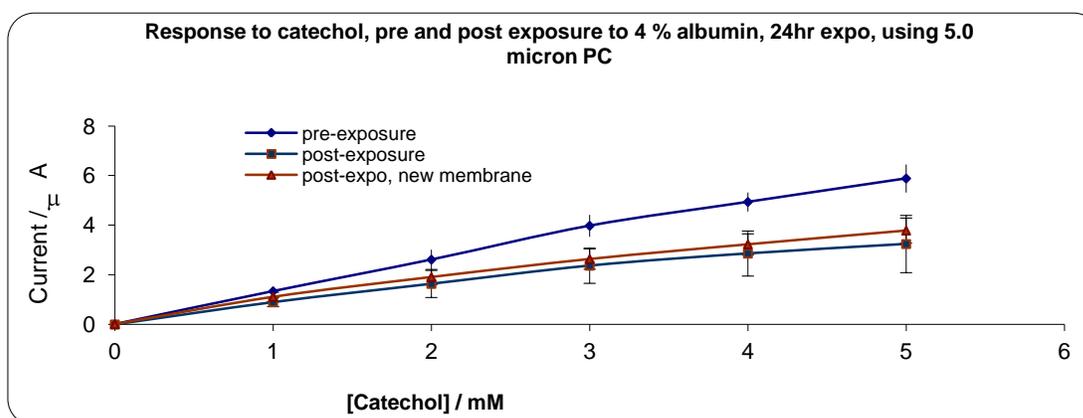


Fig. 6.38: Response to catechol, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

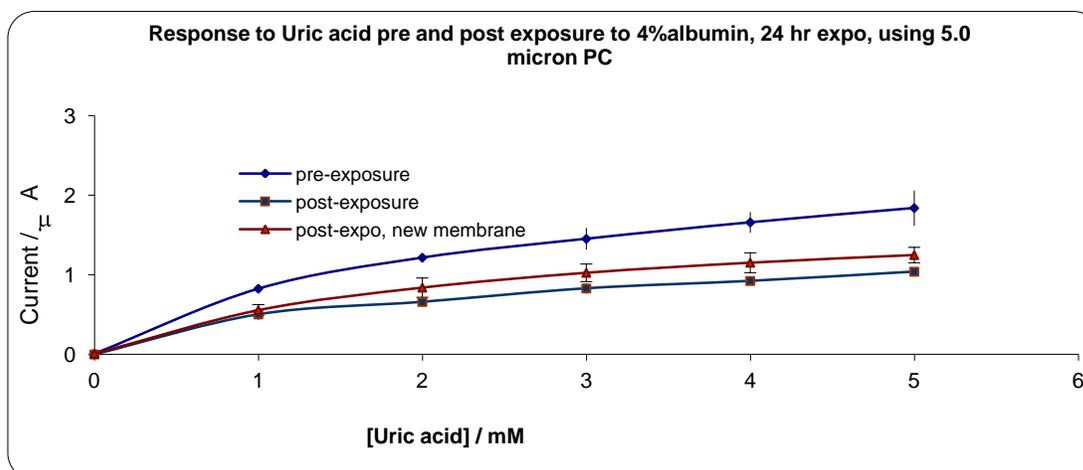


Fig. 6.39: Response to uric acid, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

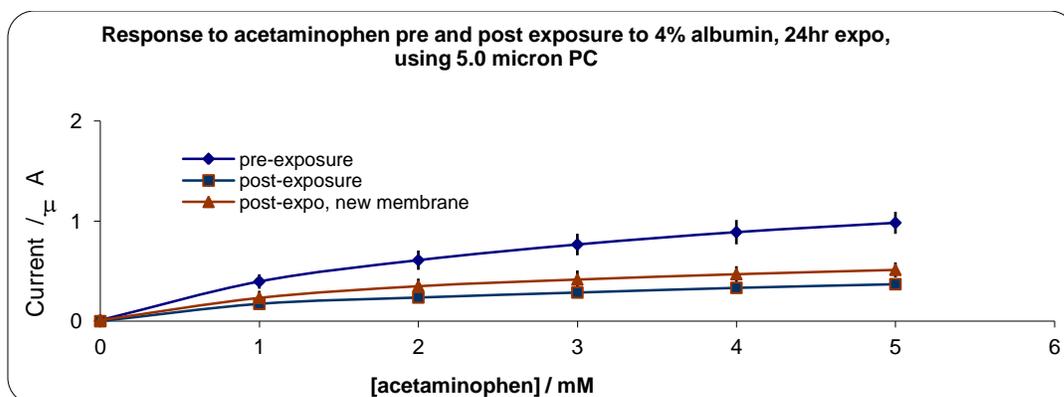


Fig. 6.40: Response to acetaminophen, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

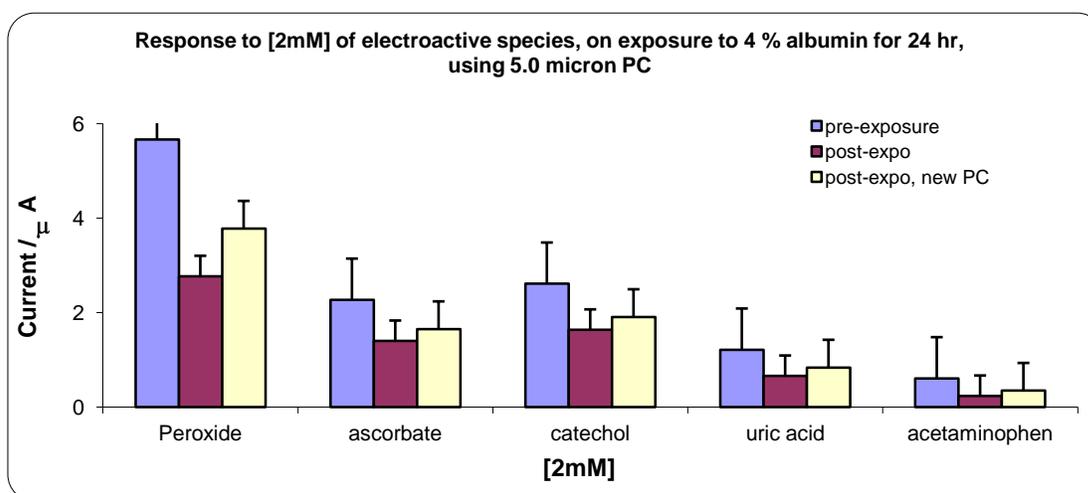


Fig. 6.41: Response to various electroactive chemicals, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v Albumin for 24 hour.

Exposure to 4 % w/v albumin solution, using a 5.0 micron pore diameter polycarbonate membrane resulted in loss in sensitivity. The response reduced to 60% of the original response for acetaminophen, 40 % for uric acid, 30 % for catechol, 35 % for ascorbic acid and 50 % for hydrogen peroxide. When a new 5.0 micron pore diameter polycarbonate membrane was applied the response improved to 70 % for all the electroactive chemicals species.

These results indicate that the albumin diffuses through the pores of the membrane to the surface of the electrode and causes electrode fouling and modifies the surface of the electrode. This modified surface of the electrode causes a reduction in the response to all electroactive chemicals but does not confer selectivity as response to all electroactive chemicals is reduced.

Membrane fouling may be less due to very large pores of the membrane. Polycarbonate membranes have vertical cylindrical pores, it is possible that the protein may cause internal fouling of the pores. This may be the reason for the loss in sensitivity and a reduced response to electroactive chemicals after exposure to protein and slight improvement in response after the application of a new membrane as the modified protein membrane would acquire charge of the protein and the negatively charged electroactive chemicals would not be selected on the basis of charge selectivity.

The results show that microfiltration polycarbonate membranes of various pore radii i.e. (0.05, 0.2 and 5.0 micron pre diameter) suffer from protein adsorption and membrane fouling on exposure to Albumin solutions. With small pore size polycarbonate membranes (0.05 micron pore diameter pc), membrane fouling is higher than electrode fouling. The protein forms aggregates and blocks the pores of the membrane. As the pore size and the exposure time are increased the extent of electrode fouling is also increased. The protein deposit forms a film on surface of the electrode, modifying the surface of the electrode, which causes a loss in sensitivity of the electrode.

The experiment was repeated with fibrinogen to study the fouling behaviour of a fibrous protein and the selectivity of thus fouled and modified polycarbonate membrane.

### **6.2.3 Fouling studies with Fibrinogen**

Fibrinogen is a fibrous protein, present in the body. In these experiments, the membrane was exposed to the protein (4 % w/v fibrinogen) for 24 hours and then the electrode was polished in order to investigate the extent of membrane fouling.

**Twenty four hour exposure to 4 % w/v fibrinogen using 0.05 micron pore diameter polycarbonate membrane:**

Results shown are averages of three experiments and standard deviations are shown.

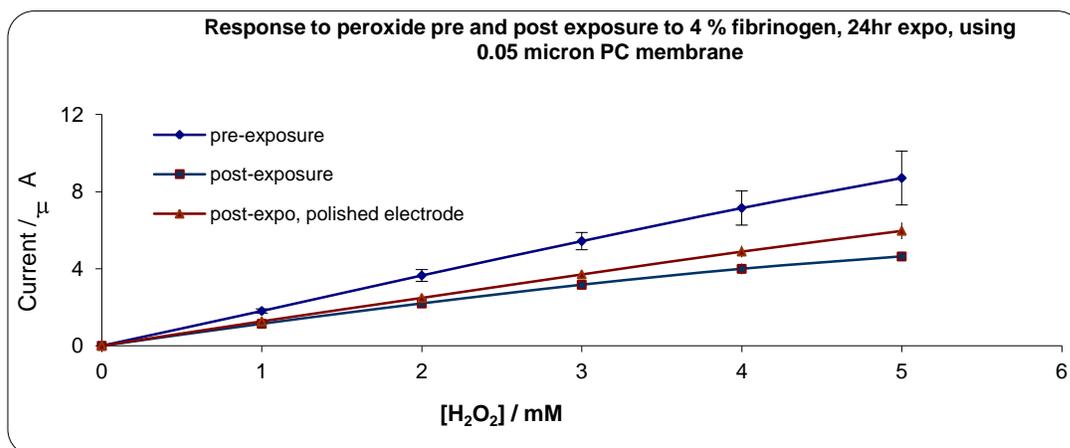


Fig. 6.42: Response to hydrogen peroxide, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour.

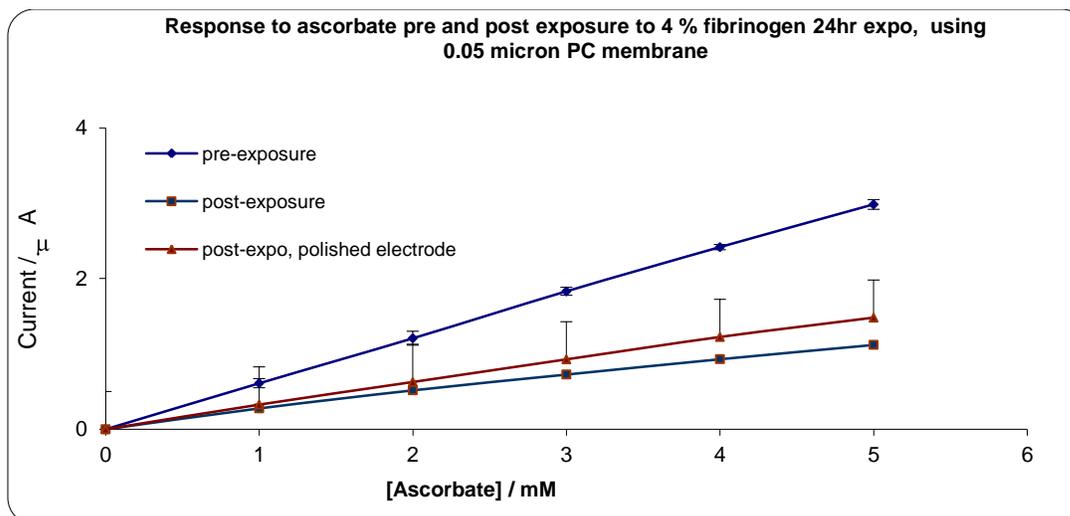


Fig. 6.43: Response to ascorbic acid, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour.

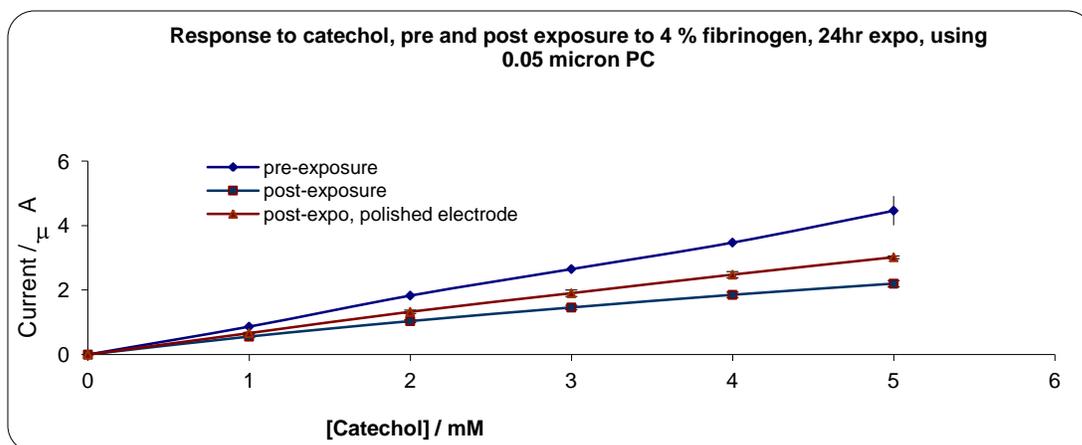


Fig.

6.44: Response to catechol, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour.

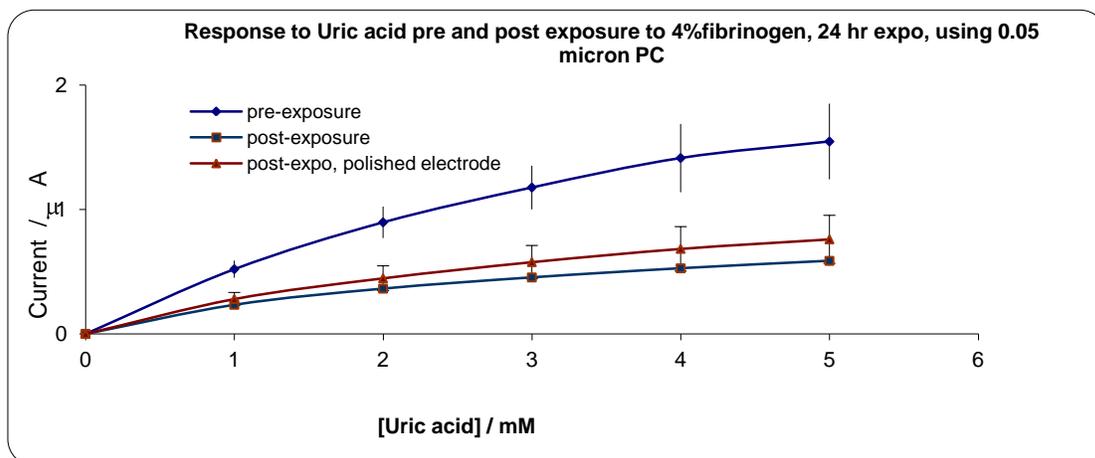


Fig. 6.45: Response to uric acid, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour.

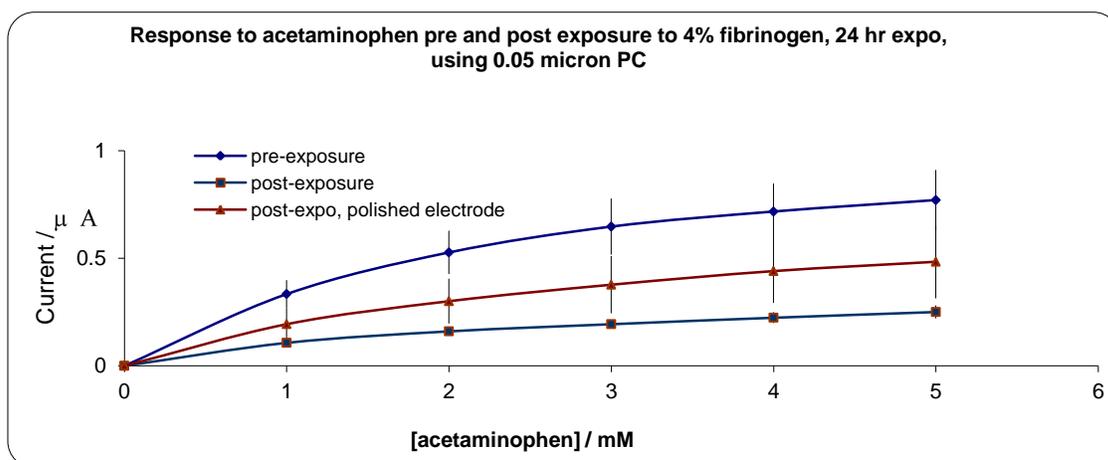


Fig. 6.46: Response to acetaminophen, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour.

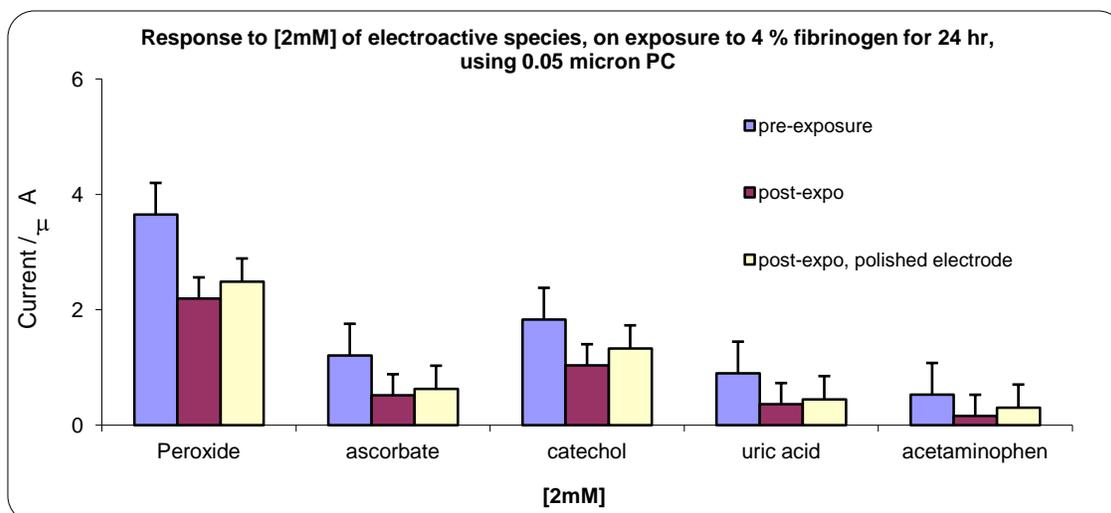


Fig. 6.47: Response to various electroactive chemicals, using 0.05 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour, n = 3.

Response to all electroactive chemicals was observed to be reduced when 0.05 micron pore diameter polycarbonate membrane was exposed to 4 % w/v solution of fibrinogen for twenty four hours. The loss in sensitivity to hydrogen peroxide was 40 %, for ascorbic acid 60%, for catechol 40%, for acetaminophen 70% and for uric acid 50% reduced response was observed. When the electrode was cleaned, the response to all electroactive chemicals improved. The improved response for hydrogen peroxide was 70%, for ascorbate 50 %, for uric acid 60%, for catechol and acetaminophen was 60% of the original response. The response for all electroactive chemicals was reduced significantly and new membrane application improved the response by 10-20% for all electroactive chemicals. The reduction in response indicates that the protein diffuses through the pores of the membrane and causes electrode fouling to some extent that results in loss of sensitivity.

Membrane fouling was more than the electrode fouling. This may be due to protein adsorption on the surface of the membrane. Fibrinogen is a fibrous protein. It may form a network of fibrous structures on the surface of the membrane and across the pores of the membrane blocking the pores of the membrane, which in turn restricts the diffusion of the analyte to the surface of the electrode and results in loss in sensitivity and reduced response to electroactive chemicals. However, this protein does not confer selectivity to

the electrode as the protein modified electrode gives a reduced response to all electroactive species.

The experiment was repeated with large pore size (5.0 micron pre diameter) polycarbonate membrane, to investigate the extent of membrane and electrode fouling on exposure to fibrinogen for twenty four hours.

### Twenty four hour exposure to 4 % W/V fibrinogen, using 5.0 micron pore diameter polycarbonate membrane

Results shown are averages of three experiments and standard deviations are shown.

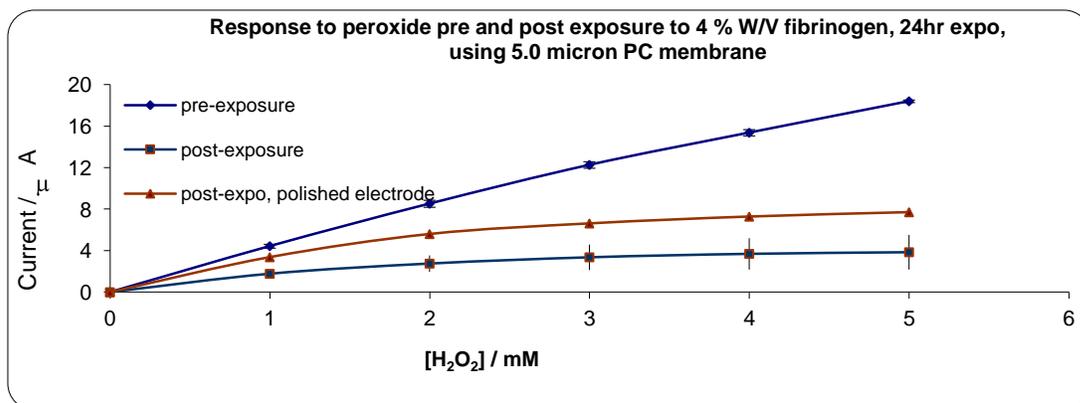


Fig. 6.48: Response to hydrogen peroxide, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour.

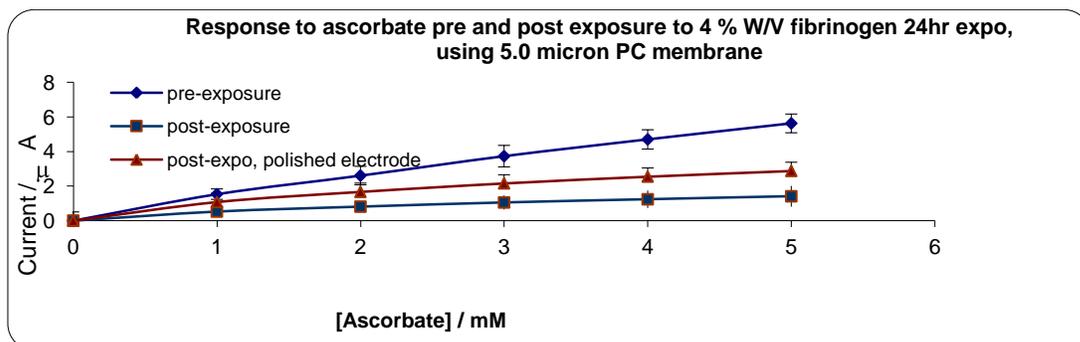


Fig. 6.49: Response to ascorbic acid, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour.

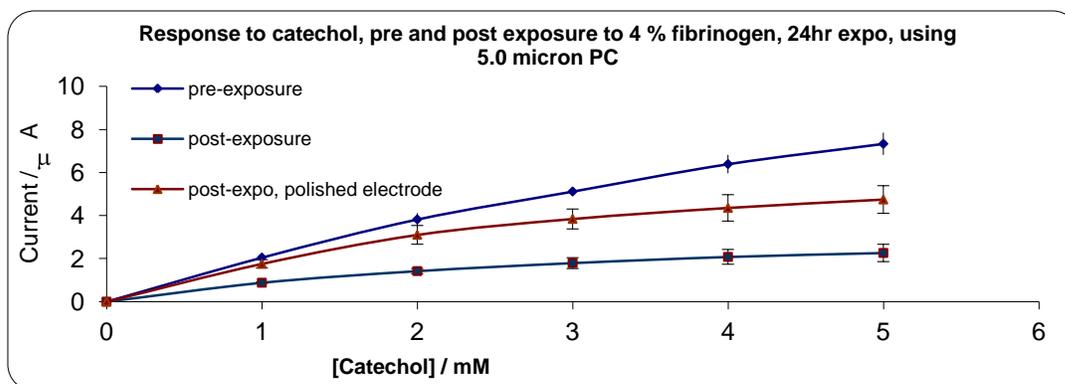


Fig. 6.50: Response to catechol, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour.

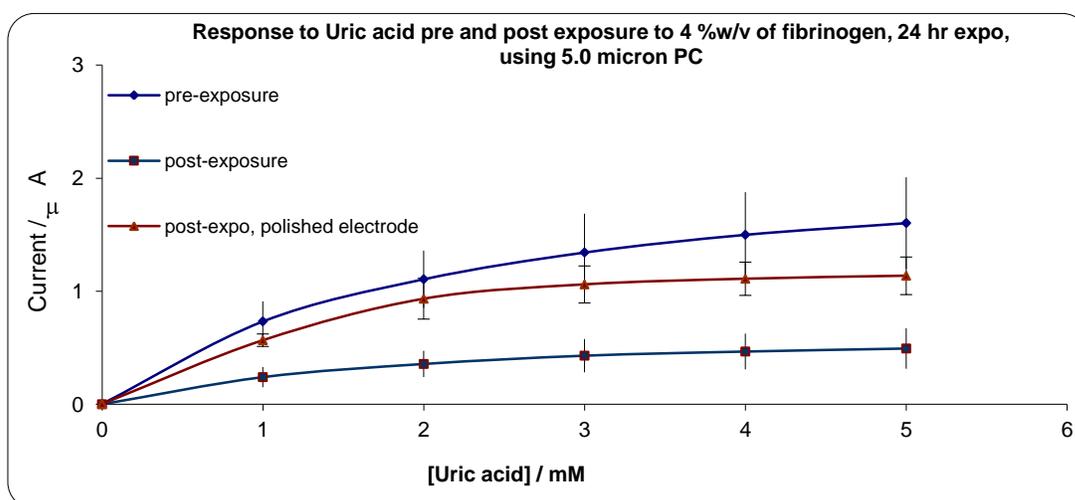


Fig. 6.51: Response to uric acid, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour.

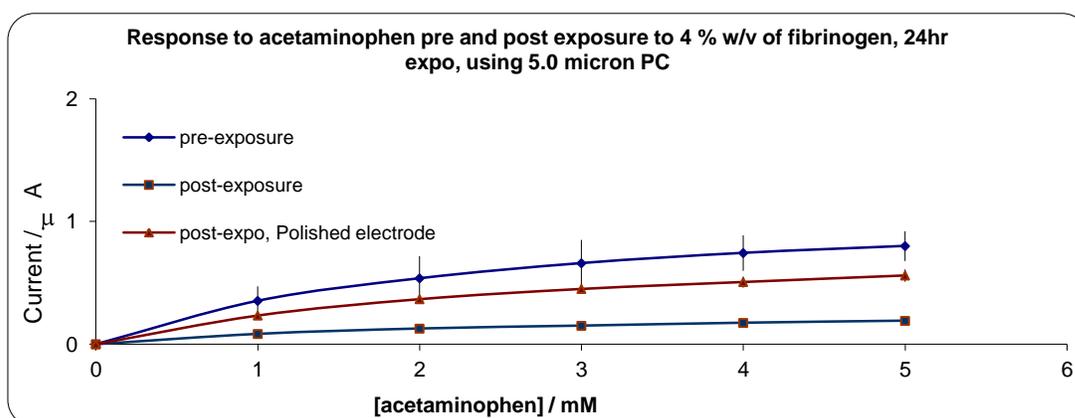


Fig. 6.52: Response to acetaminophen, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour.

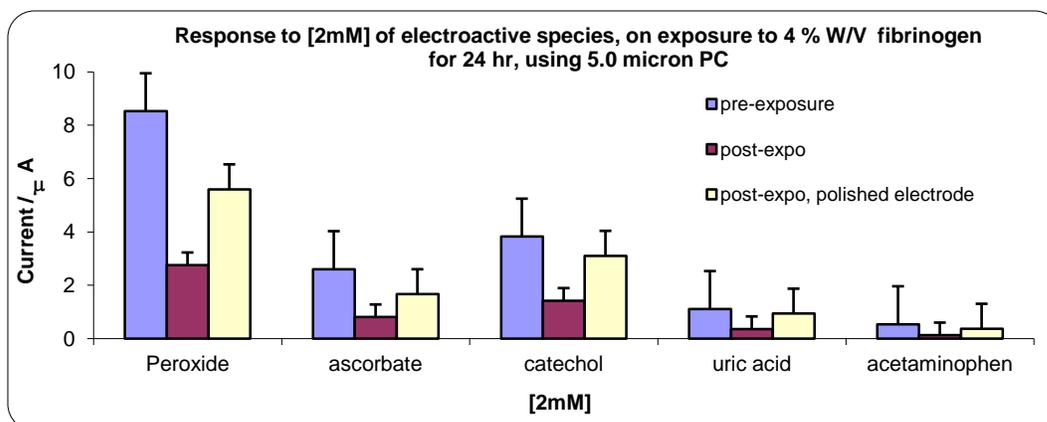


Fig. 6.53: Response to various electroactive chemicals, using 5.0 micron pore diameter polycarbonate membrane, exposure to 4 % w/v fibrinogen for 24 hour.

Results show that exposure to 4 % w/v fibrinogen for twenty four hours, using 5.0 micron pore diameter polycarbonate membrane results in loss in sensitivity. The response was reduced to 70 % for hydrogen peroxide, 60 % for catechol and 80 % for ascorbic acid and uric acid and for acetaminophen reduced to 75 %. When the electrode was cleaned, the response to all electroactive chemicals improved. The response to hydrogen peroxide went back to 75 % to the original response, 50 % for ascorbic acid and 80 % for catechol, uric acid and acetaminophen. This indicates that with large pore size polycarbonate membrane the exposure to fibrinogen results in more electrode fouling than membrane fouling as compared to small pore size polycarbonate membrane.

The protein may diffuse through the large pores of the polycarbonate membrane to the surface of the electrode. Adsorption of the fibrinogen molecules on the surface of the electrode may provide sites for further protein deposition, resulting in a layer of protein on the surface of the electrode that modifies the surface of the electrode. At the same time the protein may be adsorbed on the surface of the membrane and also on the pores of the membrane, causing external and internal fouling respectively of the membrane. The electrode and the membrane fouling together result in reduced response and loss in sensitivity to all electroactive species.

The results indicate that large pore size polycarbonate membranes allow greater protein permeation to the surface of the electrode than the small pore size polycarbonate

membrane. The membrane fouling is greater with small pore size polycarbonate membranes than with larger pore size polycarbonate membranes. The protein maybe adsorbed on, around and in the pores of the small pore size polycarbonate membranes, block the pores of the membranes, more easily than the large pore size polycarbonate membrane. Longer exposure to protein may results in total loss in sensitivity with time. The concentration of the protein can have an effect on the extent of the membrane and electrode fouling.

The effect of concentration on the electrode and membrane fouling was also investigated.

### Effect of concentration on the fouling of 0.05 micron pore diameter polycarbonate membrane

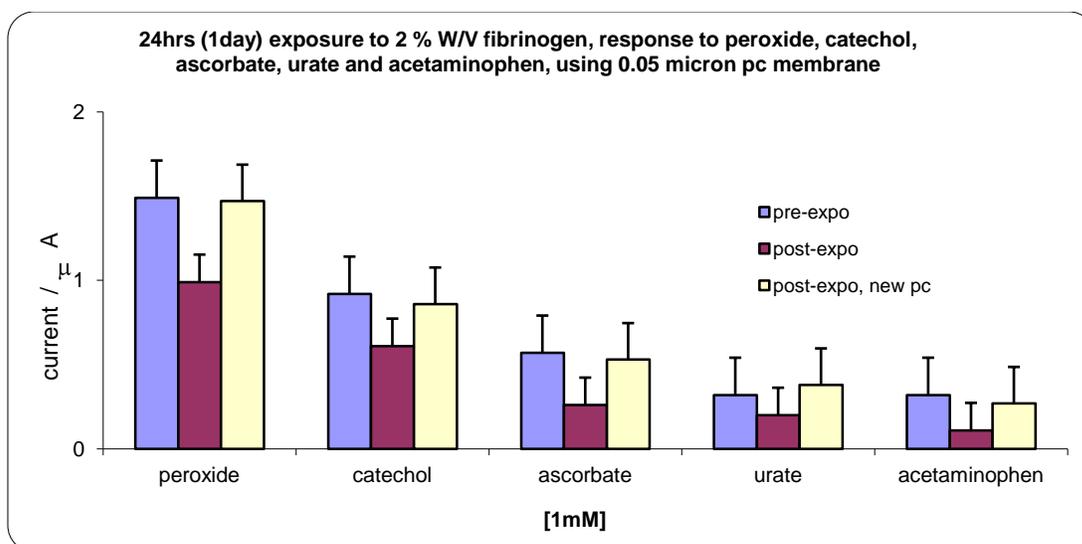


Fig. 6.54: Response to various electroactive chemicals, on exposure to 2 % w/v fibrinogen, using 0.05 micron polycarbonate membrane, n =3.

Results show that with 2 % w/v concentration fibrinogen, 24 hour exposure, fouling occurs mostly on the membrane. This result is deduced from the fact that after a new membrane is applied; the response obtained with all the electroactive chemicals is similar to the response obtained pre-exposure to the protein. Fibrinogen in low concentration

may only be adsorbed on the surface of the membrane. Blocking pores of the membrane and may not cause the fouling of the electrode.

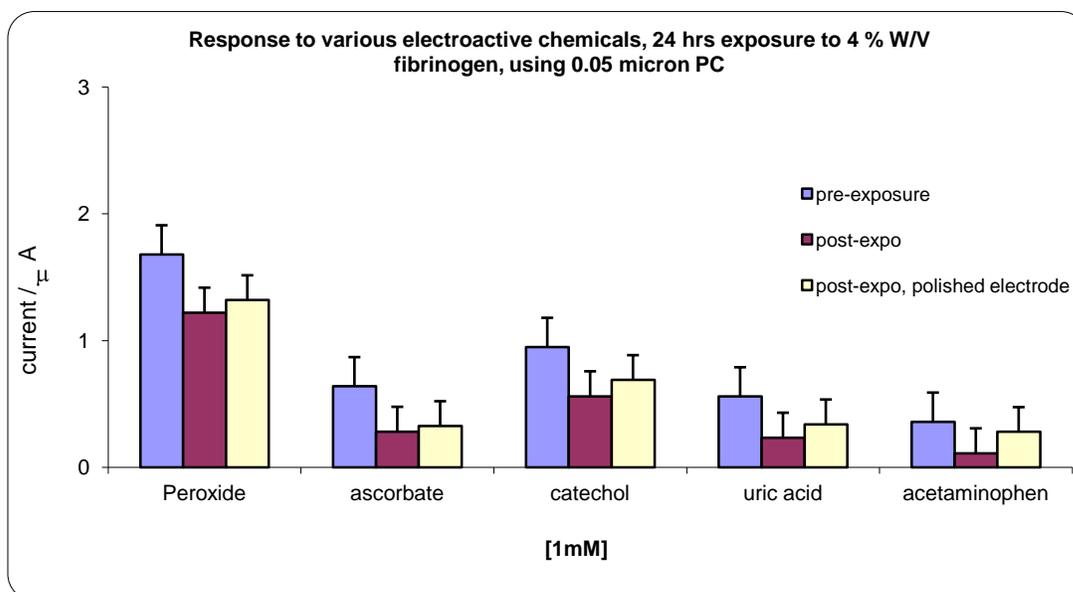


Fig. 6.55: Response to various electroactive chemicals, on exposure to 4 % w/v fibrinogen, using 0.05 micron polycarbonate membrane, n = 3.

The response obtained with a 0.05 micron pore diameter polycarbonate on exposure to the 4 % w/v fibrinogen solution, shows that the protein causes fouling of the electrode as well as the fouling of the membrane. On exposure to protein, a decreased response was obtained with all electroactive chemicals. In this experiment the fouled membrane was reapplied after polishing the electrode in order to investigate the extent of fouling of the membrane and the electrode.

Results indicate an improved response after polishing of the electrode. However, the response did not go back to the original baseline. This indicates that even though a higher concentration of fibrinogen causes electrode fouling, mostly the protein is adsorbed onto the surface of the membrane. It may be so that at the start of the experiment, the protein goes through the pores of the membrane and causes electrode fouling. It is hypothesised that most of the protein is adsorbed on the membrane, blocks the pores of the membrane and may cause cake like layer formation on the surface of the membrane. To investigate

the protein adsorption on the membrane samples of the modified membranes were studied using the field emission scanning electron microscope.

### **6.3 Membrane characterisation using Scanning electron microscopy**

The scanning electron microscope (SEM) plays a significant role in studies involving surface characterisation of materials, plant and animal tissues, cells, surface and ultra structure of implants, biomaterials and synthetic materials. It is a powerful tool in the investigation of material properties, imaging of the surface of materials and investigation of the topography of specimens. Kim et. al. (1990) studied the top layer of various ultra filtration membranes using high resolution field emission scanning electron microscope at low accelerating voltages and analysed micrographs quantitatively. Analysis and processing of SEM images using image analysing software allows quantification of the morphological parameters such as pore size distribution, pore density and average pore size quickly and effectively (Choudhari et. al. 2013).

There are many advantages of using this technique e.g. it allows higher magnification and depth of the specimen to be investigated as compared to the optical microscope. Moreover, large areas of a specimen can be investigated over considerably less time as compared to the atomic force microscope.

The scanning electron microscope was used in this study to characterise the surface of commercially available precast polymer membranes before and after exposure to macromolecules and proteins. Membranes used in these investigations were poly(carbonate) coated with poly(vinylpyrrolidone) (PVP) and also PVP free poly(carbonate) (PC) membranes.

#### **6.3.1 PVP coated Poly(carbonate) membranes**

Poly(carbonate) membranes are widely used for general filtration processes. The majority of commercially available precast poly(carbonate) membranes are coated with

A study of biointerfacing polymeric barrier membranes for sensors

poly(vinylidene pyrrolidone) as it confers good wetting properties to the membranes. These polymeric membranes are available in various pore sizes. Three different pore diameter membranes were used in this study i.e. 0.05, 0.2 and 5.0 micron pore diameter polycarbonate membranes.

The scanning electron micrographs were taken at high as well as low magnifications.

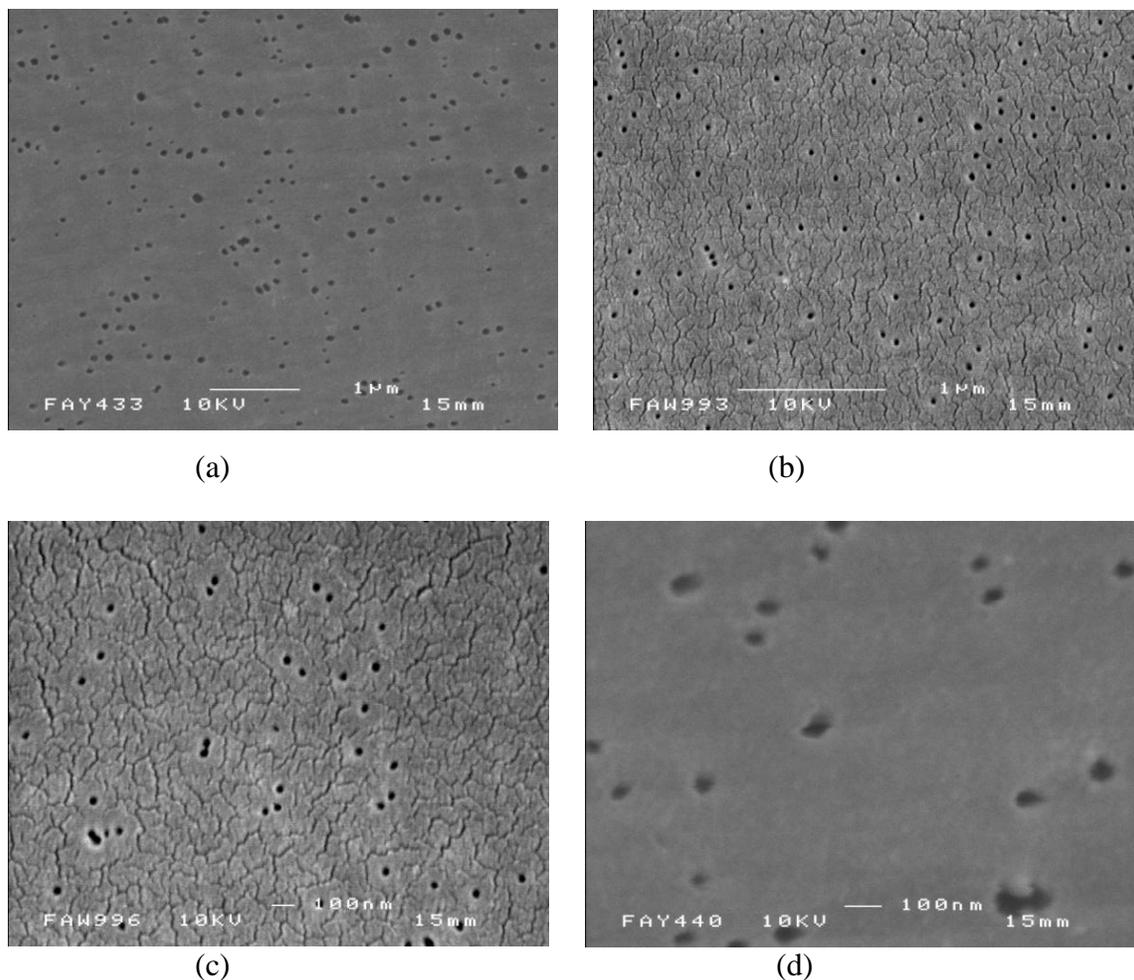


Fig.6.56: Surface of a 0.05micron pore diameter pvp-poly(carbonate) membrane. (Magnification: (a) = x 20,000 (b) = x 33, 000, (c) = x 50,000 (d) = x 80,000)

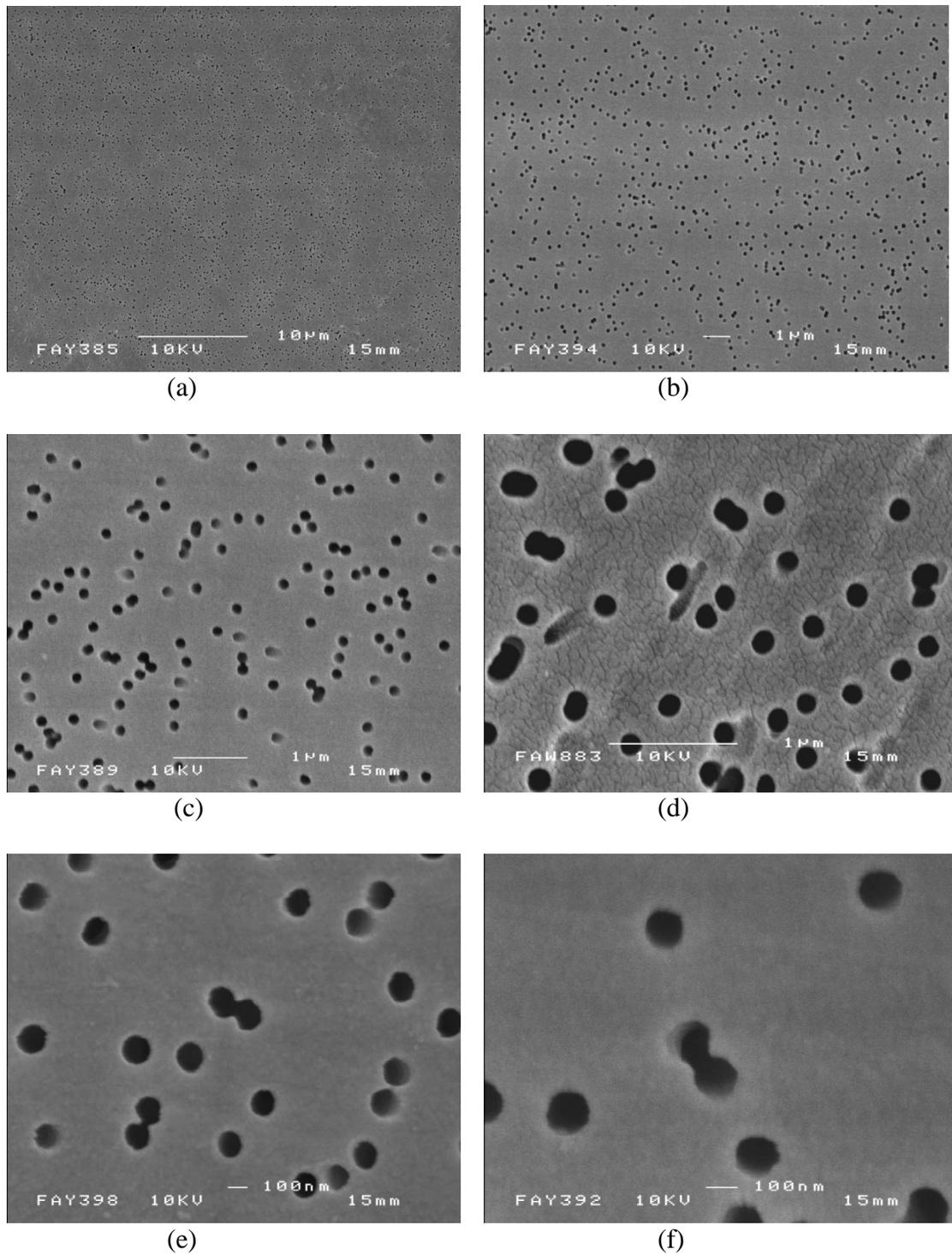


Fig.6.57: Surface of a 0.2 micron pore diameter pvp-poly(carbonate) membrane.  
(Magnification: (a) = x 3,000 (b) = x 7,000 (c) = x 20,000 (d) = x 33,000 (e) = 50,000  
(f) = x 80,000)

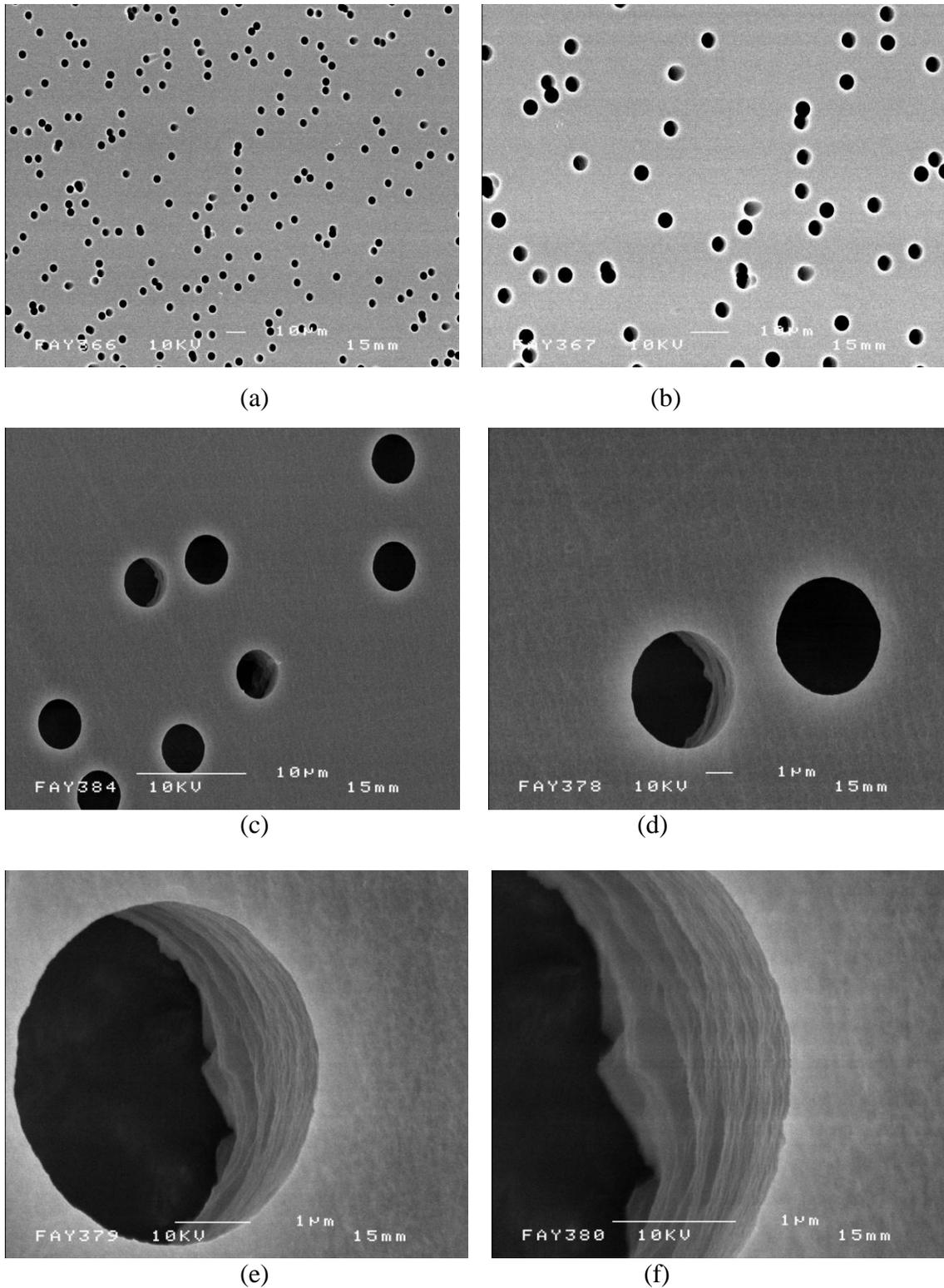


Fig.6.58: Surface of a 5.0 micron pore diameter pvp-poly(carbonate) membrane. (Magnification: (a) = x 500 (b) = x 1,000 (c) = x 3,000 (d) = x 7,000 (e) = x 15,000 (f) = x 33,000.

The micrographs (Fig. 6.56 b, d) show cracks in the surface of poly(carbonate) membranes. The surface of the PVP coated poly(carbonate) membrane coated with a thin layer of gold, cracks under vacuum conditions and also suffers from charge effects as well as beam damage. To overcome these problem the samples were coated with a thicker layer of gold, which resolved the problem of surface cracking and beam damage to some extent but caused some loss of resolution.

It is evident from the micrographs, for all of membranes investigated, that pores are not evenly distributed throughout the surface of the membrane and also that not all are cylindrical in shape or even continuous through the membrane. Some pores are very closely situated (Fig. 6.57 e). The pore diameter in some areas of the membrane is effectively double or even three times that of the supplied pore size as the pores are either too closely situated or interweave into one another. Hence, it is assumed that the pore density as well as the pore size in these PVP coated poly(carbonate) membranes is not evenly distributed throughout the entire surface of the membranes.

### 6.3.1.1 Effect of phosphate buffer on PVP-PC membranes, control

The scanning electron micrographs of PVP coated poly(carbonate) membranes were taken after exposure of phosphate buffer in order to investigate the effects of exposure of buffer on the structure of these membranes.

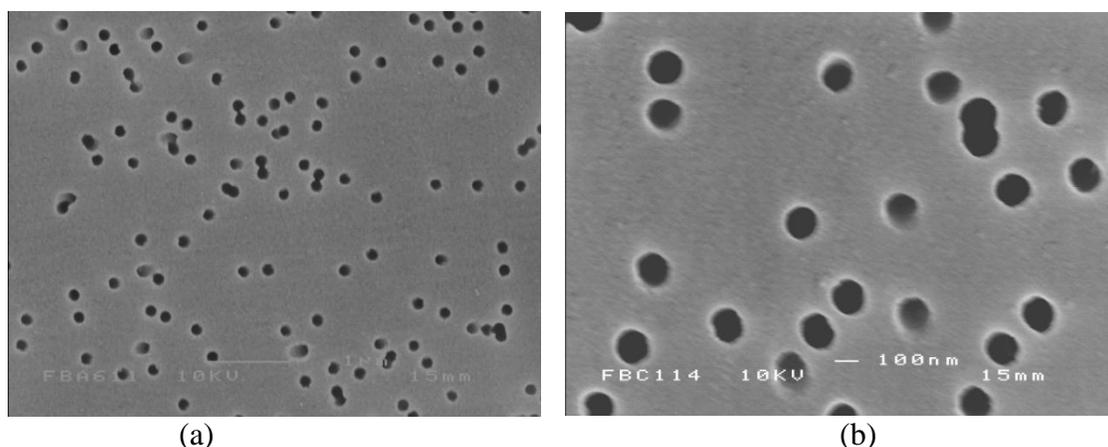


Fig.6.59: Surface of a 0.2 micron pore diameter pvp-poly(carbonate) membrane, post exposure to buffer for 1 hour.(Magnification: (a) = x 20,000; (b) = x 50,000).

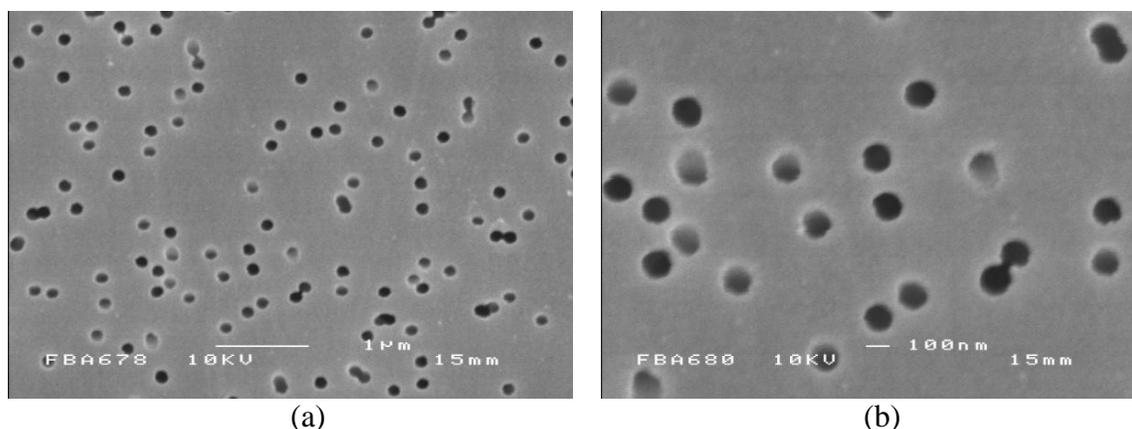


Fig.6.60: Surface of a 0.2 micron pore diameter pvp-poly(carbonate) membrane, post exposure to buffer for 24 hours.(Magnification: (a) = x 20,000; (b) = x 50,000)

The PVP coated poly(carbonate) membrane, when soaked in buffer have some changes in the surface morphology of the membrane. The surface of the membrane suffers from swelling, or water uptake which, may be due to the fact that the coating on the surface of the poly(carbonate) membrane i.e. poly(vinylidene pyrrolidone) is soluble in water and when these membranes are exposed to buffer solution the coating partially dissolves or absorbs some water. However, the swelling on the surface of the membrane is not extensive as the texture of the surface of membrane is observed to be similar after one hour and 24 hours of exposure to buffer solution (Fig. 6.59 (a) and 6.60 (b) respectively). Therefore, it is assumed that the surface of the PVP coated poly(carbonate) membrane is stable after some initial swelling.

### 6.3.1.2 PVP-PC membranes post-exposure to Albumin

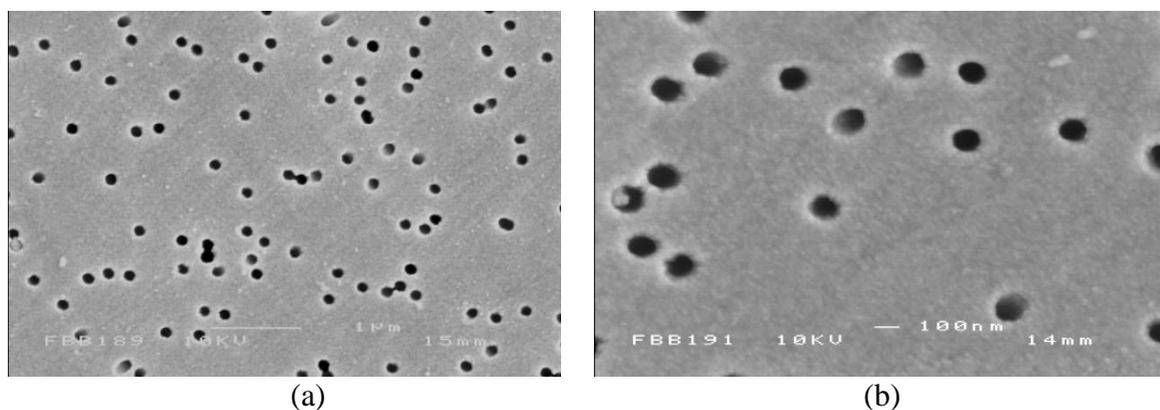


Fig. 6.61: Surface of a 0.2 micron pore diameter PVP-PC membrane after 1 hour exposure to 4 % w/v albumin solution. (Magnification: (a) = x 20,000 ; (b) = x 50,000).

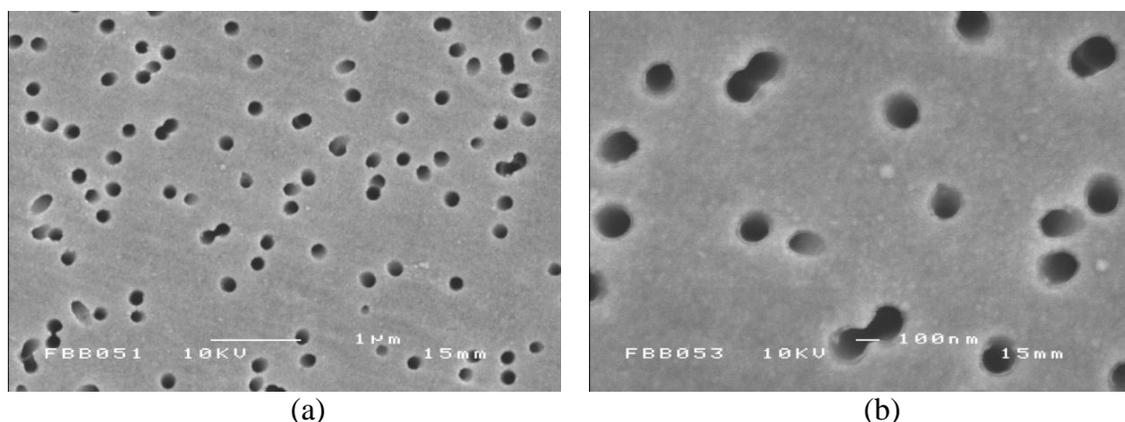


Fig. 6.62: Surface of a 0.2 micron pore diameter pvp-poly(carbonate) membrane after 24 hours exposure to 4 % w/v albumin solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

The results show that after exposure to albumin solution, the surface of the poly(carbonate) membrane is covered with a layer of protein. By comparing the micrographs, the protein layer can be seen on the membrane after one hour of exposure (Fig. 6.61a). Aggregates of protein are also visible on surface of the 0.2 micron pore diameter membrane (Fig. 6.62 a). Moreover, the protein deposit, a thick layer of protein, is observed around the edges of the pores (Fig. 6.62 b). The layer of albumin on the surface of the poly(carbonate) membrane appears to be thicker containing more aggregates after longer exposure times. It may be due to the fact that after initial protein film deposition the adsorption of further protein may be enhanced by increase in the number of sites for protein adsorption, the aggregates on the surface of membrane, after initial exposure, may also act as sites for further deposition of protein. However, longer exposure times also allows more time for more protein to deposit on the surface of the membrane. In both scenarios end result is the same, i.e. thicker adsorbate on the surface of the membrane. Therefore, it can be deduced from these results that longer exposure time results in more fouling of the surface of the membrane.

The investigation on membrane modification with proteins and macromolecules involved various proteins. The studies with albumin showed membrane modifications, hence another globular protein, Lysozyme was selected in order to investigate if all globular fouled the membranes and caused similar modification of the surface of the membrane or if there are differences in the fouling behaviour of different proteins.

### 6.3.1.3 PVP-PC membranes post-exposure to Lysozyme

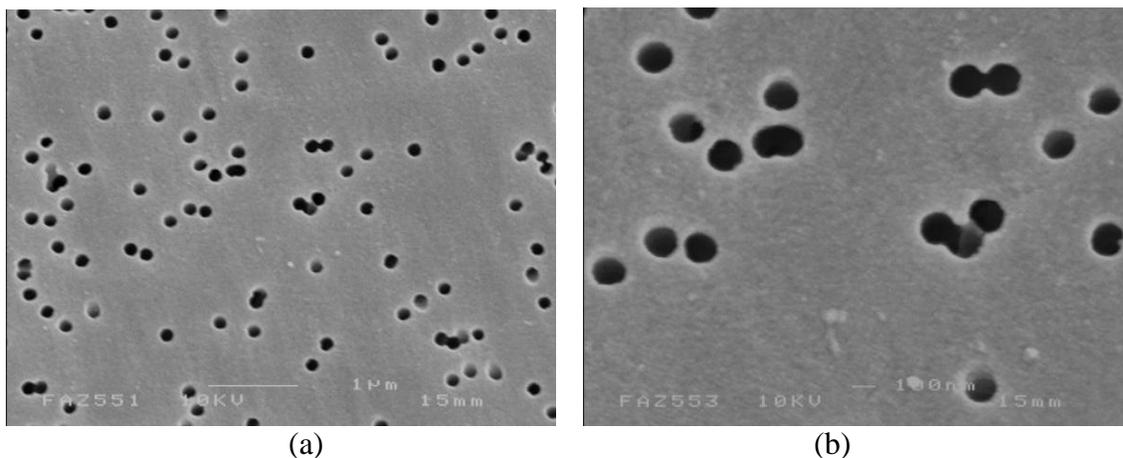


Fig.6.63: The surface of a 0.2 micron pore diameter pvp-poly(carbonate) membrane after one hour exposure to 4 % w/v Lysozyme solution. (Magnification: (a) = x 20,000; (b) = x 50,000)

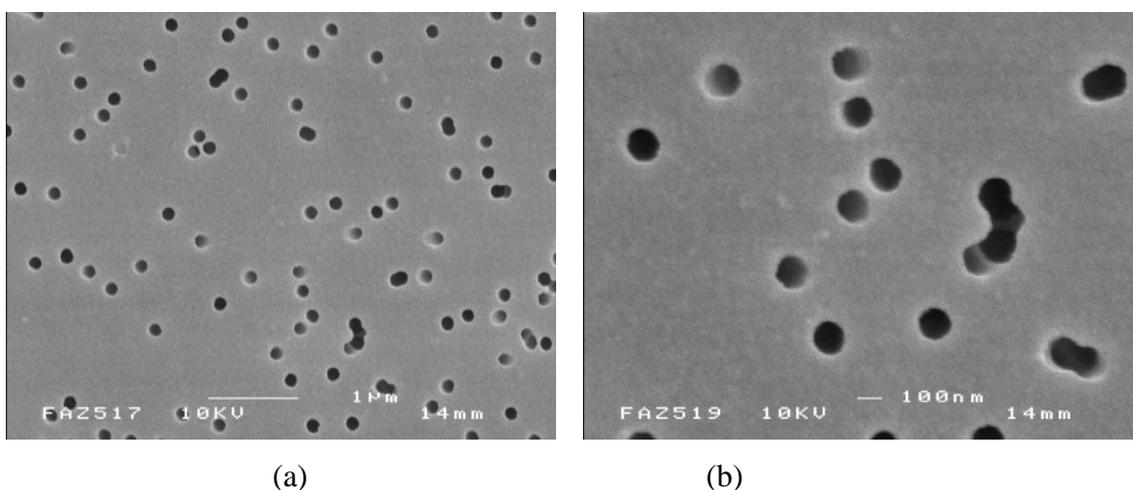


Fig.6.64: The surface of a 0.2micron pore diameter pvp-poly(carbonate) membrane after 24 hours exposure to 4 % w/v Lysozyme solution. (Magnification: (a) = x 20,000; (b) = x 50,000)

The exposure to Lysozyme solution results in deposition of a film of protein on the surface of the PVP-poly(carbonate) membrane. Aggregates of the protein can be seen more on the surface than in and around the pores after 24 hours (Fig. 6.64 b). The amount of protein (Lysozyme) and the size of these aggregates along with the thickness of protein layer on the surface of the membrane appear to stay the same after one hour and after 24 hours of exposure, unlike albumin. This could be due to many factors such as morphology of the membrane, conformation of protein in liquid and also the interaction

of protein with the surface of membrane. These results show that different proteins, despite having globular structures i.e. albumin and lysozyme foul and modify the surface of polycarbonate membranes differently. The charges carried by proteins, morphology and structure along with the constitution (amino acids that make up the protein) of each protein can affect its interaction with the polymer membrane that it is fouling. Fibrinogen was investigated in order to study the interaction of a fibrous type of protein with the polycarbonate membranes.

#### 6.3.1.4 PVP-PC membranes post-exposure to Fibrinogen

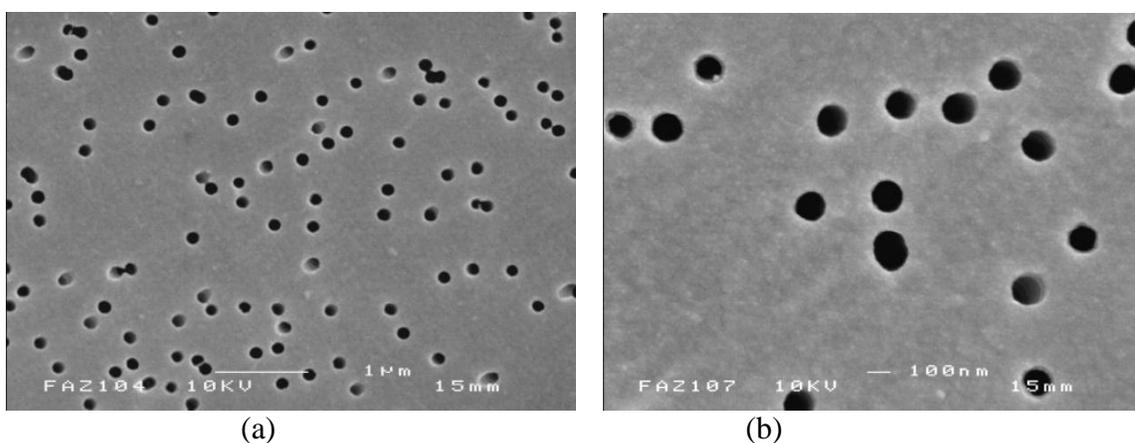


Fig.6.65: Surface of a 0.2 micron pore diameter pvp-poly(carbonate) membrane after 1 hour exposure to 4 % w/v Fibrinogen solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

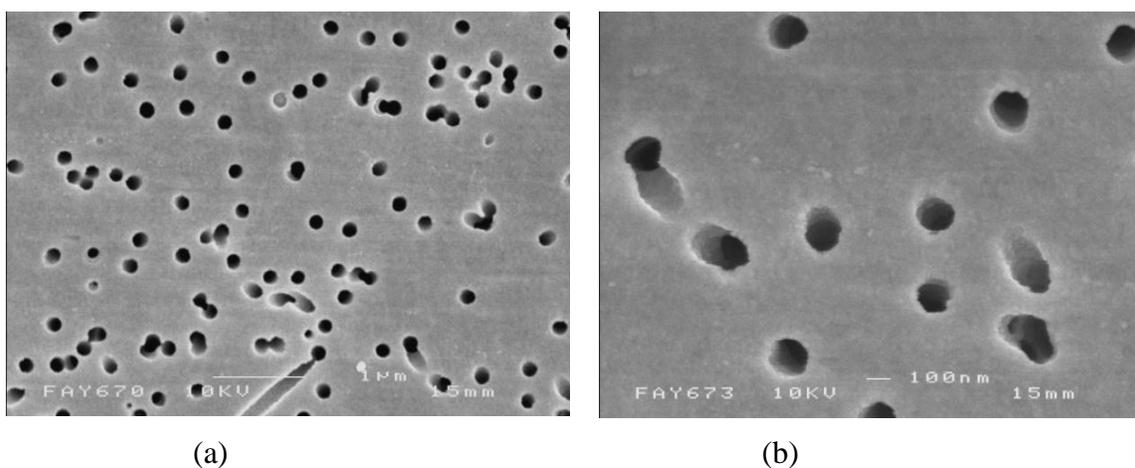


Fig.6.66: Surface of a 0.2 micron pore diameter pvp-poly(carbonate) membrane after 24 hours exposure to 4 % w/v Fibrinogen solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

Exposure to fibrinogen solution causes deposition of a protein film on the surface of the PVP-coated poly(carbonate) as can be seen from Fig. 6.65.

The surface of the membrane shows a thick layer of protein with aggregates. This layer of protein is not only on the surface of the membrane but is also present around the pores of the membrane. These aggregates of the protein grow in size with time and can be seen after 24 hours exposure, Fig. 6.66 (b). Moreover, a thick deposit of the protein is observed around the edges of the pores after 24 hours of exposure. Hence, it can be deduced that fibrinogen exposure causes fouling of the surface of the membrane, modifies the surface at short exposure times and at longer exposure times thick layer with protein aggregates forms on the surface of the polycarbonate membranes.

The investigations were continued with hyaluronan and polycarbonate membranes were used to study the interaction of hyaluronan with polymer membranes.

### 6.3.1.5 PVP-PC membranes post-exposure to Hyaluronan

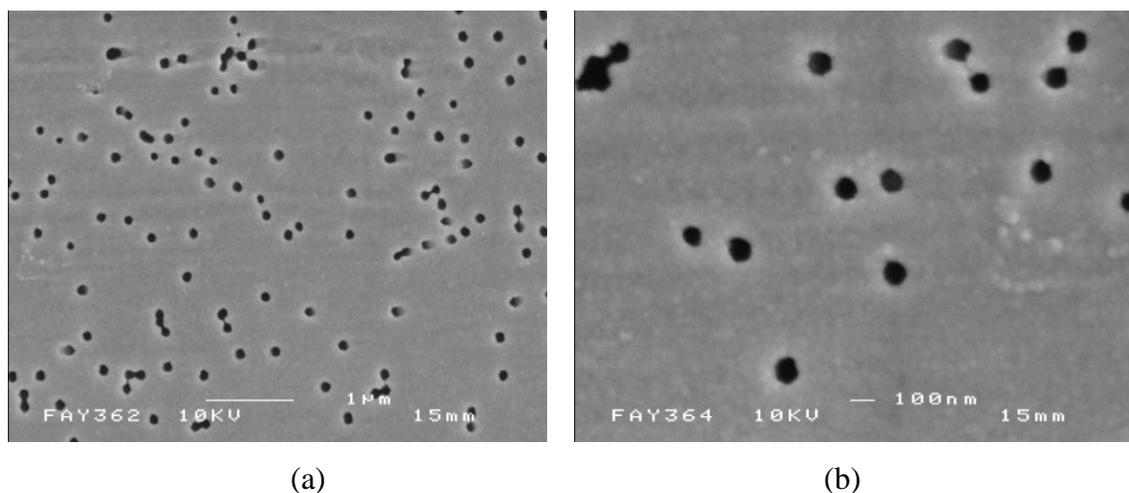


Fig.6.67: Surface of a 0.2 micron pore diameter pvp-pc membrane after 1 hour exposure to 1 % w/v Hyaluronan solution. (Magnification: (a) = x 20,000; (b) = x 50,000)

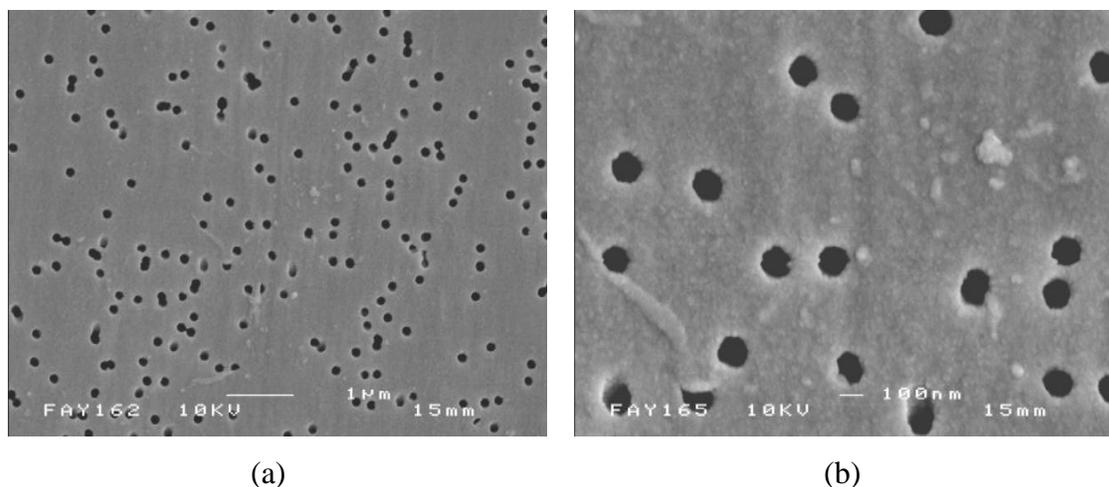


Fig.6.68: Surface of a 0.2 micron pore diameter pvp-pc membrane after 24 hours exposure to 1 % w/v Hyaluronan solution. (Magnification: (a) = x 20,000; (b) = x 50,000)

Hyaluronan is observed to adsorb on the surface of the PVP coated poly(carbonate) membrane, forming a thick grainy layer. The deposits of hyaluronan are observed after one hour of exposure on the surface of the membrane as well as around the pores in the membrane (Fig. 6.67). After 24 hours of exposure, the entire surface of the 0.2 micron pore diameter pvp-poly(carbonate) membrane is covered heavily with deposits of hyaluronan. Aggregates are large and a thicker layer of the deposit can be seen around the edges of the pores (Fig. 6.68 b).

The deposits of hyaluronan on the surface of the PVP coated poly(carbonate) membrane are much larger as compared to the deposits of other proteins investigated in our study. This is perhaps due to the structure and size of the hyaluronan as it is a polysaccharide.

Foetal calf serum was used in this study to investigate the fouling behaviour of the polycarbonate membranes as it contains a variety of proteins and macromolecules at the physiological concentrations.

### 6.3.1.6 PVP-PC membranes post-exposure to foetal calf serum

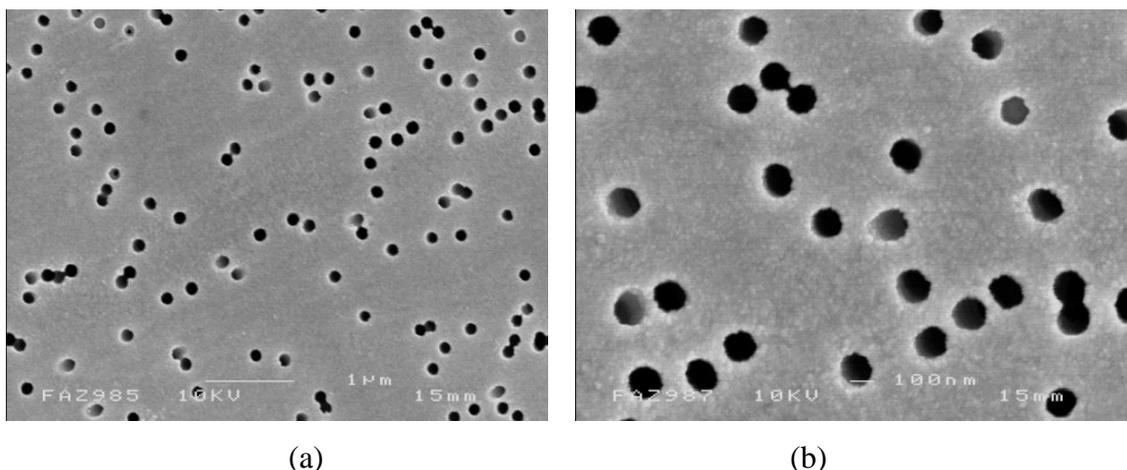


Fig.6.69: Surface of a 0.2 micron pore diameter pvp-pc membrane after 1 hour exposure to foetal calf serum. (Magnification: (a) = x 20,000; (b) = x 50,000)

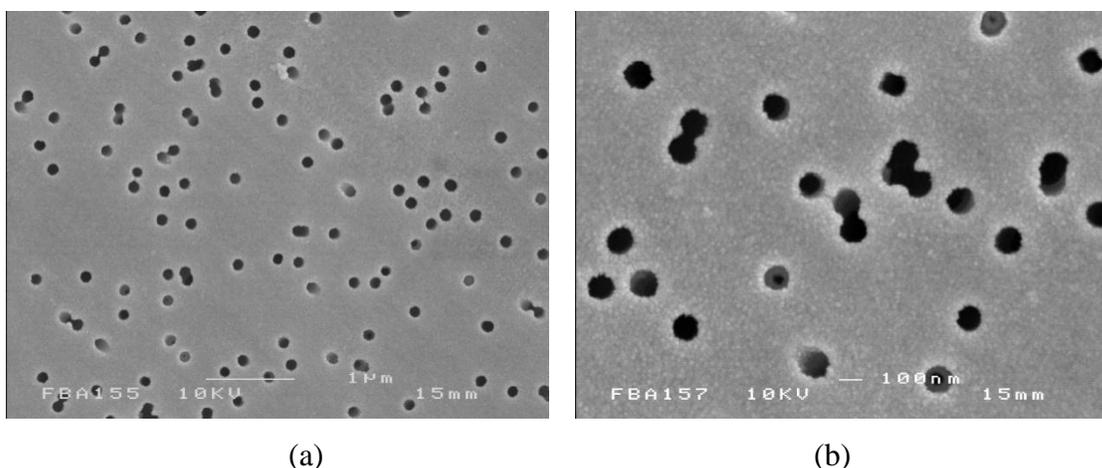


Fig.6.70: Surface of a 0.2 micron pore diameter pvp-pc membrane after 24 hours exposure to foetal calf serum. (Magnification: (a) = x 20,000; (b) = x 50,000)

Exposure to foetal calf serum even for shorter periods results in the formation of a thick film formation on the surface of the 0.2 micron pore diameter PVP coated poly(carbonate) membrane as can be seen from Fig. 6.69 (b). This can be due to a variety and higher concentrations of proteins and macromolecules that are present in the foetal calf serum. The entire surface of the membrane is observed to be covered with thick layer of adsorbed serum. The deposit layer appears to be granular in nature. The granular deposits are not only present on the surface of the membrane but also around the pore

edges. After a longer exposure time i.e. 24 hours, the membrane is observed to be covered in a thicker grainy deposit (Fig. 6.70).

The results show that commercially available PVP coated poly(carbonate) membranes undergo surface changes post exposure to various macromolecules. The macromolecules adsorb not only on the surface of the membrane but also around the pore edges. Longer exposure time results in greater deposits and larger aggregates around the pore edges of PVP coated poly(carbonate) membrane.

The PVP coating on the polycarbonate may affect the fouling behaviour of these membranes and may alter the surface modification. Hence, polycarbonate membranes without the poly(vinylidene pyrrolidone) coating were used in order to investigate if the pvp free PC membranes behaved differently and to study the fouling and modification of these membranes when exposed to various solutions of proteins and macromolecules.

### 6.3.2 PVP free Poly (carbonate) membranes

The uncoated poly(carbonate) membranes were used to investigate the phenomenon of protein and macromolecule adsorption on track etched homogeneously porous pre-cast membranes. The 0.2 micron pore diameter membranes were used in these studies.

#### 6.3.2.1 PVP free Poly (carbonate) membranes, unmodified, control

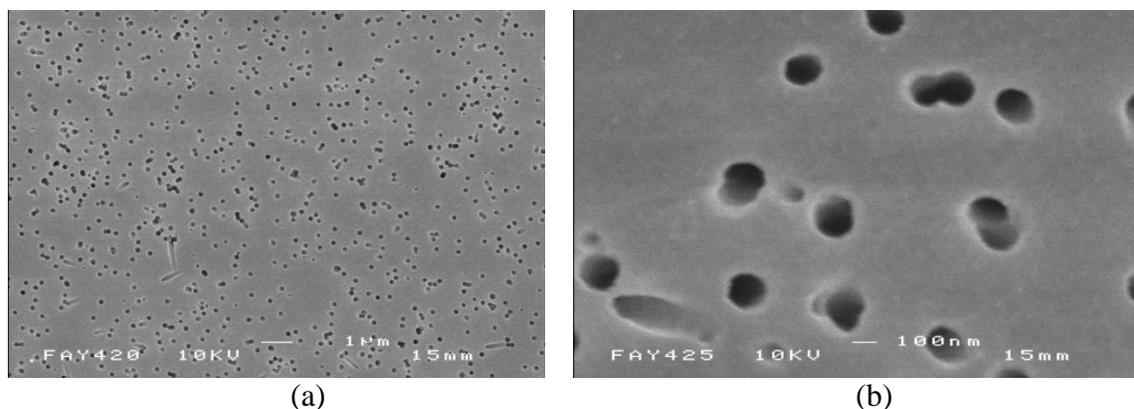


Fig.6.71: Surface of a 0.2 micron pore diameter poly(carbonate) membrane. (Magnification: (a) = x 7,000; (b) = x 50,000).

The poly(carbonate)membranes have circular, vertical, cylindrical pores on the surface of the membranes. However, not all of these pores are continuous through the membrane, Fig. 6.71 (b) or of the same size.

### 6.3.2.2 PVP free poly (carbonate) membranes, Buffer Exposure

The poly(carbonate) membranes were soaked in phosphate buffer solution at pH 7 for various duration of times. These membranes were studied under the field emission scanning electron microscope as a control (as compared to protein and macromolecule exposed polycarbonate membranes) in order to investigate if the phosphate buffer solution at pH 7 causes any changes in the morphology of the pvp free polycarbonate membranes at physiological pH and conditions.

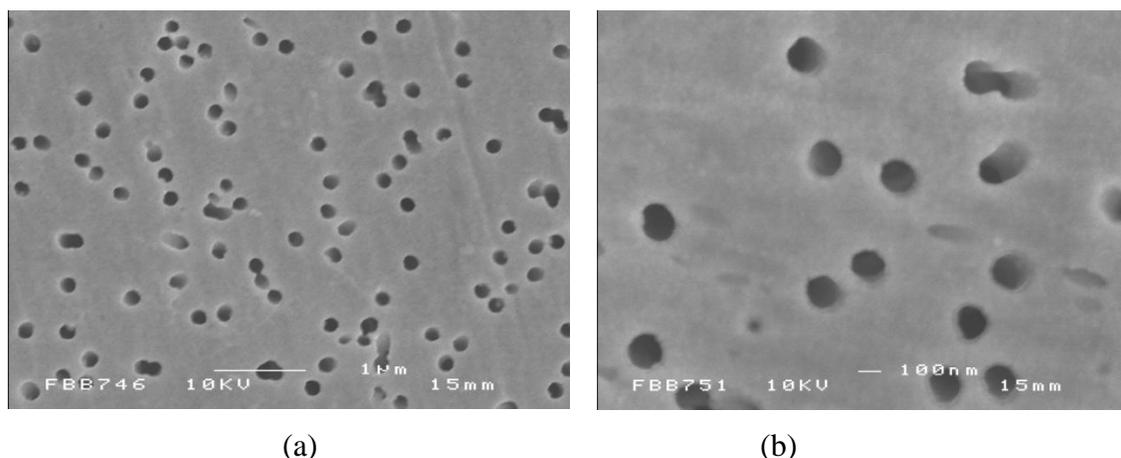


Fig.6.72: Surface of a 0.2 micron pore diameter poly(carbonate) membrane after 1hour exposure to buffer. (Magnification: (a) = x 20,000; (b) = x 50,000).

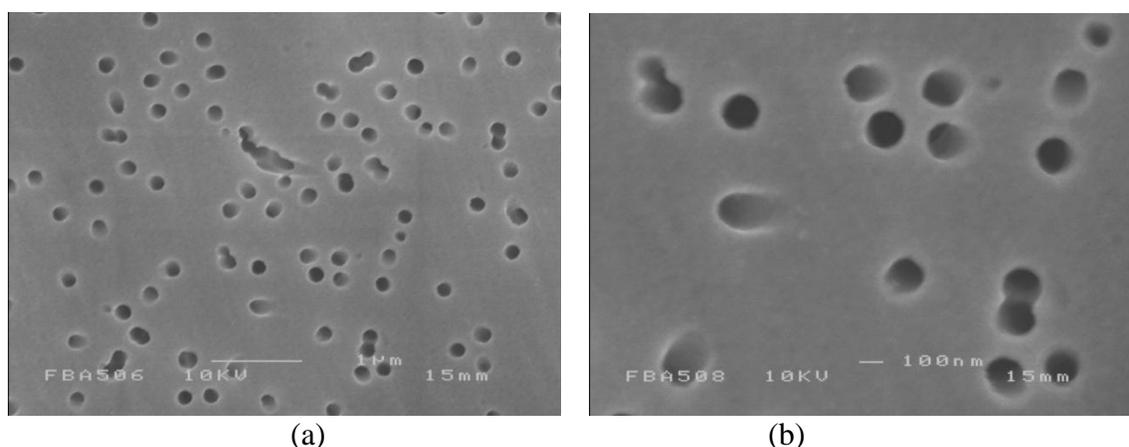


Fig.6.73: Surface of a 0.2 micron pore diameter poly(carbonate) membrane after 24 hours exposure to buffer. (Magnification: (a) = x 20,000; (b) = x 50,000).

The control experiments show that the surface of the poly(carbonate) membrane changes very slightly after exposure to the phosphate buffer solution at pH 7. These morphological changes are apparent after one hour of exposure and the membrane appears to be stable at longer exposure times, i.e. after 24 hour the surface of the membrane (Fig. 6.73 (b)) is observed to be similar to that after one hour of exposure (Fig. 6.72(b)).

The buffer exposure causes slight swelling of some areas of the membrane (Fig. 6.72 (a)). This slight change in the morphology of the surface of the polycarbonate membranes can be due to leaching of substances from the surface or water uptake by the polycarbonate membranes. However, the change in the morphology of the surface of the membrane is not significant which, indicates that the pvp free polycarbonate membranes are stable at physiological pH and conditions as they are stable in phosphate buffer solution at pH 7.

The pvp free polycarbonate membranes were then used in fouling studies that involved various proteins and macromolecules in order to investigate the fouling behaviour and interaction of the proteins and macromolecules with the surface of a polymer membrane.

### 6.3.2.3 PVP free poly(carbonate) membranes, post exposure to Albumin

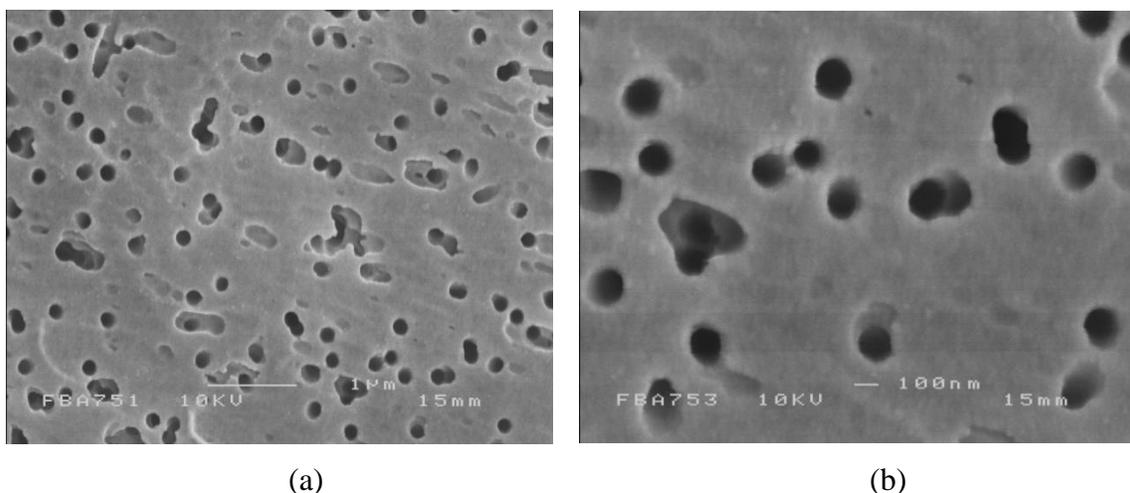


Fig. 6.74: Surface of a 0.2 micron pore diameter pvp free poly(carbonate) membrane after 1hour exposure to 4 % w/v Albumin solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

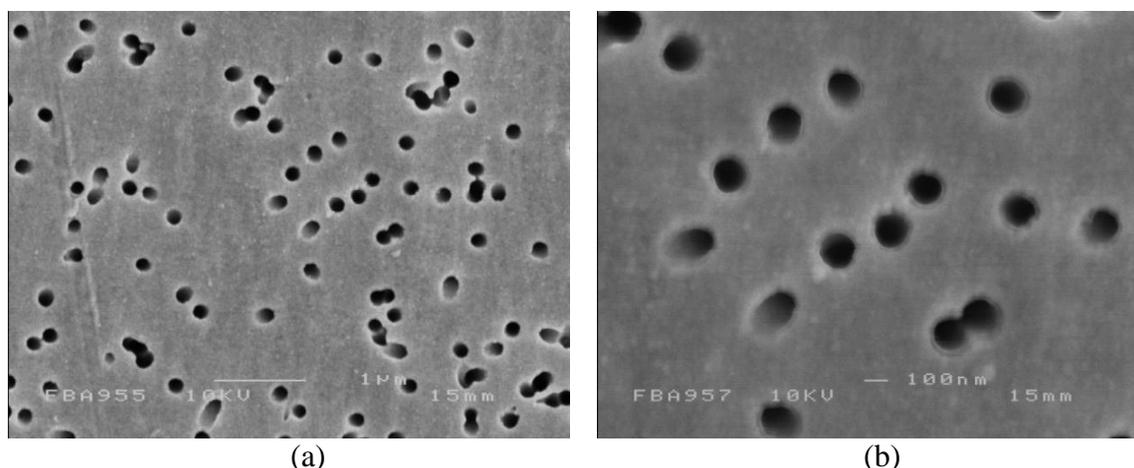


Fig. 6.75: Surface of a 0.2 micron pore diameter pvp free poly(carbonate) membrane after 24 hours exposure to 4 % w/v Albumin solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

The Fig. 6.74 and 6.75 show modified surface of the pvp free polycarbonate membranes after exposure to albumin solution. It can be observed that a layer of protein (albumin) forms on the surface of the pvp free poly(carbonate) membrane when exposed to solution of albumin. The protein adsorbs on the surface of the membrane and granular deposits of the protein are observed that grow with longer exposure times. Larger aggregates of albumin are observed after 24 hours of exposure. Some aggregates are seen around the edges of the pores as well as on the surface of the membrane (Fig. 6.75 (b)).

These results show that albumin modifies the surface of the pvp free polycarbonate membranes. The protein causes deposition of granular adsorbate on the surface of the membrane after one hour of exposure. At longer exposure times, the layer of the protein deposit grows in thickness and the aggregates are also observed to be enlarged in size.

The experiments were repeated with pvp free polycarbonate membranes using Lysozyme to foul and modify the surface of the membrane in order to study if another globular protein fouls and modifies the surface of the polymer membranes in a similar or different way to albumin and to investigate if the fouling patterns differ for different proteins.

### 6.3.2.4 PVP free Poly(carbonate) membranes, post exposure to Lysozyme

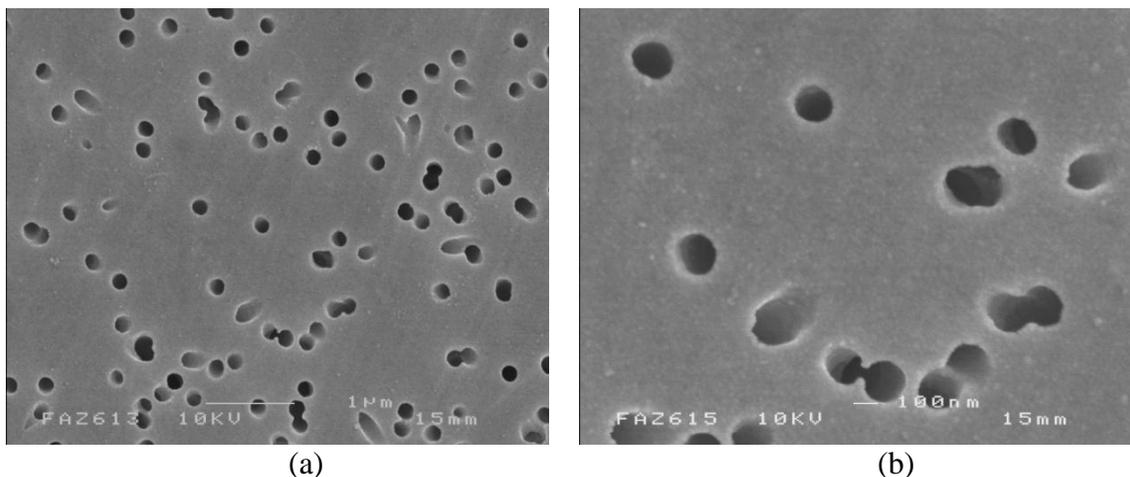


Fig.6.76: Surface of a 0.2 micron pore diameter non pvp poly(carbonate) membrane after 1hour exposure to 4 % w/v Lysozyme solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

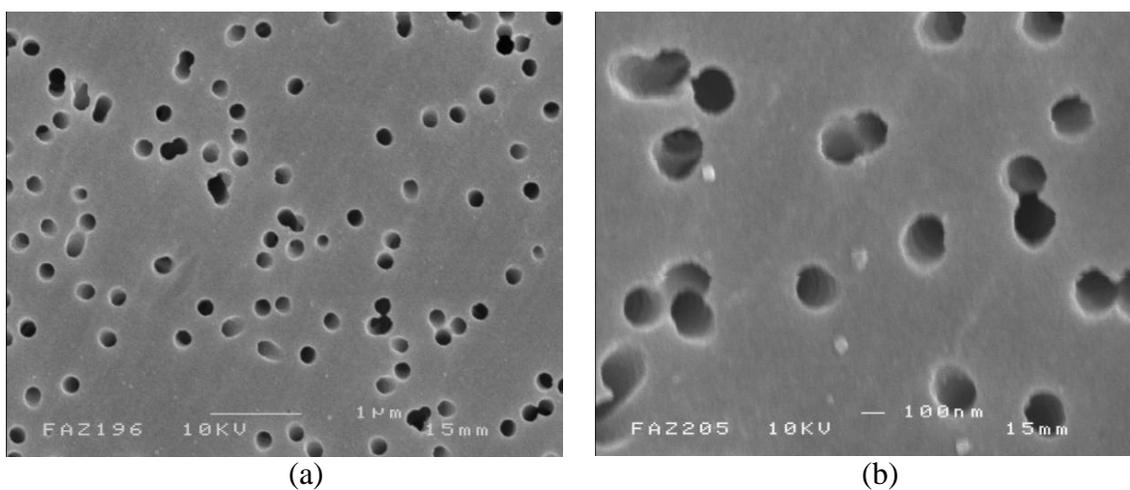
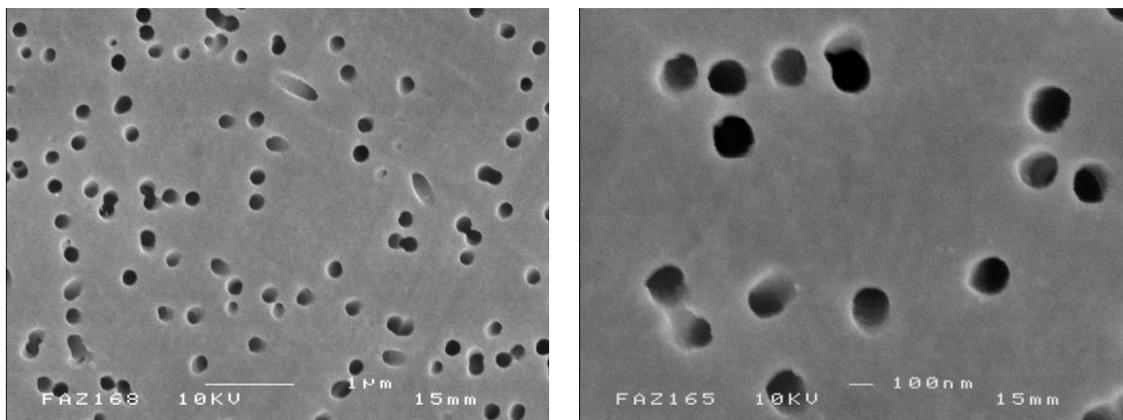


Fig.6.77: Surface of a 0.2 micron pore diameter non pvp poly(carbonate) membrane after 24 hours exposure to 4 % w/v Lysozyme solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

The exposure to a solution of lysozyme causes an initial adsorption of protein on the surface of the poly(carbonate) membrane. The protein not only coats the surface of the membrane, but aggregates are also formed that seem to be scattered over the entire surface of the membrane after only one hour of exposure (Fig. 6.76 b). However, after 24 hours of exposure only some of the aggregates are observed to grow in size (Fig. 6.77 b).

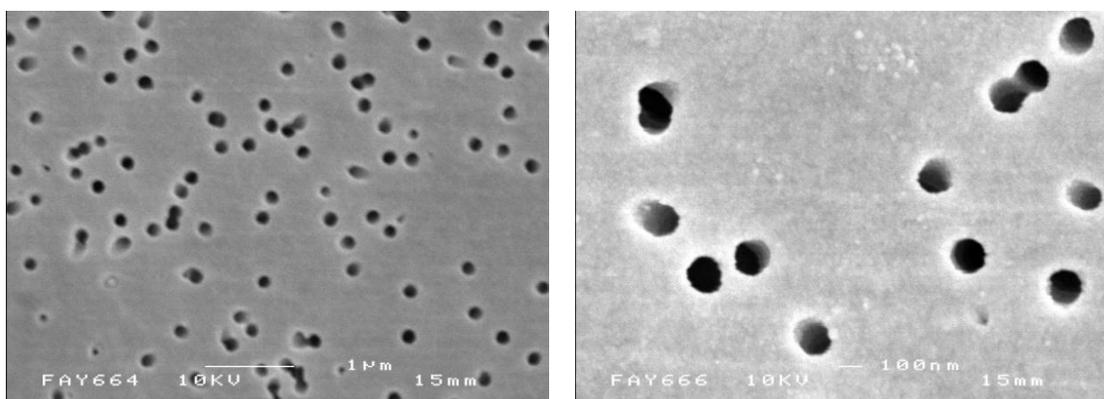
### 6.3.2.5 PVP free Poly (carbonate) membranes, post exposure to Fibrinogen



(a)

(b)

Fig.6.78: Surface of a 0.2 micron pore diameter non pvp PC membrane after 1 hour exposure to 4 % w/v fibrinogen solution. (Magnification: (a) = x 20,000; (b) = x 50,000).



(a)

(b)

Fig.6.79: Surface of a 0.2 micron pore diameter non pvp PC membrane after 24 hours exposure to 4 % w/v fibrinogen solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

The exposure to a solution of fibrinogen causes adsorption of a thick layer of protein on the surface of the membrane with some aggregates (Fig. 6.78). However, after longer exposure time, a thicker layer of the protein with more aggregates can be seen on the surface of the membrane as well as around the edges of the pores (Fig. 6.79). These results show that fibrinogen modifies the surface of the pvp free polycarbonate membranes after one hour of exposure and after longer exposure times the protein layer and aggregates grow in thickness.

The pvp free polycarbonate membranes were used to investigate the adsorption and fouling behaviour of the hyaluronan on polymer membranes.

### 6.3.2.6 PVP free Poly(carbonate) membranes, post exposure to Hyaluronan

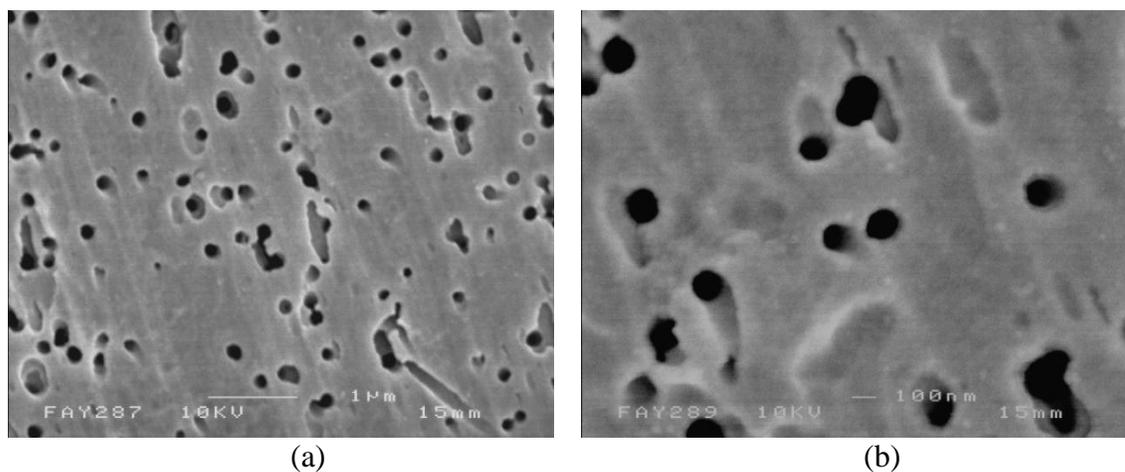


Fig. 6.80: Surface of a 0.2 micron pore diameter PC membrane after 1hour exposure to 1% w/v hyaluronan solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

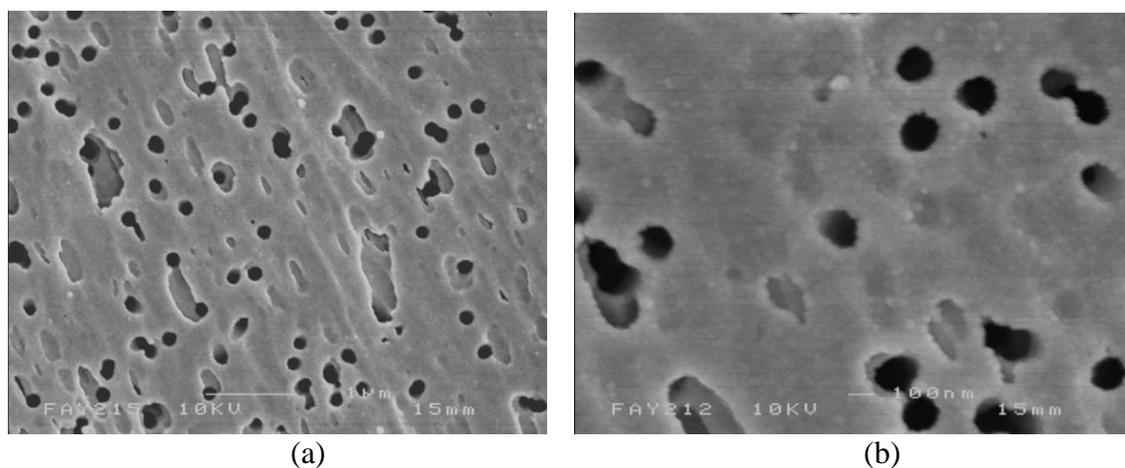


Fig.6.81: Surface of a 0.2 micron pore diameter PC membrane after 24 hours exposure to 1% w/v hyaluronan solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

The results show that exposure to the solution of hyaluronan leads to adsorption of a thick and non-uniform layer of this macromolecule on the surface of the pvp free poly(carbonate) membrane (Fig. 6.80). Longer exposure times, twenty four hours, result

in the formation of a thicker, granular layer on the surface of the membrane. The aggregates are observed to be very similar to aggregates of proteins on the entire surface of the membrane (Fig. 6.81). However, the hyaluronan coats the surface of the pvp free polycarbonate membrane entirely unlike the proteins. It can be due to the fact that hyaluronan is a polysaccharide and forms a gel like substance in solution. Moreover, the interaction between the hyaluronan and the surface of the polymer membrane would be different to that of interaction between the protein and the surface of the polymer membrane due to many factors including the constituents and the charge on the protein and hyaluronan.

The results also show a thick layer of hyaluronan deposit on the entire surface of the polymer membrane, which indicates that hyaluronan coats the entire surface as well as the pores of the polymer membrane.

The experiments were repeated with pvp free polycarbonate membranes using the foetal calf serum.

### 6.3.2.7 PVP free PC membranes, post exposure to foetal calf serum

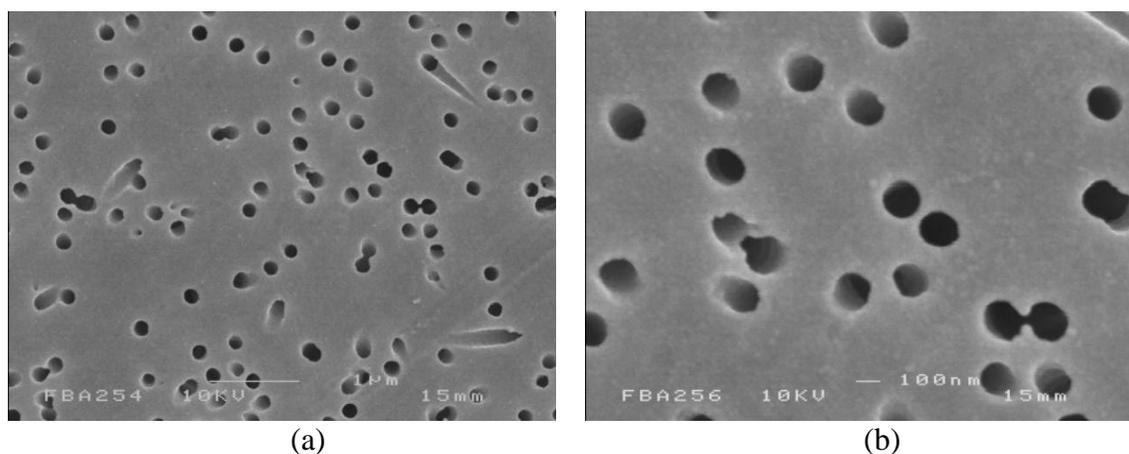


Fig. 6.82: Surface of a 0.2 micron pore diameter PC membrane after 1 hour exposure to foetal calf serum. (Magnification: (a) = x 20,000; (b) = x 50,000).

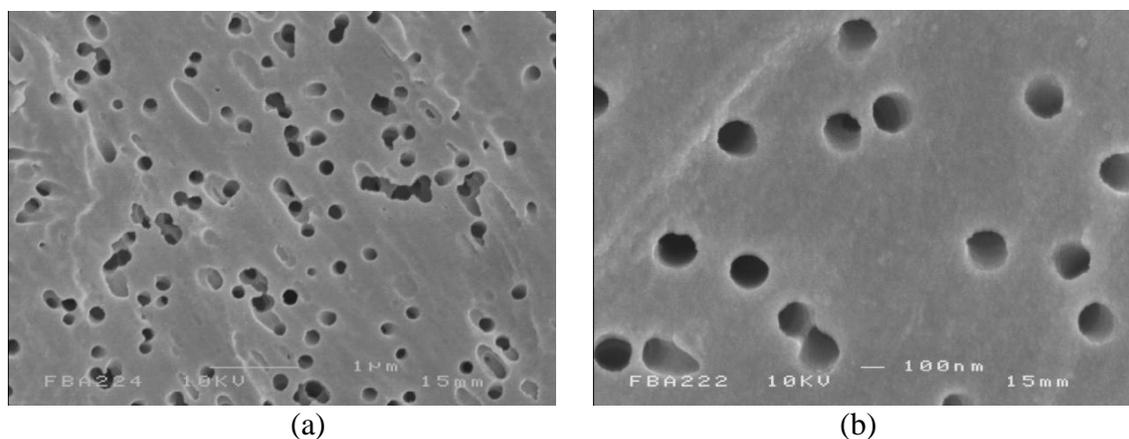


Fig. 6.83: Surface of a 0.2 micron pore diameter PC membrane after 24 hours exposure to foetal calf serum. (Magnification: (a) = x 20,000; (b) = x 50,000).

The results show that exposure to foetal calf serum leads to formation of a film on the surface of the 0.2 micron pore diameter pvp free poly(carbonate) membrane. The adsorbate is observed to be in the form of a thick grainy layer on the entire surface after one hour of exposure (Fig. 6.82). After 24 hours of exposure this layer of the deposit becomes thicker and is observed to coat the entire surface of the membrane, deposited layers are not continuous or smooth but can be seen as wave like layers on the surface of the membrane (Fig. 6.83). This can be due to the fact that foetal calf serum is contains variety of proteins and macromolecules at physiological concentrations. Different proteins interact differently with the surface of the polymer membranes differently. The thick wave like formation of the adsorbate may be the result of these proteins and macromolecule interactions with the surface of the pvp free polycarbonate membranes. There are many factors that affect the interaction and fouling behaviour of the polymer membrane. The pore size of the polymer membrane can affect how a polymer membrane is modified after being exposed to same concentrations of the same protein solution.

#### **6.4 Effect of pore size on biofouling of polymer membranes**

The pore size effect on biofouling of membranes was investigated using poly(vinylidene pyrrolidone) coated poly (carbonate) membranes. Three pore sizes, 0.05 micron, 0.2 micron and 5.0 micron pore diameter polycarbonate membranes, were used in this study.

These studies were carried out using all of the macromolecules used, control experiments were also performed, which involved unmodified and buffer exposed membranes.

### 6.4.1 Unmodified Membranes

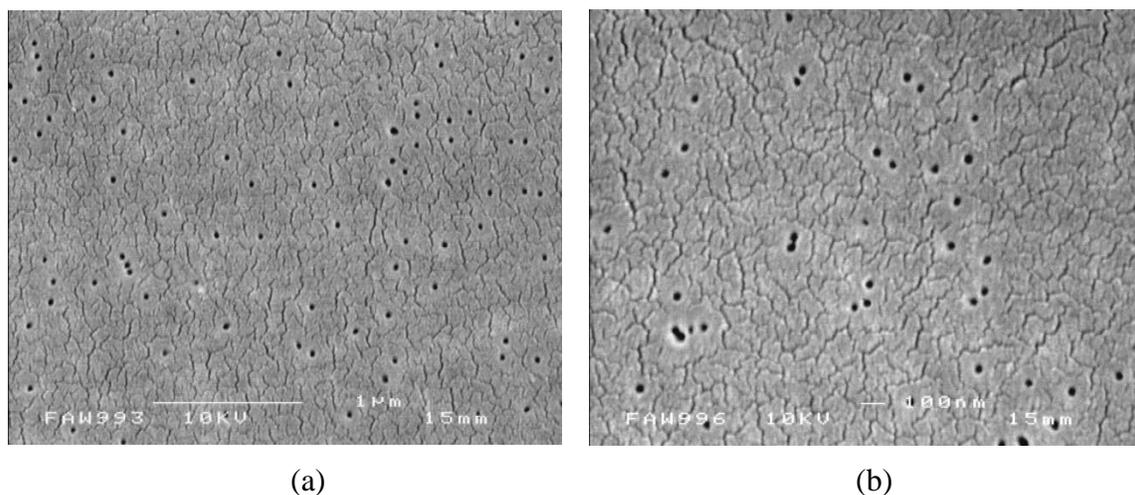


Fig. 6.84: Surface of an unmodified 0.05 micron pore diameter PVP coated poly (carbonate) membrane. (Magnification: (a) = x 33,000 (b) = x 50,000).

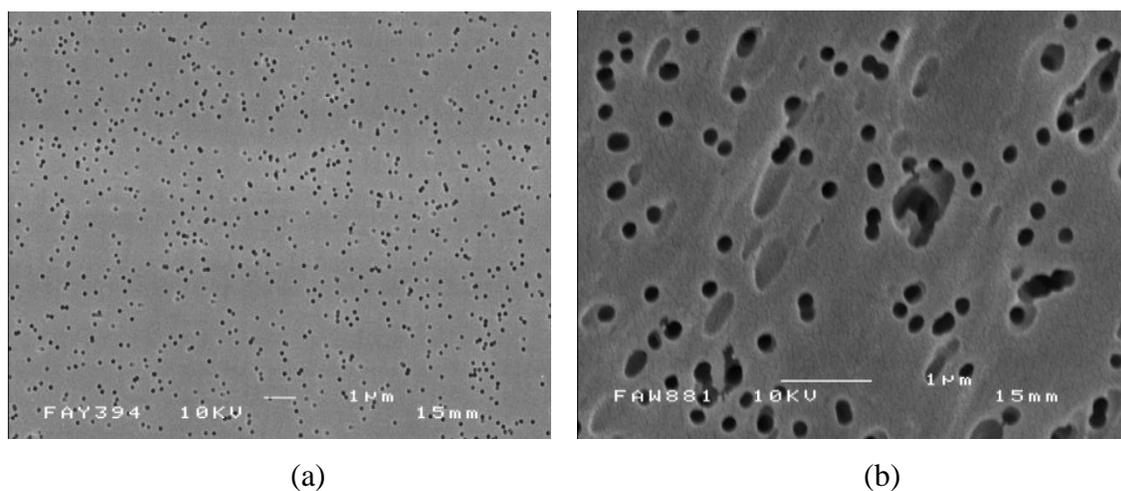


Fig. 6.85: Surface of an unmodified 0.2 micron pore diameter PVP coated poly (carbonate) membrane. (Magnification: (a) = x 7,000; (b) = x 20,000).

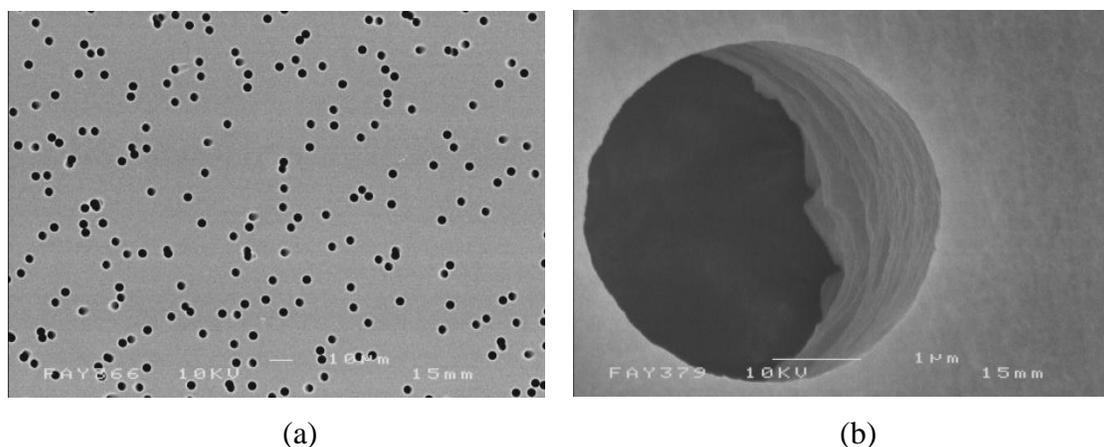


Fig. 6.86: Surface of an un modified 5.0 micron pore diameter PVP coated poly (carbonate) membrane. (Magnification: (a) = x 500; (b) = 20,000)

The 0.05 micron pore diameter PVP coated poly (carbonate) membrane was observed to have cracks more than other pore sizes. The micrographs show that regardless of the pore size, track etched PVP coated poly(carbonate) membranes all have an uneven pore density. The biggest pore size, i.e. 5.0 micron pore, diameter membrane provides an opportunity to study the inner structure of the pore. The pores seem to have a layered structure (Fig. 6.86 b). As the pores are similar for all three pore sized membranes, i.e. vertically cylindrical in nature, it can be assumed that the pore structure would also be similar if not identical for all of the membranes.

#### 6.4.2 Control – membranes post exposure to buffer solution

The membranes were exposed to buffer solution for 1 hour and 24 hours to investigate the effect of buffer solutions on the membranes.

A study of biointerfacing polymeric barrier membranes for sensors

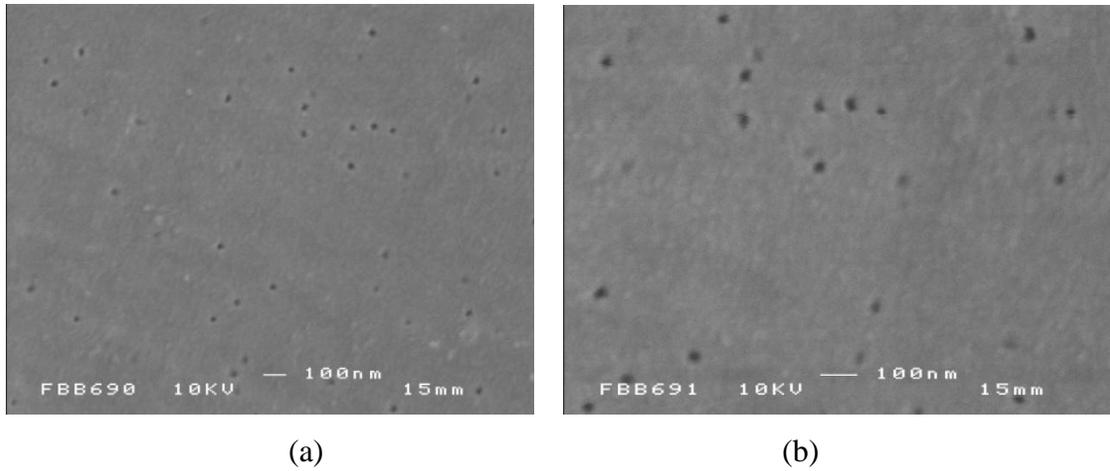


Fig. 6.87: Surface of a 0.05 micron pore diameter pvp-poly(carbonate) membrane, after 1 hour exposure to buffer. (Magnification: (a) = x 50, 000; (b) = x 80,000)

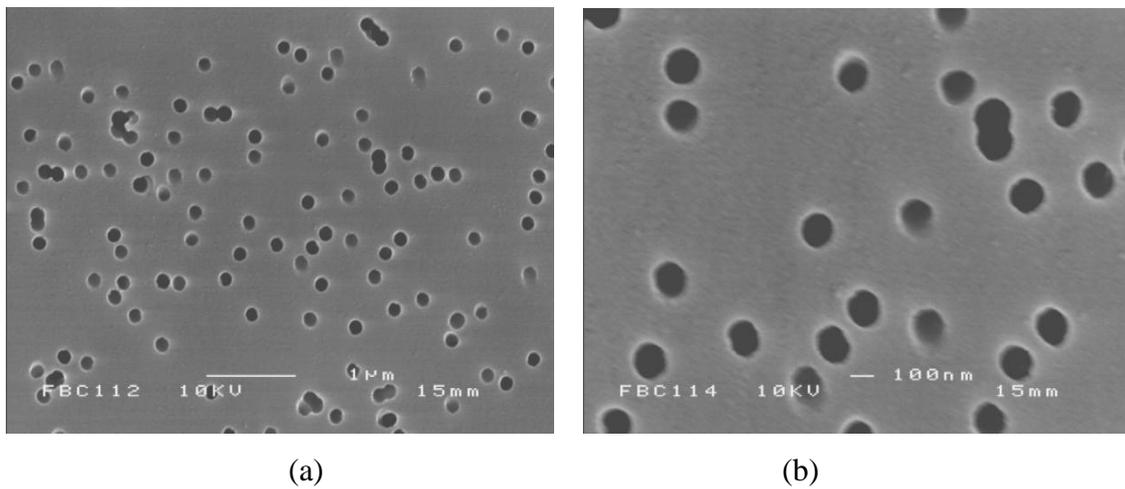


Fig.6.88: Surface of a 0.2 micron pore diameter pvp-poly(carbonate) membrane, after 1 hour exposure to buffer. (Magnification: (a) = x 20,000 (b) = x 50,000)

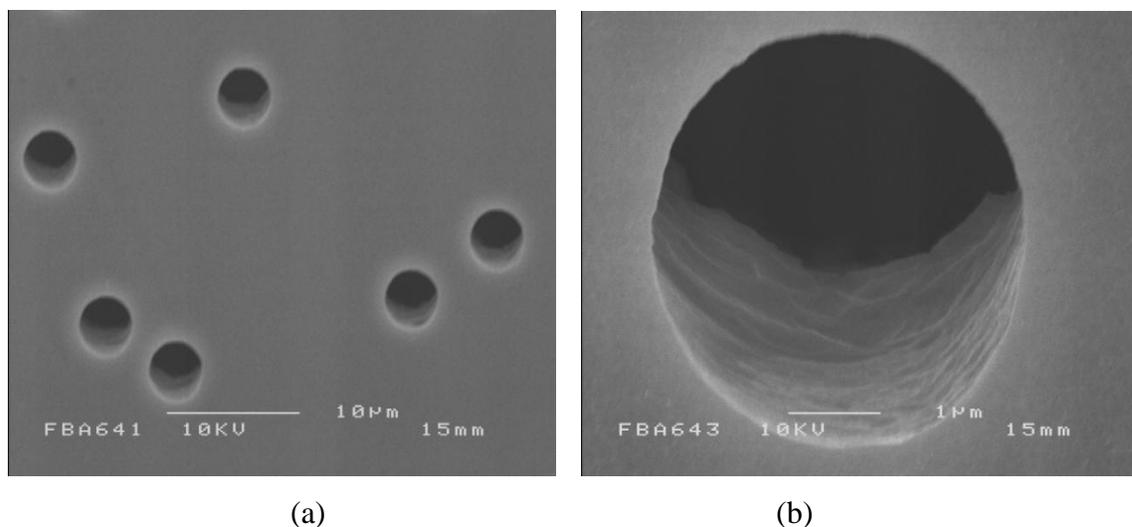


Fig. 6.89: Surface of a 5.0 micron pore diameter pvp-poly(carbonate) membrane, after 1 hour exposure to buffer. (Magnification: (a) = x 3,000 (b) = x 20,000)

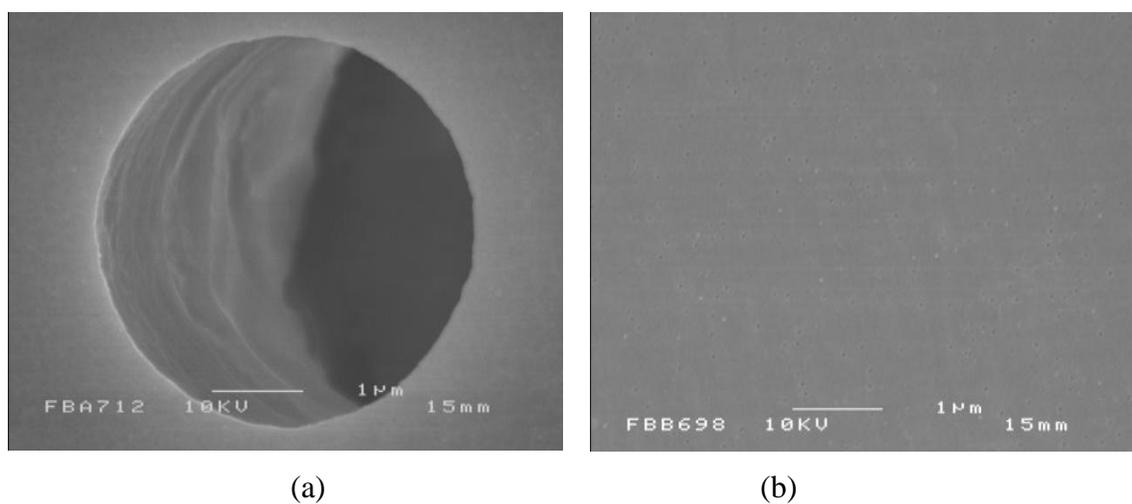


Fig. 6.90: Surface of (a) 5.0 micron and (b) 0.05 micron pore diameter pvp-pc membrane, after 24 hours exposure to buffer. (Magnification: (a) = x 20,000 (b) = x 20,000)

The results show raised features on the surface of the PVP coated poly(carbonate) membranes (Fig. 6.87(b), 6.88(b), 6.90). This could be due to water uptake at the surface of the membrane. This change in the morphology of the surface of the membrane can be more clearly seen in the smallest pore size membrane i.e. 0.05 micron pore diameter pvp-polycarbonate membrane (Fig. 6.87(a) and 6.90(b) pores are hard to see in this micrograph due to magnification). However, the results show that there are no adverse changes in the morphology of the surface of all the membranes, which indicates that the

pvp coated polycarbonate membranes of 0.05, 0.2 $\mu$ m and 5.0 micron pore diameter are stable at longer exposure times.

### 6.4.3 PVP-PC membranes, post exposure to Albumin

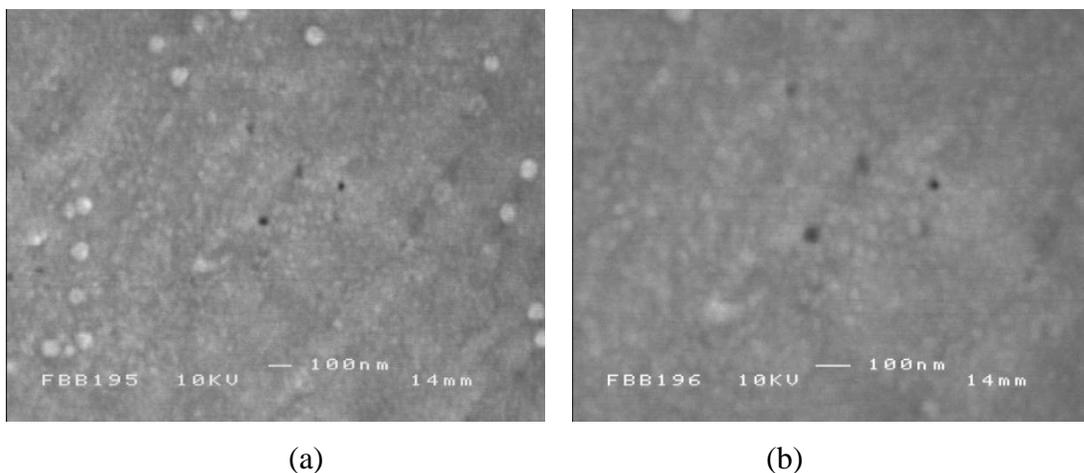


Fig. 6.91: Surface of a 0.05 micron pore diameter PVP-PC membrane, after 1 hour exposure to 4 % w/v albumin solution. (Magnification: (a) = x 50,000; (b) = x 80,000)

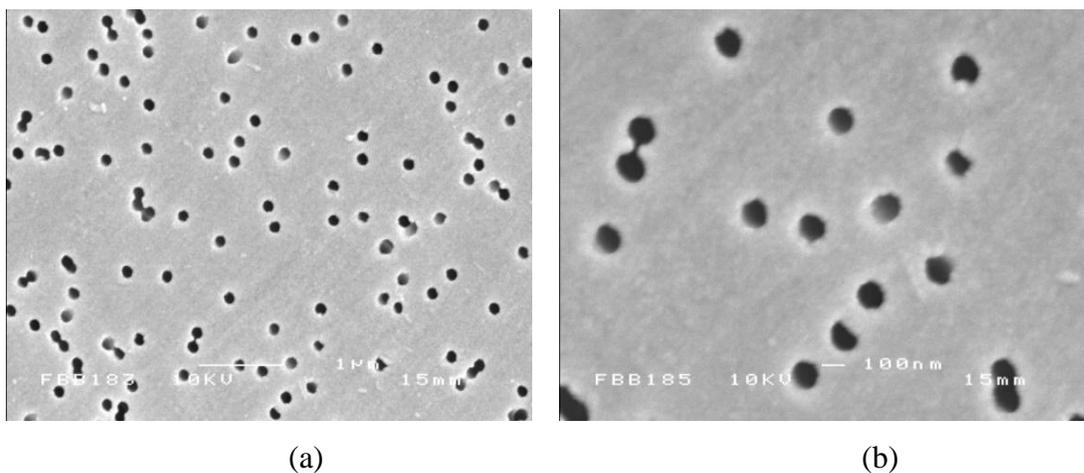


Fig. 6.92: Surface of a 0.2 micron pore diameter PVP-PC membrane, after 1 hour exposure to 4 % w/v albumin solution. (Magnification: (a) = x 20,000; (b) = x 50,000)

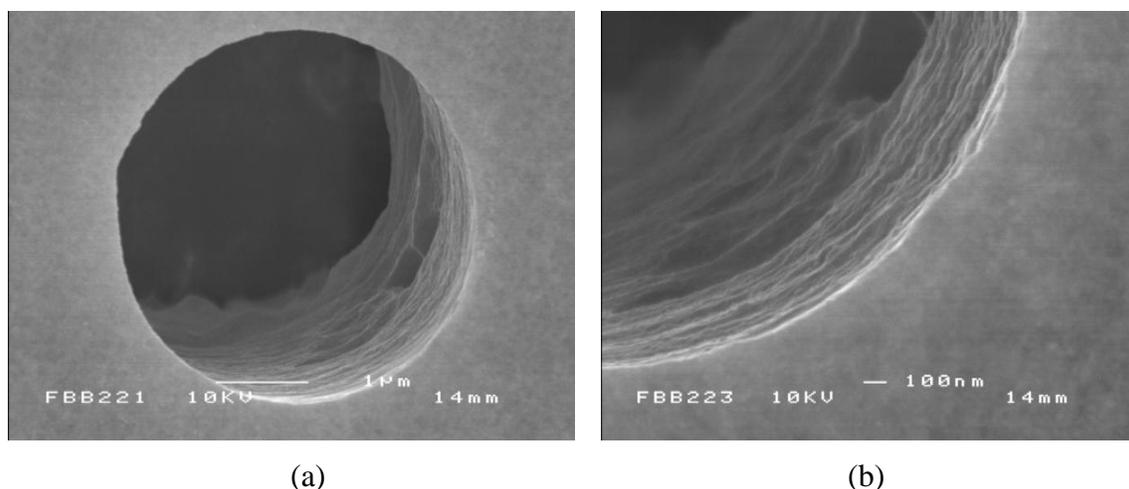


Fig. 6.93: Surface and inside of a pore of a 5.0 micron pore diameter PVP-PC membrane, after 1 hour exposure to 4 % w/v albumin solution. (Magnification: (a) = x 20,000; (b) = x 50,000)

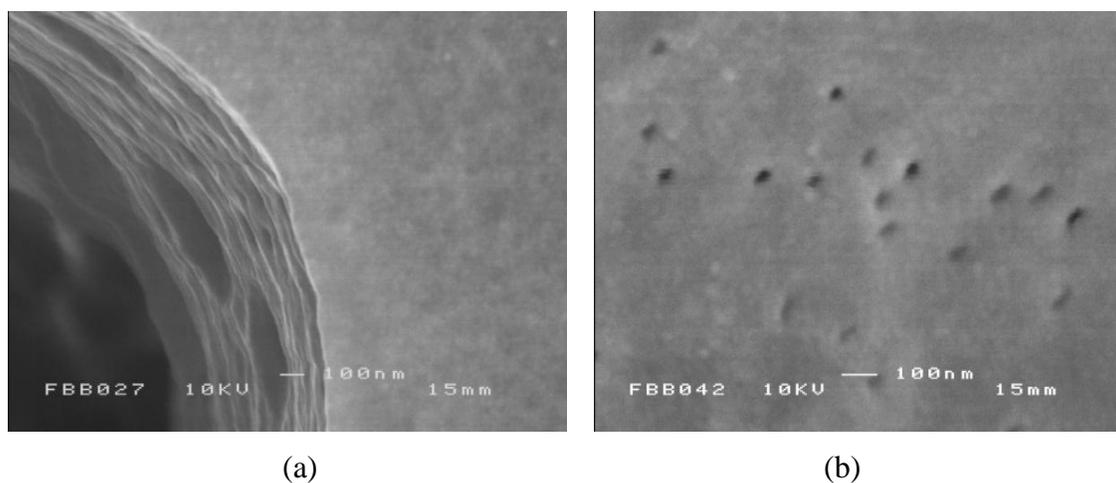


Fig. 6.94: Surface of (a) inside structure of a 5.0  $\mu\text{m}$  and (b) 0.05  $\mu\text{m}$  pore diameter PVP-PC membrane, after 24 hours exposure to 4% w/v albumin solution. (Magnification: (a) = x 50,000 (b) = x 80,000)

The images show that the surface PVP-PC membrane is covered with a layer of protein just after one hour of exposure to the solution of albumin. The pores in the lowest pore size membrane, i.e. 0.05micron pore diameter pvp-pc, are observed to be narrowing and getting blocked at longer exposure times (Fig. 6.94 (b)). The 0.2 micron pore diameter pvp-pc membrane also shows deposits of protein on the surface as well as around edges of the pores (Fig. 6.92 (b)).

Longer exposure times result in deposition of a thick cake like layer of protein on the surface of these membranes. Aggregates of the protein can be seen around the edges of the pores for all pore sizes, as well as on the surface of membranes (Fig. 6.91, Fig. 6.92, and Fig. 6.94).

The experiments were repeated with all pore sizes of pvp coated polycarbonate membranes using Lysozyme in order to investigate if other proteins have any profound effect on the fouling due to the pore size of the polymer membranes.

#### 6.4.4 PVP coated poly(carbonate)membrane with Lysozyme

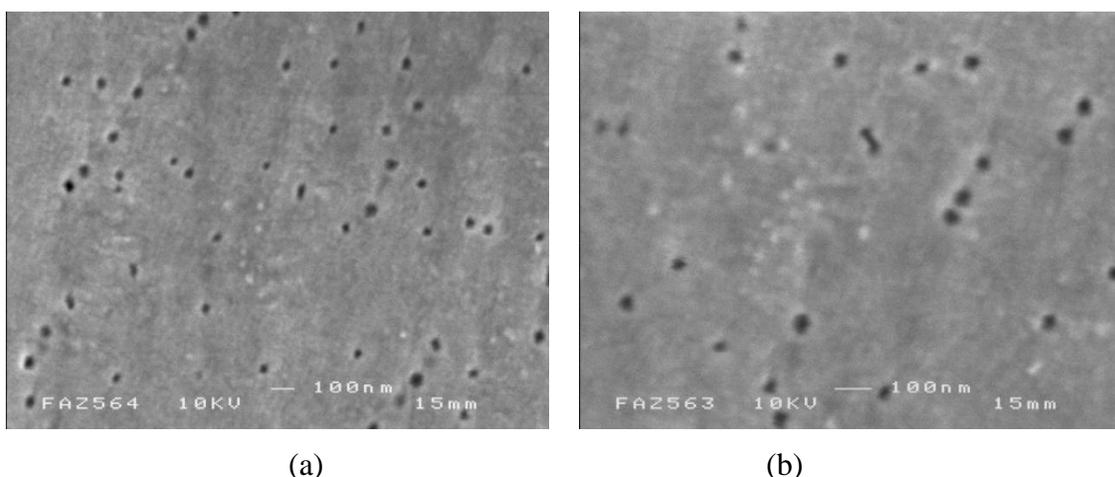


Fig. 6.95: Surface of a 0.05 micron pore diameter PVP-PC membrane, after 1 hour exposure to 4 % w/v Lysozyme solution. (Magnification: (a) = x 50,000; (b) = x 80,000)

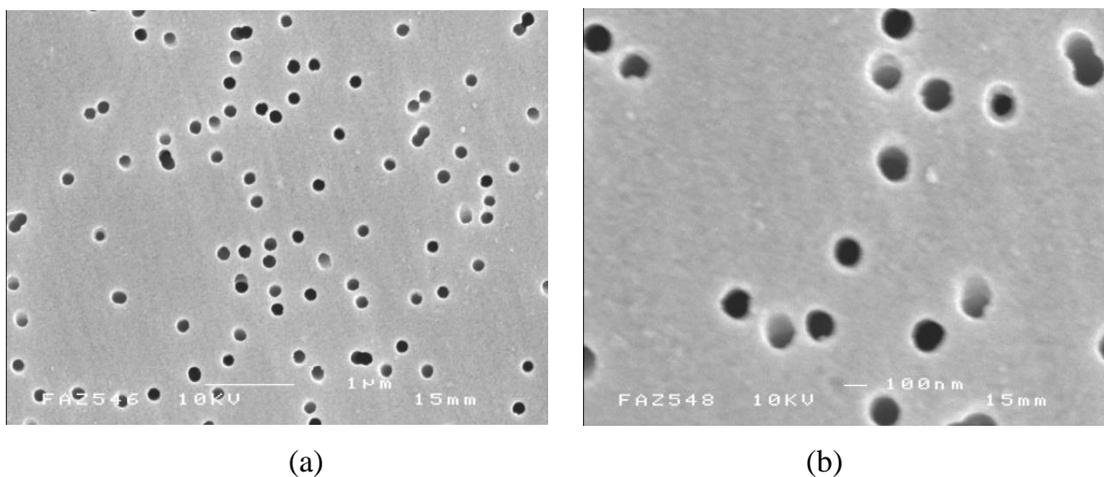


Fig. 6.96: Surface of a 0.2 micron pore diameter PVP-PC membrane, after 1 hour exposure to 4 % w/v Lysozyme solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

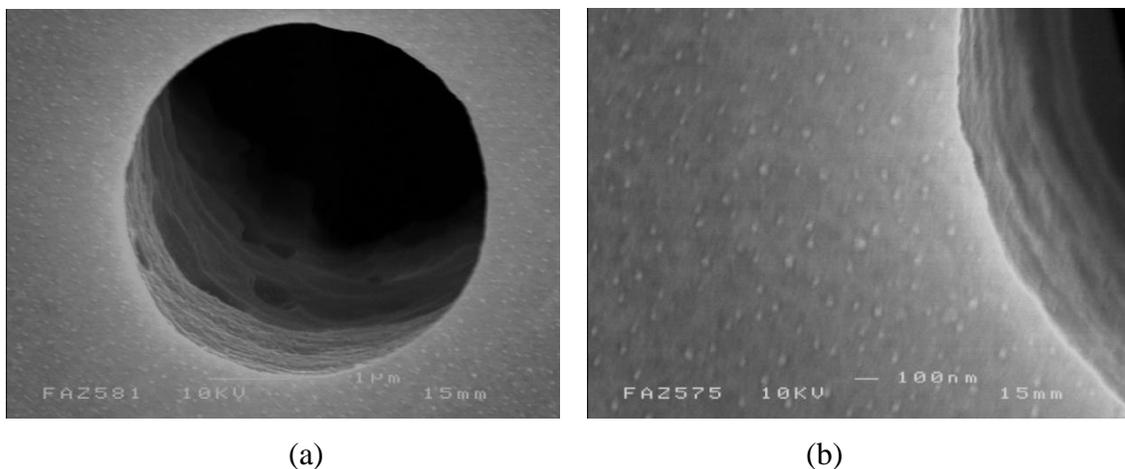


Fig. 6.97: Surface of a 5.0 micron pore diameter PVP-PC membrane, after 1 hour exposure to 4 % w/v Lysozyme solution. (Magnification: (a) = x 20,000; (b) = x 50,000).

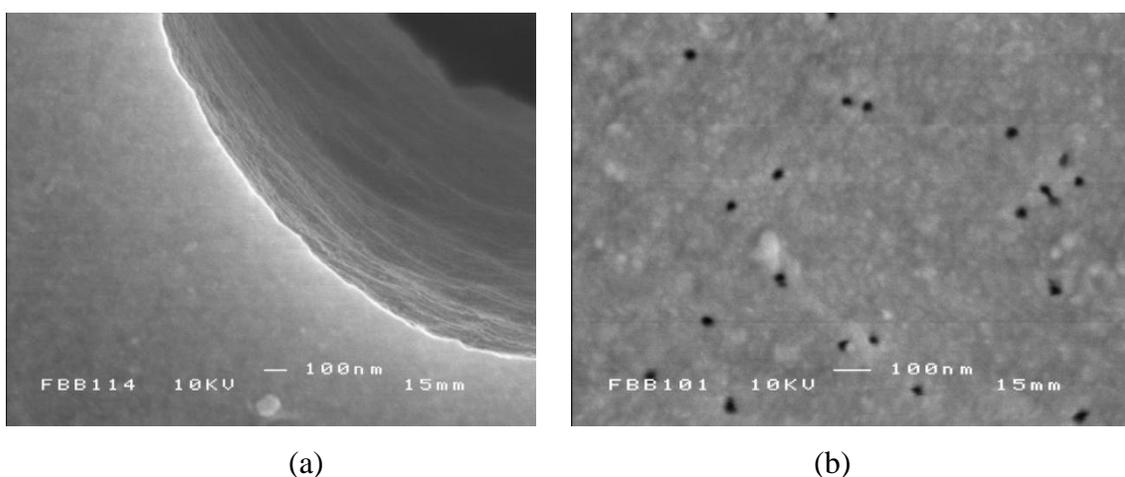


Fig. 6.98: Surface of (a) 5.0 μm and (b) 0.05 μm pore diameter pvp-pc membrane, after 24 hours exposure to 4 % w/v Lysozyme solution. (Magnification: (a) = x 50,000 (b) = x 80,000)

The results show that exposure to the lysozyme solution for one hour results in deposition of a thick layer of the protein containing aggregates on entire surface of the PVP coated poly(carbonate) membrane. The deposits and aggregates of the protein are also observed around edges of the pores regardless of pore size of the membrane. After twenty four hours the aggregates grow in size, moreover, a thicker coat of the protein layer can be observed on the entire surface of the membranes (Fig. 6.98).

The aggregates of the protein are circular in shape and are observed on the entire surface of all of the pvp-pc membranes. Moreover, on comparison of the largest and the smallest

pore size pvp-pc membranes after long exposure time (twenty four hours), both membranes are observed to be modified with protein layer covering the entire surface of the membrane. However, the 0.05 micron pore diameter pvp-pc membrane is observed to undergo narrowing and partial blockage of the pores (Fig. 6.98 (b)).

The experiments were then repeated with fibrinogen to investigate the effect of pore size on the fouling profile of pvp-pc membranes.

#### 6.4.5 PVP-PC membrane post exposure to Fibrinogen

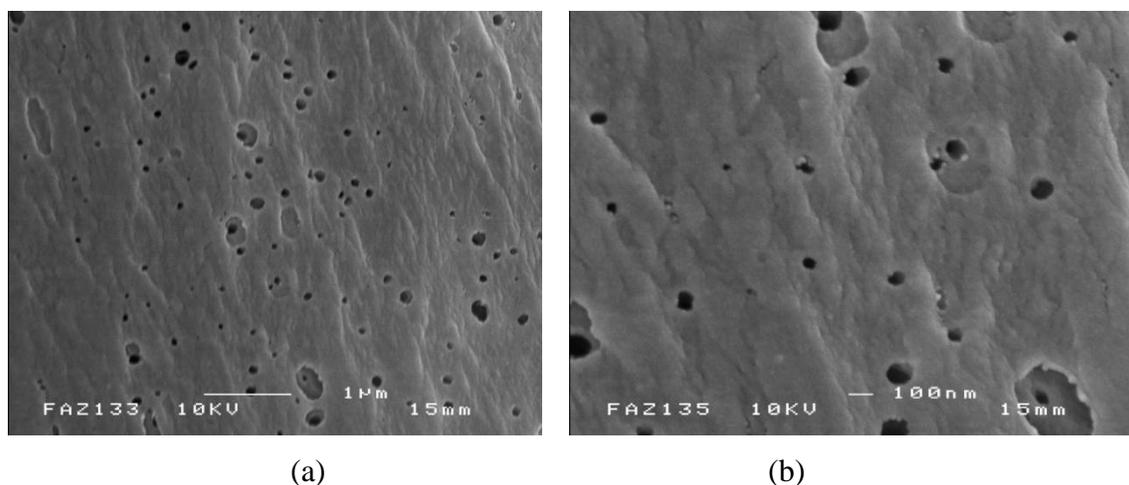


Fig. 6.99: Surface of a 0.05 micron pore diameter PVP-PC membrane, after 1 hour exposure to 4 % w/v fibrinogen solution. (Magnification: (a) = x 20,000; (b) = x 50,000)

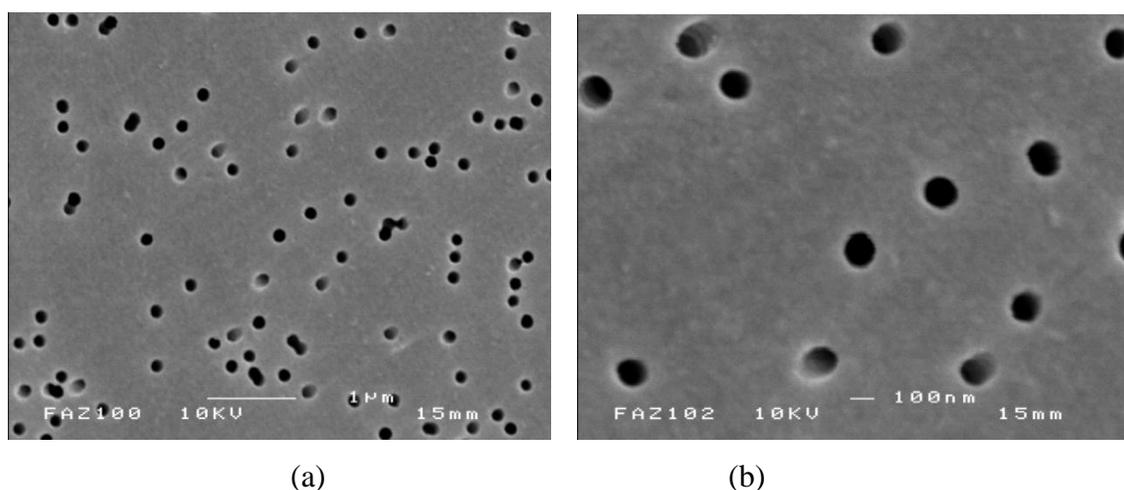


Fig. 6.100: Surface of a 0.2 micron pore diameter PVP-PC membrane, after 1 hour exposure to 4 % w/v fibrinogen solution. (Magnification: (a) = x 20,000; (b) = x 50,000)

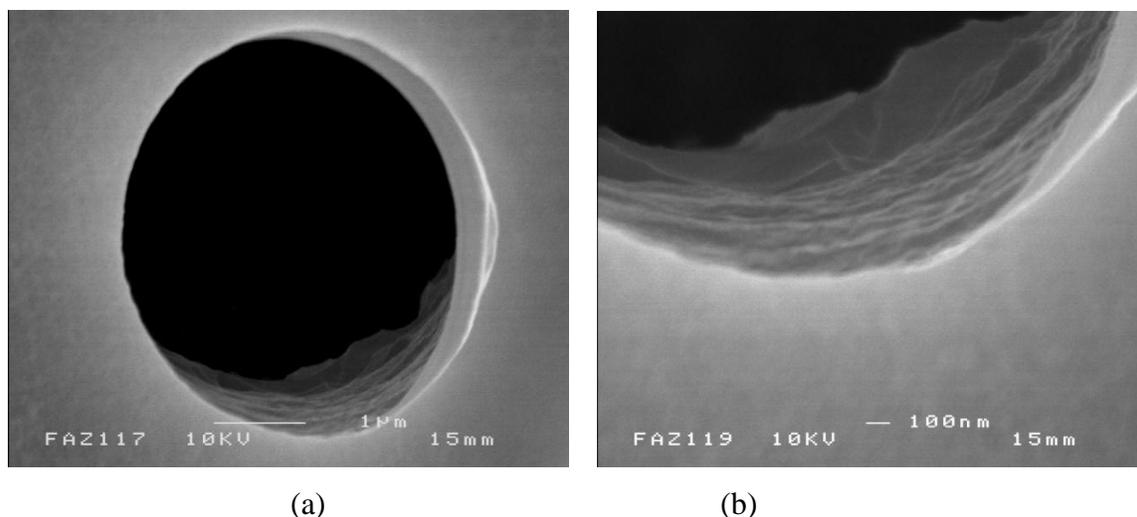


Fig. 6.101: Surface of a 5.0 micron pore diameter PVP-PC membrane, after 1 hour exposure to 4 % w/v fibrinogen solution. (Magnification: (a) = x 20,000; (b) = x 50,000)

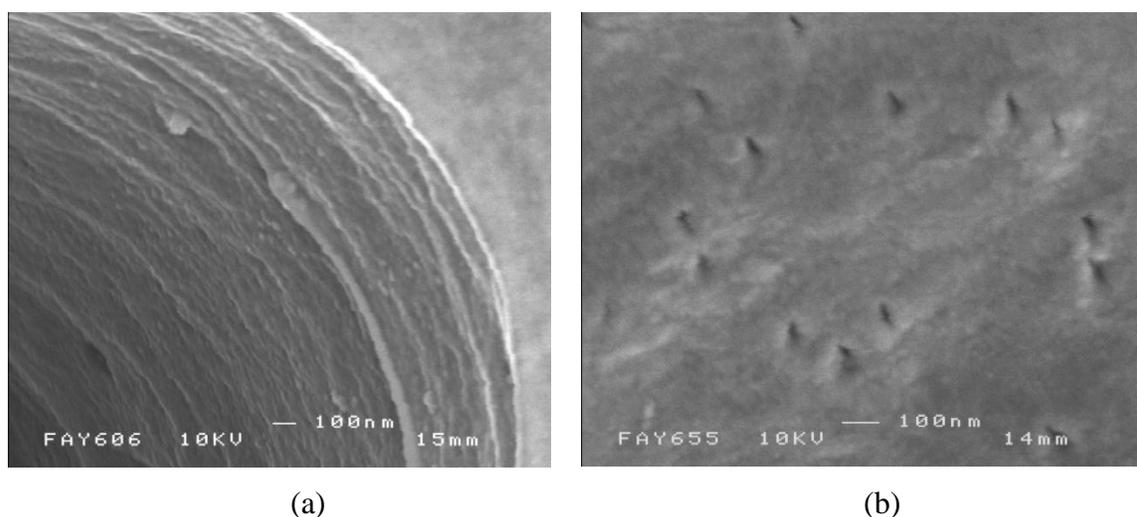


Fig. 6.102: Surface of (a) 5.0  $\mu\text{m}$  and (b) 0.05  $\mu\text{m}$  pore diameter PVP-PC membrane, after 24 hours exposure to 4 % w/v fibrinogen solution. (Magnification: (a) = x 50,000 (b) = x 80,000)

The micrographs show that exposure to the solution of fibrinogen leads to deposition of protein film on the surface of PVP coated poly(carbonate) membranes, which can be more clearly observed in lower pore size membranes. This layer of protein deposit is observed on the surface of the membrane after one hour post exposure (Fig. 6.99, 6.100).

Pore blocking is not observed after one hour on the surface of 0.05 micron pore diameter PVP coated poly(carbonate) membrane, rather formation of another membranous

structure on top of the membrane can be seen (Fig. 6.99). However, the pores are observed to be blocking up and the protein deposit appears to be thicker and non uniform after long exposure time (Fig. 6.102 b). The 5.0 micron pore diameter membrane has the least amount of protein deposited around the pores, only a few of the protein aggregates can be seen at longer exposure times (Fig. 6.102(a)).

The results show that the pvp coated polycarbonate membranes of all pore sizes i.e. 0.05, 0.2 and 5.0 micron pore diameter, are modified significantly after one hour of exposure to fibrinogen solution. At longer exposure to the solution of fibrinogen the pvp coated polycarbonate membranes with smallest pore size suffer severe fouling. However, the largest pore sized pvp-pc membrane is observed to have protein coating inside the pores of the membrane at long exposure times.

The experiments were repeated with hyaluronan using all pore sizes of the pvp coated pc membranes.

#### 6.4.6 PVP – PC membrane post exposure to Hyaluronan

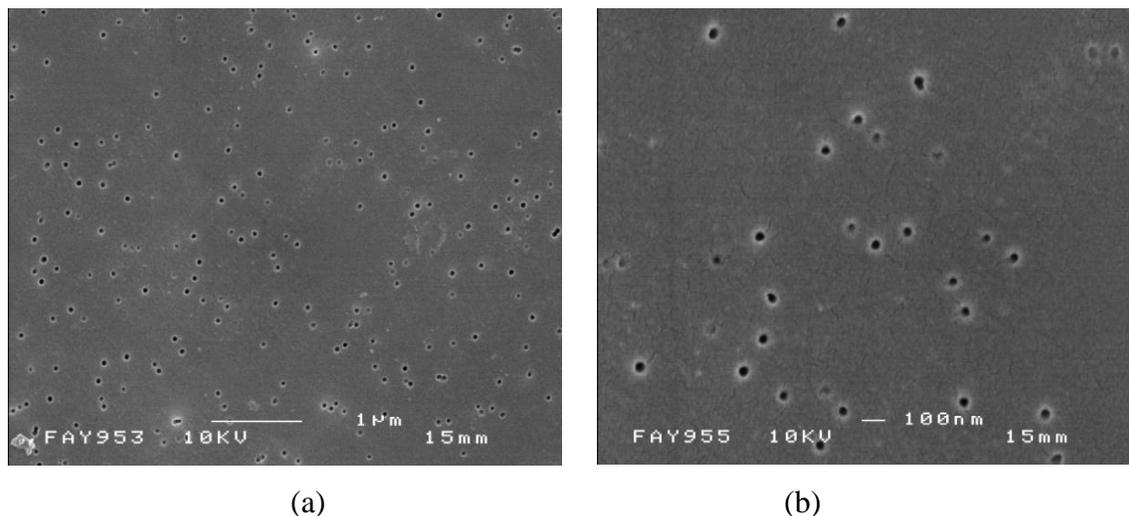


Fig. 6.103: Surface of a 0.05 micron pore diameter PVP-PC membrane, after 1 hour exposure to 1 % w/v hyaluronan solution. (Magnification: (a) = x 20,000; (b) = x 50,000)

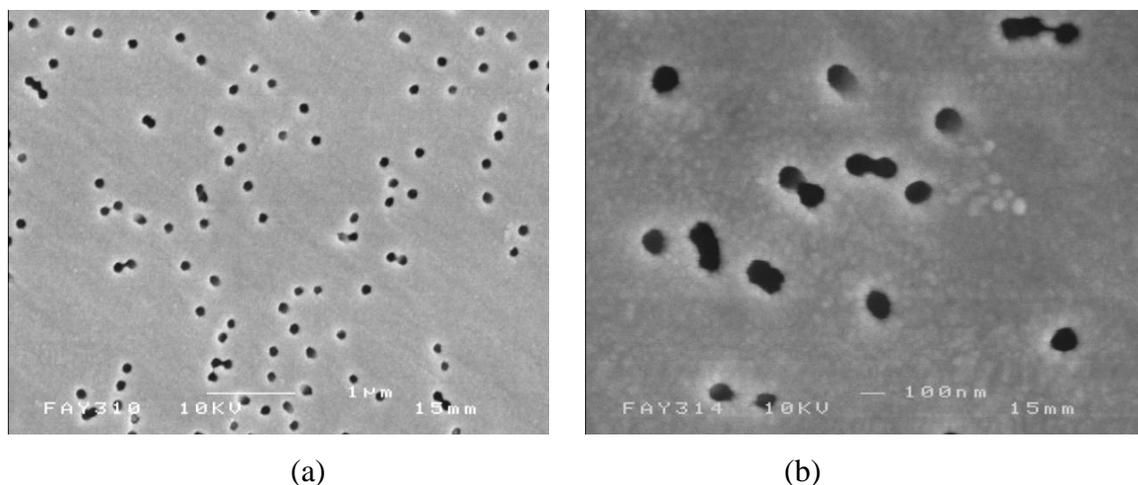


Fig. 6.104: Surface of a 0.2 micron pore diameter PVP-PC membrane, after 1 hour exposure to 1% w/v hyaluronan solution. (Magnification: (a) = x 20,000; (b) = x 50,000)

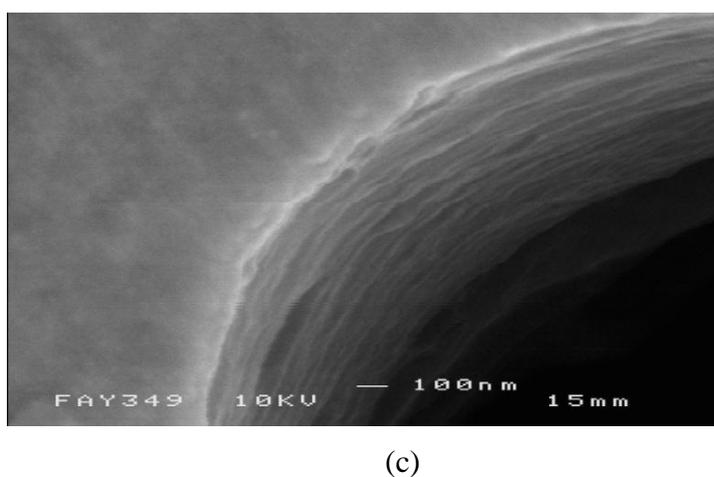
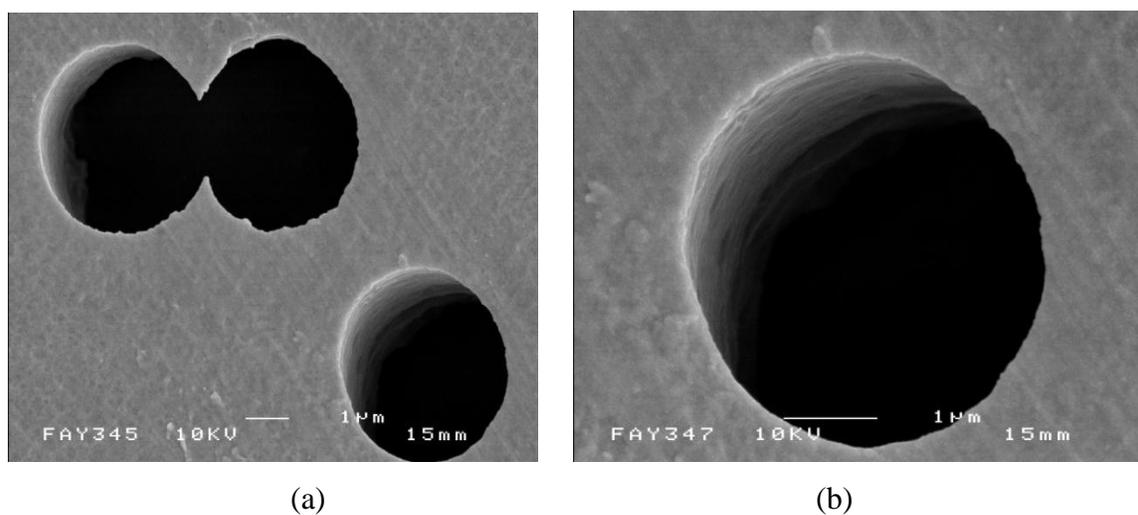


Fig. 6.105: Surface of a 5.0 micron pore diameter PVP-PC membrane, after 1 hour exposure to 1 % w/v hyaluronan solution. (Magnification: (a) x 10, 000 (b) = x 20,000; (c) = x 50,000)

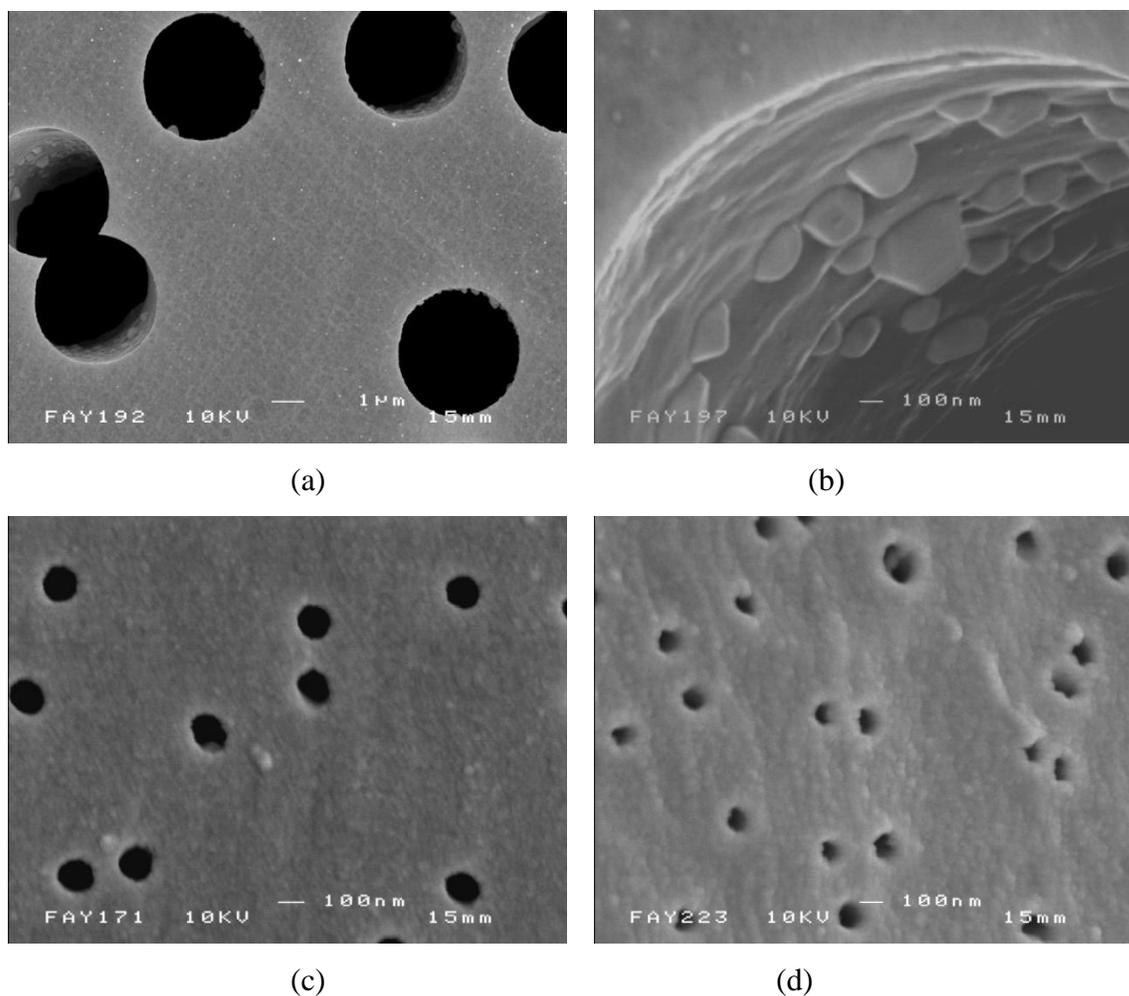


Fig. 6.106: Surface of (a) 5.0  $\mu\text{m}$  surface and (b) 5.0  $\mu\text{m}$ , inner surface of the pore, (c) 0.2  $\mu\text{m}$  surface, (d) 0.05  $\mu\text{m}$  pore diameter PVP-PC membrane, after 24 hours exposure to 1 % W/V hyaluronan solution. (Magnification: (a) = x 7,000 (b), (c) and (d) = x 50, 000)

The results show that exposure to hyaluronan solution results in deposits of hyaluronan being adsorbed on the surface of the PVP coated poly (carbonate) membranes after one hour of exposure. Aggregates as well as a thick layer of coating of hyaluronan can be seen on the entire surface of the membrane regardless of the pore size after one hour.

After 24 hours of exposure a thicker cake like granular textured deposit can be observed on the entire surface of the 0.05 micron pore diameter membrane. However, in addition to the thicker layer of the deposit on the 5.0 micron pore diameter membrane rather large

globular forms of deposits can also be seen on inside of the pores (Fig. 6.106 (a), (b)). Pores of the membrane are not observed to get completely blocked for either pore size.

The results for hyaluronan and fibrinogen are similar in that the pores of the largest pore size pvp-pc membranes are observed to be modified. Granular, circular and globular adsorbate is observed on inside of the pores as well as on the surface of the membranes. It can be due to the fact that aggregates of the proteins and hyaluronan form at short exposure times and then they grow in size at longer exposure times, modifying the entire surface as well as inside of the pores of the membranes.

The experiments were repeated with foetal calf serum using all sizes of the pvp-pc membranes.

#### 6.4.7 PVP-PC membrane, post exposure to Foetal Calf Serum

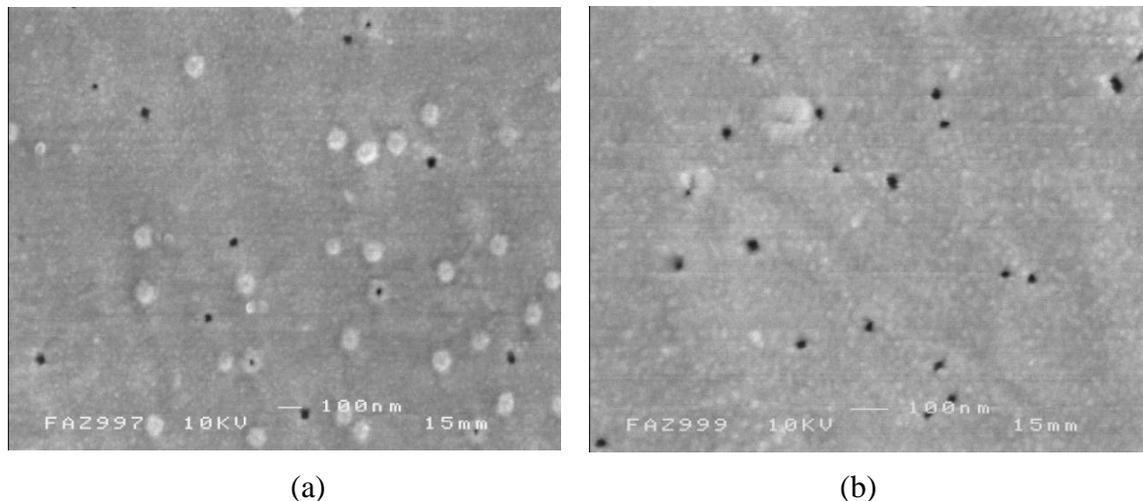


Fig. 6.107: Surface of a 0.05 micron pore diameter PVP-PC membrane, after 1 hour exposure to foetal calf serum. (Magnification: (a) = x 50,000; (b) = x 80,000)

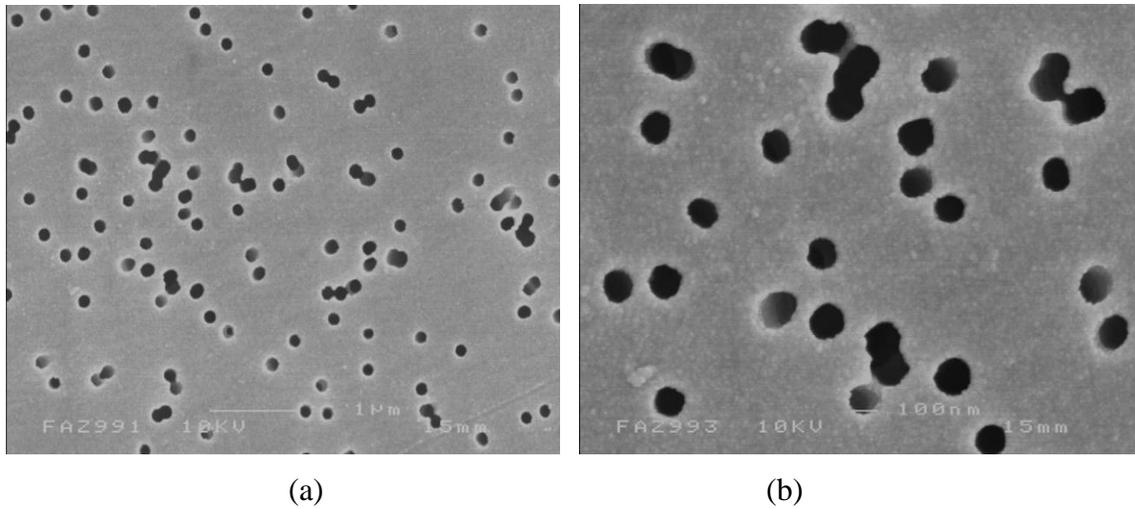


Fig. 6.108: Surface of a 0.2 micron pore diameter PVP-PC membrane, after 1 hour exposure to foetal calf serum. (Magnification: (a) = x 20,000; (b) = x 50,000)

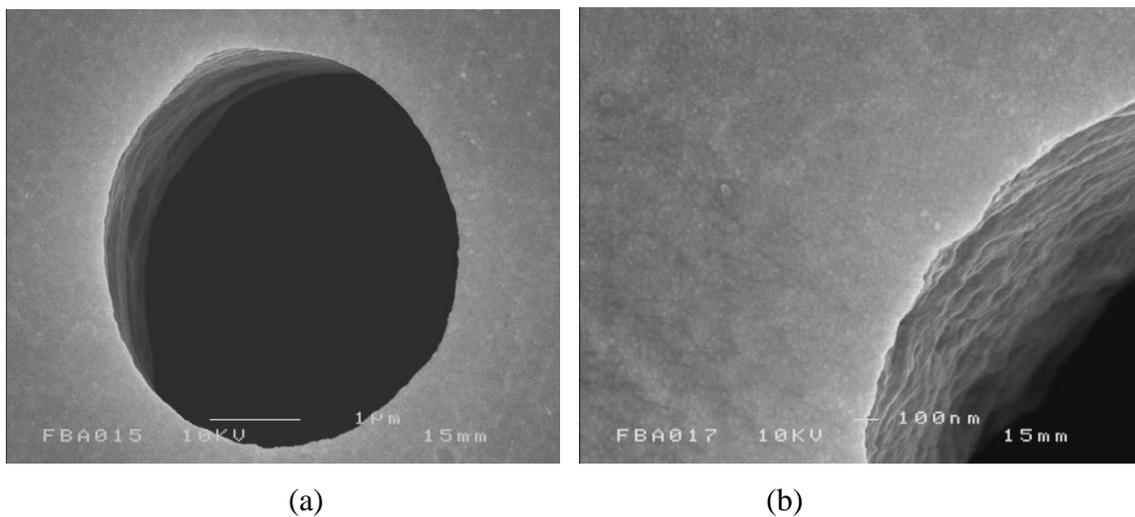


Fig. 6.109: Surface of a 5.0 micron pore diameter PVP-PC membrane, after 1 hour exposure to foetal calf serum. (Magnification: (a) = x 20,000; (b) = x 50,000)

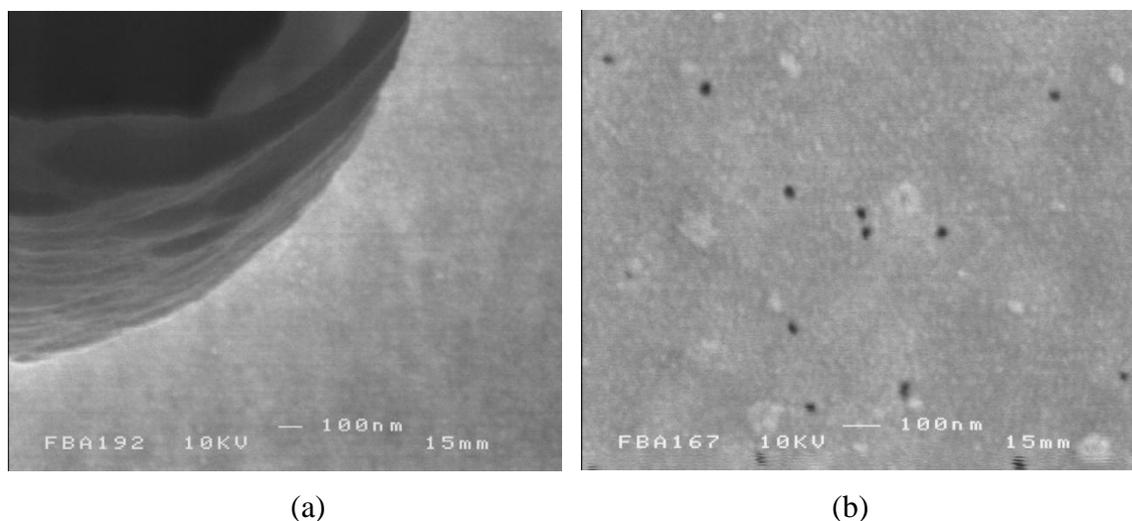


Fig. 6.110: Surface of (a) 5.0  $\mu\text{m}$  and (b) 0.05  $\mu\text{m}$  pore diameter PVP-PC membrane, after 24 hours exposure to foetal calf serum. (Magnification: (a) = x 50,000 (b) = x 80,000)

The results show that exposure to foetal calf serum results in fouling of the pvp-pc membranes. The deposits are observed on the surface of the membrane for all of the membranes investigated (Fig. 6.107, 6.108). With 5.0 micron pore diameter membrane the deposits are only observed on the surface of the membrane in the form of a cake like layer after one hour of exposure and the morphology does not appear to change after twenty four hours of exposure. However, with the 0.05 micron pore diameter membrane the longer exposure times lead to thickening of the layer of deposit on surface of the membrane and the aggregates are observed to have grown in size, moreover pore blocking seems to be taking place (Fig. 6.110(b)).

The fouling behaviour with foetal calf serum is similar to the fouling behaviour to other proteins investigated as well as hyaluronan. The smallest pore size i.e. 0.05 micron pore diameter polycarbonate membranes were observed to be fouled very extensively as compared to the larger pore sizes polycarbonate membranes.

The concentration of proteins can have an effect on the fouling behaviour of the membranes. Hence, investigations were carried out to determine how the different concentrations of the same protein fouled the membranes of the same pore sizes.

## 6.5 Effect of concentration on biofouling of polymer membranes

The field emission scanning electron microscope was used to investigate the effect of protein concentration on fouling of membranes. The albumin solution of concentration of 4 % w/v and 10 % w/v was used for these studies.

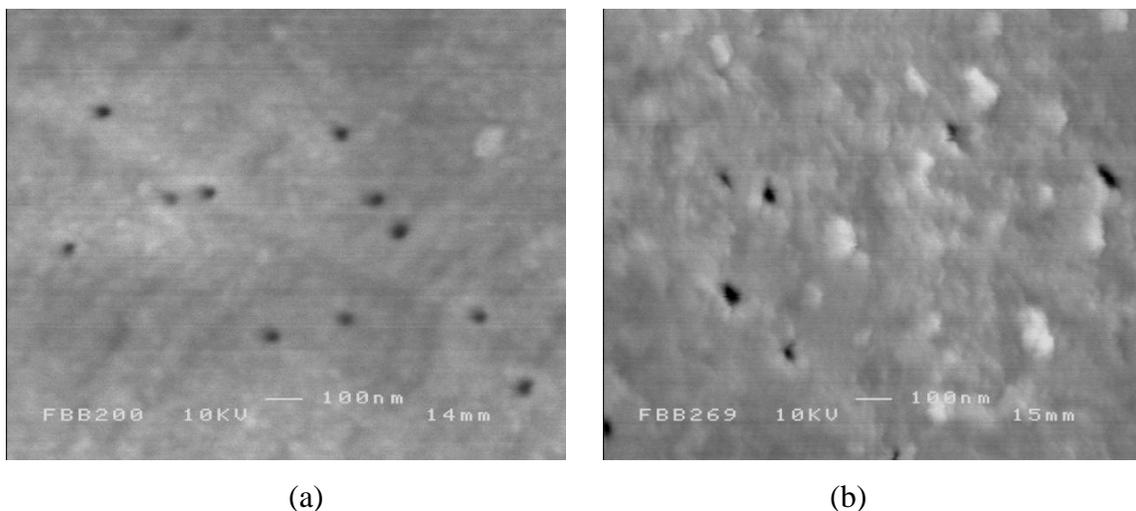


Fig. 6.111: Surface of a 0.05 micron pore diameter PVP-PC membrane, after 1 hour exposure to (a) 4 % w/v (b) 10 % w/v solution of albumin. (Magnification: x 80,000)

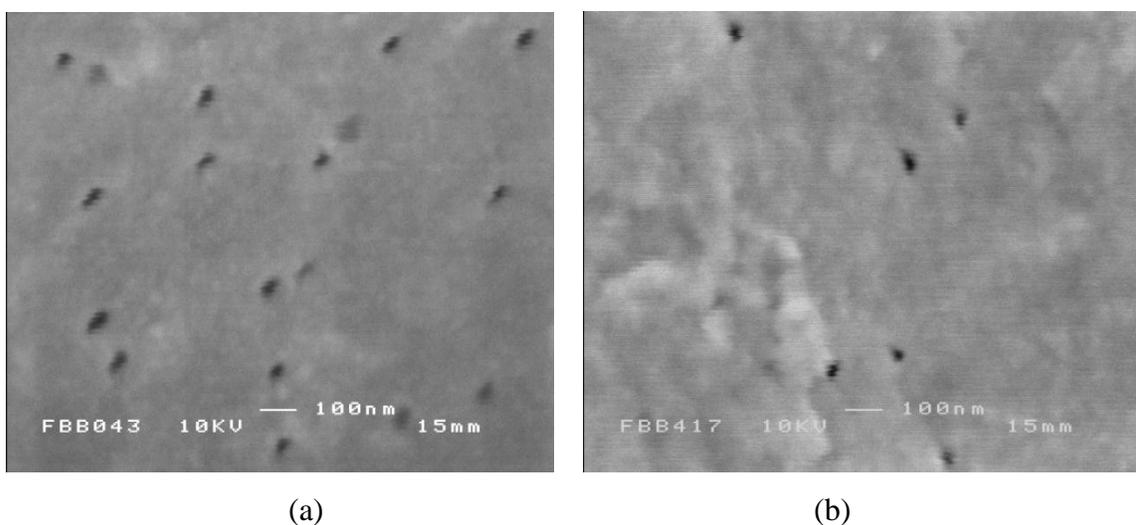


Fig.6.112: Surface of a 0.05 micron pore diameter PVP-PC membrane, after 24 hours exposure to (a) 4 % w/v (b) 10 % w/v solution of albumin. (Magnification: x 80,000)

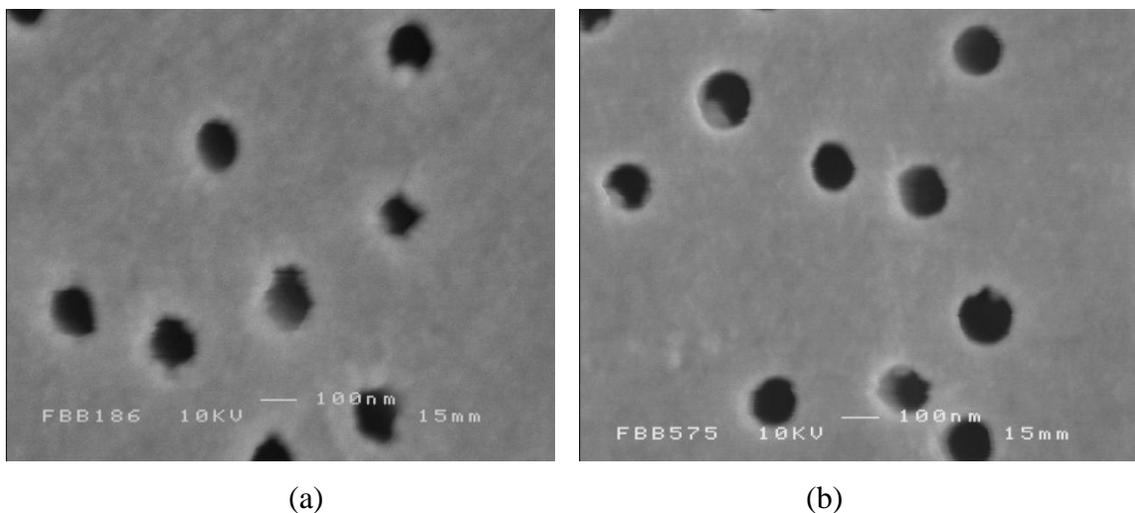


Fig. 6.113: Surface of a 0.2 micron pore diameter PVP-PC membrane, after 1 hour exposure to (a) 4 % w/v (b) 10 % w/v solution of albumin. (Magnification: x 80,000).

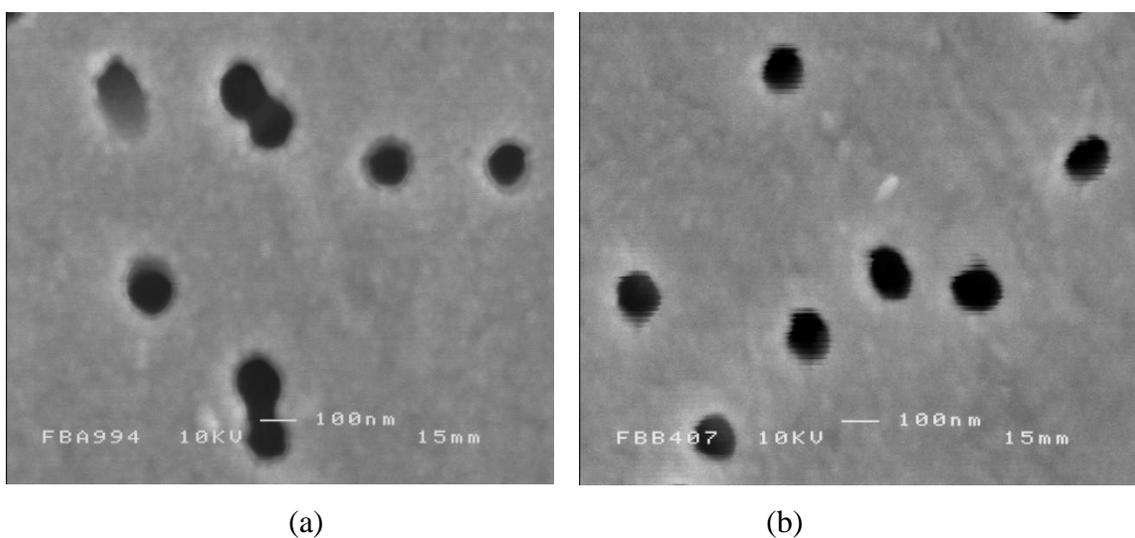


Fig. 6.114: Surface of a 0.2 micron pore diameter PVP-PC membrane, after 24 hour exposure to (a) 4 % w/v (b) 10 % w/v solution of albumin. (Magnification: x 80,000)

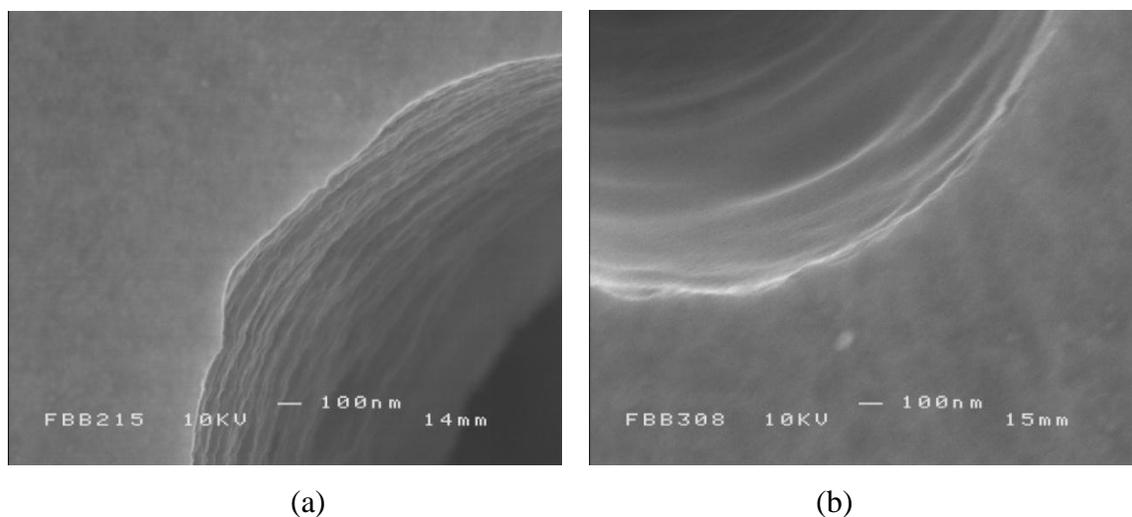


Fig. 6.115: Surface of a 5.0 micron pore diameter PVP-PC membrane, after 1 hour exposure to (a) 4 % w/v (b) 10 % w/v solution of albumin. (Magnification: x 50,000).

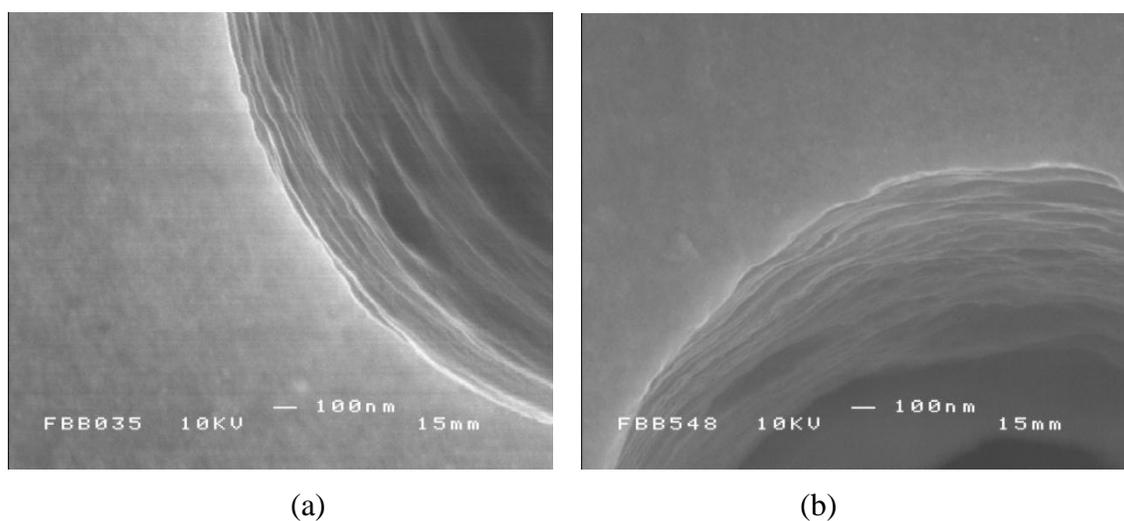


Fig. 6.116: Surface of a 5.0 micron pore diameter PVP-PC membrane, after 24 hours exposure to (a) 4 % w/v (b) 10 % w/v solution of albumin. (Magnification: x 50,000).

The results show that when lower pore size PVP-PC membranes are exposed to higher concentrations of albumin solution, the protein deposits in the form of a thick layer on the surface of the membrane. Moreover, the aggregates of the protein are deposited not only just on the surface of the membrane but also in and around pores of the membrane as can be seen in Fig. 6.111(b) and 6.113(b). With longer exposure times these aggregates of protein grow in size (Fig. 6.112(b) and 6.114(b)).

The pattern of fouling is the same for lower and higher concentration of protein, only the size of the aggregates is larger for higher concentrations along with thickness of the protein layer on surface of the membrane. For a larger pore size membrane, i.e. 5.0 micron pore diameter PVP-PC membrane, protein adsorbs on the surface of the membrane after exposure to higher concentration solution of protein, some aggregates on the surface of the membrane are observed at longer exposure times.

It can be deduced from these results that protein initially is deposited in form of a layer on the surface of an isoporous track etched membrane. Pore blocking is observed to take place after longer exposure times and higher concentrations of proteins along with a thicker cake like layer of deposit and formation of aggregates of protein.

The pH of the solution can have an effect on the fouling behaviour of a membrane by proteins and macromolecules.

## **6.6 Effect of pH on biofouling of polymer membranes**

Phosphate buffer of pH 3 was used for these studies. The 0.05 micron, 0.2 micron and 5.0 micron pore diameter PVP and non PVP-poly(carbonate) membranes were used for these investigations. The protein used was Albumin at 10 % w/v at 1 hour and 24 hours exposure times.

### 6.6.1 PVP-PC membranes post exposure to phosphate buffer at pH 3

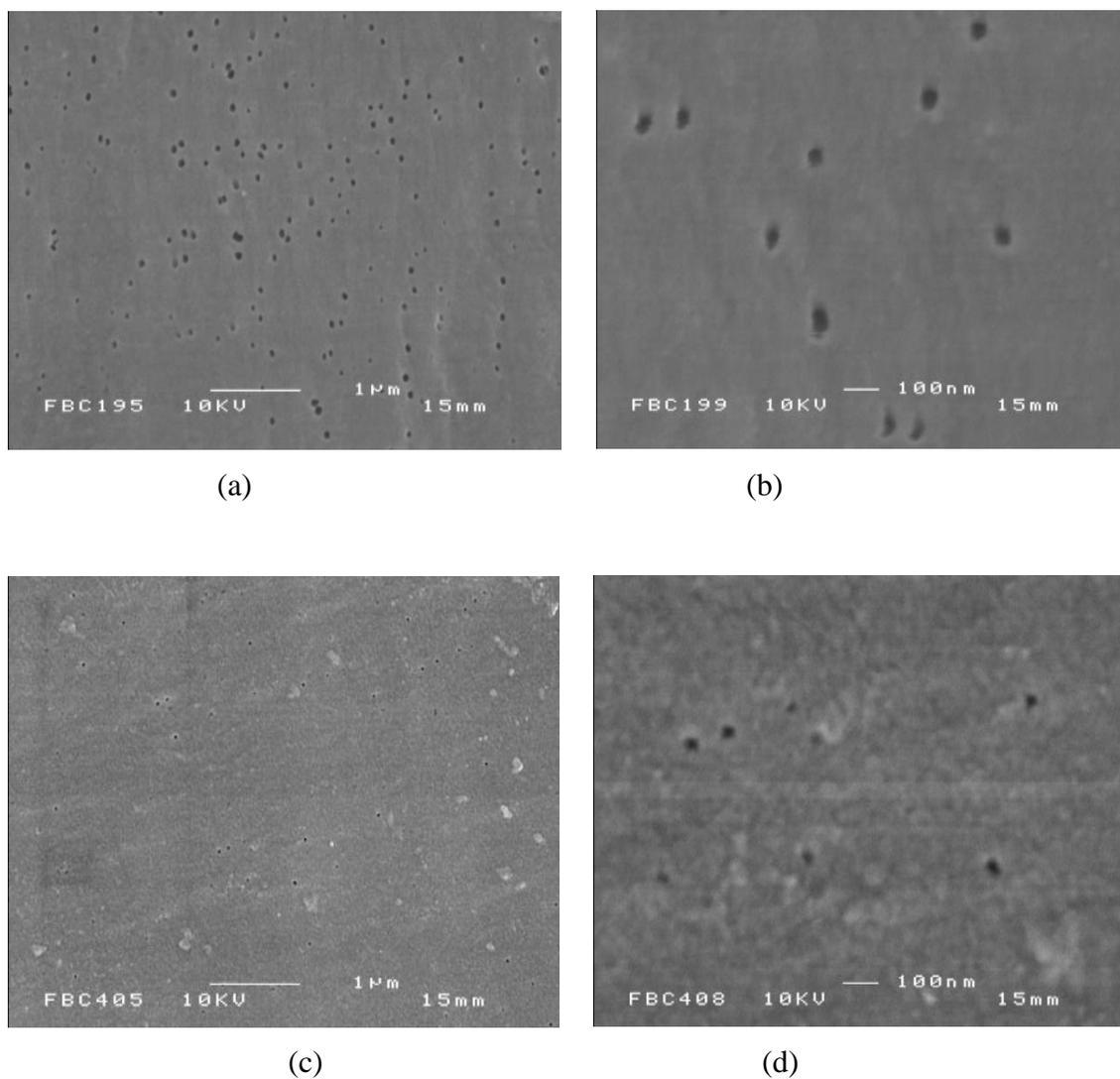


Fig. 6.117: Surface of a 0.05 micron pore diameter PVP-PC membrane after exposure to buffer pH 3 (a),(b) after 1 hour exposure; (c),(d) 24 hours post exposure (Magnification: (a), (c) = x 20,000; (b), (d) = x 80,000).

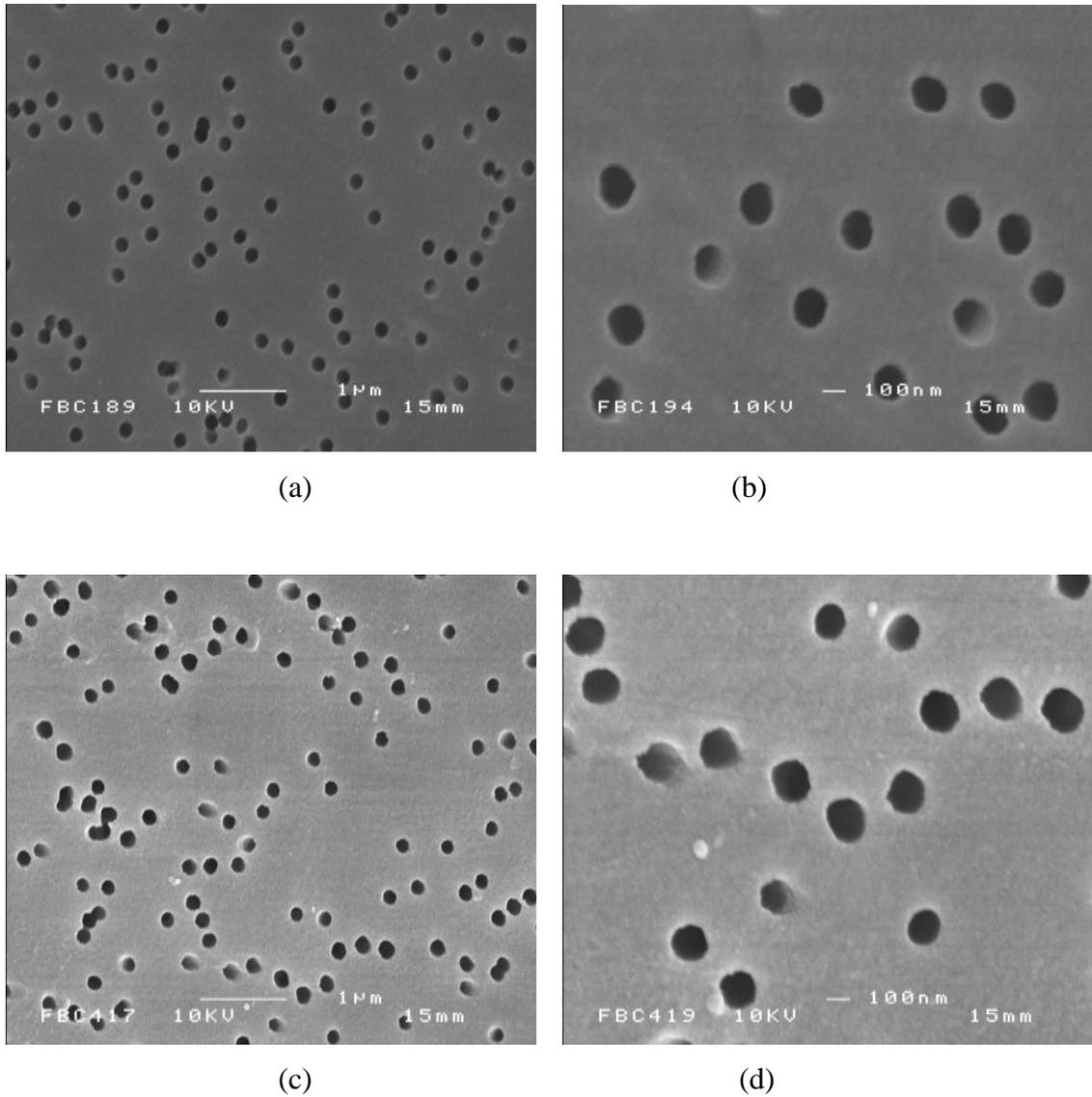


Fig. 6.118: Surface of a 0.2 micron pore diameter PVP-PC membrane after exposure to buffer pH 3 (a),(b) after 1 hour exposure; (c),(d) 24 hours post exposure (Magnification: (a), (c) = x 20,000; (b), (d) = x 50,000).

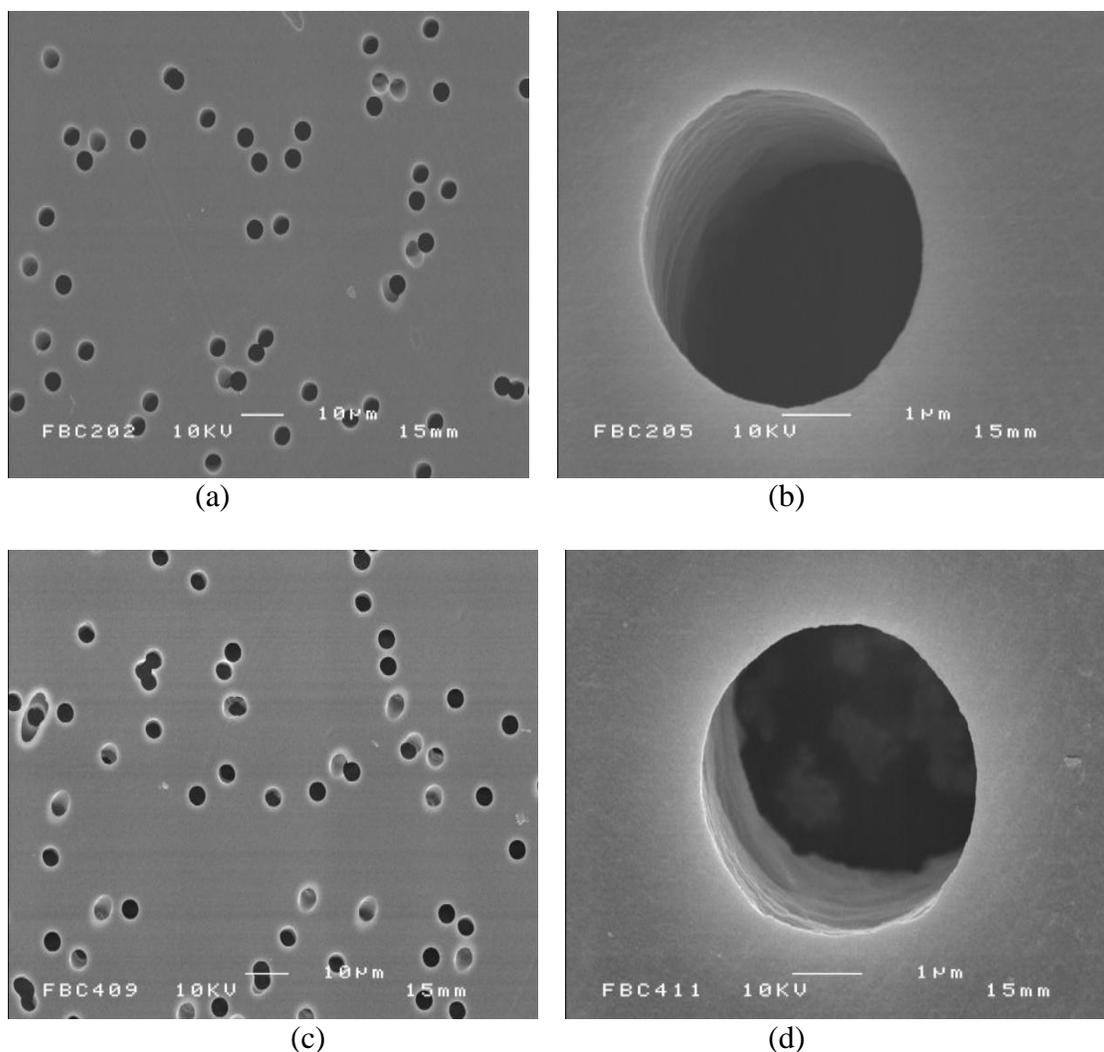


Fig. 6.119: Surface of a 5.0 micron pore diameter PVP-PC membrane after exposure to buffer pH 3 (a),(b) after 1 hour exposure; (c),(d) 24 hours post exposure (Magnification: (a), (c) = x 1000; (b), (d) = x 15,000).

The results show that the PVP-PC membranes are stable after one hour exposure to the phosphate buffer solution at pH 3. However, after twenty four hours exposure, the morphology of the membrane is observed to change and clumps of material can be seen on the surface of these membranes (Fig. 6.117(c, d), 6.118 (c, d) 6.119(d)). These features on the surface of a membrane, at longer exposure times, are more evident for smaller pore size membranes, i.e. 0.05 micron and 0.2 micron pore diameter (Fig. 6.117(c), 6.118(d)) than 5.0 micron pore diameter membranes (Fig. 6.119).

The experiments were repeated with PVP free polycarbonate membranes using phosphate buffer at pH 3 to investigate if pH had any effect on the stability of these membranes.

### 6.6.2 PC membranes post exposure to phosphate buffer at pH 3

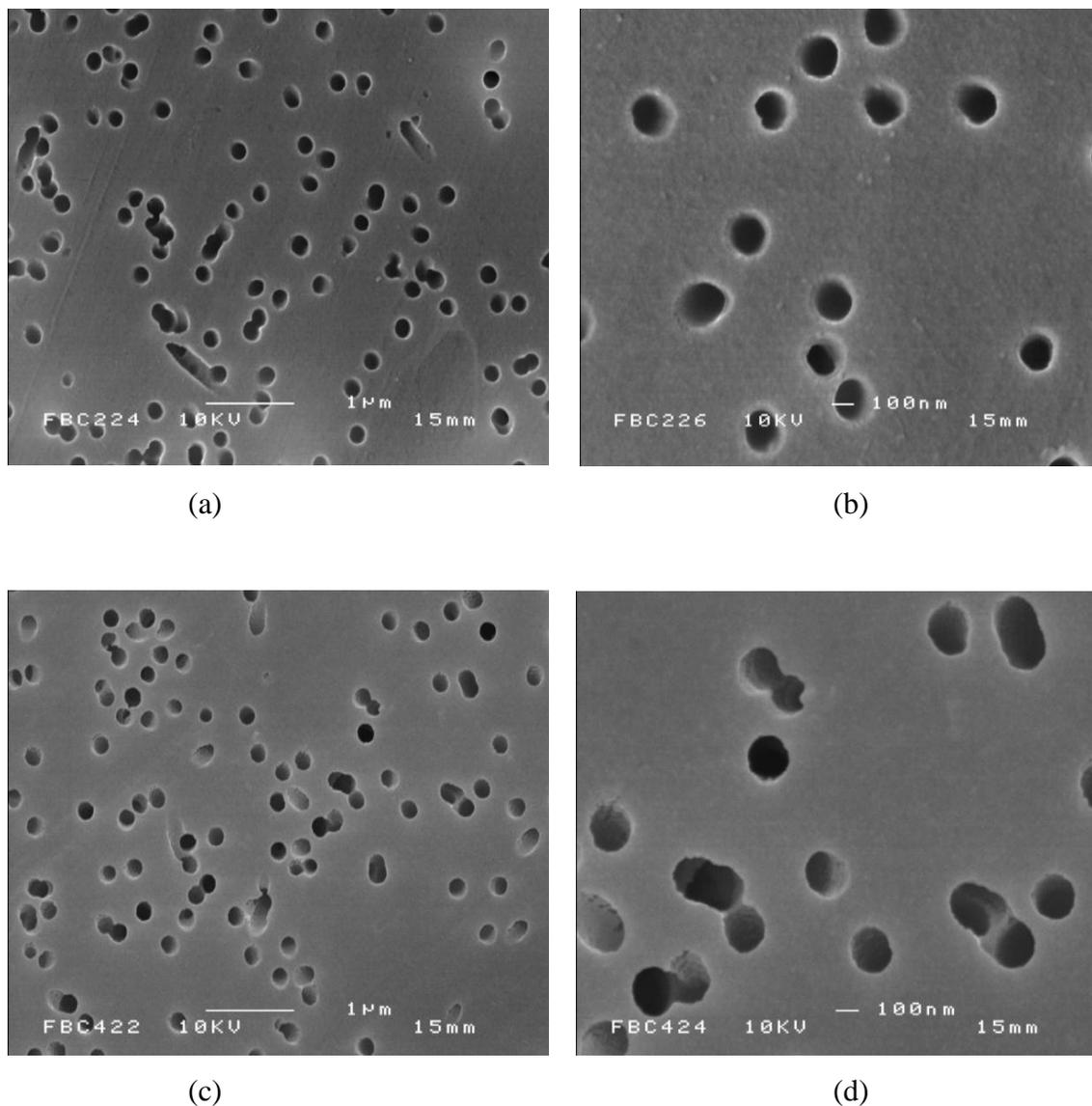


Fig. 6.120: Surface of a 0.2 micron pore diameter PC membrane after exposure to buffer pH 3 (a),(b) after 1 hour exposure; (c),(d) 24 hours post exposure (Magnification: (a), (c) = x 20,000; (b), (d) = x 50,000).

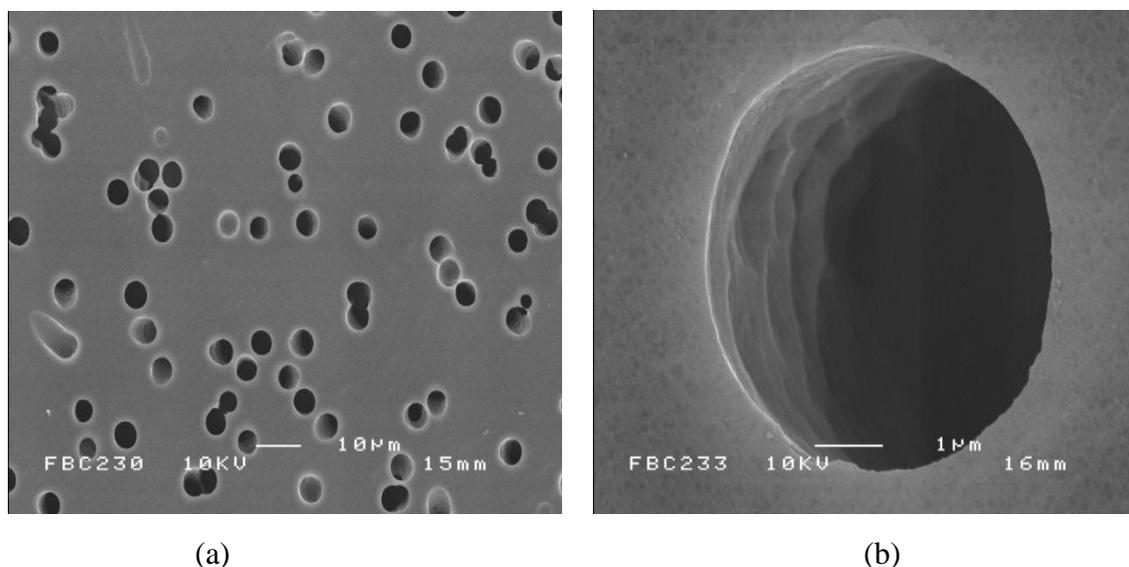


Fig. 6.121: Surface of a 5.0 micron pore diameter PC membrane after exposure to buffer pH 3 after 1 hour exposure (Magnification: (a) = x 1000; (b) = x 15,000).

The micrographs show that poly (carbonate) membranes show slight changes on exposure to phosphate buffer at pH 3. Poly(carbonate) membranes are stable at longer exposure times to phosphate buffer of pH 3 (Fig. 6.120 (c, d)) This difference could be due to the PVP coating being dissolved in the case of PVP-PC, whereas the uncoated poly(carbonate) is observed to be more stable at pH 3.

The polycarbonate membranes were then used to study the effect of the pH of the protein solution on the protein to polymer interaction and to investigate the fouling behaviour of the protein by varying the pH of the protein solution.

### 6.6.3 PVP-PC membranes post exposure to Albumin at pH 3

In this study the pvp-polycarbonate membranes were exposed to the albumin solution at high concentration i.e. 10 % w/v for one hour and twenty four hours at pH 3 to investigate if the pH of the protein solution had any effect on the fouling profile and adsorption behaviour of albumin on the pvp-polycarbonate membrane.

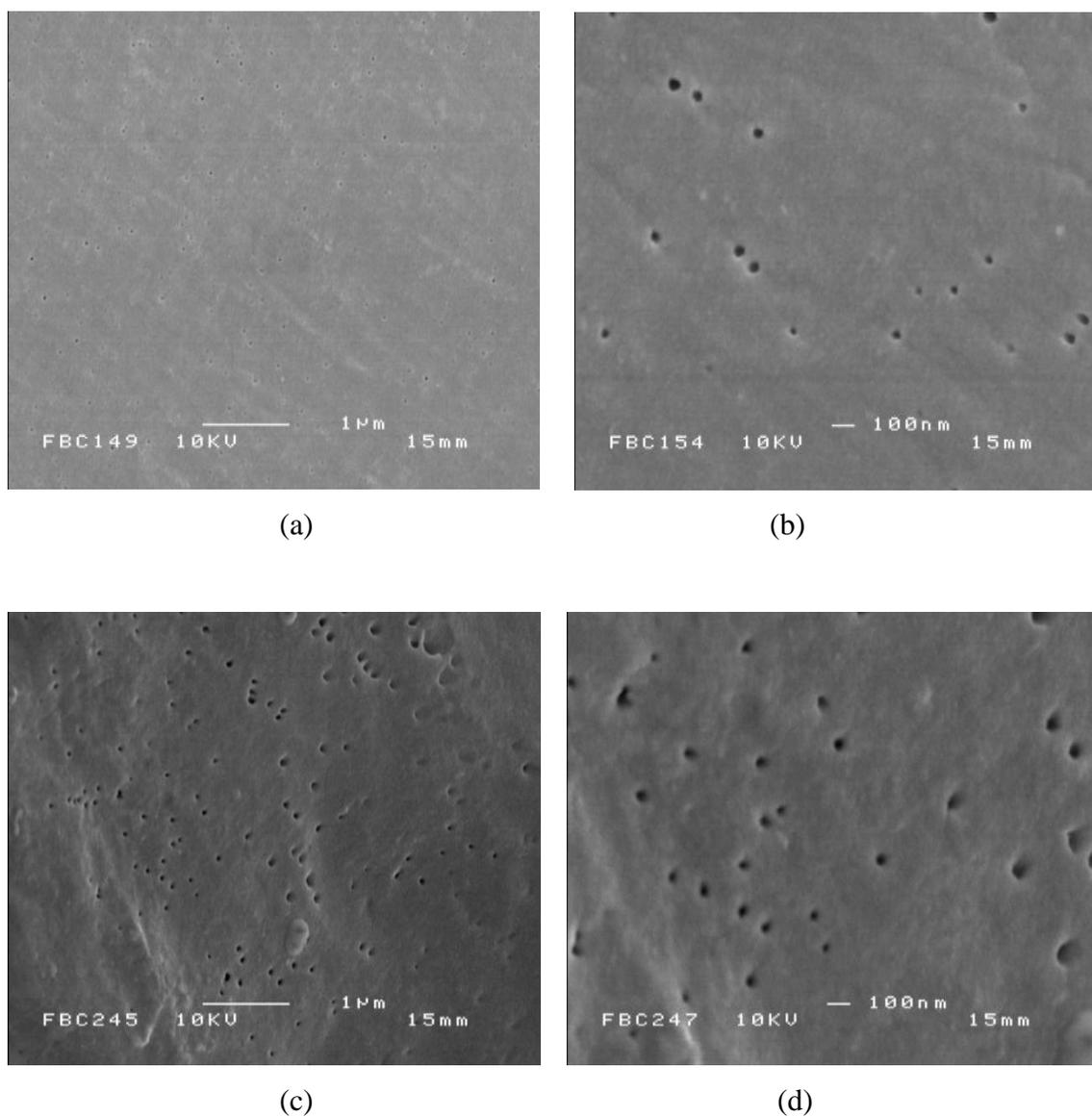


Fig. 6.122: Surface of a 0.05 micron pore diameter PVP-PC membrane after exposure to 10 % w/v Albumin solution at pH 3 (a),(b) after 1 hour exposure; (c),(d) 24 hours post exposure (Magnification: (a), (c) = x 20,000; (b), (d) = x 50,000).

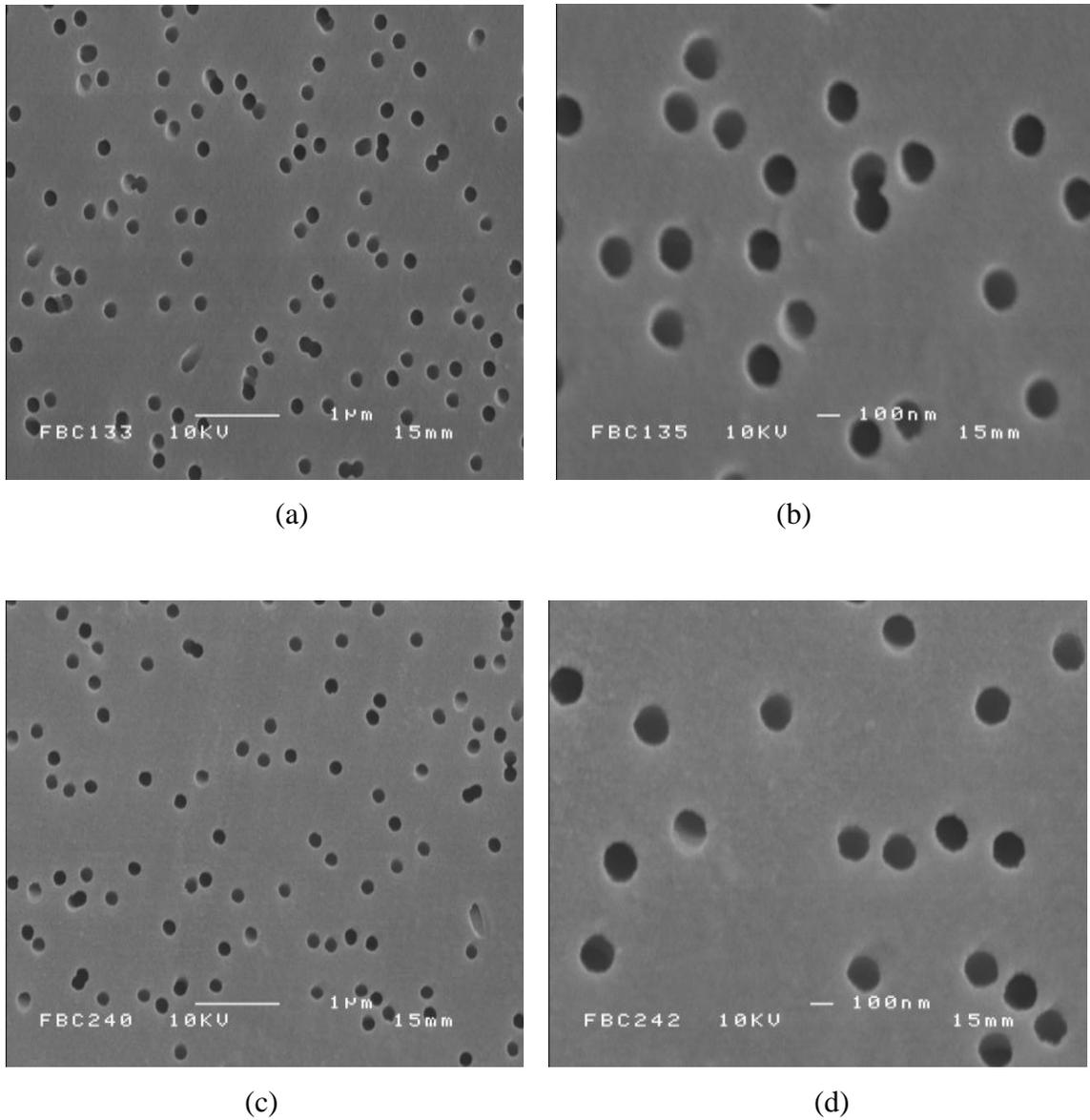


Fig. 6.123: Surface of a 0.2 micron pore diameter PVP-PC membrane after exposure to 10 % w/v Albumin solution at pH 3 (a),(b) after 1 hour exposure; (c),(d) 24 hours post exposure (Magnification: (a), (c) = x 20,000; (b), (d) = x 50,000).

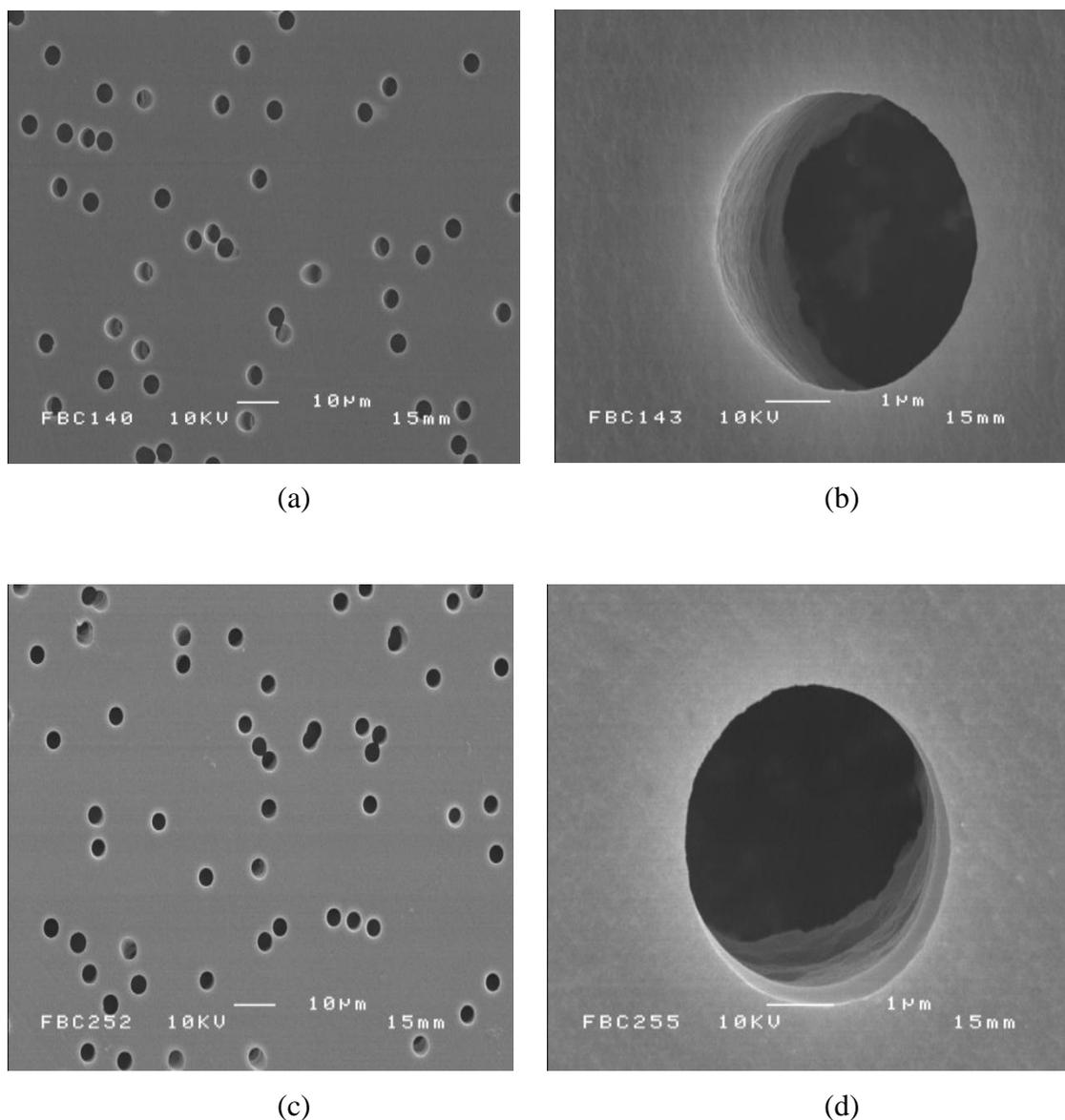


Fig. 6.124: Surface of a 5.0 micron pore diameter PVP-PC membrane after exposure to 10 % w/v Albumin solution at pH 3 (a),(b) after 1 hour exposure; (c),(d) 24 hours post exposure (Magnification: (a), (c) = x 1000; (b), (d) = x 15,000).

The results show that the surface of the PVP-PC does not undergo significant morphological changes after one hour exposure to 10 % w/v solution of albumin at pH 3. However, the surface of the 0.05micron pore diameter, i.e. the smallest pore size membrane, shows some changes, a layer of protein and some aggregates can be seen on the surface of the membrane (Fig. 6.122 (a, b)). At longer exposure times, significant changes in the morphology are observed only for the 0.05 micron pore diameter

membrane, a thick layer of adsorbed protein can be seen on the surface of the membrane (Fig. 6.122 (c, d)). Aggregates of protein can also be seen on the surface of the 0.2 micron and 5.0 micron pore diameter membranes at longer exposure times (Fig. 6.123 (d), 6.124 (d)).

The experiments were repeated with PVP free polycarbonate membranes.

#### **6.6.4 Poly (carbonate) membranes post exposure to Albumin solution at pH 3**

The pvp free polycarbonate membranes were exposed to 10 % w/v solution of albumin at pH 3 for this investigation, in order to study the effect of varying the pH of protein solution on fouling behaviour of polymer membranes. The effect of exposure time on adsorption of proteins on polymer membranes at pH 3 was also investigated.

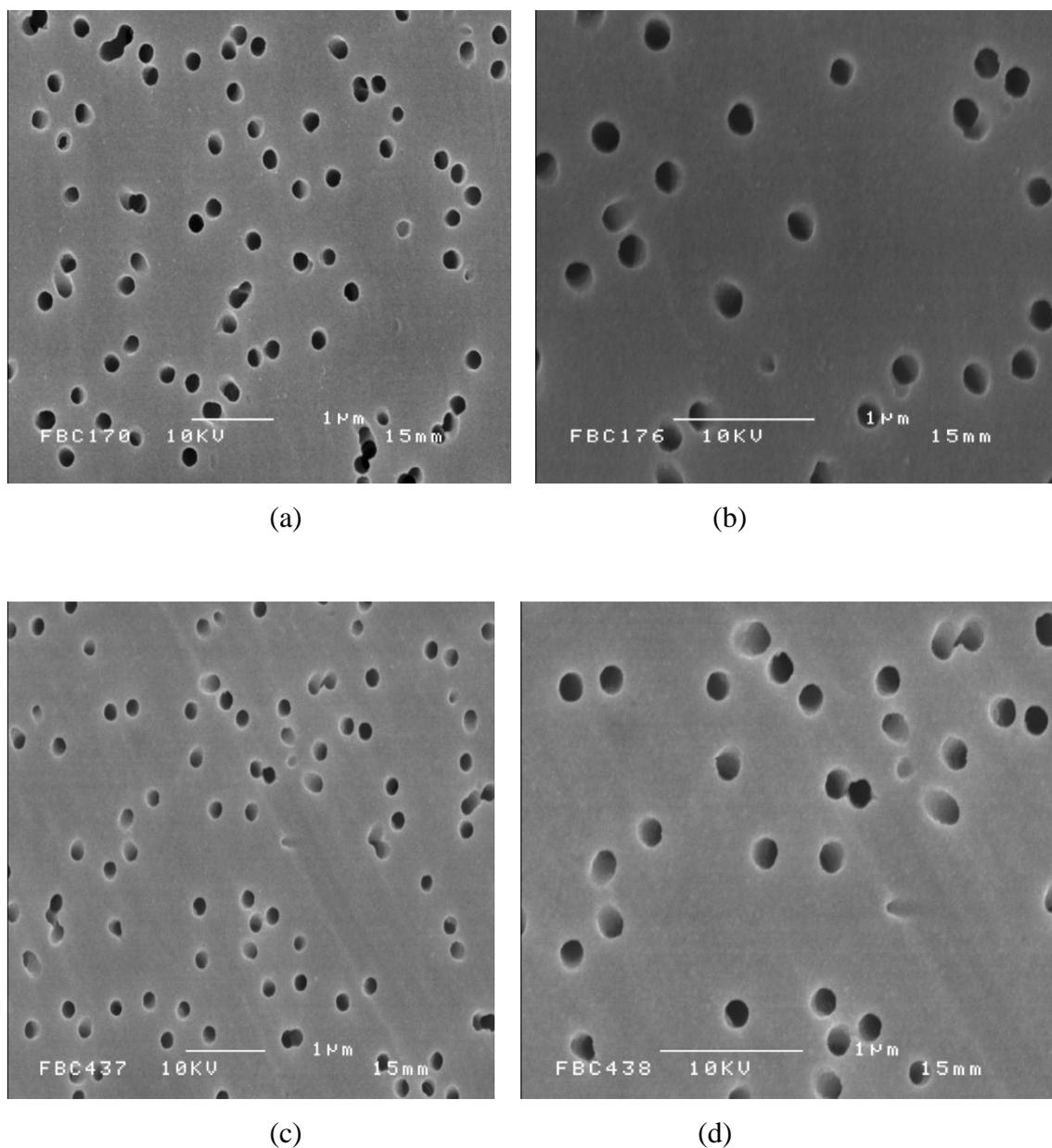


Fig. 6.125: Surface of a 0.2 micron pore diameter PC membrane after exposure to the 10 % w/v Albumin solution at pH 3 (a),(b) after 1 hour exposure; (c),(d) 24 hours post exposure (Magnification: (a), (c) = x 20,000; (b), (d) = x 33,000).

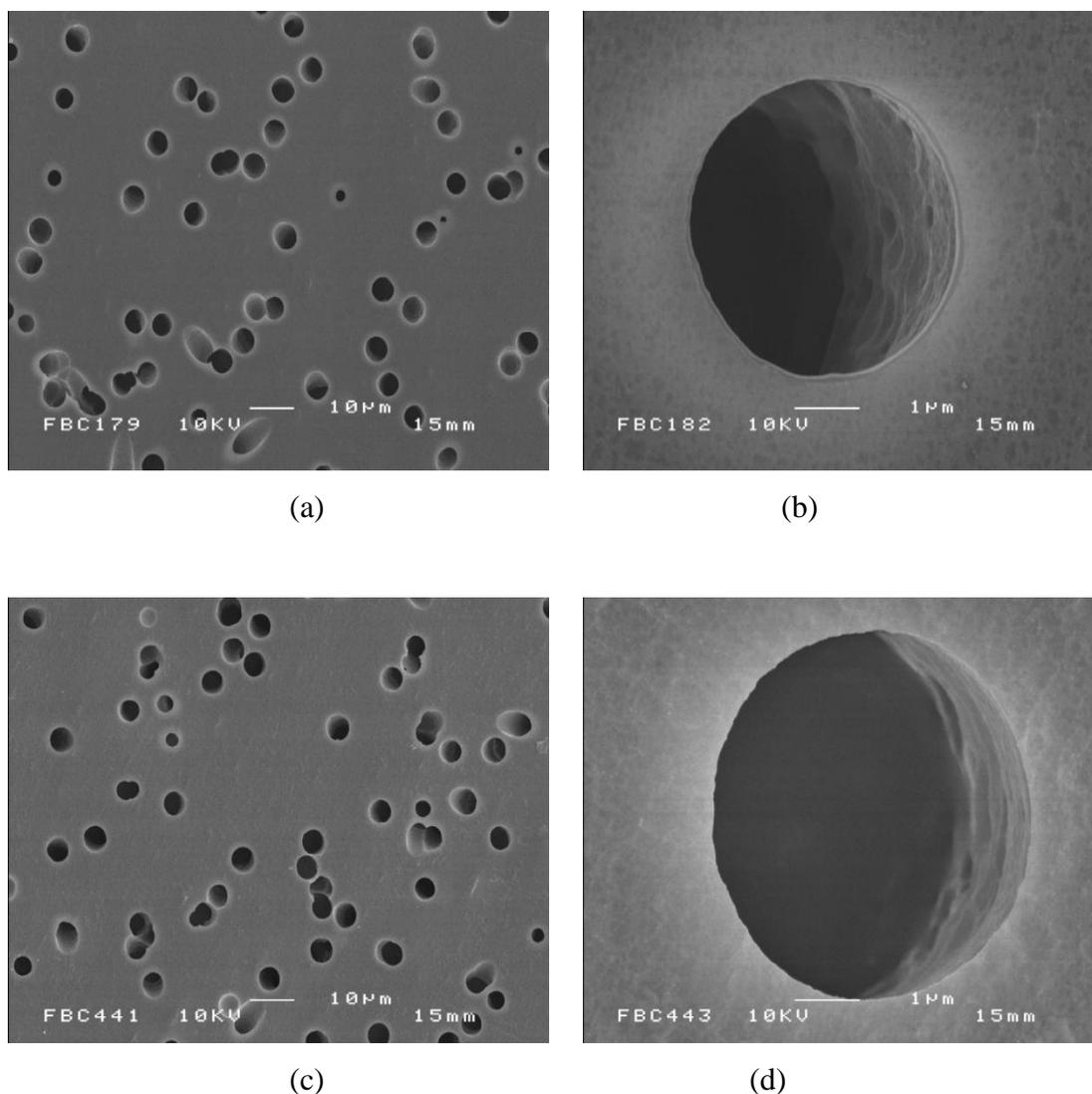


Fig. 6.126: Surface of a 5.0 micron pore diameter PC membrane after exposure to the 10 % w/v Albumin solution at pH 3 (a),(b) after 1 hour exposure; (c),(d) 24 hours post exposure (Magnification: (a), (c) = x 1000; (b), (d) = x 50,000)

The micrographs show that a layer of protein is adsorbed at the surface of poly(carbonate) membranes when exposed to albumin solution at pH 3 after one hour of exposure (Fig. 6.125 (a), 6.126 (a)). This layer of protein is observed to be thicker with some aggregates at longer exposure times, however, no deposits are observed on the inside of pores (Fig. 6.125 (d), 6.126 (d)).

The micrographs were used for statistical analysis of the adsorption phenomena using the image analysis software.

It is very difficult to study the exact nature of the adsorption of proteins and the thickness of the protein layers with the field emission microscope. Hence, further investigations were made using an atomic force microscope using polycarbonate membranes.

## **6.7 AFM study of modified PC membranes**

Visual observation of single molecules and atoms was not possible four decades ago. The groundbreaking research by Binnig et. al. (1986) opened the doors to this new dynamic dimension of atomic scale visualization of the biological systems. The atomic force microscope makes use of a probe that does not damage the surface of the material under investigation. It is the best technique available currently for visualization of biological membranes and proteins. AFM images at sub-molecular level provide a wealth of insight, allows for data to be acquired more efficiently and effectively than previously possible (Frederix et. al. 2009).

In the present study the atomic force microscope was used to visually observe the structure and morphology of the polymer membranes with and without adsorbed protein films. The images were used to study the surface roughness and the thickness of the deposited macromolecule films after a given period of time.

The 5.0 micron pore diameter membrane has been shown here for illustration of the result

E:\SARAH R\13-11-05\10%alb,5mic-pc-uncharge-1 .ffr

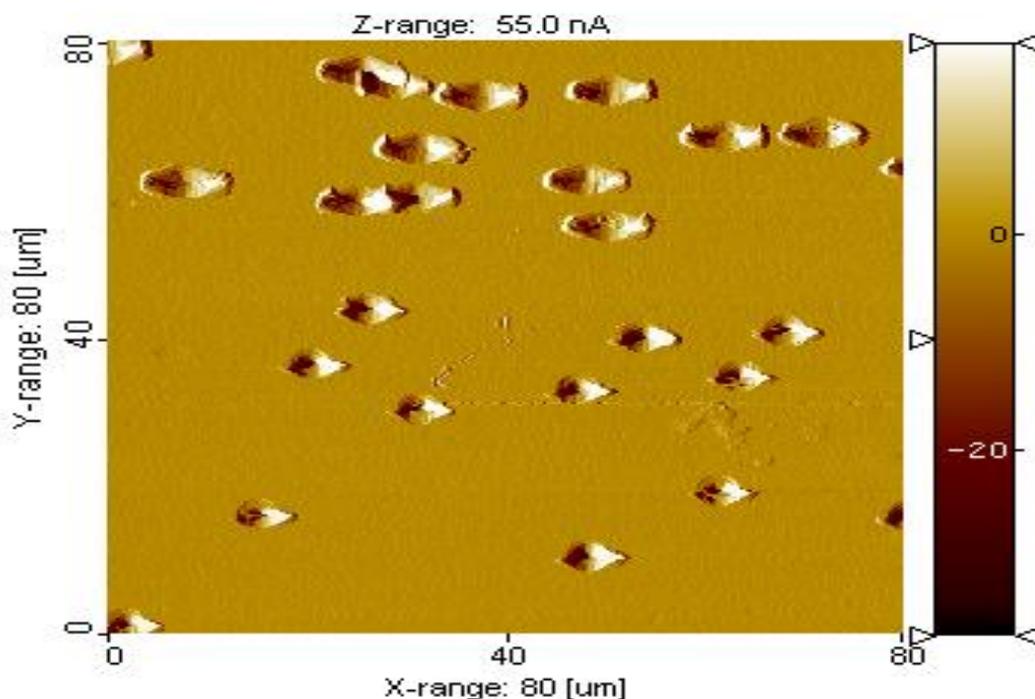


Fig. 6.127: Surface of a 5.0 Micron pore diameter PC after 1 hour exposure to 10 % w/v solution of albumin.

The results show that the protein is adsorbed on the surface of the membrane as well as around the pore edges (Fig. 6.127). The pore edges seem to be raised, which indicates that the protein is adsorbed around the edges of the pores of the polycarbonate membranes.

The AFM studies show like the SEM investigations that proteins deposit on the surface of the membranes and also in and around the pore edges. The amount of protein adsorbed depends on the concentration of protein and the time of exposure. However, the AFM images do not give any idea of how quickly the proteins adsorb in hydrated state on the surface of the polymer membranes. Hence, in order to investigate the adsorption of

proteins on the polymer membranes in real time and in hydrated state Quartz crystal Microbalance was used. Polycarbonate was used in the studies involving QCM.

## **6.8 QCM study of Macromolecules adsorption on PC membranes**

The Quartz crystal microbalance allows the monitoring of adsorption behaviour of proteins in real time and in hydrated state. The formation of protein mono layers can be investigated by using this technology.

Pastorino et. al. (2002) tested the surface density of lipase films with QCM and determined the amount of protein deposited and the mass of protein films after deposition. Nakayama et. al. (2003) examined the thickness of thin protein films (silk fibroin) with QCM and used the atomic force microscope and the field emission scanning electron microscope to study the surface roughness of these films. QCM has been widely used in the study of protein adsorption on various surfaces and is a reliable tool in determining the dynamic thickness and mass of adsorbed biological molecules.

The deposition of macromolecule films on bare gold electrodes as well as on poly(carbonate) films, in real time and in hydrated state, was investigated in our studies using the quartz crystal microbalance (QCM). The data obtained from these investigations was used to calculate the surface density and thickness of the deposited macromolecule films. The macromolecules investigated included albumin and fibrinogen at 4 % w/v solution. The effect of exposure time was also investigated.

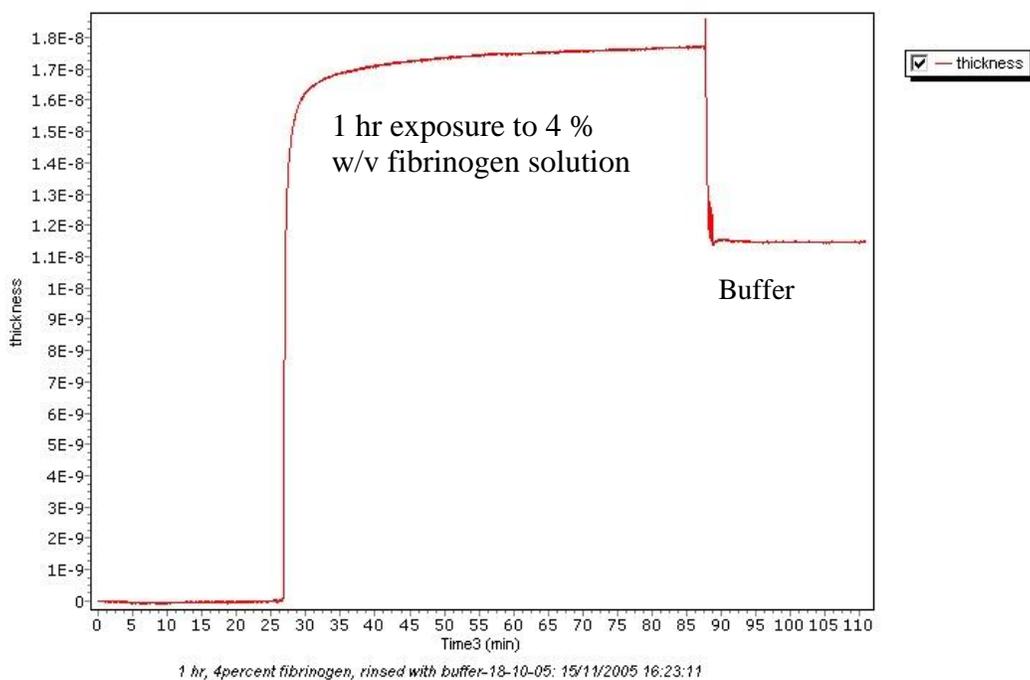


Fig. 6.128: Adsorption of albumin on PC membrane in real time in hydrated state one hour exposure to 4 % w/v fibrinogen solution.

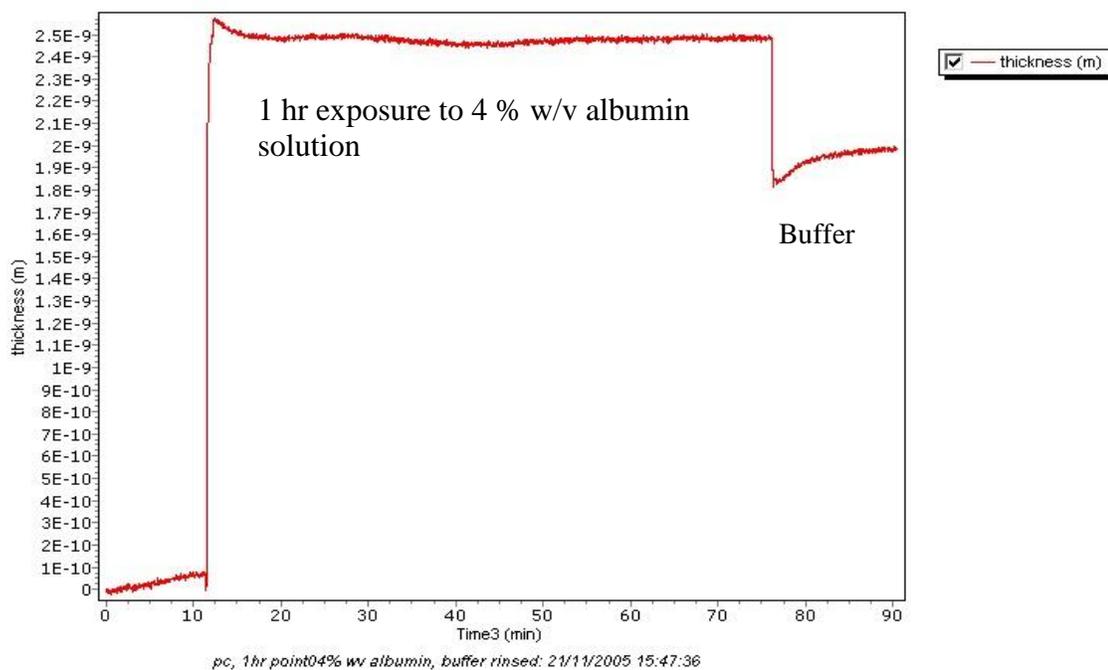


Fig. 6.129: Adsorption of albumin on PC membrane in real time in hydrated state, one hour exposure to 4 % w/v albumin solution.

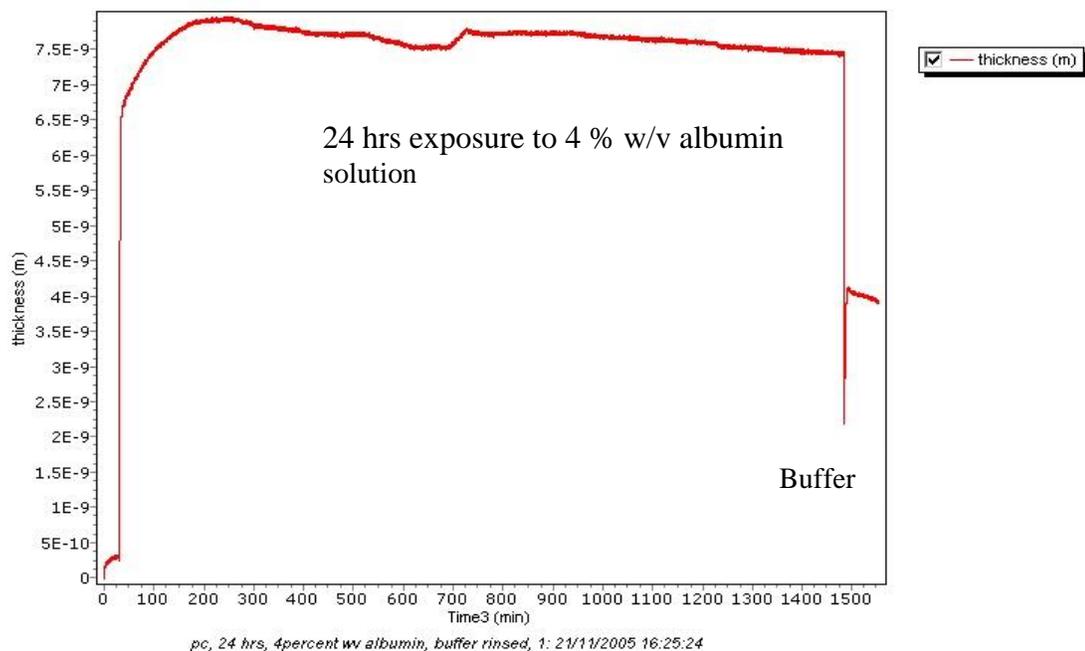


Fig. 6.130: Adsorption of albumin on PC membrane in real time in hydrated state, 24 hours exposure to 4 % w/v albumin solution.

The results show that proteins adsorb instantaneously on the surface of the polycarbonate membrane. However, the thickness of the protein film formed reduces after rinsing with buffer solution. This is due to the fact that abundant labile protein that is present in the solution is removed and only the protein that is adsorbed on the surface of the membrane remains. The adsorbed film is stable after one hour of exposure. The thickness of protein films however, differs with different exposure times. Results show that longer exposure times lead to a thicker film of the protein being formed on the surface of the polymer membranes (Fig. 6.130).

The macromolecules accumulate on surface of a membrane, they adsorb and desorb on the surface of the polymer membrane, a reversible process, until a point in time is reached when the adsorption is irreversible. At this point a stable film of macromolecules adsorbs onto and modifies the surface of the membrane irreversibly. With longer exposure times cake formation starts taking place on the surface of the membrane.

The results of the QCM study show that different proteins adsorb differently on the surface of the polycarbonate membranes. The film formed after exposure to the fibrinogen solution is thicker than the film formed after exposure to albumin (Fig. 6.128, 6.129). This can be due to dimensions of the proteins. Fibrinogen is a fibrous, long thread like oval shape protein. However, albumin is globular in nature. Moreover, the interaction of the protein with the surface of the polymer membrane may also determine the nature and adsorption pattern of the proteins involved. The proteins may adsorb end on or sideways on the surface of the polymer membrane. The charges on the proteins also determine their adsorption and fouling behaviour.

## **Chapter 7 Discussion and conclusions**

Biointerfacing barrier membranes are used in sensors in order to enhance selectivity of the desired analyte and to prevent and exclude the response from undesired electroactive agents. In the present study various polymeric membranes including PVP-PC, PC, PVDF, Cellulose acetate and electropolymerised poly(phenol) and poly(phenol red) were investigated as potential barrier membranes for sensors. The membranes were modified with various macromolecules including albumin, fibrinogen, lysozyme and hyaluronan as well as foetal calf serum and tested for selectivity to various electroactive species including hydrogen peroxide, ascorbic acid, uric acid and acetaminophen.

The selectivity studies showed that hydrogen peroxide is selected most favourably by all the membranes, i.e. PVDF, PVP-PC, PC, cellulose acetate and poly(phenol) as well as poly(phenol red), pre-exposure to macromolecules and post-modification with macromolecules. If the sensitivity to hydrogen peroxide is taken to be 100% then the sensitivity to catechol is 40 – 50%, ascorbic acid is 30 – 40%, uric acid is 20 – 30 % and sensitivity to acetaminophen is 5 – 10%. This pattern of selectivity was observed for all the membranes regardless of their pore size and duration of exposure to macromolecules. This may be due to many factors including the size and charge of the solutes, due to more activity of individual electroactive agents on the surface of the platinum electrode.

The transport of a solute across a semi-permeable membrane results from the random motion of molecules. As the solute molecules in a solution move, they collide with the membrane from time to time until they diffuse through it. Small molecules collide more frequently with the membrane. Hence, their rate of molecular diffusion through the membrane will be higher. Larger molecules moving at low velocities collide less frequently with the membrane thus their rate of migration through the semi-permeable membrane is low, even if they fit through the pores of the membrane.

The rate of transport of a solute across a semi-permeable membrane depends mainly on the size, shape and charge of the molecule. The size of a solute molecule is highly correlated to its molecular weight. As the size of the molecule approaches or exceeds the size of membrane pore, diffusion of the solute will be completely or partially prevented.

Five electroactive chemicals were used in this study namely, hydrogen peroxide, ascorbate, catechol, urate and acetaminophen.

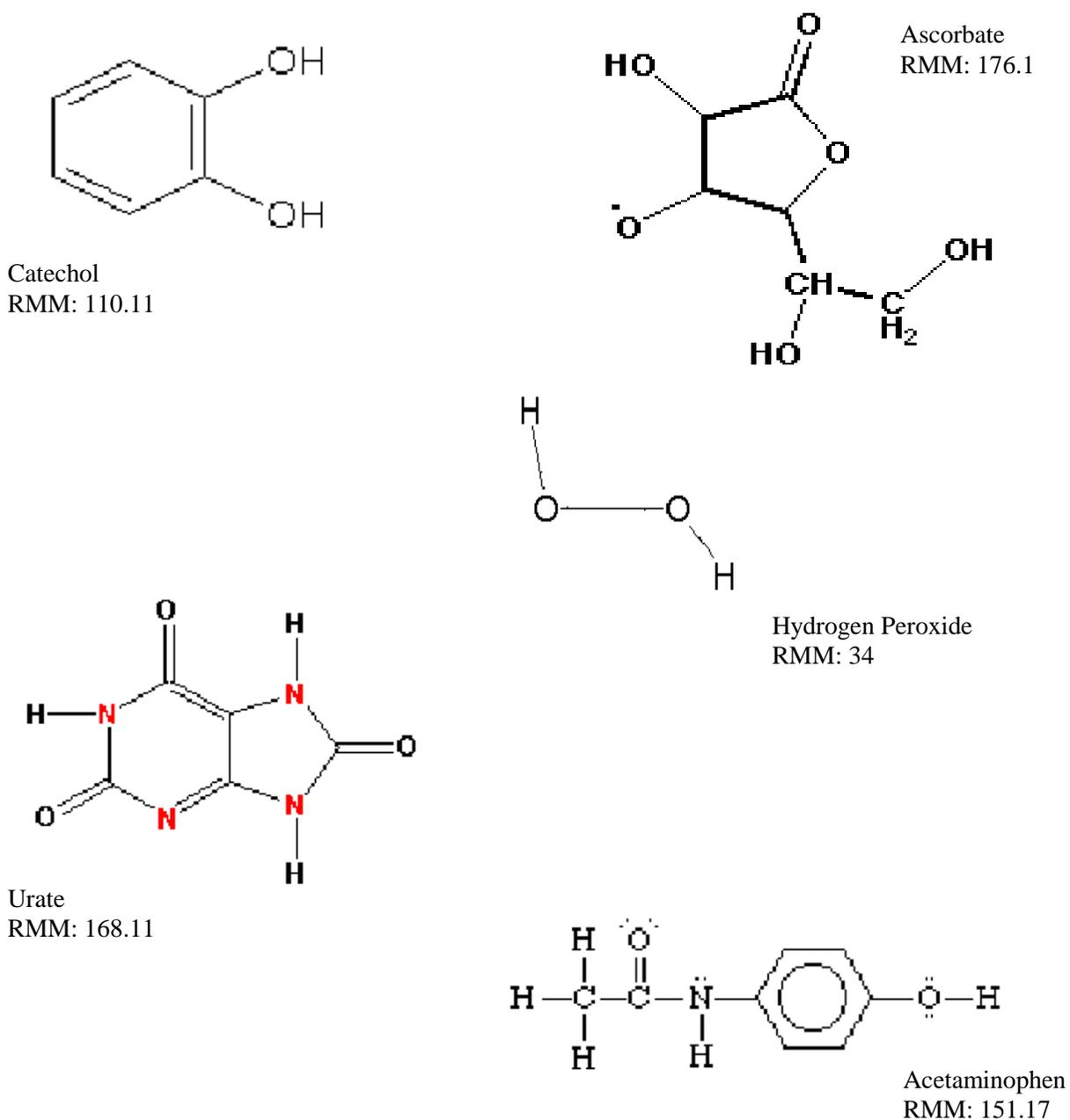


Fig. 7.1: Schematic diagram of the chemical structure of solutes.

These chemicals are all present in our body and are detected by the glucose sensors and give a higher than the true value for the analyte being monitored. These chemicals are all electroactive and are all small molecules. Hydrogen peroxide is the smallest molecule compared to other solutes as can be seen from the Fig. 7.1. It is selected due to its small size by all the polymer membranes used in this study. Hence, as expected it gives the highest response with all polymer membranes.

Results indicate that larger pore size polymer membranes give a higher response to the electroactive species as compared to smaller pore size membranes. This may be due to greater flux or transport of the analyte through the larger pore size membrane as compared with the smaller pore size membrane. One of the disadvantages associated with the commercially available polymer membranes is that the pores are not evenly spaced throughout the membrane (SEM results). Moreover, pore conditions are not always constant i.e. size range of pores in these membranes may be variable, so experiments are not always reproducible. This phenomenon was observed during the morphological studies of these membranes using the scanning electron microscopy.

The electroactive chemicals, hydrogen peroxide, ascorbate, urate, catechol, and acetaminophen, are selected by all the polymer membranes that have been investigated in this study. However, when polymer membranes are modified with macromolecules, the response to all electroactive chemicals is reduced.

It was discovered in the present study that polycarbonate membranes of smallest pore size i.e. 0.05 micron pore diameter modified with hyaluronan select hydrogen peroxide more readily than any other electroactive chemical. The polymer membranes modified with other proteins gave reduced response to all electroactive chemicals. The amperometry results showed that only hyaluronan modified membranes gave higher than original response to hydrogen peroxide as compared to protein modified membranes. The pore size of the membrane had an effect on the biofouling and modification of the membranes along with the duration of the exposure time. Longer exposure times and

higher concentrations of the proteins results in higher level of fouling of the surface of the membranes, which in turn affects the selectivity of the membranes.

The analyte-electrode interaction and the analyte-membrane interactions affect the conditions of flux. In modified membranes the flux of analyte is dependent on many factors including the fouling of membrane, exposure time, hydrodynamics of the process and physicochemical properties of the feed solution as well as of the membranes. When a polymer membrane is exposed to a solution of proteins or macromolecules, the protein or macromolecule adsorbs almost instantaneously on the surface of the membrane (QCM results). However, the proteins and macromolecules in the solution also desorb from the surface of the membrane. The process of adsorption, desorption and chemisorptions is continuous and reversible if no covalent agents are used. However, with longer exposure times or higher concentration of the proteins or macromolecules this adsorption and chemisorptions becomes irreversible and a stable layer or film of proteins or macromolecule forms on surface of the membrane. The proteins and macromolecules all either positively or negatively charged at physiological conditions and pH. Hyaluronan, albumin and fibrinogen are all negatively charged, whereas lysozyme is positively charged at pH 7. The membranes modified with these macromolecules at physiological pH and conditions, may acquire the charge of the macromolecule, which in turn would affect selectivity of the modified membrane to the electroactive chemicals.

Charge on an electroactive agent plays a key role in its selectivity through a modified membrane. The membranes get covered completely by the protein fouling layer (Huisman et. al. 2000). In present study the SEM images show that macromolecules totally cover the surface of the polymer membranes. The deposition is inside and around edges of the pores, pore narrowing is observed after exposure to macromolecules, the adsorption also takes place on the entire surface of the membranes. It is assumed that the polymer membrane thus modified, acquires charge of the macromolecules, i.e. due to electrostatic charge of the proteins or hyaluronan. Hence, the negatively charged electroactive agent would be rejected due to electrostatic repulsion and excluded.

Acetaminophen is negatively charged at physiological conditions hence it gets excluded due to charge (amperometry results).

The macromolecules used in the present study are all negatively charged at physiological conditions apart from lysozyme at pH 7. The proteins aggregate easily at or near their isoelectric point, due to reduced electrostatic repulsion. These aggregates cause fouling of the membrane and reduce the flux in general. However, as the adsorbed layer is negatively charged, i.e. for hyaluronan, albumin and fibrinogen, the response to negatively charged chemicals such as acetaminophen is reduced (chapter 6).

The dimensions and structure of the proteins can have an effect on interaction of the polymer membranes with the proteins. Three proteins were used in this study namely fibrinogen, albumin and lysozyme along with hyaluronan, which is a polysaccharide. Albumin and lysozyme are globular type of proteins. Fibrinogen is a fibrous protein it forms a network of fibrous structure during wound healing processes. Hence, it can be assumed that it may form similar fibrous structures on the mouth of the pores in the polymer membranes, causing a sort of bridging at the inner structure of the pores and fouling the membrane internally. The narrowing of the pores was observed in the SEM studies and the amperometry results show that the response to electroactive chemicals is reduced after polymer membranes are modified with fibrinogen. The electrode was polished in order to investigate if the polymer membrane was irreversibly modified with the protein layer. Polishing the electrode would only produce an active surface area for the dissociation of the analyte at the surface of the electrode. The membrane remains fouled; due to the negative charge on such modified membrane, the analyte with negative charge is excluded, this is why the response after polishing of the electrode does not improve significantly. This indicated that the membrane was irreversibly modified with the protein. Findings for hyaluronan and albumin are similar to fibrinogen; this is again due to the net negative charge on these films acquired from the electrostatic charge of the molecules in operation. However, with hyaluronan flux of hydrogen peroxide is observed not to be hindered and response similar to bare electrode is obtained, this may be due to

the faster diffusion, convection and physicochemical interactions between the surface of the electrode and the analyte as well as the analyte and the membrane.

The response to hydrogen peroxide with hyaluronan film on the surface of the electrode was of the same magnitude as that of the bare electrode. Hyaluronan modified membranes also showed greater selectivity for hydrogen peroxide than other interfering species. It is assumed that this new composite of macromolecule-polymer membrane film retains the individual characteristics of the polymer film and also of the macromolecules that are now part of this new composite film (hyaluronan in this case) thus, facilitating the flux of the desired analyte (hydrogen peroxide) to the surface of the electrode. The response to other electroactive chemicals is reduced due to the charge selection by this new composite (polycarbonate-hyaluronan) membrane, thus alleviating the problem of interference. Moreover, hyaluronan is biocompatible and is abundantly present in the body, hence the composite polymer-hyaluronan membrane when used as a biointerfacing barrier membrane for sensors may enhance selectivity and biocompatibility.

The design of a needle type of electrode was investigated in the present study and results (chapter 3) show that the 1 mm extruded tip needle type of electrode with a reference electrode away from the working electrode gives very similar current response as that of 2 mm extruded tip needle electrode of the same type. The current response is most linear with the electrode with the smallest surface area. The flat tip needle type of electrode shows the most linear response. As the surface area of a needle type of electrode increases the current density decreases. Current density is highest for the flat tip needle type of electrode. The flat tip disc shaped needle type of electrode is a better design for use in a needle than an extruded tip needle type of electrode. Inlaid gold disc electrodes that are effectively needle type of electrodes, embodying a gold filled silica capillary constituting the working electrode, have been shown to detect hydrogen peroxide very effectively (Anastasova et. al. 2012).

The flat tip needle type of electrode shows a linear current response and the highest current density compared with other needle electrodes. This can be due to the small and

flat surface area of the electrode and the disc shape of the active electrode, the diffusion to the surface of the electrode would be spherical instead of linear and the electrode response is more like that of a microelectrode than that of the large macro electrodes. The current density per unit area is large for the flat tip needle electrode. The flat tip needle type of electrode is the best design for detection of hydrogen peroxide.

It was assumed that the flat tip type needle type of electrode has similar design to the flat disc type of electrode. Hence, the Rank cell, which is a flat disc type electrode, was used for amperometry investigations on the polymer membranes, unmodified and modified with macromolecules, as it provided ease of use.

The investigations carried out using amperometry, SEM, AFM and QCM all show that macromolecules modify the polymer membranes as well as the surface of the electrode. The use of naturally available biological macromolecules as a coating material for the modification of the polymeric membranes can provide biocompatible barrier membranes that could alleviate the problem of interference. These biointerfacing polymeric barrier membranes can have significant use in sensors.

## **Conclusions**

- The flat tip needle type of electrode is the best design for use as sensor for hydrogen peroxide detection and monitoring.
- Phenol red and phenol film, poly(carbonate) and poly(vinylidene fluoride) membrane responses to electroactive species are dependent on stirring effects.
- The electropolymerisation of phenol and phenol red is a self-limiting process, once the film is formed the electropolymerisation process stops.
- The electropolymerised phenol red film is a better choice as a membrane material than the electropolymerised phenol film, as an inner membrane for use in glucose sensors due to its higher selectivity of hydrogen peroxide and rejection of interfering chemicals.

- Precast poly(carbonate) is a better membrane material than the precast PVDF, cellulose acetate and pvp coated polycarbonate membrane as it is stable at various pH levels and exposure times.
- Studies with poly(carbonate) membranes show that as the pore size of the membrane decreases the linearity of response increases.
- Exposure to proteins results in loss of sensitivity of the sensor with all proteins investigated i.e. albumin, fibrinogen and lysozyme.
- Longer exposure times to proteins result in greater fouling of all of the polymer membranes and of the electrode, which in turn gives rise to a reduced response.
- Membranes with smaller pores protect the underlying electrode from fouling.
- Large pored membranes let proteins diffuse through to the surface of the electrode that leads to loss of sensitivity and reduced response of the sensor.
- Proteins adsorb instantaneously at the surface of the polymer membranes.
- The exposure to protein solution results in the formation of a stable layer of protein on the surface of the polymer membranes.
- Higher concentration and longer exposure times cause thickening of the protein layer on the surface of the polymer membranes.
- Proteins and macromolecules cause biofouling of the surface of the membrane, modify the membrane and cause narrowing of the pores in the polymer membranes.
- The macromolecules and proteins modify the pores of the polymer membranes.
- The macromolecules form aggregates on the surface of the polymer membranes as well as on the inside of the pores of the membranes.
- The polymer membranes with small pore sizes when exposed to macromolecules solution undergo pore blocking.
- Hyaluonan causes a grainy layer like deposit on the entire surface of the polymer membranes.
- The polymer membranes modified with hyaluronan are selective of the hydrogen peroxide more than any other electroactive chemical. Hence, resolve the problem of interference from other electroactive species.

## Chapter 8 Future Work

- Further study on the membrane characterization using modeling and suitable statistical methods on the SEM and AFM images in order to investigate the dimensions of the macromolecule modified polymer membranes.
- Entrapment of proteins in electropolymerised films and testing for selectivity of such biofilms with various electroactive chemicals.
- Investigation of the overall surface charges of the macromolecule on the modified membranes using zeta potential or other suitable methods.
- Investigation of the way proteins adsorb on the polymer membranes in hydrated state in real time by using imaging techniques such as the scanning probe microscopy and fluorescence Microscopy.
- Further study of biofouling behaviour of various macromolecules in hydrated state and real time on polymer membranes using QCM, investigation of pH and time effect on the protein adsorption.
- Fouling and modification of membranes with one protein followed by another to investigate if any one protein prevents further fouling from taking place.
- Study of two or more composite of proteins on the surface of the polymer membrane, investigation of selectivity profile.
- Further studies on flat tip needle type of electrodes using various polymers and protein coatings, in order to investigate the fouling behaviour of the macromolecules on the polymers at the surface of needle type of electrodes.

## A study of biointerfacing polymeric barrier membranes for sensors

- Selectivity studies of the modified flat tip needle type of electrodes, using various naturally available proteins including heparin.
- The flat tip needle type of electrode coated with macromolecules by entrapment method using phenol red and macromolecule solution, to be investigated for potential use in glucose sensors.
- Further investigation of various polymers modified with hyaluronan using the flat tip needle type of electrodes for selectivity and biocompatibility.

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