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Development of conducting polymer membrane structures for protein and gas separation

Dezhi Zhou

University of Wollongong

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DEVELOPMENT OF CONDUCTING POLYMER MEMBRANE STRUCTURES FOR PROTEIN AND GAS SEPARATION

A thesis submitted in fulfilment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

from

UNIVERSITY OF WOLLONGONG

by

DEZHI ZHOU, B.E., M.E.
To my parents for their encouragement.

To my wife Yi Dai and my daughter Anna M Zhou
for their support and patience
DECLARATION

This is to certify that the work described in this thesis is my own work and has not been submitted for a higher degree at any other university or institution.

Dezhi Zhou
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I wish to express my sincere gratitude to Professor Gordon G. Wallace for his enthusiastic supervision and encouragement through this work.

I greatly value the supervision and the technical advice of Dr Alistair Hodges and Dr Albert H. Mau of CSIRO Division of Chemicals & Polymers. I would like to address the supervision and help of Dr Chee O. Too through this work.

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PUBLICATIONS


The development of new materials has greatly expanded the application of membrane separation technologies. Conducting electroactive polymers, such as polypyrrole, represent a new class of electrodynamic membranes with potential application in a number of areas.

A range of new electromembranes have been successfully synthesised and characterised in this work. A new electromembrane transport systems for protein separation and recovery has been devised.

Platinised electromembranes have been successfully fabricated with the use of conventional commercial membranes such as polyvinylidene fluoride (PVDF) or polysulfone membranes as substrates. Polypyrrole composite membranes as well as free-standing films have been electrochemically synthesised with a series of counterions of different molecular weights and biofunctional groups.

Electrochemically controlled protein transport and separation have been carried out with both platinised PVDF membranes and polypyrrole coated platinised PVDF composite membranes. Results show that the transport of test proteins across membranes can be electrochemically manipulated by controlling the stimuli applied to the membranes. In addition, the transport/separation of two proteins of different isoelectric points has been demonstrated with both the stationary transport cell and a flow-through cell. Separation factors of 70 were obtained for BSA over haemoglobin.
The incorporation of biofunctional groups into polypyrroles has been achieved using electropolymerisation. Interactions between test proteins, such as human serum albumin (HSA) and thrombin, with polypyrroles have been investigated using the Electrochemical Quartz Crystal Microbalance (EQCM). The binding of thrombin to polypyrrole-heparin was shown to be selective.

Studies into gas separations using polypyrrole conducting polymer membranes were also carried out. The oxygen and nitrogen permeation rates of polypyrrole membranes varied depending on the type of counterion incorporated into the polypyrrole. Polypyrrole prepared with conventional counterions such as $p$-toluene sulfonate have higher permeation rate for nitrogen then that for oxygen when tested with pure gas. Polypyrrole-polyaniline sulfonate was found to have higher oxygen selectivity over nitrogen with $O_2$:$N_2$ of 3~6:1 obtained. Polypyrrole membranes prepared with polyanions show no selectivity because of their high porosity. The separation of gas mixtures was carried out using air as the feed gas.
**ABBREVIATIONS**

A  electrode surface area  
A\(^{-}\)  doping anion  
Ab  antibody  
AFM  atomic force microscopy  
Ag/AgCl  silver and silver chloride reference electrode  
Ag  antigen  
\(\alpha\)  separation factor  
antiHSA  antibody of human serum albumin  
BS  benzene sulphonic acid  
BSA  bovine serum albumin  
C  concentration (mol l\(^{-1}\))  
C\(_{dl}\)  capacitance of double layer (F)  
CEP  conducting electroactive polymer  
C\(_{f}\)  frequency - mass conversion factor (ng Hz\(^{-1}\))  
CV  cyclic voltammetry or cyclic voltammogram  
Cyt. C  cytochrome c  
D  diffusivity (cm\(^2\) s\(^{-1}\))  
Dex  dextran sulfate  
\(\Delta E\)  potential difference (V)  
\(\Delta f\)  frequency variation (Hz)  
\(\Delta m\)  mass change (g)  
\(\Delta p\)  pressure difference (cmHg or psi)  
\(\Delta t\)  time period (s)  
E\(_{app}\)  applied potential (V)
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{p(a)}$</td>
<td>anodic peak potential (V)</td>
</tr>
<tr>
<td>$E_{p(c)}$</td>
<td>cathodic peak potential (V)</td>
</tr>
<tr>
<td>EQCM</td>
<td>electrochemical quartz crystal microbalance</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday constant C·mol$^{-1}$</td>
</tr>
<tr>
<td>$f_0$</td>
<td>resonant frequency of quartz crystal (Hz)</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>GPR</td>
<td>gas permeation rate [cm$^3$(STP) cm$^{-2}$ s cmHg$^{-1}$]</td>
</tr>
<tr>
<td>HEMO</td>
<td>haemoglobin</td>
</tr>
<tr>
<td>HSA</td>
<td>human serum albumin</td>
</tr>
<tr>
<td>$i$</td>
<td>electrode current (A)</td>
</tr>
<tr>
<td>$i_c$</td>
<td>capacitive or charge current (A)</td>
</tr>
<tr>
<td>$i_f$</td>
<td>Faradic current (A)</td>
</tr>
<tr>
<td>$J_p$</td>
<td>protein transport flux</td>
</tr>
<tr>
<td>$J_w$</td>
<td>water flux (cm$^3$ cm$^{-2}$ s$^{-1}$ kPa$^{-1}$)</td>
</tr>
<tr>
<td>$\eta_q$</td>
<td>AT-cut quartz shear modulus (dyne cm$^{-2}$)</td>
</tr>
<tr>
<td>$M^+$</td>
<td>cation</td>
</tr>
<tr>
<td>$M_w$</td>
<td>molecular weight (g)</td>
</tr>
<tr>
<td>MYO</td>
<td>myoglobin</td>
</tr>
<tr>
<td>$n$</td>
<td>number of electron</td>
</tr>
<tr>
<td>ox</td>
<td>oxidation process</td>
</tr>
<tr>
<td>PAS</td>
<td>polyaniline sulphonic acid</td>
</tr>
<tr>
<td>PE</td>
<td>polyelectrolyte</td>
</tr>
<tr>
<td>$pI$</td>
<td>isoelectric point</td>
</tr>
<tr>
<td>PPy</td>
<td>polypyrrole</td>
</tr>
<tr>
<td>PS</td>
<td>polysulphone</td>
</tr>
<tr>
<td>PTS</td>
<td>p-toluene sulphonic acid sodium salt</td>
</tr>
<tr>
<td>PVDF</td>
<td>polyvinylidene fluoride</td>
</tr>
<tr>
<td>$Q_c$</td>
<td>capacitive charge (C)</td>
</tr>
<tr>
<td>QCM</td>
<td>quartz crystal microbalance</td>
</tr>
</tbody>
</table>
Qi : gas permeation rate \([\text{cm}^3(\text{STP}) \text{ cm}^{-2} \text{ s}^{-1}]\)

R : resistance

red : reduction process

\(\rho_q\) : quartz density (g cm\(^{-3}\))

\(\rho_s\) : surface resistivity (W)

\(R_u\) : solution resistance (W)

\(\rho_v\) : resistivity (W cm)

S : solubility \([\text{cm}^3(\text{STP}) \text{ cm}^{-3}(\text{polymer}) \text{ cmHg}^{-1}]\)

SDS : sodium dodecyl sulfate

SEM : scanning electron microscopy

\(\sigma_v\) : conductivity (S cm\(^{-1}\))

\(\sigma_s\) : surface conductivity (S)

t : time (s)

\(t_{\text{film}}\) : thickness of CEP film on quartz electrode (m)

\(t_q\) : thickness of the quartz resonator (m)

q : gas permeation time lag (s)

V : electrical voltage (V)

\(V_w\) : water permeation (cm\(^{-3}\))

u : scan rate (mV s\(^{-1}\))

\(u_{\text{tr}}\) : velocity of transversal acoustic wave in quartz (m s\(^{-1}\))

Z : charge
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CHAPTER 1

GENERAL INTRODUCTION
Conducting electroactive polymers can be stimulated *in-situ* to elicit desired responses to reversibly adapt their chemical and physical properties accordingly. For example, they can respond to heat, light, or electrical stimuli. This unique dynamic property of conducting electroactive polymers qualify them to be fabricated into "intelligent materials" suitable for applications as membranes [1,2], sensors [3,4], rechargeable batteries [5,6], liquid display devices [7-9], and actuators [10-14].

One area where conducting polymers could potentially make a significant impact is that of membrane technology. Membranes are utilised to perform separations in a wide range of applications. The proliferation of membrane technology is likely to continue as more sophisticated systems are designed to suit specific applications.

One of the limitations of conventional membranes is that their physical and chemical properties are fixed and can not be changed after synthesis. In addition, these conventional membrane separation processes typically utilise a single property or driving force, such as pressure, concentration, electrical potential, or pH differential.

Contrary to conventional membranes, conducting electroactive polymer membranes are electrodynamic. By varying the applied electrical stimuli to the membrane, such as potential, electric field strength and direction, its transport property and interaction with solute can be altered. In this way, the separation properties and/or fouling characteristics of the membrane can be improved with the use of conducting polymers.

This work has focused on the development of conducting electroactive membrane systems for electrochemically controlled protein transport and separation. Procedures for synthesising conducting electroactive
membranes, such as the use of substrate to produce polypyrrole composite membranes, have been developed. Electromembrane transport and separation systems have been established and the electrochemically controlled transport and separation of proteins has been explored.

This chapter reviews the development of conventional membranes, general protein separation techniques and conducting electroactive polymers.

1.1 MEMBRANE SEPARATION

1.1.1 The Development of Membrane Technology

The history of membrane technology can be traced back to 1748 when the first membrane phenomenon, osmosis, was observed by Nollet \[^{15}\]. Early scientific research on membranes was not carried out solely by physicists and chemists, but also by scientific workers in other disciplines such as biologists, biochemists, biophysicists and zoologists in the early period. Some of the milestones in the development of membrane science and technology are summarised in Table 1.1.

The development of membrane processes for commercial or industrial applications lagged far behind scientific research. Even towards the middle of the eighteenth century, membrane phenomena were observed and studied primarily to elucidate barrier properties and related phenomena rather than to develop membranes for technical or industrial applications.

Based on Zsigmondy’s early work \[^{16}\], the first commercial membranes for practical applications were manufactured by Sartorius in Germany after World War I. However, the applications of these porous cellulose
nitrate or cellulose acetate membranes were limited to use on a laboratory scale.

**Table 1.1 Some important development in membrane science**

<table>
<thead>
<tr>
<th>Milestones</th>
<th>Membrane phenomena and theories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation:</td>
<td>Osmosis: Nollet 1748 [16]</td>
</tr>
<tr>
<td></td>
<td>Electro osmosis: Reuss 1803, Porret 1816 [17]</td>
</tr>
<tr>
<td></td>
<td>Dialysis: Graham 1861 [18]</td>
</tr>
<tr>
<td>Relation:</td>
<td>Diffusion: Fick 1855 [19]</td>
</tr>
<tr>
<td></td>
<td>Osmotic pressure: van't Hoff 1887 [20]</td>
</tr>
<tr>
<td></td>
<td>Electrolyte transport: Nernst-Planck 1889 [21]</td>
</tr>
<tr>
<td>Theoretical Considerations</td>
<td>Osmotic pressure: Einstein 1905 [22]</td>
</tr>
<tr>
<td></td>
<td>Membrane potential: Henderson 1907 [23]</td>
</tr>
<tr>
<td></td>
<td>Membrane equilibrium: Donnan 1911 [24]</td>
</tr>
<tr>
<td></td>
<td>Irreversible thermodynamics: Kedem, Katchalsky 1964 [25]</td>
</tr>
<tr>
<td>Transport model</td>
<td>Ionic membranes: Teorell 1937 [26], Meyer, Sievers 1936</td>
</tr>
<tr>
<td></td>
<td>Pore model: Schmid 1950 [27]</td>
</tr>
<tr>
<td></td>
<td>Solution diffusion model: Lonsdale 1965 [28]</td>
</tr>
</tbody>
</table>

A major breakthrough for industrial applications was the development of asymmetric membranes [29]. These membranes consist of a thin dense skin layer supported by a porous sublayer. The membrane transport properties are determined by the top skin layer and thus asymmetric membranes show much higher permeation rates or fluxes than the symmetric membranes of a comparable thickness.

Gas separation membranes for industrial applications were produced in 1979 by Henis and Triodi [30]. They placed a very thin layer of polymer with a high gas permeability on top of an asymmetric membrane. The
pores in the top layer were filled to obtain a composite membrane suitable for gas separations.

Membrane processes developed and currently utilised are summarised in Table 1.2. The search for new and better membranes continues.

**Table 1.2 Development of membrane processes**

<table>
<thead>
<tr>
<th>Membrane process</th>
<th>Country</th>
<th>Year</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microfiltration</td>
<td>Germany</td>
<td>1920</td>
<td>laboratory use (bacteria filter)</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>Germany</td>
<td>1930</td>
<td>laboratory use</td>
</tr>
<tr>
<td>Haemodialysis</td>
<td>Netherlands</td>
<td>1950</td>
<td>artificial kidney</td>
</tr>
<tr>
<td>Electrodialysis</td>
<td>USA</td>
<td>1955</td>
<td>desalination</td>
</tr>
<tr>
<td>Hyperfiltration</td>
<td>USA</td>
<td>1960</td>
<td>sea water desalination</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>USA</td>
<td>1960</td>
<td>macromolecule separation</td>
</tr>
<tr>
<td>Gas separation</td>
<td>USA</td>
<td>1979</td>
<td>hydrogen recovery</td>
</tr>
<tr>
<td>Membrane distillation</td>
<td>Germany</td>
<td>1981</td>
<td>concentration application</td>
</tr>
<tr>
<td>Pervaporation</td>
<td>Germany/</td>
<td>1982</td>
<td>dehydration of organic solvent</td>
</tr>
<tr>
<td></td>
<td>Netherlands</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from ref.[ 31].

1.1.2 Definition and Classification of Membranes

1.1.2.1 Membrane Definition

Although it is difficult to give an exact definition of a membrane, a general definition could be [32,33]:
A membrane is a 'material' or a group of 'materials' that lies between two different phases. The material is physically and chemically distinctive from both phases and due to its properties and the force applied, is able to control the mass transport between these phases.

This definition does not imply that membranes are two dimensional thin objects, although this is true for the majority of existing membranes.

A membrane, as a selective barrier between two phases in a separation process, can be thick or thin. Its structure can be homogeneous or heterogeneous. The mass transport can be active or passive, and the driving forces can be pressure, concentration, electric field, pH differential, temperature or even chemical interaction.

1.1.2.2 Membrane Classification

Membranes can be classified in numerous ways according to different points of view. One classification is by nature, i.e. synthetic membranes and biological membranes. This classification is the clearest distinction possible.

In practice, membranes can also be classified by their chemical structure or morphological structure. This classification is more illustrative because the membrane structure determines the separation mechanism and hence the application.

Classification of membranes based upon their physical properties has also been proposed [31]. Under this category, membranes can be divided into homogeneous and heterogenous membranes, or symmetric and asymmetric membranes. (Figure 1.1)
Chapter 1 General Introduction

Membranes

- synthetic membrane
- biological membrane

   living and non-living membrane...

   symmetric membrane
   - cylindrical porous membrane
   - porous homogeneous membrane

   asymmetric membrane
   - porous membrane
   - porous with top-layer
   - dense skin on porous membrane

**Figure 1.1 Classification by membrane structures**

In recent years, more and more scientists and engineers tend to use this classification due to practical considerations. It is commonly known that the performance of a membrane, in terms of flux and separation efficiency, is largely determined by the pore size, porosity and pore structures.

The symmetric membranes can be either porous or nonporous with their thickness ranging from 10 μm to 200 μm. The resistance to mass transfer is determined by the actual pore structure and the total membrane thickness. A decrease in membrane thickness results in increased permeation rate or flux.

Asymmetric membranes consist of a skin layer fabricated on top of a porous support layer with a thickness of 50 to 150 μm. The membrane performance is normally determined by this dense skin layer. The resistance to mass transfer is normally low compared to the same
thickness of a symmetric membrane because the dense skin layer is usually very thin (between 0.01 μm and 1.0 μm).

1.1.3 Membrane Separation Processes

The membrane separation process is characterised by the use of the membrane as a selective barrier to accomplish a particular separation. The membrane has the ability to transport one component more readily than another because of differences in physical and/or chemical properties between the membrane and the permeating component[32]. Transport through the membrane takes place as a result of a driving force acting on the individual components in the feed. In many cases the permeation rate through the membrane is proportional to the driving force, i.e. the flux-force relationship can be described by a linear phenomenological equation [31]. Thus the flux (J) is related to the driving force according to

$$J = -k \frac{dF}{dx}$$  \hspace{1cm} (1.1)

where k is called the phenomenological coefficient and $\frac{dF}{dx}$ is the driving force, expressed as the gradient of F (concentration, pressure or temperature) along a coordinate x perpendicular to the transport barrier. An example of such as linear relationship is Fick's law [19], which relates the mass flux to a concentration difference by the diffusion coefficient (D).

For a pure component permeating through a membrane, it is possible to employ such a linear relationship to describe transport. However, when two or more components permeate simultaneously, such relationships can not be generally applied because coupling phenomena may occur in their fluxes and forces.
Membrane processes can be classified according to the pore size of membranes and the driving forces applied (Table 1.3). When particles of diameter $>100$ nm have to be retained, it is possible to use a rather open membrane structure. The hydrodynamic resistance of such membranes is low and small driving force is sufficient to obtain high fluxes. The membrane process is then called microfiltration.

To separate macromolecules (with molecular weights ranging from $10^4$ to more than $10^6$) from an aqueous solution, the membrane structure must be more dense and hence its hydrodynamic resistance is also high. This process is usually referred to as ultrafiltration.

It is also possible to separate low molecular weight components of approximately equal size from each other. In this case a very dense asymmetric membrane is used, resulting a very high hydrodynamic resistance. Such a process is called hyperfiltration or reverse osmosis.

In addition, electrodialysis and gas separation are also important membrane processes. Electrodialysis is a membrane process in which the driving force for transport is supplied by an electrical potential gradient (or electrical field). The separation principle can be expressed by a Donnan exclusion mechanism [24]. This process occurs only when charged molecules, such as amino acids or proteins, are present in the feed solution. A typical feature of this process is that ionic groups or charged membranes (such as ion-exchange membranes) are necessary. Electrodialysis membrane processes can be found their uses in various applications such as water desalination and whey proteins separation [170] in dairy food manufacturing.

Gas separation by membrane process has also reached the stage of commercialisation although the search for new membrane materials is
still continuing. Currently there are two completely different types of membranes that can be used for this purpose: a dense nonporous membrane where transport takes place via diffusion-solution \[28\], and a porous membrane where Knudsen flow \[27\] occurs. In the first case, an asymmetric membrane is often employed to obtain high gas permeation rate. Commercial application of gas separation membranes can be found in hydrogen recovery, but the separation of oxygen/nitrogen and methane/carbon dioxide provide other examples of gas separation.

In some cases, two or more mechanisms may be involved in a single membrane process for particular separation purposes. For examples, Bowen and his co-workers have demonstrated an electrically enhanced transport system by combining electrophoresis/electrodialysis with ultrafiltration/microfiltration processes for protein separation \[34,35 \]. The driving forces in such a system include both pressure and electrical field.

**Table 1.3 Membrane separation processes**

<table>
<thead>
<tr>
<th>Membrane process</th>
<th>Membrane pore size</th>
<th>Driving force</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microfiltration</td>
<td>&gt;100 nm</td>
<td>pressure</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>1 - 100 nm</td>
<td>pressure</td>
</tr>
<tr>
<td>Hyperfiltration</td>
<td>0.1 - 1 nm</td>
<td>pressure</td>
</tr>
<tr>
<td>Electrodialysis</td>
<td>ion-exchange</td>
<td>electrical potential</td>
</tr>
<tr>
<td>Dialysis</td>
<td>porous-nonporous</td>
<td>concentration</td>
</tr>
<tr>
<td>Piezodialysis</td>
<td>nonporous</td>
<td>electrical potential</td>
</tr>
<tr>
<td>Osmosis</td>
<td>nonporous</td>
<td>osmotic pressure</td>
</tr>
<tr>
<td>Parvaporation</td>
<td>nonporous</td>
<td>concentration, heat</td>
</tr>
<tr>
<td>Thermo-osmosis</td>
<td>nonporous</td>
<td>thermo-diffusion</td>
</tr>
</tbody>
</table>

*Adapted from ref. [31]*
1.1.4 Preparation and Characterisation of Synthetic Membrane

1.1.4.1 Membrane Preparation Techniques

Generally speaking, many kinds of synthetic materials can be used for preparing membranes. Thus the materials can either be inorganic such as ceramic, glass, metal or organic including all kinds of polymers.

The basic principle involved in membrane preparation is to modify the material in such a way by means of an appropriate technique so as to obtain a membrane structure with a morphology suitable for a specific separation. The choice of material limits the preparation technique employed, the membrane morphology obtained and the separation principle allowed.

Table 1.4 Membrane preparation techniques*

<table>
<thead>
<tr>
<th>Technique</th>
<th>Materials</th>
<th>Pore size and porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sintering</td>
<td>organic, inorganic, ceramic, metal and glass</td>
<td>0.1-10 μm, 10-20%</td>
</tr>
<tr>
<td>Stretching</td>
<td>semi-crystalline polymers</td>
<td>0.1-3μm, up to 90%</td>
</tr>
<tr>
<td>Track-etching</td>
<td>polycarbonate, mica</td>
<td>0.02 - 10 μm, less than 10%</td>
</tr>
<tr>
<td>Template leaching</td>
<td>glass</td>
<td>greater than 0.05 μm</td>
</tr>
<tr>
<td>Phase inversion</td>
<td>soluble polymers</td>
<td>0.05 μm - 0.5 μm</td>
</tr>
<tr>
<td>Coating</td>
<td>polymer, metal</td>
<td>porous and nonporous,</td>
</tr>
</tbody>
</table>

*Adapted from ref [31]
A number of different techniques are available for the preparation of synthetic membranes. Some of these techniques can be used to prepare organic as well as inorganic membranes. The most important techniques are listed in Table 1.4 and include sintering, stretching, track-etching, phase inversion and coating.

1.1.4.2 Characterisation of Membranes

Membranes differ significantly in their structure and consequently in their functionality. The purpose of membrane characterisation is the determination of the physical and chemical properties of a given membrane so that the prediction of the membrane performance for a given application can be made. Membrane characterisation usually involves the measurements of (i) intrinsic properties, such as structural and morphological properties, and (ii) actual performance in a particular separation process, such as its permeability, reproducibility and chemical stability.

A number of techniques are available for the characterisation of membranes and they are briefly summarised in Table 1.5.
Table 1.5 Techniques for membrane characterisation

<table>
<thead>
<tr>
<th>Characterisation technique</th>
<th>Information obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scanning electron microscopy (SEM)</td>
<td>pore structure, surface morphology (pore size, porosity, pore distribution)</td>
</tr>
<tr>
<td>Transmission electron microscopy (TEM)</td>
<td>pore size, pore distribution, pore geometry</td>
</tr>
<tr>
<td>Permeability</td>
<td>pore size, pore distribution, permeability</td>
</tr>
<tr>
<td>Bubble point method</td>
<td>maximum pore size</td>
</tr>
<tr>
<td>Mercury intrusion</td>
<td>pore size, pore distribution</td>
</tr>
<tr>
<td>Gas adsorption - desorption</td>
<td>pore size, pore distribution, permeability</td>
</tr>
<tr>
<td>Thermoporometry</td>
<td>pore size and distribution, crystallisation</td>
</tr>
<tr>
<td>Permpormetry</td>
<td>pore size distribution</td>
</tr>
<tr>
<td>Solute rejection measurement</td>
<td>molecular weight &quot;cut-off&quot;</td>
</tr>
<tr>
<td>Differential scanning calorimetry (DSC)</td>
<td>Tg, crystallinity and density, stability</td>
</tr>
<tr>
<td>Differential thermal analysis (DTA)</td>
<td>Tg, crystallinity and density, stability</td>
</tr>
<tr>
<td>Wide angle X-ray diffraction (WAXS)</td>
<td>size and shape of crystalline, structure</td>
</tr>
</tbody>
</table>

* Adapted from ref. [31]

1.2 PROTEIN SEPARATION TECHNIQUES

A major portion of most biochemical investigations and production involves the purification of the materials under consideration because these substances must be relatively free of contaminants if they are to be properly characterised and utilised [36]. This is often a formidable task
because a typical cell contains thousands of different substances, many of which closely resemble other constituents in their physical and chemical properties. Furthermore, the material may be unstable and exists in only very small amounts. The development of efficient and satisfactory techniques for the purification and recovery of proteins is still an intellectually challenging task of both fundamental and technological importance.

**Table 1.6 Characteristics of proteins and separation techniques**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Separation techniques*</th>
</tr>
</thead>
</table>
| Charge        | Ion exchange chromatography  
                Electro dialysis  
                Electrophoresis  
                Isoelectric focusing |
| Polarity      | Adsorption chromatography  
                Paper chromatography  
                Reverse-phase chromatography  
                Hydrophobic interaction chromatography |
| Size          | Ultrafiltration membrane  
                Dialysis  
                Gel electrophoresis  
                Gel filtration chromatography  
                Ultracentrifugation |
| Specificity   | Affinity chromatography |

*From Reference [37]*

Various physicochemical properties of the proteins of interest are utilised in modern separation techniques to separate it progressively from other substances. The characteristics of proteins and other biomolecules that are utilised in the various separation procedures are solubility, ionic charge, molecular size, adsorption properties, and binding specificity for other
molecules (Table 1.6). Most commonly used techniques for proteins separation are often based on these characteristics.

Currently, protein separation and purification are carried out by chromatographic separations, electrophoresis, ultracentrifugation and membrane separation. Most of these techniques have such a high degree of discrimination that one can now separate a series of proteins with similar properties.

1.2.1 Chromatographic Methods

Modern separation methods rely heavily on chromatographic procedures [38-40]. In all of them, a mixture of substances to be fractionated is dissolved in a liquid or gaseous fluid known as the mobile phase. The resultant solution is percolated through a column consisting of a porous solid matrix known as the stationary phase. The interactions of individual solutes with the stationary phase act to retard their progress through the matrix in a manner that varies with the properties of each solute. If the mixture being fractionated starts its journey through the column in a narrow band, the different retarding forces in each component (that cause them to migrate at different rates) will eventually cause the mixture to be separated into bands of pure substances.

Chromatographic methods can be classified according to their mobile/stationary phases. For example, in gas-liquid chromatography the mobile and stationary phases are gas and liquid, respectively, whereas in liquid-liquid chromatography they are immiscible liquids one of which is bound to an inert solid support. Chromatographic methods may also be classified according to the nature of the dominant interaction between the stationary phase and the substances being separated. If the retarding force
is ionic in character, the separation technique is referred to as ion exchange chromatography, whereas if it is a result of the adsorption of the solutes onto a solid stationary phase, it is known as adsorption chromatography.

Protein mixtures may contain large numbers of different components, many of which closely resemble one another in their various properties. Therefore, the isolation procedures for most biological substances incorporate a number of independent chromatographic steps in order to purify the substance of interest according to several criteria. The power of chromatography derives from the continuous nature of the separation processes. A single purification step may have very little tendency to separate a mixture into its components. In reality this process is applied in a continuous fashion so that it is, in fact, repeated hundreds of times, and the segregation of the mixture into its components ultimately occurs.

The advantages of chromatography are its high resolution, high sensitivity and its capacity for automation. Most commonly used chromatographic methods are as follows: Ion-exchange Chromatography, Paper Chromatography, Gel Filtration Chromatography, and Affinity Chromatography. However, this separation process is limited to biochemistry laboratory use on a bench-scale biochemical separation such as for quantitative estimation of nanogram quantities of biomaterials such as vitamins, steroids, lipids, and drug metabolites. It is unsuitable for protein separation on a large scale.

1.2.2 Electrophoresis

Electrophoresis, the migration of ions of charged particles in an electric field, is widely used as an analytical separation process for biomaterials
This method is based on the law of electrostatics where the electrical force, $F_{\text{electric}}$, on an ion with charge $q$ in an electric field of strength $E$ is expressed by

$$F_{\text{electric}} = qE \quad (1.2)$$

The resulting electrophoretic migration of the ion through the solution is opposed by a frictional force ($F_{\text{friction}}$) which is given by

$$F_{\text{friction}} = vf \quad (1.3)$$

where $v$ is the rate of migration (velocity) of the ion and $f$ is the frictional coefficient. In a constant electric field, the forces on the ion balance each other:

$$qE = vf \quad (1.4)$$

so that each ion or charged particle moves with a constant characteristic velocity. An ion's electrophoretic mobility, $\mu$ is defined as

$$\mu = \frac{v}{E} = \frac{q}{f} \quad (1.5)$$

The above Equation only applies to ions at infinite dilution in a non-conducting solvent. In aqueous solution, polyelectrolytes such as proteins are surrounded by a cloud of counterions, which impose an additional electric field of such magnitude that the above Equations are only approximations of reality. To date, there is no existing theory that can accurately describe the mobility of polyelectrolytes because of the complexities of their ionic solutions. However, the Equation 1.5 correctly indicates that the molecules at their isoelectric points, $pI$, have zero electrophoretic mobility. In addition, it is clear that for proteins and other polyelectrolytes that have acid-base properties, the ionic charge and the electrophoretic mobility are a function of solution $pH$. 

[41-43]
There are several electrophoresis techniques that have been developed for the characterisation and separation of proteins and polyelectrolytes. The commonly used techniques are Paper Electrophoresis\textsuperscript{[37]}, Gel Electrophoresis, SDS-page, Isoelectric Focusing \textsuperscript{[44]}, and Capillary Electrophoresis \textsuperscript{[45]}.

Paper electrophoresis separates ions largely on the basis of their charge, in which an electric field of \( \sim 20 \) V cm\(^{-1} \) is adequate for many separations. The high diffusion of small molecules such as amino acids and small peptides, however limits their resolution in complex mixtures. This difficulty can be reduced and the separation speeded up by using an electric field of \( \sim 200 \) V cm\(^{-1} \). However, this require a means of dispassion of the heat generated by the application of such as high potential.

Gel electrophoresis \textsuperscript{[46]} is among the most powerful methods of macromolecule separation. The gel, such as polyacrylamide and agarose, have pores of molecular dimensions whose size can be specified. The molecular separations are therefore based on gel filtration as well as the electrophoretic mobility of the molecules being separated. The gel bands may be detected by staining, radioactive counting, or immunoblotting.

SDS - Page electrophoresis of proteins is based on their interaction that sodium dodecyl sulfate (SDS), a detergent that binds quite tenaciously to proteins causing them to assume a rod like shape. In addition, most proteins bind SDS in the same ratio, 1.40 g of SDS / g of protein (about one SDS molecule for every two amino acid residues). The large negative charge that the SDS imparts masks the protein's intrinsic charge so that SDS-treated proteins tend to have identical charge-to-mass ratios and similar shapes. Consequently, the electrophoresis of proteins in a SDS-page separates them in order of their molecular masses because of gel filtration effects.
Although gel electrophoresis in its various forms is a common and highly effective method for separating charged molecules, it typically requires hours for a run and is difficult to quantitate and automate. These disadvantages are largely overcome through the use of capillary electrophoresis, a technique in which electrophoresis is carried out in very thin (1 to 10 μm diameter) capillary tubes made of quartz, glass or plastic. Such narrow capillaries rapidly dissipate heat and hence permit the use of high electric field of up to 300 V cm⁻¹. This reduces the separation time to a few minutes. The rapid separations, in turn, minimise band broadening caused by diffusion, thereby yielding extremely sharp separations. Capillary electrophoresis can be automated in much the same way as HPLC, that is, with automatic sample loading and on-line detection. Since capillary electrophoresis can only separate small amounts of material, it is largely limited to use as an analytical technique rather than as a separation process for production.

1.2.3 Ultracentrifugation

Ultracentrifugation is a separation process based on the centrifugal force generated by a rotational speed (up to 80,000 rpm) [36,37]. The rate at which a particle sediments in the ultracentrifuge is related to its mass and shape. So that this allows the macromolecules to be characterised by their sedimentation rates.

Solvation increases the frictional coefficient of a particle by increasing its hydrodynamic volume. Further more, the frictional force is minimal when the particle is a sphere, as in this conformation it has the lowest surface area possible. Hence the frictional ratio is indicative of both molecular solvation and shape.
Most commonly used techniques are sedimentation and preparative ultracentrifugation. These techniques are often the choice for separating protein mixtures whose components have a range of densities and particle sizes. These substances include nucleic acids, viruses, and certain subcellular organelles. However, ultracentrifugation is rather ineffective fractionation for the separation of protein mixtures of similar densities.

1.2.4 Membrane Separation Processes

Proteins are macromolecules (with molecular weight up to 200 KD) and bear numerous ionisable groups which have a variety of pK’s. A range of membrane processes have been developed for the separation of proteins to date. Among them, ultrafiltration, dialysis and electrodialysis are frequently used processes for protein separation and recovery.

Protein separation by ultrafiltration \(^{[47,48]}\) is usually achieved with the membrane pore size of between 1 nm and 100 nm depending on the actual size of the proteins to be separated. The mechanism of ultrafiltration is based on the sieving effect, and this process is often used for the fractionation of macromolecules where large molecules have to be retained by the membrane while small molecules and solvent should permeate freely. The rejection is determined mainly by the size and shape of the solute relatively to the pore size in the membrane and the transport of solvent is directly proportional to the applied pressure. The performance of a given membrane is determined by two parameters; its selectivity and its flux (or permeation rate). The selectivity of a membrane towards a mixture is generally expressed by the retention (R) or the separation factor \(^{[154]}\).
Most of the ultrafiltration membranes used for protein separation today are asymmetric membranes with a thin active/selective layer (less than 1 \( \mu \text{m} \)) grafted on a more porous support. Because the hydrodynamic resistance is mainly determined within a small part of the total membrane thickness, they are more efficient in terms of transport flux and energy consumption.

Ultrafiltration has proven to be an effective protein separation process for fast protein fractionation on a relatively large scale. Applications can be found in fields of pharmaceutical, leather, chemical, food and dairy industries. Various applications in the food and dairy industry are the concentration of milk and cheese making, the recovery of whey proteins, the recovery of potato starch and proteins.

Ultrafiltration separation is easy to operate and has a relatively low running cost. However, it is difficult to separate proteins from a mixed protein solution merely via sieving and size exclusion according to their molecular weights. Since the molecular weight is not the only criterion determining the selectivity. Other factors, such as occurrence of concentration polarisation and the polymer molecular weight distribution, will also affect the molecular weight "cut-off" of the membrane. In addition, this process is not suitable for separating proteins of similar molecular weights or hydrodynamic sizes. So its application in protein separation is usually limited to raw biomaterials production. Further separation is often needed to obtain high purity bio-products.

Proteins are charged species in solution of different pH and their separation can also be achieved by electrodialysis - an electrically driven membrane process in which an electrical potential difference acts as the driving force on those charged macromolecules \([45,49,50]\). In an electrodialysis process for proteins separation, a number of cation- and
anion-exchange membranes are placed in an alternating pattern between a cathode and an anode. When an ionic feed solution is pumped through the cell, the positively charged ionic groups migrate towards the cathode and negatively charged ionic groups migrate towards the anode (Figure 1.2).

![Diagram](image)

**Figure 1.2** Separation of amino acid by electrodialysis

Amino acids contain both basic and acidic groups, and because of this amphoteric character, the molecule can be positively charged or negatively charged depending on the pH of the solution. At high pH, the amino acid is negatively charged and migrates towards the anode when an electrical field is applied. At lower pH, the amino acid is positively charged and migrates towards the cathode. At pH equal to the isoelectric point (pI) of the amino acid, there is no net charge and the amino acid will not migrate in an electrical field. By varying the pH of the solution in each compartment, a complete separation of a particular protein from others can be achieved. However, the transport flux of charged particles across the membrane to a large extent depends on the actual electrical field applied. As a result, a high electrical voltage between the cathode and anode is usually required to achieve a high permeation rate.
1.3 CONDUCTING POLYMER MEMBRANES

1.3.1 The Development of Conducting Polymers

Conducting polymers are a relatively new class of organic polymer with the remarkable ability to conduct electrical current \([51]\). Most conducting polymers are characterised by the existence of extensive conjugated \(\pi\)-systems, doped by oxidation or reduction so as to have unpaired electrons and alternative single and double bonds along the polymer chain (Figure 1.3). Many applications require the polymer to be capable of cycling between oxidised states and reduced states, for polymers to hold charge and discharge, and for the change of oxidation state to be accompanied by changes in electronic, optical, chemical and/or mechanical properties.

![Polyacetylene](image)
![Polypyrrole](image)
![Polythiophene](image)
![Polyaniline](image)

*Figure 1.3 Schematic of conjugated conducting polymers*

The level of excitement regarding these new materials is currently high since a plethora of new, often exotic, applications have been materialised \([52,53]\). The ability to process different forms of polymer, such as powder, electrical wires or thin membrane films will facilitate development of further application areas.
The first conducting polymer was prepared prior to 1900, but it was not until 1977 \cite{54,55}, that several major breakthroughs were made. In 1977 Shirakawa in Japan, Alan G. MacDiarmid, and Alan J. Heeger, both from the USA, discovered that partial oxidation with iodine or other reagents made polyacetylene films $10^9$ times more conductive than they were originally. This process transforming a polymer to a conductive form via chemical or electrochemical oxidation or reduction is now called doping. The conductivity of doped polyacetylene was found to be as high as $10^3 \text{ S cm}^{-1}$ which was higher than that of any known polymer at that time. The predicted theoretical limit of the conductivity of polyacetylene is about $2 \times 10^7 \text{ S cm}^{-1}$, more than one order of magnitude higher than that of copper. The conductivity of polypyrrole and some other conjugated polymers saturates at some $100 \text{ S cm}^{-1}$ or higher, but for many applications \cite{52}, even $10^{-3} \text{ S cm}^{-1}$ is sufficient, e.g. for electrostatic dissipation.

Another important discovery, the synthesis of continuous conducting polypyrrole thin films, was made in 1979 by Diaz and his coworkers \cite{56-58}. They reported that an oxidised form of continuous, smooth polypyrrole conducting polymer film could be prepared by the anodic oxidation of pyrrole monomer in an acetonitrile solution using a tetraethylammonium tetrafluoroborate electrolyte. The films prepared were stable in air and showed metallic properties with a conductivity as high as $100 \text{ S cm}^{-1}$. However, attempts to grow polypyrrole conducting polymer films galvanostatically under rigorously anhydrous and oxygen free conditions, as suggested by Diaz, were unsuccessful. It would appear that some reducible species must be added for the counter-electrode reaction to occur at acceptable potential. Later, it was found that other polymers such as polythiophene \cite{51} and polyaniline \cite{59} could also be synthesised using electropolymerisation.
Table 1.7 Applications of conducting polymers

<table>
<thead>
<tr>
<th>Applications</th>
<th>Major properties involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>electrical wire</td>
<td>conductivity</td>
</tr>
<tr>
<td>capacitor</td>
<td>conductivity</td>
</tr>
<tr>
<td>electrical shielding</td>
<td>conductivity</td>
</tr>
<tr>
<td>electrostatic dissipation</td>
<td>conductivity</td>
</tr>
<tr>
<td>rechargeable polymer battery</td>
<td>conductivity, electroactivity</td>
</tr>
<tr>
<td>anticorrosion coating</td>
<td>conductivity, electroactivity</td>
</tr>
<tr>
<td>liquid display</td>
<td>electroactivity</td>
</tr>
<tr>
<td>actuator</td>
<td>conductivity, mechanical properties</td>
</tr>
<tr>
<td>ion and molecule separations</td>
<td>membrane, electroactivity, conductivity</td>
</tr>
<tr>
<td>gas separation</td>
<td>membrane properties</td>
</tr>
<tr>
<td>sensing/biosensing devices</td>
<td>conductivity, electroactivity, stability</td>
</tr>
<tr>
<td>controlled release</td>
<td>ion-exchange</td>
</tr>
<tr>
<td>biocompatible polymer</td>
<td>physical and chemical stabilities</td>
</tr>
<tr>
<td>membrane separation</td>
<td>physical and chemical properties</td>
</tr>
</tbody>
</table>

Many applications of conducting polymers have been developed over the past 20 years [52,53]. Most of the applications involve the electroactivity and conductivity [60,61] of the polymer as well as other physical or chemical properties [62-64]. A brief summary of the development and application of conducting polymers is listed in Table 1.7.

1.3.2 Polypyrroles

Among conducting polymers known to date, polypyrrole and its derivatives have attracted the most attention and interest because of their
high electrical conductivity, environmental stability and high mechanical strength. They are also electrochemically dynamic. Polypyrroles and their derivatives can be easily synthesised in various forms by chemical or electrochemical polymerisation methods. Table 1.8 shows polypyrrole and some derivative conducting polymers.

Table 1.8 Selected data for polypyrrole and some of its derivative

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Monomer $E_{pa}$ vs SCE</th>
<th>Polymer $E_0$ vs SCE</th>
<th>Degree of oxidation</th>
<th>Density (g cm$^{-3}$)</th>
<th>Conductivity (Scm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[H]$_n$</td>
<td>1.20</td>
<td>-0.20</td>
<td>0.25-0.30</td>
<td>1.48</td>
<td>$10^2$</td>
</tr>
<tr>
<td>[Me]$_n$</td>
<td>1.16</td>
<td>0.47</td>
<td>0.26</td>
<td>1.40</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>[Et]$_n$</td>
<td>1.22</td>
<td>0.45</td>
<td>0.20</td>
<td>1.36</td>
<td>$2 \times 10^{-3}$</td>
</tr>
<tr>
<td>[Pr]$_n$</td>
<td>1.26</td>
<td>0.5</td>
<td>0.2</td>
<td>1.28</td>
<td>$1 \times 10^{-3}$</td>
</tr>
<tr>
<td>[n-Bu]$_n$</td>
<td>1.22</td>
<td>0.64</td>
<td>0.11</td>
<td>1.24</td>
<td>$10^{-4}$</td>
</tr>
<tr>
<td>[i-Bu]$_n$</td>
<td>1.24</td>
<td>0.60</td>
<td>0.08</td>
<td>1.25</td>
<td>$2 \times 10^{-5}$</td>
</tr>
<tr>
<td>[Ph]$_n$</td>
<td>1.80</td>
<td>0.65</td>
<td>0.15</td>
<td>1.42</td>
<td>$10^{-3}$</td>
</tr>
<tr>
<td>[Me]$_n$</td>
<td>-</td>
<td>-0.30</td>
<td>-</td>
<td>1.36</td>
<td>4</td>
</tr>
<tr>
<td>[Me]$_n$</td>
<td>1.00</td>
<td>-0.10</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>[Ph]$_n$</td>
<td>1.20</td>
<td>+0.50</td>
<td>-</td>
<td>-</td>
<td>$10^{-3}$</td>
</tr>
</tbody>
</table>

* Data from ref. [58-67]
1.3.3 Synthesis of Polypyrroles

Polypyrrole can be synthesised using chemical [56,57] or electrochemical polymerisation methods [48,49]. In both cases, the important step is to incorporate certain charged species into the polymer by a process called "doping". The polymerisation process is usually expressed as

\[
\text{nH} \begin{array}{c}
\text{N} \\
\text{H}
\end{array} + \text{A}^- \xrightarrow{\text{Ox}} \left(\text{\begin{array}{c}
\text{N} \\
\text{H}
\end{array}\text{A}^-}^+\right)^n + 2\text{nH}^+ + 2\text{ne} \quad (1.6)
\]

where A^- represents the counterion or so called doping anion being incorporated into the polypyrrole during synthesis, and n is the number of pyrrole units.

The precise chemical composition and structure of polypyrrole are still not fully understood. Results from X-ray photoelectron spectroscopy (XPS) [70], UV-photoemission and optical adsorption spectra [71] of polypyrrole show polypyrrole is mainly composed of linear chains of planar pyrrole rings linked in the 2,5-position with approximately two to four pyrrole units carrying one positive charge which is electrically balanced by the negative charge of the dopant.

Although most of the pyrrole rings are linked at 2,5 - positions, a significant number of the units are coupled through 2,3 - positions. Some structural disorder is evident (Figure 1.4). This introduces defects in the hypothetically ideal linear chain arrangement of the polymer, which greatly reduce its conjugation length and, therefore, its conductivity.
Polymerisation of polypyrrole is initiated by the oxidation of monomer to a radical cation. Polymerisation then proceeds via a radical-radical coupling mechanism rather than radical-monomer mechanism (Figure 1.5).
Chemical polymerisation involves mixing the pyrrole monomer with an oxidant in a suitable solvent. The chemical method enables the preparation of these polymers on a large scale without requiring special equipment. However, this method is often limited by the number of oxidants available and hence the number of counterions that can be incorporated are also limited. So far there are only a few oxidants (such as FeCl₃, Cu(NO)₃, AgNO₃, H₂O₂ and K₂S₂O₄) that have been found to be suitable for the preparation of polypyrrole. Polypyrrole prepared using the chemical method can be prepared mainly in:

(i) particle form such as powder,
(ii) water soluble polymer or colloids, or
(iii) membrane form such as a films or a composite membrane.

On the other hand, the electrochemical approach for making polypyrrole is versatile and provides a facile means to vary the properties of the polypyrrole by simply varying the electropolymerisation conditions (such as monomer, substrate, electrode potential, solvent or electrolyte) in a controlled manner. This method has been becoming an important technique in synthesising polypyrrole conducting polymer since 1979 when the first polypyrrole film was electrochemically prepared [48].

The electrochemical synthesis of polypyrrole can be carried out either potentiostatically or galvanostatically [72,73,83]. In addition, the potentiodynamic method is usually employed for the investigation of the mechanism of polymerisation or polymer formation. In this case, a dynamic potential applied could be a potential sweep or a pulsed potential, and the current generated as a function of the applied potential is obtained. The potentiodynamic growth of polypyrrole can be used to
investigate the minimum polymerisation potential (which is necessary to initiate the polymer growth on a given substrate material), the polymer formation potential and over-oxidation potential as well.

The potentiostatic method can be used for the preparation of polypyrrole thin films. As the current may vary from time to time due to the film growth, the amount of polymer deposition is usually controlled by controlling charge consumed at the electrode. Because of the variation of the anodic current, the rate of polymer deposition is not constant throughout the entire electrochemical polymerisation process. As such, this method is not very convenient for synthesis of a thin film of desired thickness. On the other hand, galvanostatic polymerisation is carried out at a constant current so that the polymer deposition on the electrode substrate occurs at a fixed rate which depends on the actual current density applied. Hence, the galvanostatic method is commonly used for the production of polypyrrole films.

It has been found that polypyrrole conducting polymers can be electrochemically synthesised with a variety of counterions [72-76]. The counterions used so far are simple anions, organics with sulphonated groups and polyanions. In some instances, macromolecular species or even living cells have been employed [77,78] and incorporated into the polymer. Polypyrrole can also be electrochemically synthesised in different forms on different substrates. Polypyrrole powder, wire, thin film and colloids have now been successfully synthesised by this method.

Such progress in the synthesis of polypyrrole has greatly heightened the research interest in using this material for various practical applications.
1.3.4 Characterisation of Conducting Polymers

The characterisation of polypyrrole conducting polymers usually involves the measurement and determination of their chemical, physical and electrochemical properties. Towards this end, a variety of characterisation techniques have been used (Table 1.9).

**Table 1.9 Characterisation of conducting polymers**

<table>
<thead>
<tr>
<th>Type</th>
<th>Characterisation techniques</th>
<th>Information obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemical methods</td>
<td>Cyclic Voltammetry</td>
<td>polymerisation mechanism, electrochemical properties</td>
</tr>
<tr>
<td></td>
<td>Potentiometry</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amperometry</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EQCM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Impedance analysis</td>
<td></td>
</tr>
<tr>
<td>Chemical composition and Structure</td>
<td>Elemental analysis</td>
<td>polymer composition, structure and counterion identification</td>
</tr>
<tr>
<td></td>
<td>NMR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>XPS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Raman Spectroscopy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FTIR</td>
<td></td>
</tr>
<tr>
<td>Physical properties</td>
<td>Scanning electronic Microscopy (SEM)</td>
<td>morphology and porous structure, conductivity, mechanical properties</td>
</tr>
<tr>
<td></td>
<td>Atomic force microscopy (AFM)</td>
<td></td>
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<tr>
<td></td>
<td>Four probe conductivity</td>
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<tr>
<td></td>
<td>Tensile strength analysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Permeability</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Porosity Analysis</td>
<td></td>
</tr>
</tbody>
</table>

In recent years, some techniques such as electrochemical quartz crystal microbalance (EQCM), electrochemical atomic force microscopy (EAFM) have been developed. These newly developed techniques enable us to characterise the conducting polymer and polymer membrane in greater detail. The application of these techniques has been found to be
very helpful for further investigation into the dynamic properties of polypyrrole.

### 1.3.5 Switching Properties of Polypyrroles

Polypyrroles are electrically conductive and electroactive. They are capable of switching between their reduced and oxidised states to hold charge and discharge. The reduction and oxidation of this polymer can be manipulated through the variation of the electrical stimuli applied. The changes in polypyrrole’s redox states are usually accompanied by cation- or anion-exchange between the solution and the polymer \([79-82]\). This is because polypyrroles can undergo the following redox reactions:

\[
\begin{align*}
\left( \begin{array}{c}
\text{N} \\
\text{H}
\end{array} \right)_n^+ + A^- & \overset{+e}{\rightleftharpoons} \left( \begin{array}{c}
\text{N} \\
\text{H}
\end{array} \right)_n^0 + A^- \\
\text{where the counterion } A^- \text{ is relatively mobile, and/or}
\end{align*}
\]

\[
\begin{align*}
\left( \begin{array}{c}
\text{N} \\
\text{H}
\end{array} \right)_n^+ + M^+ & \overset{+e}{\rightleftharpoons} \left( \begin{array}{c}
\text{N} \\
\text{H}
\end{array} \right)_n^0 A^- M^+ \\
\text{where } M^+ \text{ is a cation and the counterion } A^- \text{ is less mobile or immobile.}
\end{align*}
\]

This phenomenon is often referred to as the redox switching properties of conducting electroactive polymers. The redox switching properties can be found in almost every electrode process where polypyrrole conducting polymers or polypyrrole membranes are utilised.

An electrochemical redox switching process, as indicated by Equation (1.7) and Equation (1.8), is always associated with ion-exchange between the solution and the polymer matrix. The cation or anion move in and out of the polymer matrix to accommodate changes in the polymer’s redox
states. The ion-exchange properties of a polymer, to a large extent, are determined by the properties of the counterions incorporated into the polymer during electropolymerisation. The counterion's size, mobility and charge density are all crucial in determining the switching and/or ion-exchange properties of the resultant polymer. This phenomenon is not only of fundamental importance, but also of potential application in designing transport and separation processes with polypyrrole conducting polymer membranes.

1.3.6 Conducting Polymer Membranes

1.3.6.1 Development of Conducting Polymer Membranes

The unique redox switching properties of conducting electroactive polymers can be utilised to control transport of ionic species using conducting polymer membranes. By manipulating the redox states of the polymer membrane \textit{in situ} or \textit{ex situ}, selective transport can be achieved. This is known as electrochemically controlled transport.

Conducting polymer membrane system have been previously reported [84-89]. Most of these membranes were supported membranes due to the limited mechanical strength of the polymer. The ion permeation through polypyrrole membranes supported by gold mini grid membranes had been reported by Burgmayer and his co-workers [88,89]. They proposed that the ionic resistance or ion permeability of the polypyrrole membranes can be varied by changing the redox state of the membranes. The permeation rate through a polypyrrole membrane in the oxidised state was much higher than that through a polypyrrole membrane in the reduced state.
The use of polypyrrole free-standing films was first demonstrated by Wang and his co-workers [84,85]. The transport of monovalent cations such as H\(^+\), K\(^+\), Na\(^+\), NH\(_4^+\) between two electrolyte solutions were investigated by applying an electrical field across a polypyrrole film. They showed that the transport process depends on the ionic size and charge of the transported species and surface characteristic of the membrane.

Recently, the transport of metal ions across polypyrrole free-standing films has been reported by Zhao and co-workers [90-93]. In particular, the use of electrochemical stimuli, such as pulsed potential, to initiate and control the transport of metal ions was demonstrated. They showed that the transport of metal ions can be electrochemically switched on and off by varying the external electrical stimuli applied to the membranes. In addition, the transport of small organic molecules across polypyrrole membrane was also demonstrated by Mirmohseni from the same research group using a similar electrochemically controlled transport technique[94].

In other work, the permeation of volatile and gaseous compounds through a polypyrrole composite membrane was investigated [98]. It was found that the permeation rates depend upon the redox states of the polymer membrane; a high permeation rate was measured for reduced polypyrrole and a low permeation rate for oxidised polypyrrole [88,89].

Recently, gas separation by polypyrrole composite membranes and polyaniline free-standing films have been reported [95-97]. It was found that the subsequent dedoping/redoping process would cause permanent morphological structure changes to the polymer films which resulted in improved gas separation. For example, the selectivity between H\(_2\)/N\(_2\) and O\(_2\)/N\(_2\) were 3590 and 30 respectively for redoped polyaniline films.
1.3.6.2 Synthesis and Characterisation of Conducting Polymer Membranes

Conducting polymer membranes can be synthesised using the same procedures for preparing conducting polymers. They can be chemical or electrochemical. But factors such as uniformity, mechanical properties and processing ability as well as electrical conductivity and electroactivity have to be considered during the synthesis.

The characterisation of conducting polymer membranes can also be carried out using those methods previously described for the characterisation of conducting polymers.

1.3.6.3 Electrochemically Controlled Transport in Conducting Polymer Membrane Systems

Conducting electroactive polymers have the adaptive properties necessary to qualify them to be fabricated into electrodynamic membranes for controlled transport of charged species. These membranes can be electrochemically stimulated in-situ to elicit desired responses to reversibly adapt their chemical (e.g. ion-exchange) or physical (e.g. hydrophobic/hydrophilic) properties. These properties are particularly useful in designing and operating an electrodynamic membrane system (or intelligent membrane system) for various membrane processes.

There are several different processes involved in the transport of charged species across conducting polymer membranes. Factors such as the polymer's redox state, diffusion transport in the solution and membrane, and ion insertion/expulsion all will affect the final performance of the electrochemically controlled transport system.
The electroactivity or redox switching properties, to a large extent, depends on the nature of the counterion being incorporated into the polymer and that of the monomer being used. The size, charge density and other chemical properties are all crucial in determining the electrochemical properties of the resultant polymer membrane.

In addition, the electrochemical stimuli applied are also important in determining the transport processes. As shown in Figure 1.6, the electrochemical control can be applied in several different forms.

![Diagram of Electrochemical Control Factors](image)

**Figure 1.6** Electrochemical control factors in electrochemically controlled transport by conducting polymer membranes

Other factors such as the cell design and electrical connections to the membranes are also important in determining the final performance of the transport systems [93]
1.4 AIM OF THIS PROJECT

The aim of this work is to develop a multifunctional electromembrane system for electrochemically controlled protein transport and separation. Four different aspects have been addressed in this project:

(1) To develop electrically conducting membranes for the electrochemically controlled transport of proteins (Chapter 3). Platinised membranes have been introduced in the controlled transport and separation of proteins. Compared with conventional membranes, a multi-driving force separation can be applied to the platinised membrane separation process which can effectively enhance the transport flux, and reduce the fouling and concentration polarisation.

(2) To investigate the synthesis, characterisation and interaction of biofunctional polymers (Chapters 4 and 5). The synthesis of polypyrrole with specific biofunction is of great interest for bioseparation processes and biosensing technologies. Antibody (IgG) and heparin were electrochemically incorporated into the polypyrroles. The interactions between antibody-antigen and heparin-thrombin, were investigated with EQCM.

(3) To study the electrochemically controlled transport of proteins using polypyrrole composite membranes (Chapter 6). The transport of proteins has been carried out with polypyrrole composite membranes.

(4) To investigate the feasibility of gas separation by polypyrrole membranes (Chapter 7). Polypyrrole thin films and polypyrrole composite membranes have been studied in terms of their gas permeability and separation factor using either pure gas or gas mixtures.
CHAPTER 2

GENERAL EXPERIMENTAL TECHNIQUES
2.1 INTRODUCTION

Advances in conducting electroactive polymer (CEP) and membrane technology can be attributed largely to the development of instrumental techniques capable of probing electrode structures and interfaces in greater detail. A variety of in-situ and ex-situ characterisation techniques and methods have been developed and used for the studies of conducting polymers and polymer membranes (Table 1.9, Chapter 1). These techniques include conventional electrochemical characterisation methods as well as newly developed techniques will be discussed below. These characterisation techniques include:

(1) Electrochemical characterisation techniques, such as cyclic voltammetry (CV), potentiometry, amperometry and electrochemical quartz crystal microbalance (EQCM),

(2) Surface morphology characterisation techniques by Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM), and

(3) Electrical conductivity determination by four point probe techniques

All these methods are discussed in this chapter with a brief explanation on their theoretical aspects.

In addition, some experimental aspects relevant to membrane preparation, protein transport and gas permeation cell designs, are discussed briefly in this chapter. Detailed experimental procedures for these transport and separation processes will be given in each corresponding chapter.
2.2 CHARACTERISATION TECHNIQUES

2.2.1 Cyclic Voltammetry (CV)

Conventional voltammetric techniques \(^{[99]}\) involve the measurement of the current flowing through a working electrode as a function of the applied potential, which is controlled versus a reference electrode (such as a silver/silver chloride electrode). The electrochemical cell usually consists of three electrodes, i.e., the working, auxiliary and reference electrodes. There are several voltammetric techniques which can be used to study electrochemical processes \(^{[100,101]}\). These techniques include cyclic voltammetry (CV), staircase voltammetry (SV), and differential pulse voltammetry (DPV). Of these voltammetric techniques, cyclic voltammetry is one of the most extensively used in studying electrode structures and interfaces.

Cyclic voltammograms can provide information on the number and reversibility of different oxidation states of an electroactive species, and can be used for qualitative investigation of rates of heterogeneous and homogeneous reactions. This technique has also been widely used for studies of conducting electroactive polymers.

The excitation signal for cyclic voltammetry is a linear potential scan with a triangular potential versus time waveform (Figure 2.1). The applied potential is usually scanned linearly between the upper and lower potential limits at a given rate \(\nu\) (or \(\frac{dE}{dt}\)). The current (both cathodic and anodic segments) is monitored during the complete excursions of the applied triangular potential sweep.

The anodic and cathodic peaks do not appear exactly at the same potential. The potential difference between these peaks depends on the actual electrode process and the charge transfer rate. For a reversible system,
the separation of peak potentials is estimated to be $\Delta E = E_{p(a)} - E_{p(c)} \approx 0.058/n$ (Volt) \cite{100}; where $n$ is the number of electrons transferred in the redox system. A quasi-reversible system shows a greater separation in peak potentials than that in a reversible system, i.e. $\Delta E > 0.058/n$ (Volt). Response on the cyclic voltammograms are broaden indicating the rate of charge transfer is much slower than that in a reversible electrode process. For an irreversible process, a complete separation of anodic and cathodic peaks would occur in the cyclic voltammogram, reflecting a very slow rate of charge transfer in such an electrode process.

![Figure 2.1 The potential waveform (a) and resultant current (b) in a cyclic voltammetric experiment.](image)

A typical cyclic voltammogram of polypyrrole doped with dodecyl sulfate (PPy/DS) in 0.2 M KCl is given in Figure 2.2. This shows the redox process of PPy/DS with peak potentials $E_{p(c)}$ and $E_{p(a)}$ appearing at -0.65 and -0.43 V respectively when scanned between -1.0 V to +0.2 V. The oxidation and reduction of PPy/DS were accompanied by cation (K$^+$) expulsion and insertion respectively according to Equation 1.8, so that the overall charge neutrality of the polymer was maintained.
Figure 2.2 Cyclic voltammogram of PPy/DS in 0.2 M KCl at a scan rate of 20 mV s⁻¹. PPy/DS was prepared galvanostatically on a gold disc electrode (d=5 mm) at a current density of 1.0 mA cm⁻² for 2 minutes from a polymerisation solution containing 0.2 M pyrrole and 0.05 M DS.

It should be noted that cyclic voltammograms can be significantly affected by the existence of capacitive behaviour within the electrode interfacial region [86]. The capacitive charging current is determined by

\[ i_c = \frac{dQ_c}{dt} = C_{dl} \frac{dE}{dt} = C_{dl} V \]

(2.1)

where \( Q_c \) is the amount of dielectric charge, \( C_{dl} \) is the capacitance of the double layer in the interfacial region, and the applied potential scan rate (\( \nu \)) is represented by \( \frac{dE}{dt} \). Since \( C_{dl} \) varies with the applied potential, \( i_c \) is not constant throughout the entire electrode process. The presence of \( i_c \) places limitations on the signal to noise ratio. The peak current \( i_p \) is proportional to \( \nu^{1/2} \) in a fully reversible electrode process and its charging current \( i_c \) is proportional to scan rate (since \( i_c = C_{dl} \cdot \nu \)). This will limit the
highest scan rate which can be used because signal to noise ratio would
decrease with the increase in scan rate.

It should also be noted that the scan rate is limited by the cell time
constant $\tau$ (the product of solution resistance $R_u$ and capacitance $C_{dl}$), since
this controls how rapidly a potential change can occur. Other techniques
such as using a pulse potential or a microelectrode may be needed if
steady state voltammetry or higher signal to noise ratios are required.

2.2.2 Chronoamperometry

Chronoamperometry is a widely used technique in studying electrode
processes. This technique is usually carried out in a three electrode system
in which a potential is applied to the working electrode and the current
generated is recorded versus time. Chronoamperometry is found to be
very useful in studying the polymerisation processes of conducting
electroactive polymers.

The rate of reaction can be measured by monitoring the current that
passes through the working electrode since

$$i = \frac{dQ(t)}{dt} \quad (2.2)$$

where $i$ is the electrode current, $Q(t)$ the charge consumed in the
electrode process. During the polymerisation of conducting polymer, a
continuous rise in current indicates that the reactive species has been
deposited on the electrode resulting in an increase in the electrode surface
area. On the other hand, a decrease in current may be evidence of no
deposition or resistive material being deposited. A typical
chronoamperogram is given in Figure 2.3 in which polypyrrole - DS
(PPy/DS) was prepared by electrochemically oxidising pyrrole monomer
in 0.05 M sodium dodecyl sulfate solution at an applied potential of $E_{\text{app}}=0.55$ V versus Ag/AgCl reference electrode. The chronoamperogram of PPy/DS growth showed a transient increase of charging current upon applying the potential. The polymer growth is evidenced by the immediate steady increase of current after the transient.

![Image](image.png)

**Figure 2.3** Chronoamperogram of PPy/DS growth on a platinum disc electrode ($d=1.5$ mm). PPy/DS was prepared from a solution containing 0.2 M pyrrole monomer and 0.05 M DS at $E_{\text{app}} = 0.55$ V versus Ag/AgCl.

### 2.2.3 Chronopotentiometry

This technique involves the measurement of the potential-time variations at a working electrode during a short period of exhaustive electrolysis carried out at a constant current. The resultant potential (versus a reference electrode) is recorded as a function of time. A steady decrease or rise in potential during electrochemical polymerisation of conducting polymer, indicates that the deposited material is of good or poor conductivity respectively. If the potential remains relatively low and
stable, the polymer film deposited on the electrode is in a conductive form. On the contrary, if the potential remains relatively high or even increases with time during growth, this indicates resistive film deposition or no film deposition at all. Figure 2.4 shows the chronopotentiogram obtained for PPy/DS polymerisation at a current density of 1.0 mA cm^{-2}. The steady decrease in potential indicates that the PPy/DS film had deposited onto the electrode surface and is conductive.

![Figure 2.4](image)

**Figure 2.4** Chronopotentiogram obtained for growth of PPy/DS conducting polymer on a gold coated quartz electrode (d=5 mm) at a current density of 1.0 mA cm^{-2}. The polymerisation solution contained 0.2 M pyrrole monomer and 0.05 M DS.

### 2.2.4 Electrochemical Quartz Crystal Microbalance (EQCM)

The electrochemical quartz crystal microbalance (Figure 2.5) is, actually, an electrochemical version of the quartz crystal microbalance (QCM); which has long been used for frequency control and mass sensing [102,103].
Indeed, to a large extent, it was the first report of an EQCM study of polypyrrole film by Kaufman [104] that heightened the interest in the EQCM method for various applications such as electrodeposition [105-107] or even biosensing [108,109]. It now has emerged as an in-situ characterisation technique for conducting polymers [110,111] capable of detecting nano or even pico grams of mass changes on an electrode surface as different electrical stimuli such as potential or current is applied to the polymers.

The EQCM cell used in this investigation is shown in Figure 2.6 which consists of a gold coated quartz crystal electrode, a platinum mesh counter electrode and a Ag/AgCl reference electrode.
The EQCM cell and the quartz crystal electrode. The gold coated quartz electrode in contact with the cell solution was used as the working electrode in the electrochemical system. A platinum mesh and a Ag/AgCl electrode were used as the auxiliary electrode and the reference electrode respectively. A thin layer of conducting polymer was electrochemically deposited on gold coated working electrode.

The quartz crystal electrode used in this EQCM is a thin (0.165 mm), AT-cut quartz crystal with very thin gold electrode 'pads' on the opposite sides of the crystal. When an electrical potential is applied across the crystal using these two electrode pads, the AT-cut quartz crystal experiences a mechanical strain in the shear direction. Therefore, an alternating potential across the crystal causes the vibrational motion of the quartz crystal, which would result in a transverse acoustic wave that propagates back and forth across the crystal between its two faces (Figure 2.7 a). A standing-wave condition can be established in the quartz resonator when the acoustic wavelength in the quartz is equal to twice the combined thickness ($t_q$) of the crystal and the electrodes, i.e.

$$f_0 = \frac{V_r}{2t_q}$$  \hspace{1cm} \text{(2.3)}
where $f_0$ is the resonant frequency, $v_{tr}$ is the wavelength of the transverse acoustic wave, and $t_q$ is the thickness of the resonator.

$$fo = \frac{v_{tr}}{2\pi t_q}$$

*Figure 2.7 Acoustic wavelength in (a) quartz electrode and (b) quartz electrode coated with CEP film.*

A frequency shift will occur as a result of the deposition of CEP film on the quartz electrode (Figure 2.7 b). The frequency shift can be calculated from

$$\Delta f = \frac{v_{tr}}{2t_q} - \frac{v_{tr}}{2(t_q + t_{film})}$$

(2.4)

An assumption is made here that the velocity of the acoustic wave in the quartz crystal and the electrode is identical (while not rigorously true, for small electrode thickness the error is negligible). If we assume that the acoustic velocity and density of a foreign layer are nearly equal to those in quartz, a change in thickness of the foreign layer is tantamount to a change in the thickness of the quartz crystal. Under this condition, a fractional change in frequency is generated by the extension of acoustic wave travelling distances and the relationship between the frequency
change and the amount of mass deposited is given by the well-known Sauerbrey [88] equation:

\[
\Delta f = \frac{2f_0^2 \Delta m}{A(\rho_q \eta_q)^{1/2}} \tag{2.5}
\]

or

\[
\Delta m = \frac{\Delta f A(\rho_q \eta_q)^{1/2}}{2f_0^2} \tag{2.6}
\]

Where \( \rho_q \) is the quartz density which is 2.648 g cm\(^{-3} \), \( \eta_q \) is the shear modulus of the quartz, 2.947x10\(^{11} \) dynes cm\(^{-2} \), \( f_0 \) is the basic resonant frequency of a unloaded quartz crystal (Hz), \( A \) is the piezoelectrically active area (cm\(^2 \)), \( \Delta m \) is the mass change in (g) on the electrode surface, and \( \Delta f \) is the frequency change due to additional mass adsorbed onto the working quartz crystal electrode surface (Hz). Equation (2.6) can be further simplified to

\[
\Delta m = C_f \Delta f \tag{2.7}
\]

or

\[
\Delta f = C_f \Delta m \tag{2.8}
\]

where \( C_f \) (\( C_f = 1/C'f \)) is the mass - frequency conversion factor derived from the Sauerbrey equation which is 0.8673 ng-Hz\(^{-1} \) for a 10 MHz quartz crystal of 0.196 cm\(^2 \) electrode area.

The EQCM data interpretation can be made in a straight forward manner. The electrical charge represents the total amount of electrons transferred and corresponds to the mass change occurring at the electrode surface. Accordingly the frequency change measured with the EQCM, under ideal conditions, is proportional to the apparent molecular weight \( M_w \) [87]:

\[
\Delta f = \frac{M_w C_f Q}{nF} \tag{2.9}
\]


or

\[ i = \frac{nvF}{M_w} \frac{d(\Delta f)}{dE} \]  

(2.10)

where \( v \) is the scan rate during cyclic voltammetry (V\cdot s^{-1}), \( F \) is the Faraday constant (96480 \( \text{C}\cdot\text{mol}^{-1} \)) and \( n \) is the number of electrons transferred.

This format is particularly useful for cyclic voltammetry experiments (Figure 2.8), as \( \frac{d(\Delta f)}{dE} \) should appear in a form similar to that of the current in a voltammogram if the electrochemical event is accompanied by corresponding mass change. The peak potential \( E_{p(a)} \) and \( E_{p(c)} \) will appear at the corresponding potential where \( \frac{d^2(\Delta f)}{dE^2} = 0 \), i.e. at the potential where frequency change versus the potential (i.e. \( \frac{d(\Delta f)}{dE} \)) reaches to its maximum value according to equation (2.10).

The EQCM technique has been used in this research for the investigation of CEP electrodeposition and CEP-protein interactions. EQCM calibrations were carried out using the depositions of silver, copper or conducting polymers.
Figure 2.8 PPY/DS EQCM frequency change profile in 0.2 M KCl at the scan rate of 20 mV s$^{-1}$. PPY/DS was prepared galvanostatically on a gold coated quartz electrode (0.196 cm$^2$) at the current density of 1.0 mA cm$^{-2}$ for 2 minutes from a polymerisation solution containing 0.2 M pyrrole and 0.05 M DS.

2.2.5 Electrical Conductivity

One of most striking properties of conducting polymers is their remarkable ability to conduct an electric current. The accurate measurement of electrical conductivity is a major step in understanding CEPs. The conductivity of a conducting polymer, such as a piece of polymer membrane, can be measured at least in two different ways - the ASTM four point probe technique $^{[112]}$ and L. J. van der Pauw four point probe method $^{[113]}$. Both methods have been employed in this work.

The ASTM method is a standard method applied to all kinds of materials that exhibit surface or volume resistivity within the range of $1 \sim 10^{-7}$ (S
cm$^{-1}$). With this method, two identical electrical connectors are used. The four electrodes on each connector are arranged in a pattern as shown in Figure 2.9. The polymer film to be tested is placed in contact with the four electrodes and tightly sandwiched with the other connector. A constant current is then applied between the two outer electrodes which will generate a potential gradient along the current flow direction. According to Ohm's Law, the resistance of the material is proportional to the potential drop across the two inner electrodes. The resistivity of the polymer material can then be calculated according to the cross-sectional area of the polymer film and the resistance obtained.

![Diagram of four point probe method](image)

**Figure 2.9** Top view of the ASTM four point probe method for the measurement of the electrical conductivity of a CEP film. The distance between the two inner electrodes is 0.2 cm. The sample is sandwiched with another connector. A constant current is applied through the two outer electrodes.

According to this method, the surface conductivity and volume conductivity can be calculated as follows:

$$\rho_s = \frac{wV}{il} \quad (2.11)$$

where $\rho_s$ is the surface resistivity (Ω), $l$ is the distance between the two inner electrodes (cm), $V$ is the potential drop across the inner electrodes (V), $w$ is the sample width (cm) and $i$ is the current applied (A); or
\[ \sigma_s = \frac{il}{wV} \quad \text{or} \quad \sigma_s = \frac{1}{\rho_s} \quad (2.12) \]

where \( \sigma_s \) is the surface conductivity (S).

If the thickness (h) of the sample is considered (the sample cross section area \( S = wh \)), then the volume resistivity \( \rho_v \) (\( \Omega \) cm) is given by:

\[ \rho_v = \frac{whV}{il} = \frac{SV}{il} \quad (2.13) \]

and the volume conductivity of the material \( \sigma_v \) (S cm\(^{-1} \)) is:

\[ \sigma_v = \frac{il}{whV} = \frac{il}{SV} \quad (2.14) \]

This method is widely used for the measurement of the electrical conductivity of conducting polymers. It is required that the sample to be tested should have a uniform thickness and can be cut into a regular shape (such as a strip).

It should be mentioned that this method is based on a simple pattern of virtually parallel current stream-lines. The conductivity or resistivity of the polymers measured by this method might be affected by the uniformity of the actual current distribution across the polymer tested. A more accurate technique for measuring the electrical conductivity is the L. J. van der Pauw four probe method as described below.

Take a flat lamella, completely free of holes and provide it with four small but good contacts: M, N, O, and P, in an irregular sample (Figure 2.10). If a current between M and N, written as \( i_{MN} \), is applied, and the potential difference \( \Delta V_{po} = V_p - V_o \) is measured, the resistivity can be defined as:

\[ R_{MN,OP} = \frac{V_p - V_o}{i_{MN}} = \frac{\Delta V_{po}}{i_{MN}} \quad (2.15) \]
Analogously we define

\[ R_{NO,PM} = \frac{V_M - V_P}{i_{NO}} = \frac{\Delta V_{MP}}{i_{NO}} \]  \hspace{1cm} (2.16)

Figure 2.10 The measurement of conducting polymer conductivity by L. J. van der Pauw four probe method. M, N, O, and P represent the four small connections to the sample.

This method of measurement is based on the theorem that between \( R_{MN,OP} \) and \( R_{NO,PM} \) there exists the simple relation:

\[ \exp\left(-\left(\frac{\pi d}{\rho_v}\right)R_{MN,OP}\right) + \exp\left(-\left(\frac{\pi d}{\rho_v}\right)R_{NO,PM}\right) = 1 \]  \hspace{1cm} (2.17)

where \( d \) is the thickness of the lamella and \( \rho_v \) is the resistivity of the material (\( \Omega \) cm). So \( \rho_v \) is the only unknown quantity which equal to \( 1/\sigma_v \). But in practice, \( \rho_v \) can not be simply obtained from the above equation and is usually expressed as

\[ \rho_v = \frac{\pi df(R_{MN,OP} + R_{NO,PM})}{2\ln2} \]  \hspace{1cm} (2.18)

or

\[ \sigma_v = \frac{2\ln2}{\pi df(R_{MN,OP} + R_{NO,PM})} \]  \hspace{1cm} (2.19)
where $f$ is a factor which is a function only of the ratio of $R_{MN,OP}/R_{NO,PM}$ as plotted in Figure 2.11.

![Figure 2.11 Relationship between $R_{MN,OP}/R_{NO,PM}$ and factor $f$ for the measurement of conductivity of polymer membranes using L. J. van der Pauw method](image)

The L. J. van der Pauw four probe method can be applied to samples of any irregular shape such as a conducting polymer thin film or a composite membrane. It provides a more accurate approach in measuring the electrical conductivity of various conducting polymers.

### 2.2.6 Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM)

The morphology and microstructure of polypyrrole based conducting polymer membranes have been investigated using both scanning electron microscopy (SEM) and atomic force microscopy (AFM). The results obtained from SEM and AFM were analysed to determine the morphological microstructure and surface smoothness of these membranes.
SEM experiments (e.g. Figure 2.12) were carried out at the Department of Materials Engineering, University of Wollongong. The images obtained were recorded using the Leica S440 camera. SEM samples, such as substrate membranes, polypyrrole free standing films and supported polypyrrole films, were coated with a thin layer of gold to make them sufficiently conductive and then were cut into 0.5x0.5 cm² pieces. Up to eight samples were installed on a sample holder. The SEM images were taken at different magnifications, varying between 500 and 30,000, in order to obtain different detailed information on the samples.

![SEM image](image)

**Figure 2.12** SEM image of a polypyrrole CEP membrane at the magnitude of 28,000. The polymer membrane was galvanostatically prepared at 1 mA cm⁻² from a solution containing 0.2 M pyrrole monomer and 0.05 M benzene sulfonic acid sodium salt.
Unlike SEM, AFM can be performed without the need for the sample to be conductive. Only one sample can be tested at a time. The AFM images (Figure 2.13) can be obtained using either tapping or friction mode. Two different forms of images can be obtained i.e. (i) two dimensional surface plot images, and (ii) three dimensional image. The surface plot image is similar to those obtained with SEM while the three dimensional image can be used for the estimation of surface roughness.

Figure 2.13 AFM image of a polypyrrole CEP membrane. The polymer membrane was galvanostatically prepared at 1 mA cm\(^2\) from a solution containing 0.2 M pyrrole monomer and 2500 ppm heparin sulfate sodium salt.
2.3 SYNTHESIS OF CONDUCTING POLYMER MEMBRANES

2.3.1 Substrate Membranes and Platinum Coating

To make a CEP membrane suitable for macromolecular separation, the membrane should be mechanically strong and easy to handle. In addition, the membrane has to remain relatively porous so that the diffusion or electromigration of macromolecules can be realised. Free-standing CEP films are non-porous membranes and may have poor mechanical strength as well. They have been found to be suitable for ion transport [76-79] or even gas separation [95, 97]. But these films are not suitable for macromolecules separation if their porous structures remain unchanged. In this work, we employed substrate membranes to support the polymer films so as to enhance the porous nature, mechanical properties and process ability of the resultant structure (Figure 2.14).

![Figure 2.14 Schematic of platinised membrane and CEP composite membrane](image)

Two different types of commercially available membranes were selected as substrates in this work. One was polyvinylidene fluoride (PVDF) microfiltration membranes from Millipore. PVDF membranes of
different pore sizes, 0.22 μm, 0.45 μm and 0.65 μm, were employed in preparing platinised conducting membranes and conducting polymer membranes for protein separation as well as gas separation. The second type was polysulphone (PS) ultrafiltration membranes from Memtec Ltd with a nominal molecular weight "cut-off" of 100KD. These membranes are asymmetric in structure with a dense polymer skin supported by an open porous substructure. The skin layer of these PS membranes has a porosity of about 6%. These PS membranes were used as substrates in preparing conducting polymer membranes mainly for gas separation.

Platinised conducting membranes were formed by sputter coating the substrate with a thin layer of platinum. Platinum was selected because it is chemically stable and electroinactive (within the potential ranges to be considered).

The platinum coating was obtained with a DC sputtering technique using a Dynavac sputter coater (Figure 2.15). In this technique the substrate was placed on the sample holder with the side to be coated facing the platinum target. The chamber was first evacuated until its vacuum reached below $10^{-4}$ mbar and then was kept at a reduced pressure of argon. The cold plasma was struck by applying a DC potential between the platinum target and the second electrode on which the substrate to be coated was placed. Argon ions colliding with the platinum target knock out the platinum atoms and ions which then deposited on the substrate.
Figure 2.15 Diagram of sputter coater for platinised membrane preparation. 1. coating chamber vacuum and magnetron electrical control unit; 2. sputter coating chamber; 3. membrane or sample to be coated; 4. inert gas control valve; and 5. platinum target and magnetron head. The distance between the sample and the target is set at 12 cm.

Since the sputtering is an atom by atom deposition process it is possible for coating to follow the substrate surface morphology very closely and to get small crystallites in the deposited layer.

The platinum deposition rate during the sputter coating process was determined and optimised with respect to the sputtering current, chamber vacuum, inert gas pressure and coating time. This was carried out using the QCM technique. A quartz probe was inserted into the coating chamber with one of its mass sensing surface facing the platinum target. Distance between the quartz crystal electrode and the frequency change due to the deposition was obtained, and the platinum deposit rate was calculated based on this frequency change. The effective thickness of the platinum coating on a substrate was found to have a linear relationship to the sputtering current applied and time, as evidenced by the QCM results (Figure 2.16). The inert gas pressure showed little effect on the platinum coating when controlled between 2.0x10^{-3} to 6.0x10^{-3} mbar. Higher inert
gas pressure, e.g. 1.0\times10^{-1} \text{ mbar} resulted in a poor and high resistance coating on the substrate membrane.

![Graph showing Pt deposition rate vs. current](attachment:graph.png)

\[ y = 9.6000e-3 + 3.7220e-2x \quad R^2 = 1.000 \]

Figure 2.16 The thickness of platinum coating on PVDF substrate membrane versus sputtering current. Argon is used as the inert gas and is controlled at $2.0\times10^{-3} \text{ mbar}$ during the coating process.

The platinum deposition on a membrane is also affected by the vacuum in the chamber. The chamber should be evacuated down to below $10^{-4} \text{ mbar}$ before introducing inert gas into the chamber. This practise will effectively reduce the amount of residual oxygen inside the chamber resulting in improved quality of the platinised membrane.

In this work, all platinised membranes were prepared at a DC current of 50 mA for 6.5 minutes under $2\times10^{-3} \text{ mbar}$ argon pressure. The penetration depth of the platinum coatings into the Millipore PVDF membrane structure was estimated by measuring the capacitance of an uncoated substrate and that of platinised membrane. The penetration of platinum coating was estimated to be about 13 - 14 \% for coating on each
side. For double-sided platinised PVDF membrane, the actual distance between its two platinised layers was estimated to be around 75 to 80 μm.

2.3.2 Conducting Polymer Membrane Preparation

CEP membranes were electrochemically prepared by employing a three electrode system, as shown in Figure 2.6 and Figure 2.17, in which either platinum mesh or RVC was used as the counter electrode and a silver/silver chloride electrode as the reference. The working electrodes used varied in size and material according to different experimental purposes. A small electrode such as a platinum disc electrode or a gold coated quartz crystal was used for in situ studies into the electrochemical polymerisation. Large electrodes such as a stainless steel plate or a piece of platinised membrane were used as working electrodes for the preparation of free-standing CEP films or membrane supported CEP films. The stainless steel plate was polished with 0.3 micron alumina powder to a mirror finish before using it for the preparation of polymer films. A CEP film was electrochemically prepared by depositing the CEP on the stainless steel plate (as anode) and then carefully peeling it off. CEP films on metallised substrates were produced by the anodic oxidation of the monomer using the metallised membranes as working electrodes. The resultant polymer films usually had good adhesion to the substrates so that their mechanical properties are greatly improved over the free-standing CEP films.
2.3.3 Water Permeability

Membranes can be considered as porous permselective separation barriers, through which transport takes place when an external driving force is applied. The porosity of a membrane is an important parameter in determining the performance of a membrane in a particular separation process.

In this work, we used water permeability (as well as SEM) to characterise the porosity of the membranes. The membranes tested with this method included polyvinylidene fluoride (PVDF) from Millipore, platinised PVDF membrane, and conducting polymer deposited platinised PVDF membrane.

Water permeability tests were carried out using a water flux apparatus, shown schematically in Figure 2.18. The equipment was filled with Milli-Q water and the volume of water permeating through the membrane was
measured at the pressure of 100 kpa. The water permeation flux was calculated according to:

\[ J_w = \frac{V_w}{S \Delta t} \]  

(2.20)

where \( J_w \) is the water flux in cm\(^3\) cm\(^{-2}\) s\(^{-1}\) \( V_w \) the amount of water permeated in cm\(^3\), \( S \) the filtration area (cm\(^2\)), \( \Delta t \) the time in second.

![Diagram of water permeability test](image)

**Figure 2.18** Test for water permeability of conducting polymer membrane. 1. permeated water, 2. cell body, 3. reservoir, 4. water inlet, 5. pressure gauge, 6. nitrogen line, 7. lower cell body and porous support, 8. CEP membrane, and 9. upper cell body.

### 2.4 PROTEIN TRANSPORT AND SEPARATION

Studies into the electrochemically controlled protein transport and separation were carried out with two different cell designs:

(i) single membrane transport cell, and
(ii) a dual membrane (flow-through) transport cell. The electrochemical transport cell were driven by a Galvanostat/Potentiostat or combined with a custom made pulse generator.

2.4.1 Protein Transport Using the Single Membrane Cell

The transport behaviour of charged species across platinised membrane or conducting polymer composite membranes (both types of membranes are electrically conductive) was investigated with a typical cell design shown in Figure 2.19. This cell consists of two solution chambers with a membrane separating them.

Both the two electrode system and three electrode system have been utilised. In a three electrode system, a single-sided platinised membrane or polypyrrole composite membrane was used as the working electrode with its conducting layer faced the feed solution. The counter electrode and reference electrode were arranged in the feed solution side. In this case, an electrical connection to the conducting layer of the membrane was made via the stainless steel O-ring in the cell.

In a two electrode system, a double-sided coated membrane was used. One side of the membrane was used as the working electrode and the other side was used as the counter/reference electrode (by connecting the counter and reference electrodes from a Galvanostat/Potentiostat together). Because the two conducting layers of the membrane were insulated from each other by only a small distance (about 70 to 80 μm), an electric field could be set up within the solution filled the pores of the membrane upon applying electrical stimuli across the membrane. Thus the transport of charged molecules (proteins) can be electrochemically controlled by the electrical stimuli applied.
2.4.2 Protein Transport Using Dual Membrane (Flow Through) Cell

Protein transport and separation using the dual membrane (flow-through) cell was investigated. The purpose of this work was to try to increase the separation flux and productivity.

The conducting membranes used in a flow through cell were single-sided coated membranes. The membranes were installed with their conducting face towards the feed solution. The feed solution was delivered at various flow rates by use of a peristaltic pump, and the separated fractions A and B were collected (see Figure 2.20).
2.4.3 Protein Analysis

Proteins separated from the feed solution were analysed by using UV-visible spectrophotometry. The amount of proteins transferred from the feed solution to the receiving solution was determined by measuring the absorption of the receiving solution.
Among the proteins tested, BSA and HSA can be quantitatively determined with UV-Vis at 280 nm, while haemoglobin and myoglobin can be detected at both 280 nm and 410 nm. Therefore, it is possible to use UV-Vis spectrophotometry to quantitatively identify a protein in a protein mixture solution. For example, in a BSA and myoglobin mixture solution, myoglobin can be determined first by its absorption at 410 nm and then its absorption at 280 nm can be calculated (based on its concentration determined by its absorption at 410 nm). So that the BSA absorption at 280 nm can be obtained by deducting the absorption of myoglobin at 280 nm from the that of proteins mixture.

2.5 GAS PERMEATION AND SEPARATION

The use of polypyrrole films and polypyrrole composite membranes for the separation of oxygen from nitrogen have also been investigated. Oxygen, nitrogen and air were used as feed gases and were studied to determine their diffusivities, solubilities and permeabilities in the polypyrrole membranes.

2.5.1 Gas Separation Membranes

Polypyrrole conducting films were prepared and tested for oxygen and nitrogen separation in two different forms: (i) free standing polypyrrole films, and (ii) polypyrrole films deposited on platinised membranes. A free standing polypyrrole film was prepared by electrochemically depositing polypyrrole on a mirror finished stainless plate (4 cm x 6 cm). The polypyrrole film was peeled off from the plate after deposition. Polypyrrole film on a platinised membrane was obtained by electrochemically depositing a thin layer of polypyrrole on the platinised
membrane which was used as anode during the polymerisation process. All membranes prepared were dried overnight, so as to remove excess water entrapped inside the polymer membrane, before carrying out any further tests.

2.5.2 Gas Separation Experiments

Gas permeability through a piece of polypyrrole membrane was determined using the cell shown in Figure 2.21.

![Figure 2.21 Schematic diagram for the determination of gas permeability through conducting polymer membranes.](image)

The permeability of both nitrogen and oxygen were tested separately. The test gas (either oxygen or nitrogen) under pressure was allowed to permeate through a membrane. Upstream pressure was controlled at 76 cm Hg (or 101.3 kpa). The amount of gas permeating was measured with an Alltech digital flow meter (detection limit: 0.01 cm⁻³ per minute) at room temperature and atmospheric pressure. The results, such as gas
permeation rate (GPR), were then normalised to standard atmospheric conditions of 76 cm Hg and 273 °K.

In the gas mixture permeation experiment, permeates were carried from the lower chamber of the cell by a carrier gas (helium), and the composition of the permeates was analysed by gas chromatography (GC). Gas permeation rates (GPR) were calculated according to the composition and the gas flow rates.

The gas permeability or the gas permeation rate (GPR) was calculated according to the following equation:

\[ GPR = \frac{V_0}{S \Delta t \Delta p} \]  

(2.21)

where \( V_0 \) the total volume of gas permeated under standard atmospheric conditions (cm\(^3\)) within time \( \Delta t \) (s), \( S \) is the effective permeation area in cm\(^2\), \( \Delta p \) is the pressure difference between the upper high pressure stream and lower pressure stream across the membrane in cm Hg, and GPR is the gas permeation rate in cm\(^3\)(STP) cm\(^{-2}\) s\(^{-1}\) cmHg\(^{-1}\). From practical consideration and comparison purpose, cmHg\(^{-1}\).the GPR data in this work are expressed in cm\(^3\)(STP) cm\(^{-2}\) s\(^{-1}\) instead of SI units.

The separation factor or permselectivity between oxygen and nitrogen can be calculated based on the permeability test for each gas. This is generally expressed as the ratio of the permeabilities of these two gas components:

\[ \alpha = \frac{GPR_{O_2}}{GPR_{N_2}} \]  

(2.22)

where \( GPR_{(O_2)} \) and \( GPR_{(N_2)} \) are the oxygen and nitrogen permeabilities through the same membrane under standard atmospheric conditions.
The separation of oxygen from nitrogen and other gas components was also investigated using pressurised air as feed gas. The gases permeating through the membrane were metered and analysed using gas chromatography.
CHAPTER 3

PROTEIN TRANSPORT AND SEPARATION
USING PLATINISED ELECTROMEMBRANES
3.1 INTRODUCTION

The evolution of biotechnology as well as the need for increased efficiency in the food industry has resulted in a demand for systems capable of more effective separation of proteins and other biomaterials\textsuperscript{[114,115]}. The most popular analytical tools are those based on the principle of electrophoresis\textsuperscript{[116-119]}. In particular high performance capillary zone electrophoresis offers incredibly high separation efficiency. However, despite attempts to scale these systems up, the problems with using electrophoretic techniques have remained. Techniques such as precipitation, gel permeation \textsuperscript{[46]} and ion exchange \textsuperscript{[120,121]} have been used previously and while these are appropriate in various areas the selectivity and rate of separation attainable are limited. These are of advantage only for the particular protein for which they have been designed. More recently membrane processes including ultrafiltration and electrodialysis have been reported\textsuperscript{[45-48,122,123]}. These provide a number of potential advantages, including large scale separation of proteins, ease of operation and lower cost. However, it is difficult to separate proteins from a mixed protein solution merely via sieving of molecular weights, such as ultrafiltration. Consequently there is a need to develop new separation technologies for the recovery and purification of proteins.

In this chapter we describe a newly developed transport membrane ie. an electromembrane system for protein separation. These electromembranes were made from commercial membranes by sputter coating a thin layer of platinum on either one side of the membranes or both sides of the membranes. Since the surface of the membrane becomes electrically conductive, an electrical field can be obtained within the solution or the
pores of the membrane by applying a potential to the membrane. For a double-sided platinised membrane, the electrical field across its two faces is much higher than that produced in a conventional electrophoresis process because there is only a small distance (about 70 \( \mu \text{m} \)) between its two platinised conducting layers. In addition, the electric field across the membrane can be manipulated by varying the electrochemical stimuli so that the field strength and direction can be varied to suit different separation purposes. Such an experimental set up is expected to provide much higher transport for charged particles.

Two different electromembranes have been used in this work: (i) single-sided platinised membrane, and (ii) double-sided platinised membrane. Two different electrode systems were used according to which type of membrane was being used. The electrochemically controlled transport and separation of proteins such as bovine serum albumin (BSA), human serum albumin (HSA), myoglobin (MYO), and haemoglobin have been investigated.

### 3.2 EXPERIMENTAL

#### 3.2.1 Reagents and Materials

All inorganic reagents used in this investigation were of AR grade purity unless otherwise stated. Proteins, Bovine Serum Albumin (BSA), Human Serum Albumin (HSA), myoglobin from horse heart (MYO) and haemoglobin (HEMO) from horse heart were obtained from Sigma and used without further purification. All protein solutions were prepared with deionised Milli-Q water (18 M\( \Omega \) cm).
Millipore polyvinylidene fluoride (PVDF) membranes, with pore sizes of 0.10 µm (GVWP), 0.22 µm (GVHP) and 0.45 µm (HVHP) were chosen and used for the preparation of platinised electromembranes.

Platinised electromembranes were prepared from PVDF membranes and polysulphone membranes. The experimental procedures are described in Chapter 2. The platinisation process was carried out at a constant anodic current of 50 mA for 6.5 minutes. The argon pressure was maintained at ~2x10⁻³ mbar during the entire coating process.

The surface conductivities of these membranes were measured using the four point probe conductivity measurement technique, and their surface morphology was studied by using Scanning Electron Microscopy (SEM).

Water permeability tests on substrate and platinised membranes were carried out using a filtration cell (Chapter 2) so as to determine effects of the platinum deposition on the membranes.

### 3.2.2 Protein Transport Across Platinised Membranes

Electrochemically controlled protein transport was conducted using a transport cell (Figure 3.1). Two different membranes were employed for this investigation: (i) single-sided platinised membrane for a three electrode transport system, and (ii) double-sided platinised PVDF membranes (Type HVHP with 0.45 µm average pore sizes) for a two electrode transport system.

In the three electrode system, the platinised membrane was used as the working electrode while the counter and reference electrodes were placed
in the feed solution cell as shown in Figure 2.19. A pulsed potential was applied to the platinised membrane.

In the case of a two electrode system, the two conducting faces of the membrane were used as the electrodes and were connected as the working electrode and counter/reference electrode. A M2049 Potentiostat/Galvanostat from Princeton Applied Research together with a custom made signal generator were used and connected as shown in Figure 3.1.

**Figure 3.1** Protein transport using a double-sided platinised PVDF membrane in a two electrode system. The effective transport area of the membrane was 3.14 cm².

Most biomolecules, such as protein molecules, are charged species, depending on the solution pH and protein's pi. A protein molecule in a solution may carry positive charges at a sufficient low solution pH, and carry negative charges at sufficient high solution pH. In short, the sign of the net charge of a protein molecule is solution pH dependent. This property has been well demonstrated and utilised in all kinds of electrophoresis and isoelectric focusing for the characterisation and separation of biomolecules [40-45].
In this work, the electromigration of protein molecules was manipulated by controlling either the solution pH or the field direction. In the case of separating one protein from another (provided their isoelectric points are not too close), it is possible to have them carrying opposite signs of charge by judicially selecting the solution pH. Thus they would migrate in opposite directions in a field in the solution. The pIs of the proteins used in this study are given in Table 3.1.

**Table 3.1 Proteins used in the transport/separation studies**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Isoelectric point</th>
<th>Molecular weight</th>
<th>Molecular size (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine serum albumin(BSA)</td>
<td>4.8</td>
<td>66,000</td>
<td>-</td>
</tr>
<tr>
<td>Haemoglobin (HEMO)</td>
<td>7.1</td>
<td>67,600</td>
<td>64x55x50</td>
</tr>
<tr>
<td>Human serum albumin (HSA)</td>
<td>4.9</td>
<td>66,000</td>
<td>-</td>
</tr>
<tr>
<td>Myoglobin (MYO)</td>
<td>7.0</td>
<td>16,900</td>
<td>44X44X25</td>
</tr>
</tbody>
</table>

The protein transported from the feed solution to the receiving solution was measured by the UV-vis spectrophotometer, and the transport flux was calculated based on the amount of protein transported across the membrane per unit area per unit time, i.e.

\[ J_p = \frac{W_p}{S \Delta t} \quad (3.1) \]

Where \( J_p \) is the protein flux rate in mg cm\(^{-2}\) s\(^{-1}\); \( W_p \) is the amount of protein transported across the membrane in molar; \( S \) is the effective transport area of the membrane in cm\(^2\); and \( \Delta t \) is the time in seconds.
In the case of separation of one protein component from another protein existing in the same solution, the separation factor is determined by the ratio of their transport fluxes across the same membrane, i.e.

$$\alpha_{(p_1/p_2)} = \frac{J_{p_1}}{J_{p_2}}$$

where \( \alpha_{(p_1/p_2)} \) is the separation factor of protein 1 over protein 2 for the same membrane under the same experimental conditions, \( J_{p_1} \) and \( J_{p_2} \) are the transport flux of protein 1 and protein 2 respectively.

A UV-vis Spectrophotometer (HITACHI U-2000) was employed for determining the protein concentration in the receiving solution during transport and separation processes. Test proteins BSA and HSA were measured at 280 nm, while myoglobin and haemoglobin were analysed at both 280 nm and 410nm.

### 3.3 RESULTS AND DISCUSSION

Preliminary studies using cyclic voltammetry verified that none of these test proteins used could be oxidised or reduced at a platinum electrode or a conducting polymer modified platinum electrode under the electrochemical conditions employed in this investigation.

### 3.3.1 Membrane Characterisation

SEM studies revealed that the platinised PVDF membranes remained porous and the platinisation had little effect on the microporous structures of the substrate membrane (Figure 3.2).
The water flux tests carried out on these PVDF of different pore sizes are summarised in Table 3.2. Results indicate that the platinisation on PVDF membrane has only minor effect on their water fluxes.

The water flux of 0.10 μm PVDF membranes was reduced by 5% when single-sided platinised and by 8% when double-sided platinised. The water fluxes for 0.22 μm and 0.45 μm PVDF membranes remained unchanged when they were single-sided and double-sided platinised. Platinisation showed little effect on their water fluxes for these two types of membranes. A small water flux increase was observed for 0.45 μm PVDF membrane after platinisation. The water flux was increases by 4.5% for either single-sided or double-sided membrane, which might be induced by the decrease in its hydrophobicity after plasma coating.
Table 3.2 Water fluxes of PVDF membranes of different pore sizes

<table>
<thead>
<tr>
<th>Membrane group No.</th>
<th>PVDF Memb pore size (μm)</th>
<th>Flux of substrate ( \text{cm}^3 \text{cm}^{-2} \text{s}^{-1} )</th>
<th>Flux of one side platinised ( \text{cm}^3 \text{cm}^{-2} \text{s}^{-1} )</th>
<th>Flux of double-sided platinised ( \text{cm}^3 \text{cm}^{-2} \text{s}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.10</td>
<td>0.076</td>
<td>0.072</td>
<td>0.070</td>
</tr>
<tr>
<td>2</td>
<td>0.22</td>
<td>0.23</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>3*</td>
<td>0.45</td>
<td>0.84</td>
<td>0.88</td>
<td>0.88</td>
</tr>
<tr>
<td>4</td>
<td>0.65</td>
<td>0.95</td>
<td>0.94</td>
<td>0.94</td>
</tr>
</tbody>
</table>

* Water flux tested under 101 kpa. Membranes in Group 3 are hydrophobic type membranes which was pre-wetted with 1:1 ethanol/water before testing. Other membranes (Group 1, 2, and 4) are hydrophilic membranes.

The electrical conductivity of platinised PVDF membranes were measured using the four probe technique described in Chapter 2. The electrical conductivity of platinised PVDF membranes varied depending on the pore size of the membranes. The more porous the membrane then the lower its electrical conductivity. For example, the average electrical conductivity of the PVDF membranes was found to be about 5800 S cm\(^{-1}\) for 0.45 μm pore size, and was 12480 S cm\(^{-1}\) for 0.22 μm pore size. This was because a deeper platinum coating penetration was obtained in a more porous structure. Thus the platinum coating on this substrate was not as dense as that on a less porous membrane.

3.3.2 Electrochemically Controlled Protein Transport

3.3.2.1 Theoretical Principles

Considering the situation schematically represented in Figure 3.1, we have an aqueous solution containing a single protein, or two proteins with opposite charge \[^{37}\], in contact with a microporous membrane which has
electrodes laminated to both faces. The pores of the membrane are much larger than the size of the proteins. A potential can be applied between the electrodes by an external power supply to cause current to flow through the solution filled pores of the membrane. This results in an electric field being established within the membrane pores.

In the pores of the membrane under the conditions used in this work we will assume that there is no convection occurring. In the presence of an electric field across the membrane and in the absence of convection the flux ($J$) of a charged species across the membrane is given by equation (3.3) below [99].

$$J_j = -D_j \frac{\partial C_j}{\partial x} - z_j \frac{F}{RT} D_j C_j \frac{\partial \phi}{\partial x} \tag{3.3}$$

where $J_j$ is the transport flux of a charged species; $D_j$ is the diffusion coefficient of the charged species; $C_j$ is the concentration of the charged species; $z_j$ is the valence of the charged species, including sign of charge; $\frac{\partial C_j}{\partial x}$ is the concentration gradient of the charged species in the solution filled pores of the membrane; and $\frac{\partial \phi}{\partial x}$ is the potential gradient or electric field in the solution filled pores of the membrane.

This equation indicates that there are two contributions to the overall flux of the species; that due to diffusion in the presence of a concentration gradient represented by the first term in Equation (3.3) and that due to electromigration in the presence of an electric field, which is represented by the second term in Equation (3.3). The sign of the first term is only dependent upon the sign of the concentration gradient. Therefore, in the presence of a fixed concentration gradient, it cannot be influenced by external forces. The sign of the second term, however, is dependent upon the sign of the potential gradient and the sign of the charged species. The
sign of the potential gradient can be reversed by reversing the sign of the potential being applied across the membrane. In the case of a protein, the sign of the charge can be adjusted by adjusting the pH of the solution.

By making the migration term opposite in sign and larger than or equal to the magnitude of the diffusion term the transport of the species across the membrane will be prevented. In contrast, making the migration term the same sign as the diffusion term, the flux of the species across the membrane will be increased. This is the basis for transport and separation in this system.

Controlled current conditions were used to investigate the effect of electrical control on protein transport across platinised and conducting polymer coated membranes. Galvanostatic control rather than potentiostatic control was used in most of the experiments because the former affords a greater degree of control over the electric field applied. This is so for the following reasons:

Typically, the potential across the membrane was approximately 1.70 V. The potential drop across the solution filled pores of the membrane is only a minor fraction of the total applied potential; and it is this potential drop that determines the electric field strength in the solution within the pores of the membrane. We cannot approximate this electric field strength by taking the total applied potential and dividing by the distance between the electrodes, which is the usual practice in conventional electrophoresis.

However, it is a reasonable assumption to consider the solution as an ohmic conductor so that the relationship

\[ E = iR \]  

(3.4)
holds, where $E$ is the voltage drop across the solution, $R$ is the resistance of the solution and $i$ is the current flowing through the solution. Also of course from Equation (3.4)

$$\frac{\partial \phi}{\partial x} = \frac{iR}{d} \quad (3.5)$$

where $d$ is the distance between electrodes and $\frac{\partial \phi}{\partial x}$ is the average electric field across the membrane. So, assuming that $R$ does not vary too much with time, by maintaining a fixed $i$ the electric field will also be approximately fixed.

### 3.3.2.2 Electrochemically controlled transport across platinised PVDF membranes

Test proteins, myoglobin, haemoglobin, BSA, and HSA, have been investigated for their electrochemically controlled transport across platinised membranes. According to the isoelectric points given by Table 3.1, myoglobin and haemoglobin will carry positive charge in a solution of pH 6.5, and BSA will carry negative charge in the same solution. Therefore in a given electric field, myoglobin and haemoglobin will migrate in the same direction and BSA will migrate in the opposite direction in the solution filled pores of the membrane.

The transport of myoglobin at pH 6.5 was first studied under various transport modes. The experiment was initiated under diffusion controlled transport and followed by electrochemically controlled transport. The direction of the applied electrical field across the platinised membrane was varied for each 60 minutes period during the electrochemically controlled transport (Figure 3.3). Thus the period OA shows myoglobin
Figure 3.3 Electrochemically controlled transport of myoglobin across a double-sided platinised 0.45 μm PVDF membrane. Feed solution: 20 μM myoglobin aqueous solution. Receiving solution: Milli-Q water. O to A: diffusion. A to B, C to D and E to F: diffusion inhibited by applying a current density of -0.64 mA cm$^{-2}$. B to C and D to E: electrochemically facilitated transport by applying a current density of +0.64 mA cm$^{-2}$.

transport under diffusion control. AB, CD and EF periods show electrochemically inhibited transport when -0.64 mA cm$^{-2}$ was applied across the membrane with the feed side of the membrane negatively polarised. BC and DE periods show electrochemically enhanced transport when +0.64 mA cm$^{-2}$ was applied across the membrane with the feed side of the membrane positively polarised. As shown in Figure 3.3, the transport of myoglobin across this membrane can be repeatedly turned on and off by varying the electrical field applied to the pores of the membrane (ie. the electric field in the solution filled pores of the membrane). This could be of potential application in separating a protein of particular interest from a protein mixture by controlling the transport
of this protein. The transport flux of the test protein can be estimated from the slope of the transport concentration - time curve. The electrochemically enhanced transport, represented by periods BC and DE, was much faster than that in the diffusion controlled transport process. The transport flux became approximately zero during the periods of AB, CD and EF.

Transport of BSA, a protein carrying an opposite charge to myoglobin and haemoglobin in the same solution (pH 6.5), has also been investigated with the same experimental procedure. BSA carries about 8 negative charges at pH 6.5 \[40\]. Since BSA carries an opposite charge in the solution, its transport behaviour was opposite to that of myoglobin when the same electrical stimuli were applied (Figure 3.4). By varying the direction of the DC current applied, BSA transport can be turned on and off: (i) when the feed solution side of the membrane was negatively polarised (represented by AB and CD), BSA transport increased; (ii) when the feed solution side of the membrane was positively polarised, BSA transport stopped (BC and DE).

By comparing with the transport of myoglobin shown Figure 3.3, it is clear that BSA transport in opposite direction to myoglobin provide the same electric field applied across the membrane. These results effectively demonstrate the possibility of separating BSA from myoglobin in a BSA/myoglobin mixture by controlling the external potential applied.

The transport fluxes for both diffusion and electrochemically controlled transport can be estimated by measuring the slope of the concentration-time curve.
Figure 3.4 Transport of BSA across a double-sided platinised 0.45 μm PVDF membrane. Feed solution: 20 μM BSA water solution. Receiving solution: Milli-Q water. OA: diffusion controlled transport. AB and CD: electrochemically enhanced transport with current density of -0.64 mA cm⁻² applied. BC and DE: electrochemically inhibited transport with current density of +0.64 mA cm⁻² applied.

The transport of haemoglobin has been tested under diffusion transport and also electrochemically controlled transport. Haemoglobin has isoelectric point of 7.1, similar to that of myoglobin (pI 7.0) but about four times larger in molecular weight. Haemoglobin was positively charged at pH 6.5 and showed the same transport behaviour as that of myoglobin (Figure 3.5). During the transport, the diffusion controlled transport of haemoglobin was initiated and allowed to continue for 90 minutes. Subsequently, an electric field was applied across the membrane with feed solution side of the membrane positively polarised to enhance the transport of haemoglobin. During the electric enhanced transport period (AB), because both diffusion and electromigration are towards the
same direction. As a result, the haemoglobin transport flux became much faster than that in the diffusion controlled mode (OA) as indicated by a sharp increase in concentration. The above process was reversed when the polarity of the applied field was reversed; so that the haemoglobin transport across the membrane was completely stopped or even reversed (the concentration of haemoglobin is decreasing). It is clear that the diffusion controlled transport of haemoglobin can be either speeded up or stopped by manipulating the electrical stimuli applied to the solution filled pores of the membrane.

![Figure 3.5](image)

**Figure 3.5** Transport of haemoglobin across a double-sided platinised 0.45 μm PVDF membrane. Feed solution: 1290 ppm (20 μM) haemoglobin in Milli-Q water solution. Receiving solution: Milli-Q water. OA: diffusion controlled transport; AB: electrochemically enhanced transport with an applied current density of +0.64 mA cm⁻². BC: reversal of electrochemically enhanced transport (AB) by applying a current density of -0.64 mA cm⁻² applied.

### 3.3.2.3 Effect of Current Density

The electrochemically controlled transport of the test proteins BSA, myoglobin and haemoglobin were investigated under different applied
current densities so as to determine the effects of applied current on the transport of these proteins (Table 3.3). In general, the overall flux in electrochemically enhanced transport consists of two contributions, i.e. the contribution from diffusion (due to the presence of concentration gradient) and the contribution from electromigration. The diffusion transport is determined by the concentration gradient and the diffusion coefficient of the solute through the membrane. For a given protein solution and membrane, the diffusion flux is fixed provided no convection occurs in the solution filled pores of the membrane. Thus the actual protein transport flux depend on the direction and the rate of electromigration of the protein, which are determined by the net charge of the protein molecules and the applied electrochemical stimuli.

The BSA diffusion transport flux was found to be in the order of $0.26 \times 10^{-12}$ mol cm$^{-2}$ s$^{-1}$ which was substantially increased by up to 4 fold when a current density of $-0.64$ mA cm$^{-2}$ was applied. The net contribution from the electromigration can be obtained by deducting the diffusion transport flux from the electrochemically enhanced transport flux. The BSA transport flux reached its maximum value ($1.47 \times 10^{-12}$ mol cm$^{-2}$ s$^{-1}$) at an applied current density of 0.64 mA cm$^{-2}$. Similarly, the myoglobin transport flux also reached its maximum value ($1.81 \times 10^{-12}$ mol cm$^{-2}$ s$^{-1}$) when the same current density of 0.64 mA cm$^{-2}$ was applied. There were no further flux increases observed when higher current densities were applied.

The transport of haemoglobin across a membrane is similar to that of myoglobin. The diffusion transport flux was found to be in the order of $0.47 \times 10^{-12}$ mol cm$^{-2}$ s$^{-1}$ which is approximately the same as that of myoglobin. The highest transport flux of $2.05 \times 10^{-12}$ mol cm$^{-2}$ s$^{-1}$ was obtained at the current density of 0.64 mA cm$^{-2}$. The potential obtained
under this current density was 1.70 Volts on average. The transport flux tended to decrease if a current density higher than 0.64 mA cm\(^{-2}\) was applied.

**Table 3.3** Transport of proteins with different applied current densities

<table>
<thead>
<tr>
<th>Current density (mA cm(^{-2}))</th>
<th>0.0</th>
<th>0.32</th>
<th>0.64</th>
<th>1.28</th>
<th>1.92</th>
<th>2.56</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA Flux x10(^{12}) (mol cm(^{-2}) s(^{-1}))</td>
<td>0.26</td>
<td>0.53</td>
<td>1.47</td>
<td>1.15</td>
<td>1.03</td>
<td>-</td>
</tr>
<tr>
<td>Myoglobin Flux x10(^{12}) (mol cm(^{-2}) s(^{-1}))</td>
<td>0.46</td>
<td>1.02</td>
<td>1.81</td>
<td>1.77</td>
<td>1.64</td>
<td>1.51</td>
</tr>
<tr>
<td>Haemoglobin Flux x10(^{12}) (mol cm(^{-2}) s(^{-1}))</td>
<td>0.47</td>
<td>1.95</td>
<td>2.05</td>
<td>1.38</td>
<td>1.49</td>
<td>-</td>
</tr>
</tbody>
</table>

*Note: Transport of BSA, myoglobin and haemoglobin across double-sided platinised 0.45 pm P\(^{2}\)DF membranes was tested with BSA, myoglobin and haemoglobin. Feed solution: 20 \(\mu\)M protein aqueous solution for each protein. Receiving solution: Milli-Q water.*

The transport behaviour of the electromembrane, as indicated by the Equation 3.3, is determined by both diffusion and electromigration. The diffusion flux, which is dependent on the concentration gradient, is normally fixed for a given transport system so that the transport properties of the electromembrane is mainly determined by electromigration. With an increase of the applied current density up to 0.64 mA cm\(^{-2}\), the electrical field in between the two faces of the membrane increased with the highest protein transport flux obtained at a current density of 0.64 mA cm\(^{-2}\).

On the other hand, with a further increase of the applied current density (e.g. >0.64 mA cm\(^{-2}\)), the availability of charged protein molecules in the
interfacial region between the feed solution and the electromembrane became a limiting step for the electromigration of protein molecules. Also, the higher applied current density also resulted in a higher electrode potential. Consequently, water decomposition occurred and the gas bubbles generated in the solution filled the pores of the membrane. The pores of the membrane became partially blocked, which effectively reduced the protein transport across the electromembrane. For optimal protein transport, the applied current density should be limited to below 0.64 mA cm\(^{-2}\).

### 3.3.2.4 Effect of Applied Potential on Protein Transport

In addition to galvanostatic controlled transport, the potentiostatic method was also used for the transport of test protein, myoglobin. Different applied potentials were used so as to investigate the differences between galvanostatic controlled transport and potentiostatic controlled transport. The basic experimental set up was still the same as shown in Figure 3.1. The electrical field in the solution filled pores of the membrane was obtained by applying an external potential stimulus right across the membrane. Myoglobin, aqueous solution of 20 \(\mu\)M at pH 6.5, was used as the feed solution. The potential applied for each transport process varied from 1.5 to 2.0 Volts. The amount of charge consumed during the applied potential transport was calculated based upon the following equation:

\[
Q = \int_{n}^{n+\Delta n} i(t) dt
\]

where \(Q\) is the total amount of charge passed during the transport process (Table 3.4); \(i(t)\) is current vs time monitored during the transport process (A); and \(t\) is the time of the transport process (s).

It was found that the current slowly decreased with time during the transport process. For example, the initial current was found to be around
10.0 mA when an electrode potential of +1.70 V was applied, but the current slowly decreased to 4.2 mA in about 3 hours. The decrease in current is a direct evidence of increased electrical resistance in the solution filled pores of the electromembrane, which might be caused by (a) the decreased protein concentration gradient, (b) bubbles generated at the membrane faces, and (c) membrane fouling. Consequently, both the current and the protein flux decreased.

Table 3.4 shows the myoglobin transport flux and the electrical charges consumed under different electrode potential across the membrane. With an increase in the applied potential, the myoglobin transport flux increased. But the increase of transport flux was not in proportion to the total amount of charge passed through the electromembrane during a transport process. This is indirect evidence that other charged species such as water decomposition by-products produced at elevated applied potentials made the electrotransport less efficient.

**Table 3.4 The transport flux of myoglobin vs potential and charge**

<table>
<thead>
<tr>
<th>Applied potential (Volts)</th>
<th>1.50</th>
<th>1.70</th>
<th>2.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myoglobin Flux x10^{12} (mol cm^{-2} s^{-1})</td>
<td>1.31</td>
<td>1.51</td>
<td>1.89</td>
</tr>
<tr>
<td>Charge (C)</td>
<td>6.64</td>
<td>21.5</td>
<td>56.2</td>
</tr>
</tbody>
</table>

*Note: The electrochemically controlled transport flux of myoglobin under potentiostatic conditions was carried out by applying a potential across the membrane. The feed solution contained 20 μM myoglobin aqueous solution; receiving solution was Milli-Q water.*

In addition to extra charge carriers introduced by water decomposition at high applied potential, the bubbles generated and the change of local pH at
the membrane electrode surfaces also adversely affected the colour of the protein solutions and the bioactivity of the protein (denature). Both feed solution and receiving solution became turbid due to the presence of dissolved gas bubbles. As a result, the accurate measurement of the protein concentration with the UV-Vis spectrophotometry was also affected. For optimal operation of the electromembrane transport, a potential below 1.70 V is recommended.

3.3.3 Transport of Proteins From a Supporting Electrolyte Containing Solution

The electromigration of charged species in a given electric field depends on the charge density as well as the sign of the charge in a solution. Adding charged species into a solution will introduce extra charge carriers and will alter the charge density distribution in this solution. The extra charge introduced into the solution should reduce the transport flux of a test protein if the same current density is applied. In this section, the effect of salt concentration on the transport of myoglobin and BSA are considered by varying the concentration of sodium nitrate in the solution during electrochemically controlled transport (Figure 3.6 and 3.7). The current density applied for these transport processes was controlled at 0.64 mA cm\(^{-2}\). The feed solution contained 20 \(\mu\)M BSA or 20 \(\mu\)M myoglobin in sodium nitrate solution of different concentration varying between 10\(^{-2}\) M and 10\(^{-4}\) M. The receiving solution contained the same concentration of sodium nitrate as that in the feed solution.

The transport flux of test proteins in different sodium nitrate concentration are summarised in Table 3.5 and Table 3.6. The myoglobin and BSA transport flux was 1.81x10\(^{-12}\) mol cm\(^{-2}\) s\(^{-1}\) and 1.47x10\(^{-12}\) mol cm\(^{-2}\) s\(^{-1}\) if there were no extra charge carriers present in both feed and receiving solution. Both myoglobin and BSA transport fluxes decreased
Figure 3.6  Transport of myoglobin in different concentrations of sodium nitrate. Current density applied: 0.64 mA cm$^{-2}$ with feed solution side of the membrane positively polarised. Feed solution: 20 μM myoglobin. Receiving solution: sodium nitrate of the same concentration as the feed solution.

Figure 3.7  Transport of BSA in different concentrations of sodium nitrate. Current density applied: 0.64 mA cm$^{-2}$ with the feed solution side negatively polarised. Feed solution: 20 μM BSA in sodium nitrate. Receiving solution: sodium nitrate of the same concentration as the feed solution.
when transports was carried out with these proteins in sodium nitrate solution. The additional charged species, such as Na\(^+\) and NO\(_3^-\) introduced in the feed and receiving solutions, may affect the controlled current transport technique in two ways:

(1) Increasing the conductivity of the solution so that the potential difference across the membrane is decreased, which will effectively reduce the electric field strength in the solution filled pores of the membrane according to Equation 3.4 and Equation 3.5.

(2) Carrying part of the current applied through the membrane so that the net current carried by the transport of the protein is less.

Since simple ions, such as Na\(^+\) and NO\(_3^-\), are much more mobile than macromolecules, such as protein myoglobin and BSA, in the electromigration process the major part of the current applied is carried by the simple ions instead of proteins. As shown in Table 3.5 and Table 3.6: the higher the concentration of sodium nitrate in solution, the lower the protein transport fluxes.

**Table 3.5 Myoglobin transport in sodium nitrate solution**

<table>
<thead>
<tr>
<th>NaNO(_3) Conc (M)</th>
<th>0.0</th>
<th>10(^{-4})</th>
<th>10(^{-3})</th>
<th>10(^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flux x10(^{12}) (mol cm(^{-2}) s(^{-1}))</td>
<td>1.81</td>
<td>1.18</td>
<td>0.47</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*Note: Myoglobin transport flux across double-sided platinised 0.45 \(\mu\)m PVDF membrane under applied current in different concentrations of sodium nitrate. Feed solution contained 20 \(\mu\)M myoglobin in various concentrations of sodium nitrate. Receiving solution contained the same concentration of sodium nitrate as in feed solution. Current density applied was 0.64 mA cm\(^{-2}\).*
### Table 3.6 BSA transport in sodium nitrate solutions

<table>
<thead>
<tr>
<th>NaNO₃ Conc (M)</th>
<th>Flux x10¹² (mol cm⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1.47</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>0.18</td>
</tr>
<tr>
<td>10⁻³</td>
<td>0.13</td>
</tr>
<tr>
<td>10⁻²</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Note: BSA transport flux across double-sided platinised 0.45 µm PVDF membrane under applied current in different concentrations of sodium nitrate. Feed solution contained 20 µM BSA in various concentrations of sodium nitrate. Receiving solution contained the same concentration of sodium nitrate as that in feed solution. Current density applied was 0.64 mA cm⁻².

#### 3.3.4 Transport of Proteins at Different pH

Protein molecules owe their ionic character to the presence of weakly acidic or basic side chains of the type [37]:

\[-\text{COOH} = -\text{COO}^- + \text{H}^+\]
\[-\text{NH}_3 = \text{NH}_2^- + \text{H}^+\]
\[-\text{C}_6\text{H}_5\text{OH} = -\text{C}_6\text{H}_5\text{O}^- + \text{H}^+\]

In solutions of different pH, a protein molecule will have a different net charge Z which may be positive at sufficiently low pH or negative at sufficiently high pH. The pH at which a protein molecule carrying zero net charge is called the isoelectric point (pI). Thus the electromigration direction of a protein molecule in a given electric field can be manipulated by adjusting the solution pH to a value higher or lower than its isoelectric point.
The transport of myoglobin in a solution of pH 10.8 (both feed solution and receiving solution) is shown in Figure 3.8. A current density of 1.28 mA cm\(^{-2}\) was applied and the direction of this applied electric field was reversed for each 60 minute period. Myoglobin molecules carry a net negative charge at this pH. It is clear that the transport direction of myoglobin at this pH is different from that at pH 6.5, at which myoglobin molecules are positively charged (Figure 3.3). The electrochemically enhanced transport flux was also lower than that at pH 6.5 because of the buffer solution used. This result demonstrates that the electrochemically enhanced transport of proteins can be judicially manipulated by the proper adjustment of solution pH as well as adjusting the electrochemical stimuli. Such a result is useful and can be applied to the separation of a particular protein component which has a different pI from the rest of the
proteins. In this case, the selection of solution pH is becoming more important in a controlled transport or separation process.

3.3.5 Separation of Protein Mixtures

As previously discussed, the electrochemically controlled transport of proteins can be manipulated either by varying the electric field direction or controlling the solution pH. The separation of two proteins with different pIs has been investigated using these double-sided platinised PVDF membranes (Figure 3.9). The experimental setup was still the same as in Figure 3.1. A solution containing both proteins, HSA (pI 4.8) and myoglobin (pI 7.0) at pH 6.5 was used as the feed solution. Thus HSA is negatively charged and myoglobin is positively. In the case where separation was carried out by using a double-sided platinised PVDF membrane, the average transport fluxes of HSA and myoglobin were $1.0 \times 10^{-12}$ mol cm$^{-2}$ s$^{-1}$ and $6.46 \times 10^{-14}$ mol cm$^{-2}$ s$^{-1}$ respectively. The separation factor for HSA to myoglobin was 14.5:1 (where the separation factor is defined as the flux of HSA/the flux of myoglobin). Very little myoglobin was detected in the receiving solution.

The separation of BSA from haemoglobin was also carried out with the use of these double-sided platinised PVDF membranes (Figure 3.10). Both BSA (4.9) and haemoglobin (pI 7.1) had the same concentration of 20 μM in the feed solution at pH 6.5. Thus BSA was negatively charged and haemoglobin was positively charged. A current density of 0.64 mA cm$^{-2}$ was applied with the electric field directed towards the receiving solution, that is the feed solution side of the membrane was negatively polarised and receiving solution side of the membrane was positively polarised.
Figure 3.9 Separation of HSA from myoglobin using double-sided platinised 0.45 μm PVDF membrane. Feed solution was 670 ppm (10 μM) HSA and 175 ppm (10 μM) myoglobin mixture in Milli-Q water. Receiving solution: Milli-Q water. Current density was controlled at -1.28 mA cm⁻² with membrane anode facing receiving solution and membrane cathode facing the feed solution.

Such a field applied will enhance the transport of BSA while preventing haemoglobin transport. Quite pure BSA solution was separated out from the BSA/haemoglobin mixture. BSA concentration in the receiving solution reached 114.1 ppm during the 150 minutes separation process. But only 1.62 ppm haemoglobin was found in the receiving solution. The separation factor between BSA and haemoglobin in terms of their transport fluxes was in the order of 70, even higher than that between HSA and myoglobin.
Figure 3.10 Separation of BSA from haemoglobin using double-sided platinised 0.45 μm PVDF membrane. Feed solution: 20 μM BSA and 20 μM haemoglobin mixed aqueous mixture. Receiving solution: Milli-Q water. Current density of -0.64 mA cm$^{-2}$ was applied with cathode membrane facing feed solution.

3.3.6 Transport of Protein Using the Dual Membrane Cell

Protein transport using a dual membrane flow through transport cell was carried out with the use of myoglobin as a test case. Platinised PVDF membranes with nominal 0.10 μm or 0.22 μm pore sizes were used for this purpose. In the dual membrane flow through cell, the two pieces of membranes were installed with their platinised sides facing to the feed solution (centre chamber). An electrical current of 2.0 mA cm$^{-2}$ was applied across these two membranes, between which an electrical field could be set up within the feed solution (Figure 3.11). Electromigration of the test protein will occur upon applying the current.

In this case, two different driving forces are involved in the transport of myoglobin. The first one is the electromigration of myoglobin under the field from the solution phase to the membrane. Once the protein molecules reach the membrane's surface, they will diffuse into the
membrane. The second driving force is the hydraulic pressure difference existing across each membrane which will drive those protein molecules inside of the membrane into either Fraction A or Fraction B.

Figure 3.11 Mechanism of protein transport in a Dual membrane flow through cell. An electrical connection to the inner side of each membrane was made and through which an electrical current was applied.

During the transport, two different fractions of different concentration of myoglobin were obtained (Figure 3.12). Due to the directed electromigration of test protein, the concentration of myoglobin in Fraction A is lower than that in Fraction B. In addition, Fraction B was even more concentrated than that of the Feed solution, i.e., the test protein could be separated in a concentrated form by this dual membrane transport system.

The ratio between the myoglobin concentration in Fraction B and Fraction varies with the pores size of the membranes used. For examples, the ratio of $C_{F_B}/C_{F_A}$ are 9.3, 3.7 and 1.3 for 0.10 μm, 0.22 μm and 0.45 μm PVDF membranes respectively. Membrane of smaller pore size has
better selectivity than those of bigger pore sizes. For a membrane of small pore size, the membrane is more denser and less porous. As a result, non-selective diffusion transport has been effectively slowed down by the membrane. On the other hand, membrane of 0.45 \( \mu \text{m} \) pore shows little separation effect because the diffusion transport is much faster. The electric field applied between the membranes is less effective in controlling the protein transport towards desired direction.

![Figure 3.12: Transport of myoglobin using a dual membrane flow through cell. Myoglobin 680 ppm aqueous solution was the feed solution. The feed solution was delivered at 1.06 ml min\(^{-1}\). Membrane 1: PPy coated on 0.22 \( \mu \text{m} \) PVDF, Membrane 2: platinised 0.10 \( \mu \text{m} \) PVDF, and Membrane 3: platinised 0.22 \( \mu \text{m} \) PVDF.](image)

With the increase of the pore size of the membranes, the selectivity was reduced. There was little selectivity for these platinised PVDF membrane with a pore size of greater than 0.45 \( \mu \text{m} \) because they are too porous.

Compared with transport in a stationary cell, the dual membrane flow cell provides a continuous separation with fairly fast flow rate. For example, the transport flux for myoglobin reached 6.4x10\(^{-11}\) mol cm\(^{-2}\) s\(^{-1}\), which is 35 time higher than that obtained with stationary transport cell. In
addition, the myoglobin is separated in a concentrated form (840 ppm / 600 ppm).

3.4 CONCLUSION
Platinised PVDF electromembranes have been successfully prepared and utilised for the transport and separation of proteins. Characterisation of platinised membranes revealed that the platinisation had no effect on the porous structures and the water flux observed.

The electrochemically controlled transport of test proteins, such as BSA, HSA, haemoglobin and myoglobin, have been successfully achieved with the use of platinised PVDF membranes. The transport of test proteins can be manipulated by either varying the electric field direction or adjusting the pH of the protein solution. Studies on protein transport in a supporting electrolyte showed that the extra charge carriers introduced have weakened the electric field across the membrane (under controlled current model); as a result, the protein transport flux decreased.

The separation of protein mixtures has been achieved using the platinised membranes. As test cases, the separation of HSA from myoglobin, and BSA from haemoglobin have been successfully demonstrated with protein transport flux of \(-1.0 \times 10^{-12}\) mol cm\(^{-2}\) s\(^{-1}\) and a selectivity of 14.5 to 70 obtained.

In an attempt to improve the electrochemically controlled transport productivity, a dual membrane (flow through) transport cell was designed and tested for the transport of a test protein. The transport results demonstrated that a test protein solution can be fractionated into two fractions with one fraction being concentrated while the other fraction being less concentrated. This may be of potential application for fast and continuous proteins separation.
CHAPTER 4

STUDIES ON THE INTERACTIONS OF PROTEINS AND POLYPYRROLES USING EQCM AND CYCLIC VOLTAMMETRY
4.1 INTRODUCTION

Conducting electroactive polymers such as polypyrroles, polythiophenes and polyanilines have been utilised recently in the development of new composite materials [74,77,78], sensing devices [124-127] and separation technologies [128]. Their interaction with proteins is of particular interest to those involved in the development of new biosensing [129-133] and bioseparation techniques [134,135]. In addition, it has been revealed recently that polypyrroles have interesting properties for use as biomaterials enabled by the incorporation of biofunctional groups [136,137].

In this chapter, the incorporation of biofunctional groups, such as antibodies of serum albumin (IgG), polyelectrolytes and conventional counterions into polypyrrole conducting polymers has been investigated. The interaction of these materials with proteins has been studied using EQCM and cyclic voltammetry.

4.2 EXPERIMENTAL

4.2.1 Reagents

Pyrrole monomer from Merck was distilled and stored under nitrogen below -18 °C before use. p-Toluene sulfonic acid sodium salt (pTS) from Merck, heparin sulfate sodium salt (bovine intestinal mucosa) from Fluka and dextran sulfate sodium salt (Mr 50,000) from Sigma were used as received. Anti - human albumin developed in rabbit (IgG Fraction of
antiserum \( M_r \) 150,000 KD) from Sigma was dialysed against phosphate buffer, quickly frozen with liquid nitrogen and stored below -18 °C before use. Proteins myoglobin (MYO. \( M_r \) ~16,900) from horse heart, Cytochrome. c (Cyt. C. \( M_r \) 1,3400), human serum albumin (HSA \( M_r \) 66,000) and bovine serum albumin (BSA. \( M_r \) 66,000) all from Sigma were used as received. All inorganic chemicals used in this research were of reagent grade. Milli-Q water was used as solvent except as indicated.

4.2.2 Instrumentation and Some Practical Aspects about EQCM

The EQCM is a piezoelectric device capable of monolayer mass sensitivity. The great advantage of this method is that \textit{in situ} determination of the working electrode mass changes and electrochemical parameters can be obtained simultaneously. The operational principles of the EQCM and its experimental set up have been described previously in Section 2.2.4.

A PAR 173 potentiostat/galvanostat from Princeton Applied Research, a BAS - CV 27 from Bioanalytical Systems Inc, a four channel Maclab from AD Instruments, and a LC Macintosh computer were integrated with the EQCM device (Figure 2.5).

AT-cut gold coated quartz crystal electrodes of 10 MHz nominal resonant frequency from the International Crystal Manufacturing Company were selected for this research. The inner flag area of each gold coated quartz crystal was sealed with silastic (TM) so that electropolymerisation can only occur on the circular area - the effective sensing area of a quartz crystal electrode (Figure 2.6).
Theoretically, the custom made EQCM system used in this investigation was able to detect a 0.1 Hz frequency change. In reality, the background noise (or the variations of frequency in aqueous solution) generated by the electronic systems can be up to 2.0 Hz (Figure 4.1). Therefore, a change of less than 2.0 Hz cannot be accurately detected with this EQCM system. The error introduced by the background noise is estimated to be ±0.02% if the frequency change caused by the polymer deposition is limited to 10 kHz.

![Figure 4.1](image)

**Figure 4.1** The EQCM background noise and sensitivity detection. The minimum frequency change detectable is 0.1 Hz. The background noise variation is within 2.0 Hz or 1.73 ng in mass changes.

The EQCM was calibrated by the electrodeposition of copper. The amount of copper deposited on a gold coated quartz crystal was calculated based on the charge consumed according to Faraday’s Law. The EQCM conversion factor $C_f$ ($\Delta m/\Delta F$) obtained was then compared with that derived from the Sauerbrey equation. The *in situ* frequency change
during the electrodeposition of polypyrrole on a gold coated quartz crystal electrode was also detected and then compared with that detected after drying.

4.2.3 Synthesis of Polypyrroles

The synthesis of polypyrrole on the gold coated quartz crystal electrodes was carried out using the electrochemical polymerisation method. In this case, one face of the gold coated quartz crystal in contact with the solution was used as the working electrode. Four different counterions (shown below) were selected for the preparation of polypyrrole on the quartz crystal electrodes.

The selected counterions (Figure 4.2 a - b) were anti-human serum albumin (anti-HSA), heparin sulfate, dextran sulfate and $p$ -toluene sulfonic acid in sodium salt (pTS). Polypyrrole electrochemically doped with pTS (PPy/PTS) was prepared on the crystal from a solution containing 0.2 M pyrrole monomer and 0.05 M pTS. Polypyrrole/anti-HSA (PPy/anti-HSA) was prepared from 0.5 M pyrrole monomer and 500 ppm anti-HSA water solution. PPy/heparin was prepared from 0.2 M pyrrole and 2500 ppm heparin sulfate sodium salt and PPy/Dex was prepared with 0.2 M pyrrole and 2500 ppm dextran sulfate. The polymer deposition processes were carried out galvanostatically using a current density of 1.0 mA cm$^{-2}$ for 120 seconds.
Figure 4.2 Counterions used in polypyrrole conducting polymer preparation (a) IgG anti-HSA, the two circular areas represent the antigen binding sites, (b) heparin sulfate, (c) dextran sulfate and (d) p-toluene sulfonic acid sodium salt.

4.2.4 Interaction of Proteins and Polypyrrole

The interaction between different polypyrrole conducting polymer films and test proteins under various electrochemical conditions was investigated. Test proteins, bovine serum albumin (BSA), cytochrome C (Cyt. C), human serum albumin (HSA) and myoglobin (MYO), were investigated in respect to their specific and non-specific adsorption to the polypyrrole thin films synthesised on the quartz crystal electrodes.

The interactions between antihuman serum albumin (anti-HSA) and human serum albumin (HSA) are expected to be highly specific (The antibody will interact specifically by cross-linking its multivalent antigen binding site to the antigen to form an extended lattice). Polypyrrole immobilised anti-HSA was tested against HSA and other proteins to determine the
specificity of the PPy/antibody-antigen interaction. Cyclic voltammetric studies were also carried out on these polypyrrole conducting polymers to determine their electrochemical activity and the effect of interaction with the test proteins.

4.3 RESULTS AND DISCUSSION

4.3.1 EQCM Calibration

The EQCM calibration was first carried out by electrochemical deposition of copper ions on the working electrode (Table 4.1). The frequency change during electrodeposition was monitored and the amount of copper deposited was calculated based on the charge consumed using Faraday's law. The average mass/frequency conversion factor $C_f$ ($\mu g \text{ kHz}^{-1}$) was 0.865 $\mu g \text{ kHz}^{-1}$ which was in good agreement with that obtained using the Sauerbrey equation ($C_{fs}=0.867 \mu g \text{ kHz}^{-1}$).

The in situ frequency changes during electrodeposition were also investigated in comparison with the ex situ frequency changes after washing and drying. The purpose of this study was to verify if the frequency change of a quartz crystal was purely caused by the additional mass attached or there were other contributions from solvent such as entrapped water.

The frequency of a fresh crystal (before exposure to solution) was tested and recorded as $F_0$. The cell was then filled with solution and the deposition of either copper or polymer was performed. The in-situ frequency change caused by the deposition was written as $\Delta F_{sol}$. The solution was then removed and the cell was thoroughly washed with distilled water. The cell was dried and its frequency $F_1$ was recorded. The
frequency change $\Delta F_{\text{air}} = F_1 - F_0$ represents the frequency change caused by the extra mass deposited on the crystal free of solution. The comparison between $\Delta F_{\text{air}}$ and $\Delta F_{\text{sol}}$ was made with results shown in Table 4.2.

It has been found that the \textit{in situ} frequency changes in the polymerisation solutions were nearly the same as the \textit{ex situ} frequency changes tested after removing the solutions. The difference between the frequency changes $\Delta F_{\text{air}}$ and $\Delta F_{\text{sol}}$ was within 2%. These results suggest that the \textit{in situ} measurement of the frequency (or mass) changes did represent the amount of polymer deposited on the quartz crystal electrodes.

\textbf{Table 4.1} Determination of EQCM frequency-mass conversion factor

<table>
<thead>
<tr>
<th>Crystal No.</th>
<th>Q (mC)</th>
<th>$\Delta F$ (kHz)</th>
<th>$\Delta m$ (µg)*</th>
<th>$C_f$ (µg kHz$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.49</td>
<td>9.00</td>
<td>7.734</td>
<td>0.859</td>
</tr>
<tr>
<td>2</td>
<td>24.22</td>
<td>9.20</td>
<td>7.975</td>
<td>0.867</td>
</tr>
<tr>
<td>3</td>
<td>22.85</td>
<td>8.65</td>
<td>7.526</td>
<td>0.870</td>
</tr>
</tbody>
</table>

* Copper deposition on a gold coated quartz crystal electrode was carried out in 0.2 M Cu(NO$_3$)$_2$ solution at -0.05V vs Ag/AgCl reference electrode. The amount of copper deposited on a crystal was calculated based on the charge consumed during deposition using Faraday's Law: $\Delta m = M_w Q / (nF)$.

\textbf{Table 4.2} The in situ and ex situ frequency changes of depositions

<table>
<thead>
<tr>
<th>Materials</th>
<th>$\Delta F_{\text{air}}$ (kHz)</th>
<th>$\Delta F_{\text{sol}}$ (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPy/heparin</td>
<td>9.80</td>
<td>9.75</td>
</tr>
<tr>
<td>PPy/pTS</td>
<td>11.30</td>
<td>11.20</td>
</tr>
<tr>
<td>Copper</td>
<td>10.35</td>
<td>10.25</td>
</tr>
</tbody>
</table>

*Polymerisations were carried out at a current density of 1.0 mA cm$^{-2}$ for 120 seconds.
4.3.2 Polypyrrole Preparations

PPy/anti-HSA, PPy/heparin PPy/Dex and PPy/pTS were electrochemically prepared in the polymerisation solutions described previously. All polymer films were galvanostatically prepared at a current density of 1.0 mA cm$^{-2}$ for 120 seconds.

Figure 4.3 shows the chronopotentiogram and frequency change profile during the electrochemical polymerisation of PPy/anti-HSA. The initial polymerisation potential was 1.30 V versus Ag/AgCl reference. It decreased to below 1.05 V in about 30 seconds and remained steady throughout the rest of the polymerisation process. The average polymerisation potentials for PPy/pTS and PPy/heparin were 0.65 V and 1.2 V respectively when the same current density was used.

![Graph showing chronopotentiogram and frequency change profile of PPy/anti-HSA](image)

**Figure 4.3** Chronopotentiogram and frequency change profile of PPy/anti-HSA. PPy/anti-HSA was electrochemically deposited from a polymerisation solution containing 0.5 M pyrrole and 500 ppm anti-HSA. The current density applied was controlled at 1.0 mA cm$^{-2}$ for 120 seconds.
The frequency change and the amount of polymer deposited on each quartz crystal electrode is summarised in Table 4.3. The amount of mass deposited on each crystal during polymer growth was proportional to the amount of charge consumed as shown in Figure 4.3.

If we assume that the current efficiencies were the same during each polymer growth, the total amount of charge consumed in each polymerisation should be the same, since both the current and the polymerisation time were the same. It was found that the amount of polymer obtained per unit charge varied from polymer to polymer. This is presumably caused by the differences in the polymer charge distribution, the counterion size and the apparent molecular weight of the polymer synthesised. Hence the resultant polymers would vary in their chemical structures (such as doping levels) and physical properties.

**Table 4.3** Frequency and mass change vs charge consumed during polymer preparation

<table>
<thead>
<tr>
<th>Polymer(1)</th>
<th>ΔF in average (kHz)</th>
<th>Mass of polymer deposited (μg)(2)</th>
<th>Mass/Charge (μg mC⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPy/anti-HSA</td>
<td>9.21</td>
<td>7.99</td>
<td>0.340</td>
</tr>
<tr>
<td>PPy/Heparin</td>
<td>9.95</td>
<td>8.63</td>
<td>0.367</td>
</tr>
<tr>
<td>PPy/Dextran</td>
<td>16.41</td>
<td>14.23</td>
<td>0.605</td>
</tr>
<tr>
<td>PPy/pTS</td>
<td>11.70</td>
<td>10.15</td>
<td>0.432</td>
</tr>
</tbody>
</table>

(1) All polymers were prepared at the current density of 1.0 mA cm⁻² for 120 seconds.
(2) Mass determined by Δm (μg) = 0.867 ΔF (kHz).

As indicated by the above results the electrodeposition of PPy/anti-HSA on the gold coated quartz crystal substrate was less efficient than the other three polymers. This was presumably due to the large molecular weight (less mobile) and the solubility of the PPy/anti-HSA produced. The
preparation of PPy/Dex was found to be the most charge efficient process due to its relative high charge density.

### 4.3.3 Interactions of Proteins and Polypyrrole

The interactions of proteins with conducting electroactive polymers have been investigated in terms of both specific and non-specific interactions.

HSA was found to bind to the freshly prepared PPy/anti-HSA electrode. The interaction was initiated quickly and then proceeded to level off (Figure 4.4). It took less than 45 seconds for the HSA adsorption to reach 70% of capacity without stirring the test solution. The total amount of HSA adsorbed on the electrode was 1.33 μg cm⁻² or 2.0x10⁻¹¹ moles cm⁻² over 360 seconds.

![Figure 4.4](image)

**Figure 4.4** Adsorption of HSA to PPy/anti-HSA coated quartz crystal electrode in 20 μM HSA aqueous solution.

However, the interaction between HSA and PPy/anti-HSA observed by EQCM was not constant for repeated experiments. The amount of HSA adsorbed on PPy/anti-HSA varied between 1.33 - 1.77 μg cm⁻² or 2.0x
10^{-11} to 2.7 \times 10^{-11} \text{ mol cm}^{-2}, which might be caused by the orientation of anti-HSA immobilised in the polymer. The orientation of anti-HSA in the polymer is more difficult to control and would significantly affect the antigen-antibody (Ag-Ab) interaction.

The orientation of the anti-HSA in polypyrrole may be distributed in a random order and cannot be predicted. The actual distribution might vary from electrode to electrode, which would result in a variation in HSA adsorption on the PPy/anti-HSA coated electrode. But so far, no effective method has been developed to precisely control the antibody's orientation in the polymer using direct (counterion) incorporation.

In the case of adsorption between BSA and PPy/anti-HSA (Figure 4.5), there is no specific interaction between BSA and anti-HSA. The BSA-polymer interaction can be attributed to non-specific polymer adsorption. BSA adsorption gradually increased with time, and took from 145 seconds to 293 seconds to reach 70% of adsorption capacity.

![Figure 4.5 Adsorption of BSA to PPy/anti-HSA coated quartz crystal electrode in 20 \mu M BSA aqueous solution.](image-url)
The amount of BSA bound to the polymer after 30, 60, 120 seconds was found to be about 30%, 50% and 75% of that for HSA adsorbed within the same periods of time. In addition, the final amount of BSA protein adsorbed was also less than that of HSA on the same electrode surface area under the same concentration. The amount of BSA adsorbed was 1.12 μg cm⁻² or 1.70x10⁻¹¹ moles cm⁻². This is about 17% lower than the binding observed for HSA.

Albumin from rabbit was also tested for its adsorption to PPy/anti-HSA (Figure 4.6). As indicated by the adsorption curve, albumin from rabbit showed only a limited adsorption onto the PPy/anti-HSA. The total amount of protein adsorbed was 0.41 μg cm⁻² or 6.2x10⁻¹² moles cm⁻². This is less than 30% of that observed for either HSA or BSA.

Figure 4.6 The adsorption of rabbit albumin to PPy/anti-HSA coated quartz crystal electrode in 20 μM BSA aqueous solution.
Although BSA and HSA share a similar chemical structure, their interaction with anti-HSA occur through different mechanisms. In addition to the non-specific interaction, HSA is able to interact with PPy/anti-HSA based on the specific Ag-Ab binding between HSA and the anti-HSA immobilised in the polymer. Figure 4.7 shows HSA interaction with PPy/anti-HSA which has been previously saturated (or masked) with BSA. HSA was still capable of binding to BSA masked PPy/anti-HSA, indicating that there still existed unoccupied antigen binding sites on the polymer. The total amount of HSA bound to the polymer was 0.58 μg cm$^{-2}$ or 8.7x10$^{-12}$ moles cm$^{-2}$, more than 50% of what was observed previously.

![Graph showing the adsorption of HSA to PPy/anti-HSA coated crystal electrode after masking by BSA 20 μM BSA and 20 μM HSA aqueous solution.]

**Figure 4.7** The adsorption of HSA to PPy/anti-HSA coated crystal electrode after masking by BSA 20 μM BSA and 20 μM HSA aqueous solution.

Polymer PPy/anti-HSA in the reduced form was tested to determine the adsorption of the test proteins HSA and BSA. The reduced PPy/anti-HSA
polymer was obtained by applying a potential of -0.50 V versus Ag/AgCl for 5 minutes in an 0.2 M NaCl aqueous solution.

During the reduction, the polymer backbone was reduced to its neutral state and the negative sites of the immobilised anti-HSA were expected to be neutralised by the insertion of Na⁺ cations from the electrolyte. Without considering other effects, it is reasonable to assume that the immobilised anti-HSA would be surrounded by a high local concentration of Na⁺.

The adsorption of HSA to the BSA masked PPy/anti-HSA coated electrode (Figure 4.8) shows that the non-specific adsorption of BSA to the polymer was approximately the same as that bound to a freshly prepared PPy/anti-HSA. However, the amount of HSA adsorbed to the PPy/anti-HSA was decreased by 15% to 0.49 μg cm⁻².

![Graph showing adsorption](image)

**Figure 4.8** Adsorption of BSA and HSA to the previously reduced PPy/anti-HSA in 20 μM BSA and 20 μM HSA aqueous solution. The reduced PPy/anti-HSA was obtained by reducing the polymer at -0.50 V vs Ag/AgCl in 0.2 M NaCl solution for 5 minutes. The NaCl solution was removed after reduction and then tested with proteins BSA and HSA.
In addition to BSA and HSA, the other test proteins, cytochrome C and myoglobin, were also tested for their interaction with anti-HSA. For example, cytochrome C was found not to bind to PPy/anti-HSA (Figure 4.9), when tested with the same method used for BSA and HSA. Similar results were also obtained for myoglobin. That is, no adsorption was found between myoglobin and PPy/anti-HSA (Figure 4.10).

**Figure 4.9** Adsorption of Cytochrome C to PPy/anti-HSA coated quartz crystal electrode in 20 μM Cytochrome C aqueous solution.

**Figure 4.10** Adsorption of myoglobin to PPy/anti-HSA coated quartz crystal electrode in 20 μM myoglobin aqueous solution.
To further distinguish the anti-HSA-HSA interaction from other protein-polymer interactions, HSA and BSA were also tested for their non-specific or physical adsorption to the polypyrrole prepared with other counterions, such as heparin sulfate, dextran sulfate and pTS.

Limited adsorption of HSA or BSA to PPy/Heparin was observed (Figure 4.11 and Figure 4.12). The total amount of HSA adsorbed to PPy/Heparin was less than 0.2 \( \mu \text{g cm}^{-2} \), which is 15\% of that to PPy/anti-HSA. The adsorption of BSA to PPy/Heparin was found to be less than 0.45 \( \mu \text{g cm}^{-2} \) which is 40\% of BSA to PPy/anti-HSA.

![Graph](image.png)

**Figure 4.11** Adsorption of HSA to PPy/heparin coated quartz crystal electrode in 20 \( \mu \text{M} \) HSA aqueous solution.
The adsorption of HSA to PPy/pTS and PPy/Dex were also tested (Figure 4.13 and Figure 4.14). It was found that HSA showed little affinity to either PPy/pTS or PPy/Dex.
Chapter 4 Interaction of Polypyrroles and Proteins

4.3.4 Interaction between Proteins and Polypyrrole Under Potentiodynamic Conditions

The interaction between proteins and PPy/anti-HSA was investigated by Cyclic Voltammetry and EQCM. The polymer was cycled between the upper and lower potential limits at certain scan rates to vary the redox states of the polymer and induce any interaction between the test protein and the polymer. The polymer was first cycled without protein, and this was continued for another three cycles before adding HSA into the solution.

The cyclic voltammogram and EQCM frequency change of PPy/anti-HSA are given in Figure 4.15 and Figure 4.16 when cycled between +/-1.0 V in 0.01 M pH 4.0 phosphate buffer. The results show that adding HSA between the two cycles has only minor effects on the cyclic voltammogram. The redox peaks can be observed at -0.40 V and -0.70 V. A more narrow anodic peak can be identified in the following cycle (after
adding HSA into the solution). However, there is no major difference identified from the EQCM frequency change profile.

![Cyclic voltammograms of PP/anti-HSA on a gold coated quartz crystal electrode in 0.01 M pH 4.0 phosphate buffer solution. Potential limits: +/-1.0 V. Scan rate: 20 mV s\(^{-1}\). Cycle 1. without HSA. Cycle 2. after adding HSA. HSA concentration in cell solution: 20 \(\mu\text{M}\).](image)

**Figure 4.15** Cyclic voltammograms of PP/anti-HSA on a gold coated quartz crystal electrode in 0.01 M pH 4.0 phosphate buffer solution. Potential limits: +/-1.0 V. Scan rate: 20 mV s\(^{-1}\). Cycle 1. without HSA. Cycle 2. after adding HSA. HSA concentration in cell solution: 20 \(\mu\text{M}\).

Although the interaction of HSA and PPy/anti-HSA was not very conclusive by use of Cyclic Voltammetry, the redox property of the polymer was well characterised by the cyclic voltammogram and frequency change profile. PPy/anti-HSA was found to be electroactive with two pairs of broad peaks. Its redox process was accompanied by both anion- and cation-exchange which was well presented by the EQCM.

When scanned from +1.0 V to -1.0 V, the reduction of PPy/anti-HSA started at about +0.25 V, accompanied by the anion movement out of the polymer. The cation started to move into the polymer at a more negative potential (about -0.2 V). This process continued until the potential
reached -0.9 V where the cation stopped moving in and ready to move out on its reverse scan.

When scanned from -1.0 V to +1.0 V, the cations started to move out of the polymer from the beginning on the reverse scan until the potential reached +0.12 V. The anions in solution began to move into the polymer between the potentials of +0.12 V to 1.0 V.

A net frequency increase of ~0.25 kHz (or 1.2 μg cm⁻²) between each cycle was observed when PPy/anti-HSA was cycled between +/-1.0 V. This phenomenon could be attributed to the conspicuous acid-base property of the anti-HSA immobilised in the polypyrrole. There are some acid-base groups of PPy/anti-HSA that are protonated at pH 4.0 [109]. When the polymer underwent a redox process, the protonation/deprotonation, as well as Na⁺ insertion/expulsion, would occur at the same time. Because of the lower mobility of the Na⁺ cation, some proton sites in the polymer were gradually replaced by the cation Na⁺, which resulted in a mass increase.
Figure 4.16 EQCM frequency change profile of PP/anti-HSA on a gold coated quartz crystal electrode in 0.01 M pH 4.0 phosphate buffer solution. Potential limits: +/-1.00 V. Scan rate: 20 mV s⁻¹. Cycle 1. without HSA. Cycle 2. after adding HSA. HSA concentration in cell solution: 20 μM.

4.4 CONCLUSION

The electrochemical synthesis of polypyrrole with different counterions on gold coated quartz crystal electrode have been successfully obtained. The incorporation of anti-HSA into polypyrrole to assemble a composite polymer for the detection of HSA has also been successfully achieved by using the EQCM. Studies on the electrodeposition of polypyrrole showed that the amount of polymer deposited on a gold coated quartz electrode was linearly related to the charge consumed during the polymerisation process for all counterions employed.

The interaction of protein with polypyrrole synthesised with different counterions indicated that the chemical and physical interactions between the test protein and polypyrrole, to a large extent, depended on the type
of counterion incorporated in the polymer during its synthesis. Protein adsorption tests showed that both HSA and BSA had higher adsorption rates to the polypyrrole prepared with anti-HSA. But the adsorption of HSA to PPy/anti-HSA was much faster than that of BSA. The proteins myoglobin and cytochrome C showed no interaction with PPy/anti-HSA.

In comparison, limited adsorption was observed between HSA and polypyrroles prepared with dextran sulfate, heparin sulfate and pTS. These results suggest that the counterions or biofunctional groups incorporated into polypyrrole are crucial in determining the protein binding properties of the polymers, which might be of potential application in assembling EQCM biosensing devices.

The investigation on the interaction between test proteins and polypyrrole under potentiodynamic conditions was not conclusive. Adding test protein to an electrochemical process had little effect on the cyclic voltammogram or the EQCM frequency change profile.
CHAPTER 5

SYNTHESIS AND CHARACTERISATIONS OF POLYPYRROLE/HEPARIN -- INTERACTIONS OF THROMBIN
5.1 INTRODUCTION

Heparin is a variably sulfated glycosaminoglycan that consists predominantly of alternating $\alpha (1 \rightarrow 4)$-linked residues of D-iduronate-2-sulfate and N-sulfo-D-glucosamine-6-sulfate [37]. The structure, although not always identical, is generally depicted as follow:

![Chemical structure of heparin sulfate](image)

Figure 5.1  Chemical structure of heparin sulfate

Heparin has an average of 2.5 sulfate residues per disaccharide unit, which makes it the most negatively charged polyelectrolyte in mammalian tissues. Their average molecular weights vary between 5,000 - 30,000. Heparin occurs almost exclusively in the intercellular granules of the mast cells that line arterial walls [138], especially in the liver, lungs, and skin. Due to its unique structure and surface charge distribution, heparin is widely known for its anticoagulant property and its specific affinity to thrombin [139,140] as well as some proteins and enzymes. It inhibits the clotting of blood, and its release, through injury, is thought to prevent runaway clot formation.

A number of methods have been developed to enable heparin immobilisation on the surface of other materials [141-144] for the determination and separation of thrombin - a clotting protein of clinical importance. Most of these methods are based on either some variant of ion
exchange chromatography or affinity chromatography on matrix bound
heparin or other synthetic thrombin inhibitors [145]. More often, heparin or
other thrombin inhibitor is covalently linked to the matrix, such as to a
Sepharose gel by a reaction between an uronic acid-carboxyl group of
heparin and aminohexyl-Sepharose. Thrombin or antithrombin can be
separated and eluted, using a concentration gradient of salt, from the
Sepharose column. The affinity of immobilised heparin, to thrombin and
antithrombin vary with the immobilisation [146] methods as well as the
anion and cation in the solution[133].

Previous work [74,148,149], has shown that conducting electroactive
polymers have a range of interesting properties suitable in designing new
biomaterials. They are readily assembled into supramolecular structures
with multifunction by using simple electropolymerisation processes
(Equation 5.1). For example, polyelectrolytes and other biofunctional
groups [77,78] can be incorporated into polypyrroles during the
electropolymerisation processes according to:

\[
 n \text{[Fig.]} + \text{PE}^- \xrightarrow{\text{OX}} \left[ \text{Fig.} \right]^n_{\text{PE}^-} \quad (5.1)
\]

where \text{PE}^- represents a polyanion or biofunctional group being
incorporated in the polypyrrole matrix. Because the chemical and physical
properties of the resultant polymer are mainly determined by the
incorporated polyanion, polypyrrole of specific function can be devised by
this method.

In this chapter, the synthesis and characterisation of polypyrrole containing
heparin composite (PPy/Heparin) have been investigated in order to
develop a new composite material for thrombin separation or even sensing.
The synthesis of PPy/Heparin has been carried out using the
electrochemical method by oxidising pyrrole monomer in the presence of heparin sulfate in an aqueous solution. In doing so, heparin would be electrochemically incorporated or entrapped in the polymer. The electrochemical properties of the resultant polymer (PPy/Heparin) have been characterised using Cyclic Voltammetry (CV) and Electrochemical Quartz Crystal Microbalance (EQCM) technique. The heparin immobilised in the polypyrrole matrix has been assayed in terms of its reactivity with toluidine blue dye which has long been used as a tool for the characterisation of solid immobilised heparin [139]. The interactions of conducting electroactive polymer (CEP) such as polypyrrole-heparin (PPy/Heparin) with test proteins have been addressed in respect to the adsorption of these on PPy/Heparin. The morphology of this composite has also been studied using atomic force microscopy (AFM).

5.2 EXPERIMENTAL

5.2.1 Reagents

Pyrrole monomer from Merck Co. was distilled and stored under nitrogen below -18°C before use. Para-toluene sulphonylic acid sodium salt (pTS) from Merck and heparin sodium salt from bovine intestinal mucosa (Cat No.51536) from Fluka were used as received. Proteins, bovine serum albumin (BSA) and human serum albumin (HSA) from Sigma, were used as received. Alpha-thrombin from bovine plasma was obtained from Sigma with a specific activity of 48 NIH units per mg solid product. The thrombin was reconstituted with Milli-Q water, as described by the supplier, and the solution contained 96 NIH units per 100 μl in 0.15 M NaCl and 0.05 M sodium citrate at pH 6.5. All other inorganic chemicals were of analytical reagent grade. Milli-Q water (18 MΩ cm) was used for solution preparation and protein reconstitution.
5.2.2 Instrumentation

A four channel AD Instruments Maclab, BAS CV-27 and Macintosh computer were integrated together with the custom made EQCM (refer to Chapter 2) and used for the in-situ electrochemical polymer deposition, characterisation and in-situ protein binding tests. Gold coated AT-cut quartz crystal electrodes of nominal 10 MHz from International Crystal MFG were employed for the electrochemical deposition.

In this investigation, the amount of polymer and that of heparin in the PPy/Heparin polymer have been analysed by the EQCM technique combined with the toluidine blue assay. Since the amount of PPy/Heparin (Δm) deposited on the electrode is given by Equation 2.5 or Equation 2.7 (Chapter 2):

\[ \Delta f = \frac{2f_0^2 \Delta m}{A(\rho_q \eta_q)^{1/2}} \]

or

\[ \Delta m = C_f \Delta f \]

where Δf is the measured frequency change caused by the additional PPy/Heparin deposited on a quartz crystal electrode, \( f_0 \) is the nominal resonant frequency of the quartz crystal, \( \Delta m \) is the amount of PPy/Heparin deposited on the quartz crystal electrode, \( A \) is the piezoelectrically active area (0.196 cm²), \( \rho_q \) is the density of the quartz (2.648 g cm⁻³), and \( \eta_q \) is the shear modulus of the AT-cut quartz (2.947×10¹¹ dynes cm⁻²).

The conversion factor \( C_f \) for this EQCM, as obtained in Chapter 4, is 0.866 ng Hz⁻¹.
5.2.3 Electropolymerisation

PPy/Heparin films on various substrates were electrochemically synthesised from a polymerisation solution containing 0.2 M pyrrole monomer and 2500 ppm heparin sodium salt. The substrates used were:

1. gold coated quartz crystal electrode,
2. polished stainless steel plate, and
3. platinised PVDF membrane.

The electrochemical deposition was carried out under either potentiodynamic condition or galvanostatic control. The quantity of charge consumed during the polymerisation process was determined from the constant current applied and the polymerisation time. PPy/Heparin films deposited on electrodes were washed with Milli-Q water before any further experimentation.

The amount of PPy/Heparin produced per unit charge was determined by weighing the substrates before and after the polymerisation. The substrates (with or without polymer) were dried at 105 °C until a constant weight was reached.

5.2.4 Morphological Study Using Atomic Force Microscopy (AFM)

The morphology of PPy/Heparin was investigated by the atomic force microscopy (AFM). It allows us to produce 3-dimensional images with nanometre resolution and to quantitatively determine the surface roughness of the polymers. All AFM samples were electrochemically freshly prepared as described previously and washed with deionised water. The samples were dried in air before carrying out the AFM investigations. For comparison, AFM studies on polypyrrole prepared with other counterions (such as PPy/pTS, PPy/Dex) were also carried out at the same time.
5.2.5 Toluidine Blue Assay of Heparin Content in PPy/Heparin

Heparin contains esterified sulphuric acid and gives a metachromatic reaction to form a heparin-dye complex with toluidine blue dye (Figure 5.2).

![Chemical structure of toluidine blue dye (TB)](image)

Figure 5.2 Chemical structures of toluidine blue dye (TB).

The absorption of such a heparin-dye complex can be measured by UV-vis and is a function of the concentration of heparin and of the TB dye. But this procedure cannot be used directly because the absorption spectra of the two forms of TB dyes (heparin-dye complex and dye) overlap.

In this chapter, the heparin assay was carried out by using the colorimetric method described by F. C. Macintosh and P. K. Smith [151,152]. In this method, the heparin-dye complex is extracted with hexane and the uncombined dye remaining in the solution retains its normal tint. The amount of dye removed is proportional to the amount of heparin in the solution and can be determined by measuring the adsorption of the aqueous solution at 631 nm.

The amount of heparin incorporated into the PPy/Heparin was firstly determined using the toluidine blue assay. It was assumed that the amount of heparin incorporated into the PPy/Heparin was equal to the amount of heparin removed from the polymerisation solution by the electropolymerisation, i.e.

\[
\text{Heparin (incorporated)} = \text{Heparin (added in sol)} - \text{Heparin (left in sol)}
\]
By measuring the amount of heparin added to a polymerisation solution and that after the polymerisation, the amount of heparin immobilised by the polypyrrole can be determined.

To quantitatively determine the specific activity of heparin in PPy/Heparin (after synthesis), toluidine blue assays were carried out using the same technique. It had been found [152] that solid material immobilised heparin was still able to associate with toluidine blue dye to some extent and to form a solid heparin-dye complex. By using the above assay, the polypyrrole immobilised heparin (PPy/Heparin) was determined by measuring the absorption of the solution after removing the solid polymer-dye complex.

Two forms of PPy/Heparin were electrochemically prepared for the assays:

(i) free-standing PPy/Heparin films deposited on stainless steel plate,

(ii) PPy/Heparin deposited on platinised PVDF membranes.

PPy/Heparin membranes were weighed, cut into small pieces and then allowed to react with a known concentration of toluidine blue dye for certain period of time. After removing the PPy/Heparin-dye complex from the solution, the absorption of unreacted TB dye in solution was measured at 631 nm and the heparin content in the polypyrrole was calculated. The actual reaction time was controlled between 30 minutes to 12 hours.
5.2.6 PPy/Heparin and Protein Interactions

Interactions of thrombin and other test proteins with PPy/Heparin conducting polymer were investigated using the EQCM technique in respect to the specific and non-specific adsorption of these test proteins to PPy/Heparin polymer deposited on a gold coated quartz electrode. A quartz crystal electrode, electrochemically deposited with PPy/Heparin, was installed in the EQCM cell (Chapter 2 Figure 2.5) with the polymer deposited surface facing the cell solution. The cell was initially filled with 2 ml of buffer solution, and test protein was added after a stable baseline was obtained. The frequency change, corresponding to the amount of mass change due to the test protein adsorbed on the quartz crystal electrode, was monitored with the EQCM system. The protein adsorption was obtained from the actual frequency change before and after the addition of the test protein such as thrombin or other test proteins. No potential was applied to the electrode for these tests. It had been previously verified that the cell solution volume change (due to the addition of small amount of test protein solution into the cell) had little effect on the EQCM frequency reading. The frequency change due to this effect was less than 5 Hz [90].

PPy/Heparin in reduced and reoxidised forms were also tested for their interactions with proteins. The reduced or reoxidised PPy/Heparin were obtained by reducing or reoxidising the polymer in a supporting electrolyte containing 0.15 M NaCl and 0.05 M sodium citrate. The reduction or reoxidation of PPy/Heparin was carried out by applying -0.50 V or +0.50 V (versus Ag/AgCl reference) for 5 minutes.
5.3 RESULTS AND DISCUSSION

5.3.1 Electrochemical Deposition of PPy/Heparin

The electrochemical deposition of PPy/Heparin on gold coated quartz electrodes was carried out both potentiodynamically and galvanostatically.

The potentiodynamic growth of PPy/Heparin was carried out by varying the applied potential between -0.20 V (E_limit) and +1.00 V (E_limit) at the scan rate of 20 mV s^-1. The polymerisation solution contained 0.2 M pyrrole monomer and 2500 ppm heparin sodium salt prepared with Milli-Q water.

The cyclic voltammograms and the corresponding EQCM frequency changes during the polymerisation are given by Figure 5.3 and Figure 5.4. When scanned from E_limit to E_limit, the PPy/Heparin did not grow at potentials cathodic of 0.45 V, although a small increase in background or charging current was observed in the cyclic voltammogram. Polymer started to grow on the gold coated electrode when the potential anodically exceeded +0.45 V. At more positive potentials, both anodic current and frequency started to increase rapidly. The frequency change corresponding to the mass deposited on the electrode for each cycle was nearly identical with an average frequency increase of 1.20 kHz for each cycle, which is equivalent to 5.3 μg cm^{-2} of polymer deposited on the quartz crystal electrode. The polymer deposited in previous cycles had little effect on deposition in the following cycles (at least) within the first 3 cycles.
Figure 5.3  Cyclic voltammogram during electrodeposition of PPy/Heparin on gold coated quartz electrode under potentiodynamic conditions. The potential vs Ag/AgCl was scanned between $E_{\text{lim}}$: -0.20 V and $E_{+\text{lim}}$: +0.70 V at 20 mV s$^{-1}$. Polymerisation solution contained 0.2 M pyrrole monomer and 2500 ppm heparin.

Figure 5.4  EQCM during electrodeposition of PPy/Heparin on gold coated quartz crystal electrode under potentiodynamic conditions. The potential applied was scanned between $E_{\text{lim}}$: -0.20 V and $E_{+\text{lim}}$: +0.70 V at 20 mV s$^{-1}$. Polymerisation solution contained 0.2 M pyrrole and 2500 ppm heparin.
In addition to the potentiodynamic growth, the synthesis of PPy/Heparin on gold coated quartz crystal electrodes was also carried out galvanostatically using a current density of 1.0 mA cm\(^{-2}\). The polymerisation started immediately upon the application of the current as indicated by both the chronopotentiogram and EQCM frequency profile (Figure 5.5). The amount of PPy/Heparin deposited on the electrode surface was proportional to the polymerisation time with an average mass increase of 8.62 µg (or 9.95 kHz in frequency increase) in 120 seconds. The average charge consumed under these conditions was 23.5 mC, thus 0.366 µg PPy/Heparin was produced for each mC charge consumed. In contrast, for PPy/pTS the mass produced per unit charge was 0.489 µg mC\(^{-1}\), which indicates that the electrodeposition of PPy/Heparin is less charge efficient than that observed for PPy/pTS.
In the case of PPy/Heparin prepared on a large substrate such as a stainless steel plate, the amount of polymer (mg) produced for each unit charge consumed was monitored and quantitatively determined. The resulting mass per unit charge was different from that obtained on a quartz electrode. As is indicated by Figure 5.6, the polypyrrole-heparin was depositing at a rate of 0.513 mg/C (or μg/mC).

**Figure 5.6** PPy/Heparin mass versus charge consumed during the electrochemical polymerisation on a stainless steel plate electrode.
5.3.2 Morphology of Polypyrroles

Atomic Force Microscopy (AFM) studies revealed that the morphology of polypyrroles varied with the type of counterions being incorporated. PPy/Heparin showed a microporous and dendritic morphology (Figure 5.7a). The dendrites are estimated from the AFM images to be 200 - 300 nm long. Such a microporous structure of PPy/Heparin can provide a greater surface area as well as accessibility, which may be of advantage in sensing or separation processes.

The surface morphology of polypyrrole prepared with other counterions were also investigated with the AFM so as to compare with that obtained for PPy/Heparin (Figure 5.6 b, c and d). Polypyrrole prepared with dextran sulfate (PPy/Dex) showed a much rougher and more porous structure than that observed for heparin. The surface roughness was estimated to be around 2.0 μm. In the case of polypyrrole prepared with pTS (PPy/pTS), a much smaller counterion, the AFM image showed a more uniform polymer crystalline structure with a surface roughness estimated to be around 1 μm. Polypyrrole prepared with benzene sulphonic acid showed a similar morphological structure as polypyrrole pTS, but with a rougher surface. The surface roughness was around 1.5 μm.
Figure 5.7  AFM images of polypyrroles prepared with four different counterions (a) heparin sulfate, (b) Dextran sulfate, (c) pTS, and (d) BS. (To be continued in the next page)
Figure 5.7 Continued
5.3.3 Electrochemical Properties of PPy/Heparin

The electrochemical properties of PPy/Heparin prepared on gold coated quartz crystal electrodes were characterised using cyclic voltammetry and EQCM techniques. PPy/Heparin was galvanostatically prepared on a gold coated quartz crystal electrode as previously described. Cyclic voltammetry experiments combined with EQCM were carried out in aqueous solution containing 0.2 M KCl.

PPy/Heparin showed a typical cyclic voltammogram of polypyrrole with an immobile anion as dopant. The cyclic voltammogram of PPy/Heparin indicated that the polymer was electroactive with only one pair of broad redox peaks appearing at \( E_{p(c)} = -0.22 \) V and \( E_{p(a)} = +0.05 \) V. (Figure 5.8). The separation of peak potentials \( \Delta E = E_{p(a)} - E_{p(c)} = 0.27 \) V. The anodic and cathodic peak currents are estimated to be +110 \( \mu \)A and -115 \( \mu \)A respectively. In addition, the PPy/Heparin can be repeatedly cycled between its reduced and oxidised states within the potential limits without losing its electroactivity. The negative potentials required to "complete" the polymer reduction are indicative of a system where cation exchange is predominant according to:

\[
\begin{array}{c}
\text{PE}^- + n M^+ \\
\text{Red} \\
\text{Ox}
\end{array}
\]

where \( M^+ \) represents the cation K+ in the supporting electrolyte.
Electrochemical quartz crystal microbalance studies in conjunction with cyclic voltammetry revealed that PPy/Heparin exhibits cation exchange property upon reduction and oxidation. When PPy/Heparin was scanned from the anodic potential to the cathodic potential, the polymer backbone was gradually reduced to the neutral state. Since the counterions incorporated, i.e. the heparin anions, were large and relatively immobile inside the polypyrrole polymer matrix, the cations from the supporting electrolyte migrated into the polymer to neutralise the negative charges of heparin. Thus the EQCM detected a net mass increase (reflected by a frequency increase Figure 5.9). On the reverse scan, the polymer backbone was gradually reoxidised and the excess positive charge was removed from the polymer by cation expulsion. This was detected by the EQCM as a frequency (or mass) decrease associated with the oxidation wave.
There was a small frequency increase (up to ~1.5 kHz) between each cycle scanned. So that the actual mass on the quartz crystal electrode was slowly increasing due to the cation movement across the interface between the polymer and the solution.

It was found that the cation exchange capacity of PPy/Heparin, in terms of the net mass changes between its oxidised and reduced states, was much higher than those of polypyrrole prepared with other counterions. The total amount of mass variation for PPy/Heparin between its reduced and oxidised states reached 1.13 µg.

Figure 5.9  EQCM frequency changes of PPy/Heparin in 0.2 M KCl. Potential range: \( E_{+\text{limit}} = +0.70 \text{ V} \), \( E_{-\text{limit}} = -0.70 \text{ V} \) versus Ag/AgCl reference electrode. Initial potential: \( E_{\text{int}} = +0.70 \text{ V} \). Scan rate :10 mV s\(^{-1}\). PPy/Heparin preparation was the same as for Figure 5.4.
5.3.4 Toluidine Blue Assay of PPy/Heparin

There are two factors to be determined about PPy/Heparin by using the toluidine blue assays: (i) the heparin content in PPy/Heparin, and (ii) the specific reactivity of PPy/Heparin with toluidine blue.

The heparin content in a PPy/Heparin film was determined by combining the EQCM measurements and the toluidine blue tests. The amount of heparin being incorporated into the PPy/Heparin polymer was simply determined by measuring the difference in the amount of heparin in solution before and after the electropolymerisation. Since the total amount of PPy/Heparin polymer deposited on a quartz electrode can be conveniently determined by measuring the frequency change during the polymerisation process, so too the heparin content in a PPy/Heparin film can also be quantitatively determined (Table 5.1).

Table 5.1 Determination of heparin incorporated into Polypyrrole

<table>
<thead>
<tr>
<th>Polymerisation(1)</th>
<th>PPy/Heparin(2) prepared (μg)</th>
<th>Heparin (3) incorporated (μg)</th>
<th>Heparin/polymer % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26.0</td>
<td>8.6</td>
<td>33%</td>
</tr>
<tr>
<td>2</td>
<td>52.6</td>
<td>10</td>
<td>19%</td>
</tr>
<tr>
<td>3</td>
<td>52.6</td>
<td>16.7</td>
<td>32%</td>
</tr>
</tbody>
</table>

(1) The PPy/Heparin films on gold coated quartz crystal electrodes were prepared at 0.5 mA cm\(^{-2}\) current density from a aqueous solution containing 0.2 M pyrrole and 80 ppm (or 80 μg cm\(^{-3}\)) of heparin.

(2) PPy/Heparin was calculated based on the frequency changes obtained during the polymerisation. \(Δm (μg) = 0.867 \ ΔFreq (kHz)\)

(3) Heparin incorporated into the PPy/Heparin was determined by TB assay of heparin content in the polymerisation solution and calculated according to:

\[ \text{Heparin (incorporated)} = \text{Heparin (added in sol)} - \text{Heparin (left in sol)} \]
The EQCM and toluidine blue assay results indicate that a freshly prepared PPy/Heparin film on a quartz electrode consisted of 19% to 33% (w/w) of heparin and 67% to 81% of polypyrrole by weight (The variation between different experiments was believed to be caused by both experimental error and the actual charge distribution of heparin in the polypyrrole). That is to say there was 0.19 to 0.33 g heparin present in each gram of PPy/Heparin polymer. The heparin content in PPy/Heparin film is quite high compared to the amount of heparin immobilised by other methods, such as by Sepharose gels [1].

Toluidine blue assays on solid immobilised heparin (e.g. PPy/Heparin) were carried out using PPy/Heparin films. The films were firstly prepared by electrochemically depositing the PPy/Heparin films on a stainless steel plate, and then the film was peeled from the plate and cut into small pieces for the assay.

The reaction to form the heparin-toluidine blue complex takes place in seconds between free heparin and toluidine blue dye. But the reaction between solid immobilised heparin (such as PPy/Heparin) and the dye would take place for several minutes or even for hours, depending on the microstructure of the polymer. In this experiment, the assays were carried out for 30 minutes and 12 hours. As indicated by Table 5.2, the formation of TB-heparin (solid) complex was limited by the diffusion of toluidine blue rather than by the chemical reaction. With extended reaction time, higher percentage of heparin was detected.

Though there was about 19 - 33 % of heparin immobilised in polypyrrole, not all the heparin in the polymer was reactive or accessible to toluidine blue. This phenomenon could be caused by two reasons:
(1) Some of the heparin, as the doping anions, were electrically neutralised or balanced by the polycation (PPy+);

(2) Heparin on a PPy/Heparin film surface only represents a small portion of the immobilised heparin in the PPy/Heparin.

Part of the heparin inside the polymer can be accessible, depending on the porosity of the polymer and the time allowed for toluidine blue to diffuse through.

The toluidine blue assays conducted on PPy/Heparin films indicated that there was about 0.47% to 1.0% (w/w) of heparin detected within 30 minutes and 1.83 % within 12 hours. About 2.3% to 7.8% (w/w) of all the immobilised heparin was found to be active after polymerisation, depending on the actual reaction time.

Heparin assay using toluidine blue dye is affected by other factors such as substrate materials and polymerisation conditions during the polymer growth stage. Generally speaking, the higher the current density, the more porous the PPy/Heparin, which allows the immobilised heparin to become more accessible to the toluidine blue dye. Since heparin inside the polymer matrix was inaccessible to toluidine blue, especially in a short period, the amount of heparin detected for thick films was usually lower than that for thin films.
Table 5.2  Toluidine blue assay of polypyrrole immobilised heparin

<table>
<thead>
<tr>
<th>PPy/Heparin membrane (1)</th>
<th>Heparin : PPy/Heparin (w/w)</th>
<th>Accessible heparin : immobilised heparin (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 minutes 2 mA cm⁻²</td>
<td>1.0% (30 min)</td>
<td>4.5%</td>
</tr>
<tr>
<td>20 minutes 2 mA cm⁻²</td>
<td>0.47% (30 min) 1.83% (12 hours)</td>
<td>&gt;2.3% 7.8%</td>
</tr>
</tbody>
</table>

(1) PPy/Heparin membranes were prepared as described in polymerisation section.

5.3.5 Interactions of PPY/Heparin and Proteins

One of the main interests in this research is the interactions of PPY/Heparin with test proteins; such as thrombin, bovine serum albumin (BSA), and human serum albumin (HSA). The interactions between the polymer and proteins were investigated using the EQCM technique.

The PPY/Heparin was electrochemically prepared on a quartz crystal electrode at the current density of 1.0 mA cm⁻² for 120 seconds from a solution containing 0.2 M pyrrole monomer and 2500 ppm heparin. The total amount of PPY/Heparin deposited on the surface of the quartz electrode was 8.7 µg (or 44.4 µg cm⁻² electrode area) based on the total frequency change during the polymerisation. PPY/Heparin in different redox states was obtained by applying the appropriate potential in a given supporting electrolyte.

Thrombin was first tested for its adsorption or binding to the PPY/Heparin polymer at different redox states, i.e. the PPY/Heparin was tested in its freshly prepared oxidised form, or in the reduced state and finally the
reoxidised form. A freshly prepared oxidised form of PPy/Heparin was obtained using the polymerisation method described above. The reduced or re-oxidised form of PPy/Heparin was obtained by reducing or oxidising the PPy/Heparin in a 0.2 M NaCl supporting electrolyte at the potential of -0.50 V or +0.50 V respectively for 5 minutes.

The thrombin adsorption tests showed that freshly prepared PPy/Heparin exhibited the highest thrombin adsorption (Figure 5.10a). The maximum frequency shift caused by the adsorption of thrombin onto the PPy/Heparin quartz crystal electrode was greater than 0.30 kHz; which was equivalent to 0.26 µg net mass increase on the entire electrode surface. This corresponds to the adsorption of 1.33 µg thrombin per cm² electrode surface area or 0.064 NIH units cm⁻² (assuming 48 NIH units per mg solid thrombin as specified by the manufacturer).

![Graph showing EQCM studies of thrombin binding to PPy/Heparin of different oxidation states on a quartz crystal electrode.](image)

**Figure 5.10** EQCM studies of thrombin binding to PPy/Heparin of different oxidation states on a quartz crystal electrode. Electrode surface area: 0.196 cm². Supporting electrolyte: 0.15 M sodium chloride and 0.05 M sodium citrate at pH 6.5. Thrombin of 48 NIH units or 1 mg was added to the solution after 90 seconds. Curve a: the adsorption of thrombin to a freshly prepared PPy/Heparin. Curve b: the adsorption of thrombin to pre-reduced PPy/Heparin. Curve c: the adsorption of thrombin to re-oxidised PPy/Heparin.
Limited adsorption of thrombin to the reduced PPy/Heparin was found (Figure 5.10 b). This is explained as follows. When the polymeric backbone was reduced to the neutral state, it resulted in an excess of negative charge in the polymer matrix due to negatively charged heparin. Consequently, since the heparin counterions incorporated were relatively immobile, this excess negative charge had to be neutralised by an influx of positively charged species. As a result, in this case, there is competition between small mobile cation and large thrombin molecules for these anionic sites. The EQCM result (Figure 5.9) suggests that, in this case, cation insertion would predominate. As a result, a higher local cation concentration is produced in the polymer which would result in low reactivity and low adsorption of thrombin to the PPy/Heparin polymer.

In addition, thrombin was found to be able to adsorb onto a re-oxidised PPy/Heparin as shown by Figure 5.10 (c). In this case, the amount of thrombin adsorbed on the re-oxidised PPy/Heparin electrode was lowered to 0.19 µg (or 0.97 µg cm⁻²) which is about 73% of that adsorbed onto a freshly prepared PPy/Heparin. The result suggests thrombin has stronger affinity to oxidised PPy/Heparin and that the PPy/Heparin on an electrode surface can be regenerated by simply reducing/oxidising the polymer under certain electrochemical conditions.

Thus the affinity of PPy/Heparin, in different redox states, for thrombin is in the order:

**Oxidised > Reoxidised > Reduced**

This may be an advantage in designing a separation system capable of controlling the adsorption and desorption of thrombin based on PPy/Heparin.
The interactions of PPy/Heparin with other test proteins, such as bovine serum albumin (BSA), human serum albumin (HSA), haemoglobin and myoglobin, were also studied using the same method. Results (Figure 5.11) show negligible adsorption of BSA to PPy/Heparin, and limited adsorption of HSA to the PPy/Heparin (in both cases, concentration of BSA or HSA was 0.5 μM in test solution). For example, the frequency change caused by the HSA adsorption was less than 0.10 kHz or 0.087 μg in net mass increase on the entire electrode (equivalent to 0.44 μg per cm² electrode area). This represents less than 33% of the thrombin adsorbed on the PPy/Heparin.

![EQCM studies on the adsorption of test proteins including thrombin, to PPy/Heparin deposited quartz crystal electrode. Electrode surface area: 0.196 cm². PPy/Heparin was prepared at 1.0 mA cm⁻² current density for 120 seconds from solution containing 0.2 M pyrrole monomer and 2500 ppm heparin. Supporting electrolyte: 0.15 M sodium chloride and 0.05 M sodium citrate. Test proteins were added after 80 - 90 seconds. Thrombin curve: cell solution contained 48 NIH units of thrombin. HSA curve: cell solution contained 66 μg human serum albumin in 2 ml solution (or 0.5 μM HSA). BSA curve: cell solution contained 66 μg bovine serum albumin in 2 ml solution (or 0.5 μM BSA).](image-url)
In addition, limited adsorption of haemoglobin and myoglobin to PPy/Heparin was found. The adsorption of haemoglobin was in the order of 0.18 \( \mu g \) cm\(^{-2}\) whilst about 0.09 \( \mu g \) cm\(^{-2}\) of myoglobin adsorption was detected on the PPy/Heparin deposited on a quartz crystal electrode.

Thus the affinity of test proteins for the PPy/Heparin coated quartz crystal electrode (provided test protein of the same molar concentration are used) is in the following order:

\[
\text{THROMBIN} > \text{HSA} > \text{HEMO} > \text{BSA} \geq \text{MYO}
\]

These results suggest that immobilised heparin present in PPy/Heparin can be used as a medium for the separation of proteins. The PPy/Heparin has the highest affinity for thrombin which can be a major advantage in separating thrombin from other proteins.

The specific interaction between thrombin and PPy/Heparin was also investigated by blocking the non-specific sites with a high concentration of BSA. The BSA solution used was 2\% by weight which is equal to 20,000 ppm. The specific adsorption of thrombin to BSA blocked PPy/Heparin was then quantitatively determined by adding thrombin into the cell solution.

As indicated by Figure 5.12, the non-specific adsorption of BSA (the first sharp frequency increase) was estimated to be 2.83 \( \mu g \) cm\(^{-2}\) (0.64 kHz in frequency increase). The specific adsorption of thrombin to PPy/Heparin is indicated by the subsequent frequency shift up to 0.30 kHz increase (1.33 \( \mu g \) cm\(^{-2}\) increase). It seems that the adsorption of thrombin was hardly affected by the previous BSA adsorption when compared with that in Figure 5.10 a.
Figure 5.12 EQCM studies of thrombin binding to the BSA blocked PPy/Heparin deposited quartz crystal electrode. Electrode surface area: 0.196 cm². Supporting electrolyte: 0.15 M sodium chloride and 0.05 M sodium citrate. BSA (2%) was added for blocking the non-specific adsorption sites. Subsequently, thrombin (48 NIH units or 1 mg) was added to the solution. Polymer grown at 1 mA cm⁻² for 120 s.

5.3.6 Interaction of Thrombin and PPy/Dex and PPy/pTS

In addition to heparin, the interactions of thrombin with polypyrrole prepared with dextran sulfate and pTS were also investigated. Polypyrrole/Dextran sulfate (PPy/Dex) and PPy/pTS were electrochemically prepared using the same method under similar conditions to PPy/Heparin. PPy/Dex was prepared from a solution containing 0.2 M pyrrole and 2500 ppm dextran sulfate sodium salt. The average molecular weight of dextran sulfate is 50,000 which is similar to the average molecular weight of the heparin employed. PPy/pTS was prepared from 0.2 M pyrrole and 0.05 M pTS. Both polymers were prepared at the same current density of 1.0 mA cm⁻².
The PPy/Dex EQCM adsorption tests were carried out with exactly the same procedure as that for PPy/Heparin. Once a stable baseline was obtained, thrombin of the same quantity was added to the cell solution and the adsorption process was monitored. The results (Figure 5.13) suggest that thrombin showed neither specific affinity nor physical adsorption to the PPy/Dex polymer, even though dextran sulfate has a similar structure and molecular weight as those of heparin.

![EQCM studies of thrombin adsorption to PPy/Dex on quartz crystal electrode. PPy/Dex was prepared at 1.0 mA cm⁻² from solution containing 0.2 M pyrrole monomer and 2500 ppm dextran sulfate sodium salt. Cell solution contained 0.15 M sodium chloride and 0.05 M sodium citrate. Thrombin of 48 NIH units were added to cell solution after a stable baseline was obtained.](image)

There was a small frequency shift, drop of less than 70 Hz from the baseline upon the addition of thrombin. The EQCM curve stabilised after a few seconds. This change was believed to be caused by some unknown desorption occurring at the PPy/Dex surface.
Figure 5.14 EQCM studies of thrombin adsorption to PPy/pTS on quartz crystal electrode. PPy/pTS was prepared at 1.0 mA cm\(^{-2}\) from solution containing 0.2 M pyrrole monomer and 0.05 M p-toluene sulphon acid sodium salt (pTS). Cell solution contained 0.15 M sodium chloride and 0.05 M sodium citrate. Thrombin of 48 NIH units was added to cell solution after a stable baseline was obtained.

Similar results were also observed at the PPy/pTS polymer when brought into contact with thrombin (Figure 5.14). There was no adsorption found between PPy/pTS and thrombin.

The above results suggest that the interactions found between thrombin and polypyrrole largely depended on the type of counterion incorporated in the polypyrrole during growth. The interactions with polypyrrole immobilised heparin were highly specific, which might include both chemical and physical interactions.
5.4 CONCLUSIONS

Polypyrrole/heparin conducting polymer can be electrochemically prepared on gold coated quartz crystal electrodes. The PPy/Heparin prepared in this way was found to be electroactive with broad redox peaks appearing at -0.20 V and +0.05 V respectively. The material exhibited cation dominated ion-exchange property.

Heparin toluidine blue assays of the polymerisation solution (before and after the polymerisation) showed that the amount of heparin immobilised in the polypyrrole polymer was between 16.5% to 31% (w/w). The same assay conducted on polypyrrole immobilised heparin - (PPy/Heparin film) indicated that 2.3% to 7.8% of the polypyrrole immobilised heparin was detectable by the toluidine blue. In addition, the amount of heparin detected was also dependent on the actual reaction time used for the assay. The longer the reaction time, the more heparin can be detected.

The EQCM results on protein adsorption tests showed that thrombin had the strongest affinity to a freshly prepared PPy/Heparin, but showed very low affinity to the reduced form of PPy/Heparin. However, thrombin had strong affinity to the re-oxidised polymer; although this affinity was still less than that for a freshly prepared PPy/Heparin. In addition, other test proteins, such as bovine serum albumin and human serum albumin had low affinity for PPy/Heparin.

Compared with interactions between thrombin and PPy/Heparin, polypyrrole prepared with other counterions such as polyanion and small anion showed no affinity for the thrombin.
It has been shown that PPy/Heparin, as a newly developed conducting polymer composite material, may have potential applications for heparin immobilisation, thrombin separation and biosensing.
CHAPTER 6

ELECTROCHEMICALLY CONTROLLED TRANSPORT OF PROTEINS ACROSS POLYPYRROLE COMPOSITE MEMBRANES
6.1 INTRODUCTION

The electrochemically controlled transport of proteins across polypyrrole composite membranes was investigated in this chapter. The polypyrrole composite membranes were electrochemically synthesised on platinised PVDF membranes. The electrochemically controlled transport of proteins under different electrical stimuli, such as constant current, constant potential or pulsed potential, have been studied. In addition, the separation of protein mixtures has also been demonstrated in this chapter.

6.2 EXPERIMENTAL

6.2.1 Chemicals and Materials

Proteins, Bovine Serum Albumin (BSA), Human Serum Albumin (HSA), myoglobin (MYO) and haemoglobin (HEMO), were all obtained from Sigma and used as received. Thrombin from bovine plasma was ordered from Sigma and reconstituted as described by the manufacturer. Dextran sulfate (Dex, $M_w \sim 50,000$) from Sigma, $p$-toluenesulphonic acid sodium salt (pTS) from Merck and heparin sulfate from Fluka were used in the synthesis of polypyrrole composite membranes. Pyrrole monomer from Merck was distilled and stored below -18 °C before use. Other inorganic chemicals of AR grade were used unless otherwise stated. All aqueous solutions were prepared with Milli-Q water (18 MΩ cm).

Millipore polyvinylidene fluoride (PVDF) membranes (type HVHP) with nominal 0.45 μm pore size were chosen as substrate membranes. The PVDF substrate membranes were sputter coated with platinum on both sides using the same procedure described in Chapter 2.
6.2.2 Polypyrrole Composite Membrane Preparation

Polypyrrole composite membranes were electrochemically synthesised by depositing polypyrrole on a platinised PVDF substrate membrane (PPy/Pt/PVDF). The electrochemical polymerisation cell employed for PPy/Pt/PVDF composite membrane preparation was similar to that given in the Figure 2.16. The only difference is the cross section area is bigger, i.e. 7.1 cm² or 3 cm in diameter. During the membrane preparation, a platinised membrane was installed in between the two chambers with the side to be coated with PPy facing the solution chamber to which the polymerisation solution was added. The polymerisation was carried out in a three electrode system which was arranged as that shown in Figure 2.16.

The polymer composite membrane was removed from the cell after polymerisation and washed with distilled water thoroughly. Only one side of the membrane was deposited with polypyrrole. A schematic of the polypyrrole composite membrane prepared is given in Figure 6.1.

![Polypyrrole Composite Membrane Schematic](image)

**Figure 6.1** Schematic of polypyrrole conducting polymer composite membrane prepared for electrochemically controlled transport. (a) Side view of the composite membrane and (b) cross section of the membrane.
During the polymerisation, one side of a platinised PVDF membrane was used as the working electrode to which polypyrrole was to be deposited. The polymerisation was carried out galvanostatically at a particular current density for 30 seconds to 60 seconds depending on the actual thickness of the polypyrrole required.

Three different types of polypyrrole composite membranes were prepared and they were:

1. Polypyrrole-heparin composite membranes (PPy/Heparin/Pt/PVDF) which were prepared from an aqueous solution containing 0.2 M pyrrole monomer and 2500 ppm heparin.

2. Polypyrrole-dextran composite membranes (PPy/Dex/Pt/PVDF) which were prepared from a solution containing 0.20 M pyrrole monomer and 2500 ppm dextran sulfate, and

3. Polypyrrole-pTS composite membranes (PPy/pTS/Pt/PVDF) which were prepared from 0.20 M pyrrole and 0.05 M pTS.

6.2.3 Electrochemically Controlled Transport of Proteins

The electrochemically controlled transport of protein across polypyrrole composite membranes was carried out using a similar procedure to that previously employed for platinised membranes (Section 3.2.2). The same electrochemical transport cell was utilised in this work. In the transport experiment, the polypyrrole composite membrane was installed in position with its polymer coated side facing the feed solution. The Three composite membranes prepared, i.e. PPy/Heparin/Pt/PVDF, PPy/Dex/Pt/PVDF and PPy/pTS/Pt/PVDF membranes, were investigated.
Three different electrochemical stimuli were employed in electrochemically controlled transport studies. They were (i) controlled current, (ii) controlled potential and (iii) pulsed potential.

Five test proteins (BSA, HSA, haemoglobin, myoglobin and thrombin) were used and prepared as feed solution for controlled transport studies. In particular, thrombin was reconstituted with pH 6.5 buffer solution according to the manufacturer's instructions. As previously explained, the net charge of these proteins can be positive or negative depending upon the proteins' pI and the pH of the solution.

Electrochemically controlled transport was monitored by measuring the amount of protein transported from the feed solution to the receiving solution. The analysis of BSA, HSA, haemoglobin or myoglobin were carried out using UV-visible spectrophotometry. Thrombin was analysed using a chromogenic thrombin assay \cite{153,154} and was carried out by the Commonwealth Serum Laboratory (CSL) in Melbourne.

\section*{6.3 RESULTS AND DISCUSSION}

\subsection*{6.3.1 Transport under Controlled Current}

The transport of proteins across polypyrrole composite membranes was first carried out using the controlled current technique. A current density of +/- 0.64 mA-cm\textsuperscript{-2} was applied between the polypyrrole side in the feed solution and the platinum coated face in the receiving solution. The positive sign (+) of the applied current means the feed solution side of the electromembrane was positively polarised and the negative sign (-) of the applied current means the feed solution side of the membrane was negatively polarised. The direction of electrical current applied was
varied during transport so as to electrochemically manipulate the transport process.

6.3.1.1 Transport Across PPy/Heparin/Pt/PVDF Membrane

During the BSA experiment, BSA showed little transport without applying a current. But the transport of BSA started soon after applying the current (Figure 6.2). As indicated by the concentration vs time curve, BSA was transported from the feed solution to the receiving solution with a flux of 1.39x10^-12 mol cm^-2 s^-1 when a current density of -0.64 mA cm^-2 was applied to the membrane. The transport of BSA completely stopped when the direction of the current was reversed. The BSA transport flux across PPy/Heparin/Pt/PVDF was slightly lower than that obtained with platinised membranes (1.47x10^-12 mol cm^-2 s^-1) under the same experimental conditions. This was mainly caused by the additional polymer layer deposited on the platinised PVDF membranes.

![Figure 6.2 Transport of BSA across PPy/Heparin/Pt/PVDF membrane. BSA aqueous solution 20 μM was used as feed solution. Receiving solution was Milli-Q water. Current density applied across the membrane: I_{app} = +0.64 mA cm^-2 or -0.64 mA cm^-2. PPy/Heparin membrane was prepared on 0.45 μm Pt/PVDF membrane at 1.0 mA·cm^{-2} for 60 seconds.](image)
In the case of haemoglobin transport, both diffusion transport and electrochemically controlled transport of haemoglobin was observed in the transport studies of haemoglobin (Figure 6.3). The diffusion flux was estimated to be $1.0 \times 10^{-13} \text{ mol cm}^{-2} \text{ s}^{-1}$ about one third of that obtained by the electrochemically controlled transport ($3.1 \times 10^{-13} \text{ mol cm}^{-2} \text{ s}^{-1}$).

![Figure 6.3 Transport of haemoglobin across PPy/Heparin/Pt/PVDF membrane. Haemoglobin aqueous solution 20 µM was used as feed solution. Receiving solution was Milli-Q water. Current density applied across the membrane: $I_{\text{app}} = +0.64 \text{ mA cm}^{-2}$ or $-0.64 \text{ mA cm}^{-2}$. PPy/Heparin membrane was prepared on 0.45 µm Pt/PVDF membrane at 1.0 mA cm$^{-2}$ for 60 seconds.](image)

The transport of haemoglobin across PPy/Heparin/Pt/PVDF membrane was much slower. The transport flux of haemoglobin was only about 16% of that obtained for platinised membranes.

In addition, the transport of haemoglobin was also slower than that of BSA, even though both proteins have similar molecular weights and sizes.
Thus the controlled transport of haemoglobin or BSA cannot be merely explained by sieving effects. Other factors such as polymer interaction and charge density would affect the transport behaviour of the proteins. In the case of BSA and haemoglobin, the net charge density of BSA is higher than that of haemoglobin, because protein BSA carries 15 charges per molecule at pH 6.5, but haemoglobin carries only \(-4\) to \(-5\) net charges per molecule \([48]\).

The transport of myoglobin across PPy/Heparin/Pt/PVDF was found to be faster than both BSA and haemoglobin under the same experimental conditions (Figure 6.4). The myoglobin transport flux reached \(1.6 \times 10^{-12}\) mol cm\(^{-2}\) s\(^{-1}\) which is close to that obtained with platinised membranes. The myoglobin transport flux was about 87% of that for platinised membranes.

![Figure 6.4 Transport of myoglobin across PPy/Heparin/Pt/PVDF membrane. Myoglobin aqueous solution 20 \(\mu\)M was used as feed solution. Receiving solution was Milli-Q water. Current density applied across the membrane: \(I_{\text{app}} = +0.64\) mA cm\(^{-2}\) or \(-0.64\) mA cm\(^{-2}\). PPy/Heparin membrane was prepared on 0.45 \(\mu\)m Pt/PVDF membrane at 1.0 mA cm\(^{-2}\) for 60 seconds.](image)
6.3.1.2 Transport Across PPy/Dex/Pt/PVDF Membrane

Since BSA is negatively charged molecule, the transport of BSA across PPy/Dex/Pt/PVDF occurred upon applying a current of - 0.64 mA cm\(^{-2}\), i.e., the feed solution side of the membrane was negatively polarised (Figure 6.5). The BSA transport flux was found to be \(1.01 \times 10^{-12}\) mol cm\(^{-2}\) s\(^{-1}\), which is lower than that obtained for PPy/Heparin/Pt/PVDF membrane. The transport can be terminated by reversing the electrical current applied.

The transport of haemoglobin and myoglobin was also obtained (Figure 6.6 and Figure 6.7). The transport of haemoglobin across this type of membrane was found to be \(2.84 \times 10^{-13}\) mol cm\(^{-2}\) s\(^{-1}\) which is slightly lower than that for PPy/Heparin composite membrane.

The myoglobin transport flux observed was \(1.6 \times 10^{-12}\) mol cm\(^{-2}\) s\(^{-1}\) for PPy/Heparin composite membrane and \(1.08 \times 10^{-12}\) mol cm\(^{-2}\) s\(^{-1}\) for PPy/Dex composite membrane (Figure 6.7).

For all test proteins, the fluxes across PPy/Dex/Pt/PVDF composite membranes were found to be slower than those obtained for PPy/Heparin composite membranes; especially in the case of myoglobin. The decreased transport flux through PPy/Dex composite membranes could be caused by a denser PPy/Dex layer deposited on the substrate during its synthesis. As suggested by the EQCM results in Chapter 4 (Table 4.3), the charge efficiency for PPy/Dex was higher than that for PPy/Heparin in terms of the amount of polymer deposited per unit charge. The mass/charge ratio is \(0.605\ \mu\text{g mC}^{-1}\) for PPy/Dex and \(0.367\ \mu\text{g mC}^{-1}\) for PPy/Heparin, i.e. more polypyrrole has been deposited on the substrate when prepared with dextran sulfate under the same electropolymerisation condition.
Figure 6.5 Transport of BSA across PPy/Dex/Pt/PVDF membrane. BSA aqueous solution 20 μM was used as feed solution. Receiving solution was Milli-Q water. Current density applied across the membrane: $I_{\text{app}} = +0.64 \text{ mA cm}^{-2}$ or $-0.64 \text{ mA cm}^{-2}$. PPy/Dex membrane was prepared on 0.45 μm Pt/PVDF membrane at 1.0 mA cm$^{-2}$ for 60 seconds.

Figure 6.6 Transport of haemoglobin across PPy/Dex/Pt/PVDF membrane. Haemoglobin aqueous solution 20 μM was used as feed solution. Receiving solution was Milli-Q water. Current density applied across the membrane: $I_{\text{app}} = +0.64 \text{ mA cm}^{-2}$ or $-0.64 \text{ mA cm}^{-2}$. PPy/Dex membrane was prepared on 0.45 μm Pt/PVDF membrane at 1.0 mA cm$^{-2}$ for 60 seconds.
Figure 6.7 Transport of myoglobin across PPy/Dex/Pt/PVDF membrane. Myoglobin aqueous solution 20 μM was used as feed solution. Receiving solution was Milli-Q water. Current density applied across the membrane: \( I_{\text{app}} = +0.64 \text{ mA cm}^{-2} \) (with feed solution side of the membrane positively polarised) or \(-0.64 \text{ mA cm}^{-2}\) (with feed solution side of the membrane negatively polarised). PPy/Dex membrane was prepared on 0.45 μm Pt/PVDF membrane at 1.0 mA cm\(^{-2}\) for 60 seconds.
6.3.2 Transport under Controlled Potential

The electrochemically controlled transport of BSA, myoglobin and haemoglobin across PPy/Dex composite membranes was also investigated by controlling the potential $E_{\text{app}}$ across the two faces of the membrane. A constant potential was applied across the two conductive faces of the membrane. The potential applied was

(i) $E_{\text{app}} = +1.50 \, \text{V}$ (with the side of the membrane facing feed solution positively polarised) or

(ii) $E_{\text{app}} = -1.50 \, \text{V}$ (with the side of the membrane facing feed solution negatively polarised).

The electric field direction within the membrane could be changed by reversing the polarity of the potential applied.

Similar to those under controlled current transport, the transport of BSA, haemoglobin and myoglobin across PPy/Dex membranes under controlled potential showed good on/off switching transport properties (Figures 6.8, 6.9 and 6.10). The transport of proteins can be turned on and off by changing the electrical polarity. The transport fluxes for BSA was $8.7 \times 10^{-13} \, \text{mol cm}^{-2} \, \text{s}^{-1}$ compared with $1.39 \times 10^{-12} \, \text{mol cm}^{-2} \, \text{s}^{-1}$ obtained under controlled current density of $0.64 \, \text{mA cm}^{-2}$. The transport flux of haemoglobin was found to be $3.04 \times 10^{-13} \, \text{mol cm}^{-2} \, \text{s}^{-1}$ which was nearly equal to that obtained under controlled current density of $0.64 \, \text{mA cm}^{-2}$. The myoglobin transport flux was $1.51 \times 10^{-12} \, \text{mol cm}^{-2} \, \text{s}^{-1}$; similar to that obtained under controlled current density of $0.64 \, \text{mA cm}^{-2}$ which was $1.60 \times 10^{-12} \, \text{mol cm}^{-2} \, \text{s}^{-1}$. 
Figure 6.8 Transport of BSA across PPy/Dex/Pt/PVDF membrane under applied potentials of $E_{\text{app}} +1.5 \text{ V}$ and $-1.5 \text{ V}$. Feed solution: 20 $\mu$M BSA aqueous solution. Receiving solution: Milli-Q water. Membrane PPy/Dex/Pt/PVDF was prepared at a current density of 1.0 mA cm$^{-2}$ for 60 seconds.

Figure 6.9 Transport of haemoglobin across PPy/Dex/Pt/PVDF membrane under applied potentials of $E_{\text{app}} +1.5 \text{ V}$ and $-1.5 \text{ V}$. Feed solution: 20 $\mu$M haemoglobin aqueous solution. Receiving solution: Milli-Q water. Membrane PPy/Dex/Pt/PVDF was prepared at a current density of 1.0 mA cm$^{-2}$ for 60 seconds.
Figure 6.10 Transport of myoglobin across PPy/Dex/Pt/PVDF membrane under applied potentials of $E_{app} +1.5$ V and $-1.5$ V. Feed solution: 20 µM myoglobin aqueous solution. Receiving solution: Milli-Q water. Membrane PPy/Dex/Pt/PVDF was prepared at a current density of 1.0 mA cm$^{-2}$ for 60 seconds.

6.3.3 Transport Under Pulsed Potential

Transport of BSA and myoglobin under pulsed potential was also investigated using the PPy/Dex/Pt/PVDF composite membrane as a test case. A pulsed potential of 20 seconds pulse width was applied across the two faces of the membrane. So the direction of the electric field in the solution filled pores of the membranes varied alternately every 20 seconds. The charged protein molecules inside the pores of the membrane would move back and forth in a pumping manner. As results, proteins concentration polarisation and their adsorption to the membrane are effectively reduced.

The transport fluxes were affected by the potential range applied. For example (Figure 6.11), the transport flux of BSA under the pulsed
potential +/-1.50 V of 20 second pulse width was higher than that obtained under +/-1.00 V pulsed potential of the same pulse width. The transport fluxes of BSA were found to be $3.90 \times 10^{-13}$ mol cm$^{-2}$ s$^{-1}$ under +/- 1.50 V pulsed potential, and $2.87 \times 10^{-13}$ mol cm$^{-2}$ s$^{-1}$ under +/-1.00 V pulsed potential. These results clearly indicate that the electrical field still play a key roles in determining the transport of proteins across a double sided membrane.

![Figure 6.11](image_url)

**Figure 6.11** Transport of BSA across PPy/Dex/Pt/PVDF membrane under pulsed potential. PPy/Dex was deposited on 0.45 μm Pt/PVDF membrane at the current density of 1.0 mA cm$^{-2}$ for 60 seconds. Feed solution: BSA 20 μM aqueous solution. Receiving solution: Milli-Q water. (a) Transport under pulsed potential of +/-1.0 V with 20 seconds pulse width; (b) Transport under pulsed potential of +/-1.5 V with 20 seconds pulse width.
Transport of myoglobin across PPy/Dex/Pt/PVDF membrane under pulsed potential control was also studied. In this case, a pulsed potential of 20 seconds pulse width was applied to the composite membrane with its potential varied between +/- 0.70 V to +/- 1.50 V. The transport results suggest that transport under a pulsed potential of +/- 0.70 V or +/- 1.0 V proceeded at a constant flux. This is apparent in Figure 6.12 a and b, which shows that the corresponding transport curves were linear with respect to time. The transport fluxes under pulses +/- 0.70 V and +/- 1.00V were $5.69 \times 10^{-13}$ mol cm$^{-2}$ s$^{-1}$ and $6.63 \times 10^{-13}$ mol cm$^{-2}$ s$^{-1}$ respectively. On the other hand, transport using a higher potential range +/- 1.50 V resulted in a higher transport flux. But the transport flux was not constant throughout the entire transport process, as indicated by the concentration curve (Figure 6.12). The initial transport flux was high but this was not sustained. The average transport flux was $8.35 \times 10^{-13}$ mol cm$^{-2}$ s$^{-1}$.

The transport flux under pulsed potential was lower when compared with that under constant potential. For example, the BSA flux was $3.9 \times 10^{-13}$ mol cm$^{-2}$ s$^{-1}$ under pulsed potential of +/-1.5 V, while the BSA flux was $8.7 \times 10^{-13}$ mol cm$^{-2}$ s$^{-1}$ under 1.5 V constant potential. The lower transport flux is believed to be caused by the alternately changed direction of the electric field applied.
Figure 6.12 Transport of myoglobin across PPy/Dex/Pt/PVDF membrane under pulsed potential. PPy/Dex membrane was deposited on 0.45 μm Pt/PVDF membrane at 1.0 mA cm⁻² for 60 seconds from 0.2 M pyrrole monomer and 2500 ppm dextran sulphate aqueous solution. Myoglobin feed solution: 20 μM aqueous solution. Receiving solution: Milli-Q water. (a) Transport under pulsed potential of +/-0.70 V. (b) transport under pulsed potential of +/-1.0 V. (c) Transport under pulsed potential +/-1.5 V 20. Pulse width of 20 seconds was used for all transport under pulsed potential.

The effects of membrane thickness on the transport of myoglobin across PPy/Dex membrane was also investigated. A pulsed potential of +/-1.0 V with a pulse width of 20 seconds was used. Two membranes of different thicknesses were prepared by controlling polymerisation time (30 and 60 seconds) at the current density of 1.0 mA cm⁻².

As expected, the thinner of the polypyrrole films facilitated a higher flux (Figure 6.13). The average myoglobin transport fluxes were found to be 1.1x10⁻¹² mol cm⁻² s⁻¹ for the thinner membrane (Curve b) and 6.6x10⁻¹³ mol cm⁻² s⁻¹ for the thicker membrane (Curve a).
Figure 6.13 Transport of myoglobin across PPy/Dex/Pt/PVDF membrane of different thickness. Pulsed potential of +/-1.00 V with a pulse width of 20 seconds was applied to each side of the membrane. (a) PPy/Dex membrane was grown for 30 seconds at 1.0 mA cm$^{-2}$ on 0.45 μm Pt/PVDF membrane, and (b) PPy/Dex membrane was grown for 30 seconds at 1.0 mA cm$^{-2}$ on 0.45 μm Pt/PVDF membrane at 1.0 mA cm$^{-2}$ for 60 seconds. Polymerisation solution containing 0.2 M pyrrole monomer and 2500 ppm dextran sulphate aqueous solution. Feed solution: 20 μM myoglobin aqueous solution. Receiving solution: Milli-Q water.

6.3.4 Transport of Thrombin Across PPy/Heparin/Pt/PVDF Composite Membranes

The interaction between PPy/Heparin and thrombin has been previously investigated using the EQCM technique in Chapter 5. These results indicated that the interaction between PPy/Heparin and thrombin is high in terms of their adsorption, which might have potential application in thrombin separation or purification processes.
In this section, we used PPy/Heparin/Pt/PVDF composite membranes to study the thrombin transport properties across PPy/Heparin. Due to the highly specific thrombin binding to the heparin immobilised by the polypyrrole, it is reasonable to expect that thrombin would be retained by the PPy/Heparin composite membrane, while other proteins would go through the membrane via either diffusion or controlled transport.

For this work, PPy/Heparin membranes were freshly prepared from 0.20 M pyrrole monomer and 2500 ppm heparin sulfate aqueous solution. The current density was controlled at 1 mA cm⁻² for 1 minute.

The feed solution was prepared by reconstituting thrombin with 0.15 M sodium chloride and 0.15 M sodium citrate in pH 6.5 phosphate buffer solution. The receiving solution was the same as the feed solution but free of thrombin.

Transport of thrombin was carried out using two methods:

(i) diffusion transport and
(ii) electrochemically controlled transport.

In each case, the transport process lasted for 2 hours. The thrombin in both feed solution and receiving solution was assayed in order to characterise the transport properties of thrombin across PPy/Heparin membrane. The mass balance for thrombin was also determined so as to ascertain the amount of thrombin adsorbed on to the membrane.

Diffusion transport was observed with feed solution containing 10.38 IU cm⁻³ (or 165 IU in 15 cm³ feed solution). The thrombin transport results in Table 6.1 show that there was less than 0.60 IU cm⁻³ thrombin found in the receiving solution after two hours of normal diffusion. On the other hand, the amount of thrombin in the feed solution had been reduced to
6.71 IU cm\(^{-3}\), indicating that the adsorption of thrombin to the PPy/Heparin membrane had occurred. Based on the thrombin mass balance, there was about 46 IU of thrombin had been adsorbed on the PPy/Heparin membrane (or 14.6 IU per cm\(^2\) of membrane).

In the case of electrochemically controlled transport, the membrane was repeatedly switched between its oxidised and reduced states under a pulsed potential of +/-0.70 V with a 20 second pulse width. Thrombin assays revealed that more thrombin had bound to the PPy/Heparin composite membrane during this period. Almost all the thrombin added to the feed solution was adsorbed by the membrane, i.e. 124 IU out of 142 IU thrombin in the feed solution had been adsorbed on the PPy/Heparin membrane. This is equivalent to 39.5 IU per cm\(^2\) of PPy/Heparin membrane.
Table 6.1  Transport of thrombin across PPy/Heparin composite membrane

<table>
<thead>
<tr>
<th>Transport</th>
<th>Description</th>
<th>Thrombin IU·cm⁻³ (³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport of thrombin under diffusion(¹)</td>
<td>Feed solution before transport</td>
<td>10.38</td>
</tr>
<tr>
<td></td>
<td>Feed solution after transport</td>
<td>6.71</td>
</tr>
<tr>
<td></td>
<td>Receiving solution after transport</td>
<td>&lt;0.59</td>
</tr>
<tr>
<td>Transport of thrombin under pulsed potential(²)</td>
<td>Feed solution before transport</td>
<td>9.47</td>
</tr>
<tr>
<td></td>
<td>Feed solution after transport</td>
<td>&lt;0.59</td>
</tr>
<tr>
<td></td>
<td>Receiving solution after transport</td>
<td>&lt;0.59</td>
</tr>
</tbody>
</table>

(¹) Diffusion transport of thrombin across PPy/Heparin was carried out with feed solution containing 165 IU of thrombin in 15 cm³ of 0.15 M NaCl and 0.05 M sodium citrate solution. Receiving solution contained the same supporting electrolyte but free of thrombin.

(²) Transport of thrombin under pulsed potential was carried out under +/-0.70 V and 20 second pulse width. Feed solution contained 142 IU of thrombin in 15 cm³ of 0.15 M NaCl and 0.05 M sodium citrate solution. Receiving solution contained the same supporting electrolyte as feed solution but free of thrombin.

(³) Thrombin assay carried out by CSL

These results suggest that thrombin can be effectively retained by the PPy/Heparin composite membrane because of their specific affinity to the PPy/Heparin composite membrane.
6.3.5 Separation of Protein Mixtures

The separation of protein mixtures (HSA and myoglobin) has been demonstrated using polypyrrole composite membranes. An aqueous HSA and myoglobin mixture was prepared and used as the feed solution. HSA is negatively charged and myoglobin is positively charged in this solution (pH 6.5). By applying an electrical field between the two sides of the membrane with the feed side polarised negatively, HSA would transport towards the receiving solution while myoglobin would be retained.

For the transport experiment, PPy/pTS composite membranes were prepared. The membrane was installed in position with its PPy/pTS coated side facing the feed solution. Feed solution and receiving solution were then introduced into the two respective chambers.

Results (Figure 6.14) show that the average transport flux for HSA was around $1.08 \times 10^{-12}$ mol cm$^{-2}$ s$^{-1}$, whilst that for myoglobin was undetectable ($< 0.2$ ppm). Nearly one third of the BSA in the feed solution had been transported to the receiving solution within six hours. Compared with platinised membranes, the PPy/pTS composite membrane showed even higher selectivity for HSA to myoglobin. With the increase in selectivity, the average transport flux has been reduced.

Scanning Electron Microscopy (SEM) results show that the pores of the substrate membranes have been effectively bridged or blocked by the additional deposition of polypyrrole. This would tend to reduce water flux and protein transport flux.
Figure 6.14 Separation of HSA from myoglobin using PPy/pTS conducting polymer coated 0.45 µm PVDF membrane. Feed solution was 670 ppm (10 µM) HSA and 175 ppm (10 µM) myoglobin mixture in Milli-Q water. Receiving solution: Milli-Q water. Current density was controlled at -1.28 mA cm\(^{-2}\) with cathode facing the feed solution. No myoglobin was detected (<0.2 ppm) in receiving solution side. A PPy/pTS conducting polymer was deposited for 1 minute on one side of the Pt/PVDF substrate under the conditions described in the membrane preparation section.

6.4 CONCLUSION

Electrochemically controlled transport of proteins using polypyrrole composite membranes under different experimental conditions has been demonstrated. The transport of proteins across these composite membranes can be electrochemically manipulated by applying constant potential, constant current or pulsed potential. Table 6.2 shows some of
the protein transport flux versus the different polypyrrole composite membrane used in the electrochemically controlled transport.

The separation of protein mixtures has also been developed using the electrochemically controlled transport technique. A high purity of a particular protein can be selectively separated using the polypyrrole composite membranes.

Table 6 Protein transport fluxes vs polypyrrole composite membranes

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Protein transport flux x10^{13} (mol cm^{-2} s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPy/Heparin</td>
</tr>
<tr>
<td>BSA</td>
<td>13.9</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>3.1</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>16</td>
</tr>
<tr>
<td>Thrombin</td>
<td>none</td>
</tr>
<tr>
<td>HSA</td>
<td>-</td>
</tr>
</tbody>
</table>

The protein thrombin was strongly retained by the polypyrrole-heparin composite membrane due to the specific affinity with the heparin immobilised in the polypyrrole. Other proteins such as BSA, myoglobin and haemoglobin can be transported through such a membrane. This shows the potential of developing a new thrombin separation technique.
CHAPTER 7

GAS PERMEATION AND SEPARATION BY POLYPYRROLE CONDUCTING MEMBRANES
7.1 INTRODUCTION

Membrane based gas separations have tremendous potential as energy efficient alternatives to cryogenic separation. Within the last decade a number of membrane gas separation systems and technologies have emerged as commercial. \[155-157\]. This led to intensified academic interest in improving the selectivity and permeability of these systems. The synthesis of new polymeric materials as well as asymmetric membranes, in which a thin skin of polymer with gas selectivity is grafted on to a porous structural support, have been developed. In reality, the thickness of the polymer skin is a few hundred to a few thousand angstroms; which is crucial in obtaining a membrane separation process with a high permeability \[158,159\].

Considerable effort has been invested in understanding the mechanisms of gas permeation through polymeric membranes\[160-164\]. There are, in general, three mechanisms for selective gas permeation through membranes: (1) Knudsen flow through porous membranes, where rate of gas permeation is according to its molecular weight and size; (2) Surface flow through porous membranes, where gas permeation varies with diffusivity as well as size exclusion; and (3) Solution-diffusion flow through non-porous membranes, where gas permeation depends on both solubility and diffusivity in the polymer.

In general, the selectivity of a gas separation membrane increases with a decrease in the pore size of the membrane. A dense and nonporous membrane, through which gas permeation is via the "solution-diffusion" mechanism, has the best selectivity of all.

A variety of non-porous polymer membranes have potential uses for gas separation. But with a decrease in pore size, the permeation rate through
the membrane is also decreased. In practice, membranes are designed and made as asymmetric membranes. A thin gas selective layer is grafted on to a porous membrane support so as to increase its permeability.

Although gas separation membranes have been developed for years, current membrane systems still have limitations for practical use. For example, the morphology of a polymeric membrane is difficult to predict prior to its manufacture, and they cannot be adjusted consistently once the polymer film is formed. The separation factors (such as between oxygen and nitrogen) obtained are still not satisfactory. The search for a gas separation membrane system with high selectivity and high permeability still remains to be fulfilled.

Gas permeation and separation by conjugated polymer membranes had been reported by some researchers\[95-97,166\]. Most of these gas permeation membranes were chemically synthesised based on either polyaniline or polypyrrole \[163,164\].

One of the greatest advantages of using conducting polymer membranes is that the dynamic properties and microstructure of a conjugated polymer membrane can be precisely controlled during synthesis or even after it. The selectivity and permeability of conducting polymer membranes depend on the nature of the monomer being used, and, especially, that of the doping anion being incorporated in the polymer. Subsequent dedoping and redoping will also affect the selectivity and permeability of the membranes. The separation factors between oxygen and nitrogen have been reported to be 3 and 5 for polypyrrole and polyaniline membranes respectively. In some cases, higher separation factors of up to 30 have also been reported for redoped polyaniline membranes \[165\].
The best gas separation membranes are dense polymer films [165]. Less dense films are porous to all gases and therefore have little or no selectivity. Polyacetylene possess a fibrillar morphology where \((\text{CH})_x\) chains occupy only one third of the films [168]. Electrochemically prepared polyaniline films are also porous with fibrillar morphology [169] and they are not suitable for gas separation. Electrochemically prepared polypyrrole membranes can be considered to be nonporous polymer membranes with an average density of 1.2 to 1.5 g cm\(^{-3}\) [51]. The scanning electron microscope (SEM) revealed that polypyrrole conducting polymer has a compact morphology rather than fibrillar and porous structure [72]. The polymer consists of well ordered crystalline microdomains separated by disordered regions. The irregular structures and these disordered regions allow gases to dissolve and diffuse through the polymer membrane, usually in a selective way [52,53].

It has been demonstrated the polypyrrole conducting polymer can be easily synthesised as a thin film by electrochemical polymerisation. The chemical or physical properties of polypyrrole membranes can be controlled by the judicious selection of the electrochemical polymerisation conditions and the type of counterions to be incorporated. In addition, polypyrrole membranes in their doped (oxidised) form are stable in air, and their microstructures can be varied by subsequent dedoping or redoping.

In this chapter, polypyrrole membranes have been electrochemically synthesised with or without substrate support and investigated in terms of gas permeability and selectivity. Oxygen and nitrogen gases have been selected as test gases because their separation always represents the greatest challenge for all gases separation membrane systems. (The kinetic diameters of these two gases differ only by a few tenths of an angstrom;
O₂ diameter is 3.46 Å and N₂ is 3.64 Å [156]). The separation of gas mixtures was also investigated using air as the feed gas.

7.2 EXPERIMENTAL

7.2.1 Chemicals and Materials

Pyrrole monomer from Merck Co. was distilled and stored under nitrogen at -18 °C before being used for polymer membrane preparations. Para-toluene sulfonic acid sodium salt, also from Merck, was used as received. Dextran sulfate sodium salt with an average molecular weight of 50,000 was ordered from Sigma and used as received. Polyaniline sulfonic acid kindly provided by Nitto Chemical Industry Co., Ltd. Japan, was also used in polypyrrole membrane preparations. All other chemicals were of laboratory reagent grade and used as received.

PVDF membrane (with nominal 0.22 μm pore size) from Millipore and polysulphone membrane (with 100 KD molecular weight cut off) from Memtec were chosen as porous supporting substrates for the polypyrrole composite membrane synthesis.

A gas permeation system, previously described in Chapter 2, was used in this research work. This permeation cell has a 15.9 cm² effective permeation area. Bottled oxygen, nitrogen and air were used as feed gases. Gas permeated through a membrane was metered by an Alltech digital flow meter (minimum flow rate: 0.01 ml min⁻¹) and analysed by gas chromatography.
7.2.2 Gas Permeation Membrane Preparation

Two types of polypyrrole conducting polymer membranes were prepared and experimented with in the oxygen/nitrogen permeation and separation. They were (i) free standing polypyrrole films and (ii) thin films supported by polysulphone or PVDF membrane substrates. The substrate membranes were first sputter coated with a thin layer of platinum using a Dynavac Magnetron Sputter coater under the following conditions: 50 mA and 2.0x10^{-3} mbar Argon for 6.5 minutes. These conditions allowed a 780 Å thick platinum coating to be deposited on these substrate membranes.

Polypyrrole-pTS (PPy/pTS) free standing thin films were first prepared galvanostatically by oxidising pyrrole monomer in the presence of p-toluene sulfonic acid sodium salt (pTS). The electrochemical polymerisation process was carried out using a three electrode system and applying a current density of 2.0 mA cm^{-2} in a solution containing 0.20 M pyrrole monomer and 0.05 M pTS for 10 to 20 minutes. A uniform PPy/pTS film deposited on the working electrode (a stainless steel plate) and peeled off. The PPy/pTS films prepared were washed and rinsed with distilled water to remove the excess solution absorbed and then dried in air before further permeation tests. Free standing films prepared with other dopants were also prepared but the films were either too brittle for practical use or too adherent to peel off from the electrode surface.

Polypyrrole films prepared with chloride (PPy/Cl) and dextran sulfate (PPy/Dex) were too weak to whereas films prepared with benzene sulfonic acid sodium salt (PPy/BS) and polyaniline sulfonic acid (PPy/PAS), were too adherent to the plate to peel off. Therefore, all these polymer films had to be synthesised on either polysulphone or PVDF substrates to improve both mechanical strength and handling properties.
PPy/Cl membranes were prepared on PVDF substrates galvanostatically at the current density of 2.0 mA cm\(^{-2}\) for 10 to 20 minutes from a solution containing 0.20 M pyrrole and 0.05 M NaCl. PPy/Dex was synthesised at 1.0 mA cm\(^{-2}\) for 10 to 20 minutes from 0.5 M pyrrole and 5% dextran sulfate sodium salt. PPy/PAS was galvanostatically prepared at the current density of 1.0 mA cm\(^{-2}\) for 10 to 15 minutes from a solution containing 0.20 M pyrrole and 1.0% polyaniline sulfonic acid sodium salt. Polyaniline sulfonic acid membranes were also prepared using the chemical method by casting the polyaniline sulfonic acid (without cross linker) onto both PVDF and polysulphone substrates.

### 7.2.3 Gas Permeation through Polymer Membranes

The mechanism of permeation of gas molecules through a nonporous membrane is different from that of a porous membrane; in which gases are separated by differences in their molecular weights or sizes according to Knudsen diffusion flow. It is generally accepted that \cite{28,97}, in nonporous polymer membranes such as conducting polymer membranes, the gas molecules actually dissolve and diffuse through the membrane matrix. This mechanism consists of adsorption, activated diffusion and dissolution and evaporation. This is known as "solution - diffusion". Gas permeability depends on both solubility and diffusivity of a gas in the polymer membrane, which is given by

\[
P \text{ (permeability)} = S \text{ (solubility)} \times D \text{ (diffusivity)}
\]  

\( (7.1) \)

The solubility in the polymer can be obtained from Henry's law:

\[
C = Sp
\]

\( (7.2) \)
where \( C \) is the local concentration of gas in the polymer membrane in cm\(^3\) (STP) per cm\(^3\) polymer, \( S \) is the solubility of the gas in the polymer in cm\(^3\) (STP) per cm\(^3\) polymer, and \( p \) is the gas partial pressure in cm Hg. A typical solubility at standard conditions is 0.01 cm\(^3\) gas per cm\(^3\) polymer.

The diffusivity can be obtained from Fick's first law \([19]\), i.e.

\[
Q_i = -D_i \frac{dc}{dx}
\]  

(7.3)

where \( Q_i \) is the permeation flux in cm\(^3\) (STP) cm\(^{-2}\) s\(^{-1}\), and \( D_i \) is the diffusion coefficient of the permeating species in cm\(^2\) s\(^{-1}\), \( x \) is the direction of permeation in the membrane in cm.

The separation of gas 1 from another gas 2 is usually expressed by the separation factor \( \alpha_{1/2} \), i.e.

\[
\alpha_{1/2} = \frac{P_1}{P_2}
\]  

(7.4)

By combining Equations (7.2) and (7.3), the permeability of the polymer membrane can be rewritten as

\[
P_i = \frac{-Q_i l}{\Delta p_i}
\]  

(7.5)

where \( l \) is the thickness of the polymer membrane in cm and \( \Delta p_i \) is the pressure difference between the low pressure side and high pressure side in cmHg. For most permanent gases, the permeability \( P_i \) is pressure independent for all practical purposes.

Both \( P \) and \( D \), and therefore \( S \), can be determined in a single experiment by measuring the "time lag" for the pressure increase in the low-pressure side of the membrane after a high pressure has been applied to its other side (see Figure 7.1). The time lag is related to the time required by the
gas to establish a state of equilibrium in a degassed membrane and is given by [156]

$$\theta = \frac{I^2}{6D}$$  \hspace{1cm} (7.6)

where $\theta$ is the time lag, and $I$ is the membrane thickness, and $D$ is the diffusivity of the test gas. The time lag is found by extrapolating the linear portion of the pressure versus time curve to the time axis after the stationary state has been attained. It has been shown that the steady state is reached after a period amounting to three times the time lag; so extrapolation to the time axis should only be made from measurements taken after that time.

![Figure 7.1: Transient and steady state permeation through a thin film.](image)

The permeability ($P$) is given by the slope of the linear portion of the curve, and the solubility ($S$) can be calculated by dividing the permeability by the diffusivity ($D$) according to Equation 7.1.
7.2.4 Gas Permeation Rate (GPR) Test

The gas permeation tests were carried out with either pure gas, or gas mixtures for determining the permeation of a particular gas component with or without interference from other gas components.

7.2.4.1 Permeation of Pure Oxygen and Nitrogen

Bottled pure oxygen and nitrogen gases were used and tested separately for their permeation rates through polypyrrole conducting polymer membranes. The gas permeation tests were carried out with the cell design shown in Figure 2.18. The actual permeation area of each membrane is 15.9 cm². Gas pressure at the feed side was controlled by a regulator valve within the range of 76 to 304 cm Hg (i.e. 101 to 404 kpa), depending on the actual permeability of each membrane tested. Higher pressure may be needed for membranes with low permeability. The permeate at the low pressure side was collected and measured using a bubble meter under atmospheric pressure at room temperature.

7.2.4.2 Permeation of Mixed Gases

The permeation of a mixture of gases (air) was investigated with the same test system. The permeation flow rate was gauged with the bubble meter (Alltech) and the permeated gases through the polymer membrane were analysed by gas chromatography. The detector used for the GC was a thermal conductivity cell. All gas components, including carbon dioxide in the permeate were quantified. The separation factor between oxygen and nitrogen was calculated based on the concentration of each gas present in the permeate.
7.3 RESULTS AND DISCUSSION

7.3.1 Polymer Membranes

Polypyrrole and polyaniline conducting polymer membranes were electrochemically prepared with selected counterions. Polypyrrole membranes were found to have space filling microstructure whereas polyaniline showed a fibrillar microstructure. The crystalline nodule sizes of polypyrroles ranged from less than 1 µm to 7 µm depending on the type of doping anions being used during their synthesis.

Polypyrrole prepared with counterion Cl\(^-\) shows uniform morphological structure. The SEM image of PPy/Cl indicates that the PPy/Cl polymer nodules are 1.5 µm in size, and they are linked together in a certain orientation.

Both PPy/BS and PPy/pTS show a much wider nodule size distribution. The nodule size ranges from 2 to 5 µm for polymer PPy/pTS and from 1 to 7 µm for polymer PPy/BS. Due to their wide nodule size distribution, the free volume of the polymer membrane are filled by those small nodules, which make these membrane to be denser and mechanically stronger. However, PPy/BS film was too adherent to the electrode to peel off. As a result, PPy/BS free-standing films of large size suitable for gas separation could not obtained.

Polypyrrole synthesised with large anions, such as polyanions dextran and heparin sulfate, show uniform pore structures with their polymer nodule sizes being found between 2 to 2.5 µm. However, these polymer membranes are poor in mechanical strength. The PPy/Heparin image (Figure 7.2 e) shows that a PPy/Heparin membrane was cracked after drying at room temperature. Membrane supports have to be used so as to enhance their mechanical properties for further investigation.
Figure 7.2  SEM Images of polypyrrole and polyaniline membrane. (a) PPy/BS, (b) PPy/Cl, (c) PPy/pTS, (d) PPy/Dex, (e) PPy/Heparin and (f) PAn/Cl. Continued next page
Figure 7.2 Conti.
Polyaniline structures are different from these electrochemically prepared polypyrrole. The electrochemically prepared polyaniline have a fibrillar microstructures (Figure 7.2 f). These membranes have been found to be poor in mechanical strength and not suitable for gas separation.

Polypyrrole membranes were also synthesised using polyaniline sulfonic acid as counterion (PPy/PAS). The electrochemically prepared polymer films have higher water content and gel-like surface morphology. The polymer can be dehydrated at room temperature after preparation, but can not be subsequently rehydrated.

With all the conducting polymer membranes synthesised, the thickness of the polymeric films was found to be dependent upon the type of doping anions incorporated during their synthesis (Table 7.1). In general, a polypyrrole membrane prepared with small anions is much denser and has a smoother surface than those membranes prepared with large anions such as dextran sulfate.

**Table 7.1 Physical properties of polypyrrole and polyaniline**

<table>
<thead>
<tr>
<th>Polymer membrane</th>
<th>Nodule size (µm)</th>
<th>Film thickness/charge density (µm C⁻¹ cm⁻²)</th>
<th>Mechanical properties of free-standing film</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPy/BS</td>
<td>1.0 - 7.0</td>
<td>3.3</td>
<td>good smooth</td>
</tr>
<tr>
<td>PPy/Cl</td>
<td>~1.5</td>
<td>3.5</td>
<td>poor</td>
</tr>
<tr>
<td>PPy/Dex</td>
<td>2.5</td>
<td>4.8</td>
<td>poor and brittle</td>
</tr>
<tr>
<td>PPy/Heparin</td>
<td>2.0</td>
<td>4.4</td>
<td>poor and brittle</td>
</tr>
<tr>
<td>PPy/PAS</td>
<td>-</td>
<td>10.8</td>
<td>good smooth</td>
</tr>
<tr>
<td>PPy/pTS</td>
<td>1.5 - 5.0</td>
<td>3.3</td>
<td>good smooth</td>
</tr>
<tr>
<td>PAN/Cl*</td>
<td>-</td>
<td>-</td>
<td>poor, powder-like</td>
</tr>
</tbody>
</table>

*Fibrillar structure.
PVDF and polysulphone membranes were chosen as substrates for CEP film growth so that the resultant composite membranes had the necessary mechanical strength for gas separation studies. The electropolymerisation conditions employed were still the same as those for the free-standing polymer films. Thus the effective active polypyrrole layer was estimated to be the same as that of a free standing film. The supported CEP films were easy to handle and their mechanical properties were obviously improved.

7.3.2 Oxygen and Nitrogen Permeation

Oxygen and nitrogen permeation tests were carried out as described in the experimental section. A polypyrrole film was tested for its oxygen and nitrogen permeation separately.

The oxygen permeation rate was found to be lower than that of nitrogen for those polypyrrole films prepared with BS\(^{-}\), Cl\(^{-}\) and pTS\(^{-}\) as counterions, i.e. \( \text{GPR}_{\text{N}_2} > \text{GPR}_{\text{O}_2} \).

PPy/BS/Pt/PVDF membranes (polypyrrole films deposited on Pt/PVDF substrates) had a gas permeation rate (GPR) of around 1.92x10\(^{-7}\) \( \text{cm}^3(\text{STP}) \text{ cm}^{-2} \text{ s}^{-1} \text{ cmHg}^{-1} \) with a nitrogen to oxygen separation factor of 1.6.

PPy/Cl/Pt/PVDF membranes were found to have higher gas permeation rates than PPy/BS/Pt/PVDF but had a similar separation factor. The typical GPR for PPy/Cl/Pt/PVDF was in the range of 3.17 - 2.05 x10\(^{-6}\) \( \text{cm}^3(\text{STP}) \text{ cm}^{-2} \text{ s}^{-1} \text{ cmHg}^{-1} \). The separation factor was found to be 1.5 for nitrogen/oxygen.
The PPy/pTS free standing-films were found to have better selectivity over PPy/pTS/Pt/PVDF. The gas permeation rate was around $8.1 \times 10^{-8}$ cm$^3$(STP) cm$^{-2}$ s$^{-1}$ cm Hg$^{-1}$ with N$_2$/O$_2$ separation factor of up to 1.7-1.9. On the other hand, PPy/pTS/Pt/PVDF membranes were more porous which resulted in higher gas permeation rate. The GPR of PPy/pTS/Pt/PVDF was $8.52 \times 10^{-6}$ cm$^3$(STP) cm$^{-2}$ s$^{-1}$ cm Hg$^{-1}$; that is the factor of 100 higher than that of PPy/pTS free-standing polymer films. However the nitrogen/oxygen separation factor of PPy/pTS/Pt/PVDF was only 1.2; far below that of the free-standing polymer films.

PPy/Dex/Pt/PVDF membranes were found to be too porous to be used for gas permeation and separation studies. Test gases, oxygen and nitrogen, passed through the membrane at a much higher flow rate ($>10^{-4}$ cm$^3$(STP) cm$^{-2}$ s$^{-1}$ cmHg$^{-1}$ without any selectivity.

PPy/PAS was found to be different from other polypyrrole membranes in respect to its gas permeation properties. PPy/PAS polymer membranes exhibited a higher oxygen permeation rate over nitrogen. The oxygen GPR varied with the substrates and the aging time of the polymer membranes. Freshly prepared PPy/PAS membranes usually have a high oxygen permeability and higher separation factor. The separation factor $\alpha = \frac{GPR(O_2)}{GPR(N_2)}$ at the initial stage was around 5.0 to 6.5 with an average O$_2$ permeability of between $1.36 \times 10^{-8}$ cm$^3$(STP) cm$^{-2}$ s$^{-1}$ cmHg$^{-1}$. The separation factor $\alpha$ slowly decreased with aging time to a value between 1.6 to 2.8. The oxygen flow rate also decreased with time. The separation factor $\alpha = \frac{GPR(O_2)}{GPR(N_2)}$ ranged between 1.6 to 3.2 for PPy/PAS/PS membrane, and 2.8 to 6.5 for PPy/PAS/Pt/PVDF membrane. The above results are summarised in Table 7.2.
**Table 7.2 Permeation through polypyrrole membranes.**

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Polymerisation conditions</th>
<th>$\text{GPR(O}_2\text{)} 10^{-7}$ cm$^3$(STP)/(cm$^2$ s cmHg)</th>
<th>$\text{GPR(N}_2\text{)} 10^{-7}$ cm$^3$(STP)/(cm$^2$ s cmHg)</th>
<th>$\frac{\alpha (\text{N}_2/\text{O}_2)}{\alpha (\text{O}_2/\text{N}_2)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPy/BS/Pt/PVDF</td>
<td>2 mA cm$^{-2}$ 20 min</td>
<td>1.9 ($\pm$0.1)</td>
<td>3.1 ($\pm$0.1)</td>
<td>1.6 ($\pm$0.2)</td>
</tr>
<tr>
<td>PPy/Cl/Pt/PVDF</td>
<td>2 mA cm$^{-2}$ 20 min</td>
<td>21 ($\pm$1)</td>
<td>32 ($\pm$1)</td>
<td>1.5 ($\pm$0.2)</td>
</tr>
<tr>
<td>PPy/Dex/Pt/PVDF</td>
<td>1 mA cm$^{-2}$ 20 min</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PPy/PAS/PS 100KD*</td>
<td>1 mA cm$^{-2}$ 15 min</td>
<td>0.9 ($\pm$0.1)</td>
<td>0.4 ($\pm$0.1)</td>
<td>2.4$\pm$1.1</td>
</tr>
<tr>
<td>PPy/PAS/Pt/PVDF</td>
<td>1 mA cm$^{-2}$ 15 min</td>
<td>1.9 ($\pm$0.1)</td>
<td>0.5 ($\pm$0.2)</td>
<td>3.8($\pm$2.8)*</td>
</tr>
<tr>
<td>PPy/pTS film</td>
<td>2 mA cm$^{-2}$ 20 min</td>
<td>0.8 ($\pm$0.1)</td>
<td>1.4 ($\pm$0.2)</td>
<td>1.7 ($\pm$0.3)</td>
</tr>
<tr>
<td>PPy/pTS/Pt/PVDF</td>
<td>2 mA cm$^{-2}$ 20 min</td>
<td>85 ($\pm$7)</td>
<td>102 ($\pm$6)</td>
<td>1.2 ($\pm$0.1)</td>
</tr>
</tbody>
</table>

* PPy/PAS is an oxygen enrichment membrane. The separation factor is expressed as $\alpha (\text{O}_2/\text{N}_2)$. 

### 7.3.3 Mixed Gases Permeation

The separation of gas mixtures by PPy/pTS membranes had been tested so as to determine the gas permeability and separation factor in the presence of other gas components for a given membrane. In this case, PPy/pTS membranes of different thicknesses on two different substrates were prepared and tested with an air mixture (N$_2$:O$_2$ equals to 78:21) as the feed gas. Gases permeating through a polymer membrane were metered and analysed with Gas Chromatography.

The oxygen selectivity of PPy/pTS increased with the increase in the membrane's thickness (Table 7.3). For examples, with the increase of the
polymerisation time from 3 minutes to 10 minutes, both oxygen and nitrogen permeation rates were reduced. The separation factor between oxygen and nitrogen increased from 1.04 to 1.19.

Gas permeation through PPy/pTS was also dependent upon the current density used during the preparation of PPy/PTS membranes. Lower current density usually produced a dense PPy/pTS films with increased separation factor. For example, the oxygen GPR was $4.9 \times 10^{-6}$ cm$^3$(STP) cm$^{-2}$ s$^{-1}$ cmHg$^{-1}$ for the PPy/pTS membrane prepared under 2.0 mA cm$^{-2}$ for 10 minutes; but the oxygen GPR dropped down to $3.7 \times 10^{-6}$ cm$^3$(STP) cm$^{-2}$ s$^{-1}$ cmHg$^{-1}$ for PPy/pTS prepared under 1.0 mA cm$^{-2}$ for 20 minutes (The total amount of charge consumed for both electropolymerisation processes was the same).

The substrates membranes used had little effect on either gas permeation rate or the selectivity of the membranes. Polypyrrole membranes prepared on the PVDF substrates have almost the same permeation rates (for both oxygen and nitrogen) as those prepared on the polysulfone membranes.

The overall selectivity between oxygen/nitrogen obtained so far for PPy/pTS is around 1.5 when tested with air mixture as the feed gas. Compared with others' work [95-97], the selectivity obtained with this membrane is not very satisfactory.
Table 7.3 Gas permeation by PP/pTS composite membranes

<table>
<thead>
<tr>
<th>No.</th>
<th>Membranes* preparation conditions</th>
<th>GPR+ x10^{-5} [cm^3(STP) cm^{-2} s^{-1} cmHg^{-1}]</th>
<th>Separation factor</th>
<th>α (O₂/N₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 min, 2 mA cm^2</td>
<td>O₂ 2.44</td>
<td>N₂ 2.35</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>4 min 2 mA cm^2</td>
<td>1.14</td>
<td>1.07</td>
<td>1.1</td>
</tr>
<tr>
<td>3</td>
<td>5 min, 2 mA cm^2</td>
<td>0.97</td>
<td>0.90</td>
<td>1.2</td>
</tr>
<tr>
<td>4</td>
<td>8 min, 2 mA cm^2</td>
<td>0.76</td>
<td>0.68</td>
<td>1.1</td>
</tr>
<tr>
<td>5</td>
<td>10 min, 2 mA cm^2</td>
<td>0.72</td>
<td>0.61</td>
<td>1.2</td>
</tr>
<tr>
<td>6</td>
<td>10 min, 1 mA cm^2</td>
<td>0.49</td>
<td>0.38</td>
<td>1.3</td>
</tr>
<tr>
<td>7</td>
<td>20 min, 1 mA cm^2</td>
<td>0.37</td>
<td>0.29</td>
<td>1.3</td>
</tr>
<tr>
<td>8</td>
<td>20 min, 1 mA cm^2</td>
<td>0.37</td>
<td>0.29</td>
<td>1.3</td>
</tr>
<tr>
<td>9</td>
<td>30 min, 1 mA cm^2</td>
<td>0.21</td>
<td>0.14</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* All membranes used here were electrochemically prepared on platinised substrate membranes. Platinised PVDF membranes of 0.45 μm pore size were used in membranes for No. 1 to No. 7. Platinised polysulfone membranes (100KD) were used for No 8 to No. 9.

7.3.4 Other Factors Affecting Gas Permeation and Separation

It has been found that the gas permeation rate and permselectivity were affected by the morphology and the defects introduced during the synthesis of the polymer membranes. A membrane with denser and smoother surface usually had lower permeability and higher selectivity. On the other hand, a membrane with a rough surface or defects more often has higher permeability and very poor selectivity. Among all these polymer membranes prepared, PPy/BS, PPy/pTS and PPy/PAS had the
most uniform and smoothest morphology. The permeabilities of these membranes were in the range of $10^{-7}$ to $10^{-8}$ cm$^3$(STP) cm$^{-2}$ s$^{-1}$ cmHg$^{-1}$; Their permselectivity between oxygen and nitrogen were relatively high (1.6 ~ 6.5). PPy/Cl had a rough surface with reasonable permeability $3.17 \times 10^{-6}$ cm$^3$(STP) cm$^{-2}$ s$^{-1}$ cmHg$^{-1}$ and fair selectivity (1.5). PPy/Dex and PPy/Heparin were found no selectivity and not suitable for gas separation.

Membrane permeability and perm selectivity were also affected by the types of counterions incorporated during the electropolymerisation process. Polypyrrole synthesised with smaller doping anions, such as Cl$^-$, usually had lower gas permeation rate and higher perm selectivity than those prepared with relatively large anions (e.g. dextran polyanion).

### 7.4 CONCLUSIONS

The use of polypyrrole conducting membranes for gas separation has been investigated. Several doping anions had been used for the synthesis of polypyrrole conducting membranes. The surface smoothness, density and microstructure of the resultant polypyrrole membranes vary with respect to the types of doping anions incorporated into the polymer. These factors affect both permeability and selectivity.

Gas permeability and permselectivity of the polymer membrane also depend on the particular doping agent used. Polypyrrole electrochemically synthesised with conventional counterions showed higher nitrogen permeability with, for example, separation factors up to 1.9 obtained for PPy/pTS membranes. Polyanions dextran and heparin sulfate were found not suitable as counterions in preparing gas separation membranes because of their high porosity. Polypyrrole doped with
polyaniline sulfonic acid has uniform surface and higher permeability for oxygen. The separation factor was found to be 3.0 and higher with this work.

The redox states of polypyrrole conducting polymers also affect gas permeability and selectivity. A polymer membrane in its reduced state tends to have high permeability and lower selectivity because of the chain extension and the removal of dopant counterions.

Gas permeability and permselectivity were affected by the thickness of the polymer membrane employed. The thicker the polymer membrane, the lower the gas permeation rate but, as a result, higher selectivity is obtained. It seemed very difficult to produce a polymer membrane that has both high permeability and selectivity. The trade-off between permeability and selectivity always exists.

7.5 SUGGESTIONS FOR FUTURE WORK

Polypyrrole conducting polymer show higher oxygen perm selectivity when doped with polyaniline sulfonic acid. While other polymer membranes showed high permeability for nitrogen instead of oxygen when tested separately; which had also been indicated by some other researchers. In order to find an effective oxygen selective membrane, more work should be done on polypyrroles prepared with polyaniline sulfonic acid counterion or other substitutes. Factors such as the preparation methods and redox states of the polymer need to be studied further.
CHAPTER 8

GENERAL CONCLUSIONS
The development of electromembrane systems with dynamic controllable transport and separation properties was the main objective of the current studies. Generally, the physical and chemical properties of conventional membranes are fixed during their synthesis. Conducting polymer membranes, such as polypyrrole free standing thin films, have emerged as a new class of membranes with inherent electrodynamic properties. These membranes are capable of responding to external electrical stimuli by varying their chemical properties (e.g. ion-exchange) or physical properties (hydrophobicity). But the lack of good mechanical properties is always a major concern for any practical application.

In this work, methods for fabricating platinised membranes and polypyrrole composite membranes have been established. The same principles can also be used in preparing other metallised membranes by using other electrochemically inert metals. Polypyrrole composite membranes were also electrochemically synthesised based upon platinised PVDF membranes. The mechanical properties of polypyrrole conducting polymer membranes have been greatly improved by using these platinised membranes.

The transport and separation of proteins across platinised electromembranes and polypyrrole composite membranes were carried out to electrochemically control the rate of protein transport. By applying electrical stimuli, the transport of test proteins can be turned on and off \textit{in situ}. Protein transport fluxes up to $2 \times 10^{-12}$ mol cm$^{-2}$ s$^{-1}$ have been achieved with such a membrane system. The separation of protein mixtures was also successfully demonstrated using either the platinised electromembranes or the polypyrrole composite membranes. The
separation factors between test proteins were relatively high so that quite pure protein products can be obtained with this electromembrane separation system. The use of a flow through transport system was also demonstrated with particular test proteins. Results show that electrochemically controlled transport can be operated as a continuous process for protein separations.

The synthesis of biofunctional polypyrrole and polypyrrole membranes was also addressed. By incorporating biofunctional groups, such as antibody, into polypyrrole during its synthesis, a polymer with both electroactivity and bioactivity can be obtained. For example, the EQCM experiments indicate that the selectivity of PPy/anti-HSA to HSA was highly specific among all proteins tested. Transport of KCl and HSA indicated that KCl was able to transport across the PPy/anti-HSA composite membranes, while HSA was retained by the membrane. A decrease of KCl flux after adding HSA to the transport solution was observed.

The incorporation of other functional molecules, such as polyelectrolytes, into polypyrrole films and membranes was also carried out. For example, the incorporation of heparin, a polyelectrolyte of both clinical and commercial importance, has been successfully demonstrated and utilised for the detection and separation of thrombin. EQCM studies showed that the interaction between PPy/Heparin and thrombin is highly specific. Transport experiments indicated that thrombin cannot transport across the PPy/Heparin membrane while other proteins can.

The use of polypyrrole as gas permeation membranes was investigated. The permselectivity of polypyrrole varied when different counterions had
been incorporated into the polypyrrole membranes. Polypyrrole membranes synthesised with small anions, such as those sulphonated organic anions, usually have limited selectivity between oxygen and nitrogen. But when synthesised with polyaniline sulphonic acid, higher selectivity and permeability were obtained. On the other hand, polypyrrole prepared with those polyanions, such as dextran sulfate and heparin sulfate, were found to have no selectivity for gas separation.

Although the results presented in this thesis have added a new dimension to the protein separation technology, some problems are still to be resolved in future research. These include:

(i) The maximum potential applied across a membrane is limited because water decomposition occur if extreme potentials are applied.

(ii) To separate one protein component from another, a constant potential across the membrane is necessary. But with polypyrrole composite membrane, overoxidation might occur which would result in the degradation of polypyrrole.

(iii) To broaden the applications of conducting polymer for gas separation, other conducting polymer materials and membrane processing methods should be considered in the near future. One of the promising materials for gas separation is polyaniline based conducting polymer membranes as indicated by the results in this research - the membrane’s selectivity for oxygen was higher when polyaniline sulphonic acid was incorporated into polypyrrole.
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