1995

Chromatographic studies on the properties of polypyrrole and polyaniline

Hawick Chriswanto

University of Wollongong
NOTE

This online version of the thesis may have different page formatting and pagination from the paper copy held in the University of Wollongong Library.

UNIVERSITY OF WOLLONGONG

COPYRIGHT WARNING

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site. You are reminded of the following:

Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.
Chromatographic Studies on the Properties of Polypyrrole and Polyaniline

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

DOCTOR OF PHILOSOPHY

from

THE UNIVERSITY OF WOLLONGONG

by

Hawick Chriswanto, M.Sc.

Chemistry Department

1995
Acknowledgement

I wish to express my sincere thanks to Professor G.G. Wallace, my supervisor, for his valued advice, assistance, appreciation, and patience during the present course.

I also acknowledge the technical assistance of all the staff of the Inteligent Polymer Research Laboratory in particular, and of the Department of Chemistry for their general assistance. Special thanks is expressed to Ms Kerry Gilmore for her patience and willingness in providing helpful assistance.

I would like to thank Anthony Hodson, Norman Barisci, Mark Imisides, Trevor Lewis, and Daniela Ongarato. Their assistance in preparation and proof reading of this thesis is greatly appreciated.

Friendly support from fellow students is also highly appreciated.

I am grateful to my wife, Marna, for her overwhelming support and encouragement. Her patience and persistence in typing this thesis is especially appreciated.

Finally, I wish to express my appreciation to the AIDAB for financial support in the form of EMMS scholarship and to the PPPTMGB-LEMIGAS, Jakarta, Indonesia for the nomination.
Abstract

This thesis describes a study of the physicochemical properties of conductive polymers. Although these polymers have been investigated extensively for more than a decade, studies of their properties employing chromatographic methods are still very limited. In this work high performance liquid chromatography was employed to investigate the properties of polypyrrole and polyaniline. Their performance as a chromatographic stationary phase was also examined.

For the purpose of this study polypyrrole containing chloride and dodecylsulfate, and polyaniline with chloride, were chemically synthesized and deposited on to silica particles. The surface coating was determined by elemental analysis. Series of standard test compounds were used for chromatographic characterisation. The effect of the exposure of the polymers to redox reagents was also chromatographically examined.

Both reversed-phase and anion exchange chromatographic behaviour were observed on the polymeric stationary phases. It was found that the incorporated dodecylsulfate counterions induced unique microstructure in the polypyrrole matrix, as reflected by its chromatographic behaviour toward planar molecules. The results also indicated that the interaction mechanism involved during chromatographic elution for each of the polymers investigated might be different from each other. There was
indication that the polymer exposure to redox reagents changed its properties.

This study indicated that it is possible for conductive polymers to be used for chromatographic applications. The problem of low efficiency, however, should be addressed first.
Publications


# Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )</td>
<td>selectivity</td>
</tr>
<tr>
<td>Å</td>
<td>angstrom (( =10^{-10} ) m)</td>
</tr>
<tr>
<td>( A^- )</td>
<td>counter anion</td>
</tr>
<tr>
<td>( \alpha' )</td>
<td>dipole angle</td>
</tr>
<tr>
<td>A.C</td>
<td>alternating current</td>
</tr>
<tr>
<td>AGP</td>
<td>advanced gradient pump</td>
</tr>
<tr>
<td>AR</td>
<td>analytical reagent</td>
</tr>
<tr>
<td>As</td>
<td>asymmetry factor</td>
</tr>
<tr>
<td>BPP</td>
<td>bonded-phase packing</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>( \chi )</td>
<td>thermal conductivity</td>
</tr>
<tr>
<td>( C^+ )</td>
<td>counter cation</td>
</tr>
<tr>
<td>( C_{18} )</td>
<td>octadecyl</td>
</tr>
<tr>
<td>( C_m )</td>
<td>concentration of a component in the mobile phase</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>( C_s )</td>
<td>concentration of a component in the stationary phase</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammetry</td>
</tr>
<tr>
<td>DEP</td>
<td>diethylphthalate</td>
</tr>
<tr>
<td>DMA</td>
<td>N,N-dimethylaniline</td>
</tr>
<tr>
<td>DME</td>
<td>dropping mercury electrode</td>
</tr>
<tr>
<td>DMP</td>
<td>dimethylphthalate</td>
</tr>
<tr>
<td>DS</td>
<td>dodecylsulfate</td>
</tr>
<tr>
<td>E</td>
<td>potential</td>
</tr>
<tr>
<td>( \varepsilon )</td>
<td>dielectric constant</td>
</tr>
<tr>
<td>e</td>
<td>electron</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>ECLC</td>
<td>electrochemically controlled liquid chromatography</td>
</tr>
<tr>
<td>EDA</td>
<td>electron donor-acceptor</td>
</tr>
<tr>
<td>E°</td>
<td>standard potential</td>
</tr>
<tr>
<td>F</td>
<td>volumetric flow rate</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>h</td>
<td>peak height</td>
</tr>
<tr>
<td>HETP</td>
<td>height equivalent to a theoretical plate</td>
</tr>
<tr>
<td>HIC</td>
<td>hydrophobic interaction chromatography</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HSA</td>
<td>human serum albumin</td>
</tr>
<tr>
<td>IEC</td>
<td>ion-exchange chromatography</td>
</tr>
<tr>
<td>φ</td>
<td>volume fraction</td>
</tr>
<tr>
<td>K</td>
<td>partition coefficient or distribution coefficient</td>
</tr>
<tr>
<td>k'</td>
<td>capacity factor</td>
</tr>
<tr>
<td>k_w</td>
<td>extrapolated-to-water capacity factor</td>
</tr>
<tr>
<td>L</td>
<td>column length</td>
</tr>
<tr>
<td>λ</td>
<td>wavelength</td>
</tr>
<tr>
<td>L/B</td>
<td>length-to-breadth ratio</td>
</tr>
<tr>
<td>LR</td>
<td>laboratory reagent</td>
</tr>
<tr>
<td>M</td>
<td>molar</td>
</tr>
<tr>
<td>MeCN</td>
<td>acetonitrile</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>mg</td>
<td>milligram(s)</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>ml</td>
<td>milliliter(s)</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter(s)</td>
</tr>
<tr>
<td>Mp</td>
<td>molecular weight of pyrrole</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>mV</td>
<td>millivolt(s)</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>N</td>
<td>number of electrons</td>
</tr>
<tr>
<td>N</td>
<td>theoretical plates number</td>
</tr>
<tr>
<td>n.a</td>
<td>not available</td>
</tr>
<tr>
<td>nm</td>
<td>nanometer(s)</td>
</tr>
<tr>
<td>NPLC</td>
<td>normal-phase liquid chromatography</td>
</tr>
<tr>
<td>ODS</td>
<td>octadecilsilyl</td>
</tr>
<tr>
<td>Ox</td>
<td>oxalate</td>
</tr>
<tr>
<td>PAH</td>
<td>polyaromatic hydrocarbon</td>
</tr>
<tr>
<td>pI</td>
<td>isoelectric point</td>
</tr>
<tr>
<td>PMSP</td>
<td>polymer-modified-silica packing</td>
</tr>
<tr>
<td>PP</td>
<td>polypyrrole</td>
</tr>
<tr>
<td>PAnCl</td>
<td>polyaniline chloride</td>
</tr>
<tr>
<td>PPCl</td>
<td>polypyrrole chloride</td>
</tr>
<tr>
<td>PPDS</td>
<td>polypyrrole dodecylsulfate</td>
</tr>
<tr>
<td>ppm</td>
<td>part per million</td>
</tr>
<tr>
<td>PSDVB</td>
<td>poly(styrene divinylbenzene)</td>
</tr>
<tr>
<td>r_A</td>
<td>atomic hybrid component</td>
</tr>
<tr>
<td>RI</td>
<td>retention index</td>
</tr>
<tr>
<td>Rp-C_{18}</td>
<td>reversed-phase C_{18}</td>
</tr>
<tr>
<td>RPLC</td>
<td>reversed-phase liquid chromatography</td>
</tr>
<tr>
<td>RSD</td>
<td>relative standard deviation</td>
</tr>
<tr>
<td>RVC</td>
<td>reticulated vitreous carbon</td>
</tr>
<tr>
<td>S</td>
<td>slope of the plot</td>
</tr>
<tr>
<td>σ</td>
<td>molal surface tension increment</td>
</tr>
<tr>
<td>s</td>
<td>spin density</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecylsulfate</td>
</tr>
</tbody>
</table>
TFA: trifluoroacetic acid

t₀: dead time

tᵣ: retention time

Trp: L-tryptophan

Tyr: L-tyrosine

UV: ultra violet

Vm: volume of the mobile phase

V₀: dead volume of the column

Vᵣ: retention volume

Vs: volume of the stationary phase

Vw: van der Waals volume

W₁/₂: half peak width

Z: charge on the ion

µ: dipole moment of molecule

µm: micro meter

δ: polarizability

Δ: difference

ΔG°: Gibbs free energy

°C: centigrade
List of Contents

Acknowledgement i
Abstract ii
Publications iii
Abbreviations iv
List of Contents ix

Chapter 1

General Introduction ......................................................... 1

1.1 Fundamentals and Development of Liquid Chromatography ................. 2
  1.1.1 Introduction .................................................... 2
  1.1.2 Historical background ........................................... 3
  1.1.3 Basic concepts ................................................... 5
  1.1.4 Fundamental molecular interactions ......................... 10
  1.1.5 Modes of chromatography .................................... 15

1.2 Column Packing Materials ............................................ 21
  1.2.1 Silica ............................................................ 21
  1.2.2 Bonded-phase packings (BPPs) ................................ 23
  1.2.3 Polymer-based (PBPs) and polymer-modified-silica packings (PMSPs) .. 24
  1.2.4 Conductive polymers .......................................... 26

1.3 Electrochemically Controlled Liquid Chromatography .................... 34

1.4 Inverse Chromatography ............................................. 37

1.5 Aim of the Project ..................................................... 40
**Chapter 2**

**Polypyrrole-Coated Silica**

2.1 Introduction .................................................. 43

2.2 Experimental .................................................. 44
   2.2.1 Reagents and materials .................................. 44
   2.2.2 Instrumentation ......................................... 48
   2.2.3 Preparation of column packings ....................... 49
   2.2.4 Packing procedure ...................................... 49
   2.2.5 Chromatographic measurements ....................... 49

2.3 Results and Discussion ...................................... 50
   2.3.1 Column packings ........................................ 50
   2.3.2 Retention of small molecules at fixed eluent
        composition ............................................... 51
   2.3.3 Reversed-phase behaviour .............................. 55
   2.3.4 Column performance for small molecules .......... 59
   2.3.5 Hydrophobicity parameter determinations .......... 62
   2.3.6 Effect of eluent composition on selectivity ...... 69
   2.3.7 Chromatographic studies using PAHs ................. 76

2.4 Conclusion .................................................... 83

**Chapter 3**

**Polyaniline-coated Silica**

3.1 Introduction .................................................. 87

3.2 Experimental .................................................. 88
   3.2.1 Reagents and materials ................................ 88
   3.2.2 Preparation of column packing ...................... 89
   3.2.3 Chromatographic measurements .................... 89
   3.2.4 Instrumentation ....................................... 90
Chapter 3

3.3 Results and Discussion ................................................................. 90

3.3.1 Chemical composition of column packing .................. 90

3.3.2 Retention of small molecules at fixed eluent composition ................................................................. 92

3.3.3 Column performance for small molecules ......................... 94

3.3.4 Reversed-phase behaviour .................................................. 97

3.3.5 Effect of eluent composition on selectivity .......................... 99

3.3.6 Hydrophobicity parameter determination ......................... 103

3.3.7 Chromatographic studies using PAHs .............................. 109

3.3.9 Ion-exchange studies ...................................................... 118

3.4 Conclusion ................................................................. 122

Chapter 4

Chromatographic Study on the Interactions between Proteins and Polypyrrole and Polyaniline ................................................. 125

4.1 Introduction ................................................................. 126

4.2 Experimental ................................................................. 127

4.2.1 Reagents and materials .................................................. 127

4.2.2 Preparation of column packings ........................................... 128

4.2.3 Chromatographic measurement ........................................... 129

4.2.4 Instrumentation ............................................................. 130

4.3 Results and Discussion ................................................................. 131

4.3.1 Chemical composition of column packings .................. 131

4.3.2 Isocratic elution .............................................................. 132

4.3.3 Protein recoveries on isocratic elution .......................... 138

4.3.4 Gradient elution .............................................................. 143

4.3.5 Effect of salt ................................................................. 151

4.4 Conclusion ................................................................. 161
Chapter 5
Chromatographic Studies on the Effect of Exposure to Redox Reagents on the Properties of Polypyrrole Chloride and Polypyrrole Dodecylsulfate ..................................... 164
5.1 Introduction ................................................................................. 165
5.2 Experimental ............................................................................. 166
  5.2.1 Reagents and Materials ............................................................. 166
  5.2.3 Chromatographic measurements .......................................... 168
  5.2.4 Potentiometric measurements .............................................. 169
5.3 Results and Discussion ............................................................... 171
  5.3.1 Redox switching on conductive polymers ............................ 171
  5.3.2 Potentiometric measurement .............................................. 176
  5.3.3 Chemical composition of column packings .......................... 179
  5.3.4 Retention behaviour of benzene and derivatives .................. 179
  5.3.5 Retention behaviour of PAHs ................................................. 187
  5.3.6 Retention behaviour of basic drugs ....................................... 190
  5.3.7 Retention behaviour of amino acids ..................................... 196
  5.3.8 Reversibility of the effect of the chemical treatment .......... 203
5.4 Conclusion ................................................................................ 206

Chapter 6
General Conclusions ...................................................................... 208
References ..................................................................................... 213
List of Figures ................................................................................ 230
List of Tables .................................................................................. 237
Chapter 1

General Introduction
1.1 Fundamentals and Development of Liquid Chromatography

1.1.1 Introduction

Chromatography in its many forms is widely used as a separative and analytical technique. The separation efficiency depends on the equilibrium distribution of the analyte molecules between two phases, one stationary and the other (mobile) phase flowing over this. Solutes which are preferentially distributed in the mobile phase will pass through the system faster than those which are preferentially distributed in the stationary phase.

A column is normally used to contain the stationary phase, while the mobile phase is allowed to flow through the column either under gravity or under pressure. The sample is placed at the head of the column using a suitable sample introduction device and moves through the column under the influence of the mobile phase flow. The separated components which leave the column are detected using a suitable detector, the output of which is displayed on, for example, a strip chart recorder. A block diagram illustrating the various components of the chromatographic system is shown in Figure 1.1.
1.1.2. Historical background

Column chromatography was invented and named by the Russian Mikhail Tswett at the turn of this century [1]. He employed the technique to separate plant pigments in a column packed with calcium carbonate powder. The name chromatography was derived from two Greek words; chroma for colour, and grapern for write which were selected to account for the separated colour bands in the column. The application of chromatography grew tremendously after the work of Martin and Synge on liquid partition chromatography [2]. Those who employed ion-exchange chromatography for the first time, however, were Taylor and Urey [3] to separate Li⁺ and K⁺ isotopes using columns packed with zeolite. Synthetic ion-exchange resins then became commercially available after Samuelson demonstrated the potential of such resins for analytical application [4]. A growing interest in chromatography application in biomedical field was triggered by the report from Moore and Stein in 1948 [5] based on the separation of amino acids using ion-exchange chromatography. Another important development in liquid chromatography was the work by Tiselius, a Swedish biochemist, who
pioneered, with his colleagues, a chromatographic method called adsorption chromatography in the 1940s [6]. Gel filtration chromatography which now has become an important technique for the separation of high-molecular weight substances evolved from the work of Porath and Flodin [7].

In classical liquid-column chromatography two very important modifications were developed in the postwar decade, reversed-phase and gradient-elution chromatography. Modern high-performance liquid chromatography would be impossible without such a development. Reversed-phase chromatography, which was developed by Martin and Howard [8], is the technique in which the mobile phase is more polar than the stationary phase. Gradient-elution chromatography is the technique in which the composition of the mobile phase is changing with time during the course of the chromatographic separation. The original idea behind this method was to keep the rear of a chromatographic zone in a stronger eluting medium than the front causing the rear boundary of the zone to move faster resulting in a more compact zone. This technique which proves itself to be extremely useful was developed by Alm, Williams, and Tiselius [9].

The classical techniques used in liquid chromatography have several limitations such as the requirement of large columns, resulting in the wastage of a large amount of solvent and adsorbent, as well as the usually labourous and time consuming methods required. Because of such limitations, a number of closely related methods were developed such as paper chromatography [10] and thin-layer chromatography; the latter having its origin in the work of Izmailov and Shraiber [11] using a thin
layer of alumina on a glass plate. While the resolving power of such methods is not as high as column techniques they are low cost and rapid.

The more recent developments in column chromatography have been dealing with the transition from slow and laborious methods to sophisticated instrumental methods. This transition was mainly brought about by the increased separating efficiency of the column packings especially with the introduction of chemically-bonded stationary phases [9]. The theory of chromatography also underwent major development. Many researchers gave contributions in establishing the theory of column efficiency in relation to other chromatographic parameters, for example Martin and Synge [2]; Lapidus and Amundson [12]; Van Deemter, Zuiderweg, and Klinkenberg [13].

1.1.3 Basic concepts

**Distribution Coefficient.** Chromatographic separations are based on differences in the extent to which solutes are partitioned between the mobile and the stationary phase. The distribution or partition coefficient, \( K \), is then defined to quantitatively describe the solute equilibrium between the two phases and is expressed as:

\[
K = \frac{C_S}{C_m}
\]  

(1.1)

where \( C_S \) is the concentration of the solute in the stationary phase and \( C_m \) is the concentration in the mobile phase. Differential migration, accordingly, is dependent upon the experimental variables that affect the
distribution, i.e. the composition of the mobile and stationary phase and
the temperature.

Retention time and retention volume. Retention time (t<sub>R</sub>) is a quantitative
expression of the degree of retention of a particular compound during the
course of chromatographic elution. Experimentally, retention time is
defined as the time that elapses from the moment the sample is introduced
to the moment it reaches the detector at the end of a column (Figure 1.2).

\[ k' = \frac{V_R - V_0}{V_o} = \frac{t_R - t_0}{t_o} \]

**Figure 1.2** The measurement of retention.

The retention volume (V<sub>R</sub>), which is considered to be more appropriate,
is equal to the total volume of the mobile phase needed to elute the centre
of chromatographic band. It can be calculated from t<sub>R</sub> and volumetric
flow rate, F, according to :

\[ V_R = t_R F \]  

(1.2)
V\textsubscript{R} is directly related to the distribution or partition coefficient, K, through the following expression:

\[ V\textsubscript{R} = V\textsubscript{m} + KV\textsubscript{S} \quad (1.3) \]

where \( V\textsubscript{m} \) is the volume of the mobile phase and \( V\textsubscript{S} \) is the volume of the stationary phase within the column. When a compound does not interact with the packing in the column, it passes through without retention and is said to elute in the void volume (or dead volume), \( V\textsubscript{O} \), of the column. Physically \( V\textsubscript{O} \) represents the interstitial spaces between the packing particles and any readily accessible pores within the packing material itself which are occupied by the mobile phase.

*Capacity factor.* The capacity factor, \( k' \), is a constant that is related to the migration rate of the solute. It is defined as:

\[ k' = \frac{V\textsubscript{S}}{V\textsubscript{m}} \quad (1.4) \]

The expression indicates that the retention of a solute can be varied by (i) a change in the chemical nature and or temperature of the two phases, and (ii) changes in the volume of the phases.

Experimentally capacity factor can be calculated from retention volume and is defined by the expression:

\[ k' = \frac{V\textsubscript{R} - V\textsubscript{O}}{V\textsubscript{O}} \quad (1.5) \]
If the flow rate remains constant throughout the elution of the sample, expression (1.5) can be presented as:

\[ k' = \frac{t_R - t_o}{t_o} \]  

(1.6)

where \( t_R \) and \( t_o \) are the retention times of a retained and non-retained sample respectively.

**Selectivity.** The selectivity or the separation factor, \( \alpha \), is the ratio of capacity factors and is expressed as:

\[ \alpha = \frac{k'_2}{k'_1} \]  

(1.7)

where \( k'_2 \) is the capacity factor for the component with the longer retention time. The separation between two components will only be possible if \( \alpha \) has a value greater than unity. A favourable value, however, does not necessarily indicate that a good separation is really achieved. With \( \alpha \) larger than 1, a separation can only be achieved if the individual component bands are contained in a small volume of mobile phase; small enough that the bands do not overlap.

**Column efficiency.** To describe the width of chromatographic peaks, the concept of theoretical plates, \( N \), is commonly used. Column efficiency can be expressed as the theoretical plate number (\( N \)) or the height equivalent to a theoretical plate (HETP or simply H). The number of theoretical plates is given by:
\[ N = 5.54 \left( \frac{t_R}{W_{1/2}} \right)^2 \]  

(1.8)

where \( W_{1/2} \) is the width of a peak at half its maximum height, while HETP is expressed as:

\[ H = \frac{L}{N} \]  

(1.9)

where \( L \) is the length of the column.

**Asymmetry.** Theory assumes a Gaussian shape and that peaks are symmetrical. The peak asymmetry factor is the ratio (at 10% of the peak height) of the distance between the peak apex and the back side of the chromatographic curve to the distance between the peak apex and the front side of the chromatographic curve (Figure 1.3). A value > 1 is a tailing peak, while a value <1 is a fronting peak.

![Picture of peak asymmetry](image)

**Figure 1.3** Determination of peak asymmetry
1.1.4 Fundamental molecular interactions

In a chromatographic column the solutes separate from each other because individual solutes interact with the stationary and mobile phase to different degrees. Those solutes that interact more strongly with the stationary phase will move more slowly and will be retained to a greater extent in the column. Those solutes that interact more strongly with the mobile phase than with the stationary phase will be carried through the column faster. Due to the different rates in movement among the solutes separation occurs. The nature and the extent of the molecular interactions between the solutes and each phase, therefore, will determine the relative retention of solutes and column selectivity.

In general the molecular forces which can lead the solute to interact with the two phases, i.e the stationary and mobile phase, can be classified into three categories, i.e ionic, polar, and dispersive.

Ionic interactions.

Ionic interactions result from the electrical charges residing on the molecule, thus the interactions occur between ions, i.e between molecules that have a net positive or negative charges. The energy (E) between a pair of charges, \( q_1 \) and \( q_2 \), separated by a distance \( r \) in a medium that has a dielectric constant \( \varepsilon \) is given by:

\[
E = k \frac{q_1 q_2}{\varepsilon r} \quad (1.10)
\]

where \( k \) is a constant. The force is characterised by being nondirectional and depending only on the distance of separation and is inversely
proportional to \( r \). These kinds of interactions are exploited in ion-exchange chromatography.

**Polar interactions.**

In polar interactions at least one participant must be a molecule which is a dipole. A dipole is a molecule that does not carry a permanent charge. However, because the distribution of the electrons within the molecule is not even, one end of the molecule is more positive than the other end.

*Ion-dipole interactions.* This kind of interaction involves a molecule with permanent charges and a dipole. The potential energy of an ion-dipole interaction is expressed by:

\[
E = -k^2 Z\mu \frac{\cos \alpha'}{\varepsilon r^2}
\]  

where \( k \) is a constant, \( Z \) is the charge on the ion, \( \mu \) is the dipole moment of the neutral molecule, \( r \) is the distance, and \( \alpha' \) is the dipole angle.

*Dipole-dipole interactions.* Dipole-dipole interactions involve the attraction of the positive end of one polar molecule for the negative end of another polar molecule. This kind of interaction is also known as orientation interaction or Keesom effect. The dependency of energy of the interaction on the distance separating the interacting molecules can be expressed as \( E \sim 1/r^2 \). Examples of molecules that have permanent dipoles would be aldehydes, alcohols, and ketones.

Hydrogen bonding is an especially strong kind of dipole-dipole attraction. It is formed between a covalently bonded hydrogen atom on a donor
group and a pair of non-bonding electrons on an acceptor group. Such intermolecular interactions are much stronger than other dipole-dipole interactions. The best known proton donor groups are -OH, -NH, and -SH, while the most important hydrogen bond acceptors are the oxygen atoms in alcohols and carbonyl compounds, and nitrogen atoms in amines.

_Dipole-induced dipole interactions._ Dipoles can also be induced in molecules by an electric field resulting from neighbouring molecules that have a permanent dipole. A molecule in which a dipole can be induced in that way is said to be polarisable. The higher the polarizability of the non-polar molecule, the larger the induced dipole moment will be. This kind of interaction is also known as the Debye effect and is shorter range than dipole-dipole interactions (E ~ 1/r^5). Examples of polarizable substances are benzene and other aromatics.

Polar interactions are exploited in normal-phase chromatography to separate polar or polarisable substances. By employing nonpolar or less polar mobile phase, the interactions between the solutes and polar stationary phase are encouraged.

_Dispersive interactions._

These interactions involve molecules that have neither net charge nor a permanent dipole. When two molecules of this kind approach very closely, they synchronise their vibrations so as to give a net attractive force. Such attractions are called Van der Waals or dispersion forces. These dispersion forces have a very short range (E ~ 1/r^6); they act only between the portions of different molecules that are in close contact. They can become very strong when two planar molecules stack on one another.
The examples of these interactions are those that occur between hydrocarbon molecules or the hydrocarbon chains of polar molecules.

In chromatography to allow the dispersive selectivity to predominate in the stationary phase, this phase must be free from polar substances and contain only hydrocarbon-type materials, and the mobile phase must be more polar than the stationary phase. These conditions can usually be found in reversed-phase chromatography.

The molecular interactions just described above are summarised in Table 1.1.

**Hydrophobic interactions.**

Hydrophobic interactions, in simplified terms could be defined as the interactions between two non-polar molecules in water. The driving force that brings the molecules to interact with each other is usually called the hydrophobic effect. Generally, hydrophobic interactions increase with the ionic strength of the medium [14,15]. It diminishes, on the other hand, if the water is diluted by the addition of less polar solvents such as alcohols.

In any system it is unlikely that only one type of interaction takes place. If it does, it will almost certainly be dispersive in nature. Ionic interactions are almost always accompanied by both polar and dispersive interactions and polar interactions are always accompanied by dispersive interactions.
<table>
<thead>
<tr>
<th>Type of interaction</th>
<th>Example</th>
<th>Dependence of energy on distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionic</td>
<td>$^+\text{NH}_3$</td>
<td>$\frac{1}{r}$</td>
</tr>
<tr>
<td>Ion - dipole</td>
<td>$^+\text{NH}_3$ $\overset{-}{\text{O}}$ $^+\text{H}$</td>
<td>$\frac{1}{r^2}$</td>
</tr>
<tr>
<td>Dipole - dipole</td>
<td>$^+\text{NH}_3$ $\overset{-}{\text{O}}$ $^+\text{H}$  $\overset{-}{\text{O}}$ $^+\text{H}$</td>
<td>$\frac{1}{r^3}$</td>
</tr>
<tr>
<td>Ion-induced dipole</td>
<td>$^+\text{NH}_3$</td>
<td>$\frac{1}{r^4}$</td>
</tr>
<tr>
<td>Dipole-induced dipole</td>
<td>$^+\text{NH}_3$</td>
<td>$\frac{1}{r^5}$</td>
</tr>
<tr>
<td>Dispersion</td>
<td></td>
<td>$\frac{1}{r^6}$</td>
</tr>
<tr>
<td>Hydrogen bond</td>
<td></td>
<td>bond length</td>
</tr>
</tbody>
</table>
1.1.5 Modes of chromatography

Chromatographic separation of two components depends on their having differing $k'$ values which in turn depends on their having differing distribution ratios between the stationary and mobile phases. There are a great variety of stationary phases used in liquid chromatography which could result in various separation modes. Some general aspects of these modes are addressed below.

Normal-phase liquid chromatography (NPLC). NPLC, until recently, normally refers to the use of unmodified adsorbents, such as silica. Although it can offer impressive selectivity for separation of positional isomers for example, it has some practical limitations. Foremost among these is the need to control carefully the water content of the mobile phase to get reproducible results [16]. Retention on bonded phases, in contrast, is generally more consistent because they are less sensitive to small concentrations of water in the sample or the mobile phase [17]. Furthermore the problems such as highly retentive polar compounds on unmodified silica, are less pronounced on bonded-phase columns [18].

The retention mechanism occurring in NPLC depends on the type of stationary phase and the mobile phase composition employed. If the stationary phase is a solid one such as unmodified silica, the sample retention is governed by adsorption. Solvent molecules in the mobile phase compete with the solute molecules for sites on the adsorbent. For retention to occur, a sample molecule must displace one or more solvent molecules from the stationary phase. This mechanism is called the displacement effect. The other effect which is important for positional
isomeric selectivity is localized adsorption due to the existence of discrete adsorption sites. These sites could fit a particular adsorbing molecule with suitable positional configuration but less fit for the others [19]. The effect of discrete adsorption sites on the selectivity is illustrated in Figure 1.4.

![Figure 1.4](image)

**Figure 1.4** Effect of discrete adsorption sites on selectivity

Optimum interaction between a solute molecule and the adsorbent surface takes place when the functional group(s) of the solute overlap the adsorption sites (represented as points A). This overlapping is readily possible for mono functional solutes, but not for polyfunctional ones. Certain polyfunctional solutes, however, will be better matched to the adsorbent surface than their isomeric counterparts resulting in preferential retention of the solute that best fits the surface [16].

When a binary solvent system is used as the mobile phase both adsorption and partitioning are possible in silica as reported by Shatz and Kazoka [20]. As reported, when the solubility of the polar modifier (eg methanol) is high, characteristic adsorption behaviour is observed. When the polar modifier (eg ethylene glycol) is less miscible, however, a liquid stationary-phase layer apparently forms on the surface of silica which results in a mixed adsorption-partition mechanism.
In partition chromatography the eluate is dispersed in the mobile phase and its components are partitioned between the stationary and mobile phases according to their partition coefficients. This partitioning process results in a differential rate of migration leading to separation. Huber et al. [21, 22] has demonstrated that correlation between chromatographic retention and partition is feasible. The partition mechanism was originally considered to occur in liquid-liquid chromatography where the liquid stationary phase is mechanically immobilized on the support. Although the actual retention mechanism in bonded-phase columns has not been definitely established, some form of partition is also considered to occur between the stationary and mobile phases [16,19].

Silica gels contain numerous hydrated silanol groups on its surface which are strongly polar. Thus the driving force for the adsorption of the solutes on to the silica surface should be polar interactions such as hydrogen bonding and dipole-dipole interactions [23]. Similar interactions should take place in partition mechanisms as well. Unlike the adsorption mechanism, however, all of the solute surface area would be available for partitioning within the interface [24]. In adsorption only a fraction of the solute surface area is involved in the interaction with the stationary phase [24].

Reversed-phase liquid chromatography [RPLC]. These days RPLC in various forms dominates the application of HPLC. Nonionic, ionic and ionizable compounds can often be separated using a single column and mobile phase. In RPLC the stationary phase is relatively non-polar with respect to the mobile phase and usually made from hydrocarbonaceous materials such as octadecylsilane. Retention is generally based on the
hydrophobic character of the solute. The most polar components elute first and increasing the mobile phase polarity increases the retention time.

The actual retention mechanism in bonded-phase columns is still in controversy. Some workers considered that partitioning takes place between the hydrocarbon layer on the surface of the packing and the mobile phase [25]. Others believed that retention is governed by adsorption of solutes on the hydrocarbonaceous surface layer [26, 27]. A mixed-mode of adsorption and partition has also been proposed [28, 29].

Generally hydrophobic interactions between the solute molecule and the stationary phase play a major role in RPLC. The force driving such interactions may be explained using the solvophobic theory developed by Sinanoglu et al. and then adapted by Horvath et al. [27, 30] to apply more specifically to RPLC system. The theory postulates that hydrophobic interactions result from repulsive forces between a polar solvent and the non-polar solute and stationary phase. The binding of the solute to the stationary phase is due to the decrease in the area of the non-polar segment of the solute exposed to the solvent. A recent report, however, stated that the solvophobic theory was not an accurate description of the retention process [31]. The authors described that most of the free energy of retention arose from net attractive forces in the stationary phase.

There is a variant of RPLC which is called Hydrophobic-Interaction Chromatography (HIC). As in RPLC, solute retention in HIC is also based on the hydrophobic character of the solute. While elution with RPLC is based on increasing the concentration of organic solvent in the mobile phase, elution with HIC is advanced using a decreasing salt concentration.
Although they look different in practice, the elution works in the same principle, i.e. the elution occurs with a decrease of surface tension of the eluent.

HIC has become an important separation technique for the purification of biomolecules such as proteins. In comparison to RPLC, HIC is performed in milder conditions. In RPLC the proteins are usually denatured due to the more strongly hydrophobic stationary phase as well as the denaturing effect of the eluent which normally consists of acidic water-organic solvent system. HIC employs totally aqueous mobile phases with a weakly hydrophobic stationary phase [32]. It should be noted, however, that even in HIC protein denaturation can still occur depending on the nature of the proteins, stationary phase, and elution conditions [33].

Ion-exchange chromatography (IEC). The retention mechanism involved in this mode of chromatography is simple ion-exchange. Interactions between charged functional groups of a solute and sorbent are the basis for adsorption. Usually the active sites for strong cation exchangers are the sulfonic acid group -SO_3^-H^+; and for the weak cation exchangers the -COOH group is employed. The quaternary ammonium group, -(CH_2)_2NR_3^+OH^-, is normally the active sites for a strong anion exchanger while a primary amine group, -NH_2OH, is the active site in a weak anion exchanger. Chromatographic separation thus relies on the strength of the interaction between the sample ions and the exchange sites. Ions which interact weakly with the exchange site will elute earlier and vice versa.
In ion-exchange separations the retention and selectivity are dependent on the pH of the eluent especially for samples which are only partially ionized such as organic and biomolecules. For example, a weak acid that is less ionized at low pH will be retained shorter in anion-exchange columns. A weak base, on the other hand, that is ionized more at low pH will be retained longer in cation-exchange columns. Thus increasing pH for weak acids or decreasing pH for weak bases increases competition of the charged solute ions for exchange sites.

The ionic strength of the buffer or the mobile phase is also an important factor for controlling the solute retention. Ionic strength of the eluent can be varied by either changing the buffer concentration (holding pH constant) or by the addition of another salt. In either way, solvent strength, defined as the ability of a solvent to elute a particular solute or compound from a column, in ion exchange chromatography normally increases with ionic strength. Increasing solvent strength decreases the retention of sample ions because these ions cannot compete so well with the mobile phase ions for the exchange sites within the packing.

Many ion-exchange sorbents are based on polymeric materials such as polystyrene-divinylbenzene and polyacrylates which have a hydrocarbon backbone. Since the application of IEC for biomolecules, such as proteins, is increasingly popular the possibility of non-specific hydrophobic interactions between protein and the polymer matrix should be also considered. This kind of interaction would normally take place at high salt concentration leading to lower protein recovery. Addition of organic modifiers, such as propanol, to the mobile phase buffers can minimize the undesired solute-sorbent hydrophobic interactions [34]. Floyd et al [35],
on the other hand, describe the use of mixed-mode stationary phases for the separation of biomolecules. The stationary phases are composed of bonded silanes having both ion-exchange and hydrophobic functionalities.

The development of new stationary phases using conductive materials for ion separations has generated increasing attention recently. The use of conductive polymers, i.e polyaniline, as an anion-exchange stationary phase was reported by Syed and Dinesan [36]. Its chromatographic performance was tested for the separation of chloride, bromide and iodide ions. The application of polyaniline as an ion-exchanger has also been described by Kumar et al [37]. They used it for separation of noble metals from meteorite samples.

1.2 Column Packing Materials

1.2.1 Silica

Silica gel is at present the most commonly employed stationary phase in liquid chromatography. It is not only used as a stationary phase but also as a substrate for many bonded phases which are widely used in many applications today. It gains its present status as the most popular chromatographic support because it is easily derivatised and is available commercially in spherical particles with different specifications [38]. Silica as an absorbent has found an unchallenged position in adsorption chromatography. Snyder has provided rigorous explanations for almost every aspect of the method [39].
Chemically, silica consists of several type of silanol groups which could include free or isolated silanols, hydrogen-bonded silanols, and geminal silanols; and polysiloxane groups [40,41] as illustrated in Figure 1.5.

![Silanol Groups on Silica]

**Figure 1.5** Surface silanol groups on silica.
(A) free silanol, (B) geminal silanols, (C) hydrogen-bonded silanols, (D) siloxane.

In the presence of water most of these groups have water associated with them. This water can be removed by prolonged heating at 150-200 °C. The hydrogen-bonded silanols lose water to produce the siloxane group on heating above 200 °C [42]. The silanol groups are considered to be very important in adsorption chromatography as well as to be those which are reactive for the covalent bonding of organic species to the surface. The latter property is important for bonded-phase preparations. The presence of hydroxyl groups on silanols means that the surface of silica behaves in an acidic manner [43] which in turn enables it to act as a weak cation exchanger [40]. The isoelectric point of silica is between 2 and 3. At a pH above 4, therefore, the silica particles bear a negative charge [44]. At higher pH (8-9) it starts to dissolve. Another consequence brought about by the acidity of the silica surface is that basic analytes are strongly retained and may be irreversibly adsorbed or eluted as badly tailing peaks [45].
Physically, chromatographic silica gels are amorphous, porous solids, they can be prepared in a wide range of surface areas and pore diameters. The surface areas of commercial silica range from 100 to 860 m²/g, and the average micropore diameter ranges from 35 to 330 Å. The average particle diameter of packings is typically between 3 and 10 μm with a narrow size distribution. Both spherical and irregular shapes are available. Today most packings are based on spherical silica particles which have good mechanical strength. Spherical particles which have a narrower size distribution are believed to give more uniform packings [46]. Columns packed with irregularly shaped silica have shorter life time due to gradual dissolution of the bonded organic moieties on the sharp edges [47].

1.2.2 Bonded-phase packings (BPPs)

Based on relative polarities of the mobile phase and stationary phase BPPs are often divided into two types: normal phase and reversed-phase [6]. They are grouped into normal BPPs when the predominant functional group of the stationary phase is more polar than the mobile phases commonly used in the technique. Some typical examples of normal BPPs are those of packings containing cyanopropyl and amino propyl [48] or diol group [49]. With reversed-phase the mobile phases, which are usually mixtures of water and water-miscible organic solvents (such as methanol and acetonitrile), are more polar than the stationary phase which is usually a hydrophobic bonded phase such as octadecylsilyl (ODS) silica.
Classification of BPPs can also be based on the type of bond between the organic moiety and the silica support [50]. There are essentially four groups of different bonded phases:

- ester phases \( \equiv Si - O - R \)
- amino phases \( \equiv Si - NR_2 \)
- carbon phases \( \equiv Si - CR_3 \)
- siloxane phases \( \equiv Si - O - Si - CR_3 \)

However the commercially available BPPs are mostly based on reactions of organochlorosilanes or organoalkoxysilanes with surface silanol groups to form siloxane phases.

### 1.2.3 Polymer-based (PBPs) and polymer-modified-silica packings (PMSPs)

Although chemically bonded-phase silica has proven useful in many applications, there are still several problems. For example, ODS-silica columns have limited chemical stability and peak tailings or irreversible adsorption is observed in the case of basic compounds. These problems are generally considered to be due to the presence of residual silanol groups [40, 51-54], and the chemical stability of bonded phases is the highest for the silica with the highest concentration of hydrogen-bonded silanols [55]. Attempts have been made to overcome the problems by end capping the unreacted silanol groups. Although this procedure works to some extent, it does not eliminate all problems [56]. In further efforts to
solve the problems polymer-based stationary phases have attracted some interest as alternatives to silica-based materials. They are more stable, have the homogeneity of hydrophobic surface without strong binding sites and also can be derivatised to produce stationary phases with different polarity and functions [57]. However, they also have some disadvantages such as weaker pressure resistance, and swelling or shrinking in certain solvents [56].

The approach of coating silica makes use of the advantages of both silica particles (mechanical strength and performance) and polymer structure (chemical stability). Synthesis and characterisation of amino-functionalised materials derived from polybutadiene epoxide-coated silica has been described [58]. Polyethylene-coated silica has also been prepared and the properties compared with that of a conventional ODS-column [59]. Reversed-phase column packings with different degrees of hydrophobicity have been prepared by coating silicas with polymethacrylate-based polymers. The effect on the selectivity of protein separation was described [60]. Polymer coating of bare silica followed with derivatisation to form ion-exchange groups is another approach to the design of new phases. Huhn and coworkers prepared a low-capacity, strong-acid cation exchanger by polymer coating silica gel with either polystyrene or poly (glycidyl methacrylate) and then sulfonating the polymer [61]. The column performance was tested for the separation of metal ions in tap and mineral water as well as grape juice samples. In earlier reports Schomburg et al. described the preparation of different types of polymer-coated stationary phases [62,63]. In their methods, various polymers were synthesized at first before being chemically immobilized on the surface of the supports.
There have recently been some reports describing the preparation, characterisation and application of conductive-polymer-coated silica packing materials [64-66]. Polypyrrole-based polymers were used for coating; both reversed-phase and anion-exchange chromatographic interactions were reported [65,66]. Ge and coworkers reported that polypyrrole is an effective stationary phase for basic drugs in the presence of proteins [64]. Compared to other types of polymers, conductive polymers, such as polypyrrole, have unique properties in that they are electrically conductive and electroactive [67, 68].

1.2.4 Conductive polymers

A conductive polymer is an organic polymer that has electrical, electronic, magnetic, and optical properties similar to those of a metal in addition to the other properties commonly associated with a conventional polymer. As a group, conductive polymers have one thing in common: all of them contain extended \( \pi \)-conjugated-systems, single and double bonds alternating along the polymer chain [69].

Many of these new polymers are derived from pyrroles and thiophenes, a wide variety of which have been found to polymerise when oxidised either chemically or electrochemically [70]. The polymers become conductive only if they are in a "doped" state which can be achieved by oxidation (p-doping) or reduction (n-doping) [71]. In the case of polyaniline, however, the doping can also be carried out by protonation of the imine nitrogen [72].
According to Baughman et al. [73] conductive polymers can generally be grouped into two classes. Class I includes those polymers which simply undergo electron transfer processes upon doping to produce the conducting polymer system. The examples which belong to this class are polyacetylene, polyphenylene and polypyrrole. The polymers which undergo chemical modification, such as C-C formation, belong to the class II. The examples are poly(p-phenylene sulfide) and poly(m-phenylene).

Since the discovery of highly conducting organic polymers by successfully doping "Shirakawa" polyacetylene in 1977 [74], the field of conducting polymers has grown rapidly. It was catalysed by the promise that this new class of polymers could act as a synthetic replacement for metal in many applications.

Polypyrrole. The polypyrrole system (shown below) has attracted considerable attention and has been subjected to extensive studies. The attractiveness of this conductive polymer comes from several factors: their chemical and thermal stability [74, 75], polymers with a great variety of properties can be produced by resorting to derivatives [70, 76], copolymers [77,78], or incorporation of particular anions [79, 80] during synthesis.

Polypyrrole is formed in situ by oxidative polymerisation of the monomer. The oxidation can be carried out either chemically or electrochemically [68].
It is generally believed that the electrochemical polymerisation of pyrrole requires the coupling of two pyrrole radical cations [81, 74]. Diaz and Lacroix summarized the mechanisms involved in the polymerisation steps as follows [82]:

1. Oxidation of monomer to produce radical cations,
2. Dimerisation of two radical cations to produce dications of dihydrodimer followed by elimination of two protons to produce aromatic dimer,
3. The dimer is further oxidised, coupled, and deprotonated to produce the next higher homolog,
4. Polymer oxidation accompanied by incorporation of counter anions that results in the polymer-anion composite film,
5. The final step may involve some reaction with water to produce oxygenated material.

According to Street et al. [74], the chain growth is terminated either when the radical cation of the growing chain becomes too unreactive or, more likely, when the reactive end of the chain becomes sterically blocked from further reaction. The simplified scheme for the mechanism of formation of polypyrrole is presented in Figure 1.6. It has been suggested that monomeric units in polypyrrole are $\alpha,\alpha'$-bonded since polymerisation experiments with various substituted pyrrole monomers showed that only $\alpha$-substituted derivatives did not polymerise [83]. Further studies using IR spectrometry supported this proposition [84], i.e the structure of the polymer is predominantly an $\alpha,\alpha'$-bonded planar linear chain, in which the orientation of the pyrrole units alternate (Figure 1.7).
Waltman and Bargon [85], however, stated that whereas initially the oxidative coupling of the pyrrole monomer will mainly result in $\alpha,\alpha'$-linkages, increasing chain length of the growing oligomer will render linkages to the $\beta$-positions of the pyrrole units competitive.
Counter anions (or dopants) from the surrounding electrolyte are incorporated during the polymerisation process. Usually the polymer incorporates the anions in a ratio 3 to 4 monomer units per anion [68, 74]. The characteristics of the dopants will influence the properties of the polymer [86]. The incorporation of organic anions, for example, will generally result in smooth films. If the anion is hydrophilic, such as \( \text{ClO}_4^- \), the polypyrrole film formed is hydrophilic in contrast to that in which a hydrophobic polymeric dopant, eg. poly(styrene sulfonic acid), has been incorporated.

The incorporated dopant ions can undergo anion-exchange with other anions from the surrounding electrolyte solution. The exchange process can take place under two different mechanisms, i.e. self-diffusion (scheme 1.12) [87 - 89] or redox-controlled ion-exchange (scheme 1.13) [90].

\[
\begin{align*}
\text{(PPy)}^+ A^- (f) + B^-(s) & \rightleftharpoons \text{(PPy)}^+ B^- (f) + A^-(s) \tag{1.12} \\
\text{(PPy)}^+ A^- (f) + B^-(s) + e^- & \rightarrow \text{(PPy)}^0 + A^- (s) + B^- (s) \rightarrow \text{(PPy)}^+ B^- (f) + A^- (s) \tag{1.13}
\end{align*}
\]

where \( s \) and \( f \) represent solution and film respectively. Under self-diffusion exchange (1.12), the incorporated anion \( A^- \) is gradually replaced by anion \( B^- \) from the solution. In (1.13) the polypyrrole-coated electrode containing dopant ion \( A^- \) is immersed in a solution containing anion \( B^- \). When the film is electrochemically reduced, dopant ion \( A^- \) is expelled into the solution. When it is reoxidised anion \( B^- \) moves in instead of anion \( A^- \) because the concentration of anion \( B^- \) in the solution is higher.
The conductivity of polypyrrole arises from long chains of conjugated double bonds within the polymer backbone. The dopants change the configuration of the existing overlapping molecular orbitals to create a polaron or radical cation that is free to move under the influence of an electric field. Further oxidation can directly oxidise the polaron to form a dicationic bipolaron. It is these positive charges created on the polymer backbone that are the charge carriers for electrical conduction [91, 92]. The mechanism of formation of polarons and bipolarons is shown in Figure 1.8.

![Figure 1.8 Formation of charge carrier in polypyrrole (ref. 92)](image)
Polyaniline. Polyaniline has attracted considerable interest in the field of conductive polymers. The interest comes from the fact that a variety of different ring and nitrogen-substituted anilines can be readily synthesized. A variety of different dopants can also be doped in each derivative that can exist in a number of different oxidation states [71]. The conductive form of polyaniline has excellent chemical stability combined with relatively high levels of electrical conductivity [93] and good environmental stability [94].

The polymerisation of aniline can be carried out either chemically or electrochemically. Most of the studies in the mechanisms of polyaniline formation have focused on electrical polymerisation [95-97]. It is generally accepted that the initial oxidation step results in radical cations which then form an intermediate dimer, p-aminodiphenylamine. The incorporation of monomeric aniline into the oligomeric species that are formed is believed to auto-accelerate the electrochemical polymerisation of aniline [98]. Mohilner et al [95] proposed the similarity in the polymerisation path between chemical and electrochemical polymerisation from aniline to emeraldine.

Polyanilines are basically poly(p-phenyleneamineimine) [99]. Depending on their oxidation states, polyaniline occurs in three forms: (i) pernigraniline, i.e. fully oxidised polyaniline, (ii) emeraldine, i.e. 50% oxidised polyaniline, and (iii) leucoemeraldine, i.e. fully reduced polyaniline [100]. Of the three only the emeraldine is conductive. This form can be achieved through two independent routes [93]:

(1) oxidation of leucoemeraldine base (insulator) either
electrochemically or chemically,

(2) protonation of emeraldine base by exposure to protonic acids.

Both routes produce conductive polyaniline termed the *emeraldine salt*. In (1) the emeraldine salt formation involves no change in the number of hydrogen atoms attached to nitrogen atoms. In (2) there is no change in the formal oxidation state of the polymer. The protons instead, partly depopulate the $\pi$ system with a concomitant increase in conductivity [101]. Usually the general formula of polyaniline is represented as either emeraldine base or emeraldine salt. Figure 1.9 shows polyaniline in both forms.

![Chemical structure of polyaniline](image)

*Figure 1.9* The chemical structure of polyaniline

According to Yoon et al. [102] the electrical conduction in polyaniline occurs through polaron mechanism which is supported by the fact that:
(i) plotting log [s] versus $\chi$ results in very good linear relationship at pH <2, where [s] is spin density and $\chi$ is thermal conductivity.

(ii) when thermal conductivity ($\chi$) is plotted against electrical conductivity, very good correlation is produced.

The polaron model is also supported by Watanabe et al [103]. According to this group the formation of a radical cation creates a defect in the polymer structure which in turn induces energy levels which are symmetrically located between the valence band and the conduction band.

1.3 Electrochemically Controlled Liquid Chromatography

Electrochemically controlled liquid chromatography (ECLC) is a separation technique in which an electrical potential is applied to a specially designed chromatographic column that contains a conductive or redox-active stationary phase.

There is a significant difference between ECLC and conventional chromatographic techniques. In conventional techniques, the binding sites on the stationary phase are fixed so that the separation is optimised by changing the properties of the mobile phase. In ECLC, on the other hand, the binding sites on the stationary phase are reversibly changeable so that the optimum separation could be achieved by manipulating the stationary phase, not the mobile phase, by imposing an external potential to it.
In 1992 Nagaoka et al [104] described the separation of inorganic cations and anions using microporous glassy carbon powder packed into a glass tube column. They found that the retention of ions varied with potential applied to the column. It was observed that the retention of the cations increased when the potential was decreased and the variation in retention was greater for divalent and trivalent cations compared with the monovalent ions. In the case of inorganic anions, on the other hand, the retention time increased with an increase in the applied potential.

Later they described the use of crown ether modified carbon particles for cation separation using ECLC technique [105]. The crown ether was physically immobilized on the surface of the carbon particles because it was not immiscible with the aqueous mobile phase. It was observed that the use of crown ether as the stationary phase modifier improved its selectivity because one cation was transferred into the modifier layer more easily than the others.

Ghatak-Roy and Martin [106] described the use of a vinylferrocene/maleic anhydride copolymer modified carbon particles as the stationary phase for ECLC. They tested the potential of the system by examining the retention behaviour of methylviologen cations as a function of the applied potential, and found that the retention varied with potential. They proposed that it was the ferrocene moieties that governed the separation mechanism in this system.

Recent reports have described the use of conductive polymers in this area. Ge and Wallace [107] reported a preliminary study in the use of conductive polymer as a stationary phase in liquid chromatography. They
coated reticulated vitreous carbon (RVC) particles with polypyrrole-based conductive polymer which was then packed into a column cartridge. The performance of the column was tested with the use of several polar compounds and their retention behaviour upon the application of external potential was measured. They found that the retention of basic compounds increased when more negative potential was applied, while the retention of more acidic ones decreased. The trend of the retention was reversed when more positive potential was applied. Later they found that separation selectivity for a mixture of caffeine and theophylline improved when a negative potential was applied to the stationary phase which was prepared from polypyrrole-coated RVC [108].

In 1991 Deinhammer et al [109] proposed a technique for separating relatively large anionic compounds using a stationary phase prepared by coating glassy carbon particles electrochemically with polypyrrole. This technique made use of irreversible uptake of the anions into the film. The elution was made by changing the positive charge density of the stationary phase by using voltage steps and linear voltage sweeps. The separation was achieved because the anions had different net negative charges.

ECLC using polyaniline stationary phase has also been reported. Nagaoka et al [105] described the separation of anions on the column packed with polyaniline-coated glassy carbon particles. They found that for small anions, such as Br\(^-\), I\(^-\) and SCN\(^-\), the retention increased with potential with the larger ions being retained longer. It was observed, however, that there was no retention for large organic monoanions within the range of applied potential investigated. They proposed that the diffusivities of the anions in the polymer film had an important role for retention.
From those limited examples discussed above, it is clear that ECLC, especially in combination with the use of conductive polymers, has some potential in chromatographic applications.

1.4 Inverse Chromatography

The introduction of the term “inverse chromatography” dates back to the mid sixties when Davis and Petersen published their work on asphalt by gas chromatography [110]. They used solute probe retentions to measure polarity change in the stationary phase - the asphalt - with helium as the carrier gas. The study was considered “inverse” because, unlike usual chromatographic techniques, the main subject of investigation was the stationary phase not the solutes. The idea has been adapted in the area of liquid chromatography, in which a series of standard test compounds with known functionalities are used to compare and investigate the properties of different stationary phases.

Gonnet et al [111] using the separation factor for toluene/benzene to compare various commercial C18 columns and found that the selectivity varied significantly. They also used the pair caffeine/theophylline to compare the selectivity of the columns toward polar compounds and found that the selectivity varied significantly from 1.4 to 4.5.

Engelhardt and Junghein [112] compared the properties of more than 60 reversed-phase columns using a series of standard test compounds with different functionalities. To measure the strength of hydrophobic
interactions and selectivity, the pair ethylbenzene-toluene was used. They found that column hydrophobicities varied significantly and that the hydrophobic selectivity \( \frac{k'_{\text{ethylbenzene}}}{k'_{\text{toluene}}} \) was a function of carbon content up to a certain value. They also used neutral polar compounds such as phenol and ethylbenzoate to examine neutral polar interactions. Some basic compounds, such as aniline and N,N-dimethyl aniline, were also used for characterisation. Such compounds highlighted the differences between columns which reflected the variation in silanolphilic interaction.

According to Walters [113] a series of substituted benzene compounds is applicable to compare various C\(_{18}\) columns in reversed-phase system. The use of relative retention, expressed as \( k'/k'_{\text{benzene}} \), eliminated the effects of minor variations in chromatographic conditions on the retention values and emphasized the interactions due to the substituent groups. He found that non polar substituents on the benzene ring increased the retention while polar substituents resulted in decreased retention relative to benzene.

Polyaromatic hydrocarbons (PAHs) have also been widely used as molecular probes to investigate the properties of stationary phases. These compounds are usually used to measure shape selectivity of the phase and this reveals information on the physical microstructure. One of the PAH descriptors which is often used to measure the shape selectivity of a stationary phase is length-to-breadth ratio (L/B). The L/B is defined as the ratio of the longest axis of the molecule, L, and its longest perpendicular axis, B. The structures are assumed to be planar [114]. Wise et al [114] exhaustively studied the relationship between the L/B ratio and the retention of PAHs on reversed-phase C\(_{18}\) column. They found that for
PAHs with the same molecular size, the retentions increased with increasing L/B ratios.

The other major factor influencing the retention of the unsubstituted PAHs is planarity. The nonplanar conformation of the compound is usually due to intramolecular steric affects [115] as illustrated in Fig. 1.10. The most strain takes place in molecules which have a dibenzo(c,g)phenanthrene substructure. Strain can also be caused by the presence of a benzo(c)phenanthrene substructure, but not as much as the former structure. Three-sided bay regions such as the one in phenanthrene can also cause strain even though less important.

![Substructure that can affect the degree of PAH planarity.](image)

Figure 1.10 Substructure that can affect the degree of PAH planarity.

(A) dibenzo(c,g)phenanthrene, (B) benzo(c)phenanthrene, (C) phenanthrene.

Sander and Wise [116] proposed a "Slot model" to describe the effect of L/B ratio and planarity of PAHs to their retention on reversed-phase column. Nonplanar molecules are hindered from penetration into narrow slots, and therefore reduce the retention. On the other hand, planar molecules penetrate more easily into the slots resulting in increased
retention. Similarly, long narrow molecules, i.e those with relatively large L/B ratio, fit into existing slots more readily than square-shaped molecules, i.e those with relatively small L/B ratio. Example of PAHs that are often used as the molecular probes to measure shape selectivity capability of a stationary phase is the pair triphenylene-o-terphenyl [117, 118]. Both have the same molecular weight and similar L/B ratio; triphenylene is a planar compound while o-terphenyl is not. On a typical monomeric ODS stationary phase the selectivity factor (\(\alpha_{\text{trip}/o-\text{ter}}\)) ranges from 1.0 to 1.7, while on a polymeric ODS phase it lies between 2.0 and 2.7 [118].

1.5 Aim of the Project

The general aim of the project is to study the properties of conductive polymers by chromatographic methods and to explore the feasibility of using conductive polymers for chromatographic separations. Polypyrrole and polyaniline were chosen for this study for the following reasons:

1. the polymers have been extensively studied and a large amount of background information is available,
2. they can be synthesized easily using chemical method,
3. the monomers are liquids having relatively high boiling points and soluble in many organic solvents,
4. the polymer film can be grown easily on the surface of silica particles.
The work can be divided into three sections.

1. *Chromatographic characterisation*

In this section the basic properties of the stationary phases prepared from polypyrrole and polyaniline were investigated. A group of test compounds have been used as molecular probes to elucidate the molecular interactions, retention mechanism and chromatographic behaviour of the columns. This part includes chapter 2 and 3.

2. *Protein separation*

The chromatographic separation of biological compounds, such as proteins, has been generally considered to be a challenging task as revealed by continuing development in this field. This section devoted to studying the feasibility of using conductive-polymer-modified silica as column packings for protein separation. A set of protein samples with a wide range of molecular weight was used for this purpose. Effect of salt on protein retention was investigated. The work is presented in Chapter 4.

3. *Redox chromatography*

It is well known that the redox state of conductive polymers can be altered by subjecting the polymers to oxidative or reductive treatment either by electrochemical or chemical methods. In this section the feasibility of using chemical method to manipulate the chromatographic properties of the stationary phases prepared from polypyrrole was investigated. A series of test compounds have been used to probe the effectiveness of the approach. This section is described in Chapter 5.
Chapter 2

Polypyrrole-Coated Silica
2.1 Introduction

This chapter describes an investigation into the properties of polypyrrole using the chromatographic method. In this study it was the stationary phase which was the main target of investigation. To do so polypyrrole coated silica stationary phases were prepared. In addition to gaining information on the fundamental properties of the polymeric phases, the practical utility for chromatographic applications was also examined.

To study the properties of different stationary phases two approaches are usually employed, i.e. non-chromatographic and chromatographic methods. Elemental analysis can provide information on the polymer coverage of silica as well as the chemical composition. The chromatographic characterisation of the stationary phases usually makes use of the information obtained from the retention behaviour of some selected compounds with known properties. For example, benzene, toluene, naphthalene, and anthracene have been used to study hydrophobic interactions [119, 120]. Several other compounds have also been used to study polar interactions in reversed-phase chromatography: aniline, N,N-dimethyl aniline, and phenol [112]; and dimethyl and diethylphthalate [119]. Basic drugs, such as caffeine [121] have also been chosen to study particular molecular interaction. The ability of the stationary phases to discriminate samples on the basis of shape selectivity has been studied using a series of polyaromatic hydrocarbons [115, 122-124]. Thus, the
molecular interactions and retention mechanisms involved in the chromatographic process can be investigated using a properly selected set of standard compounds.

In the course of this work column packing materials were prepared by coating silica gels with polypyrrole chloride and polypyrrole dodecylsulfate. The polymers were prepared by chemical polymerisation. Elemental analysis was used for surface characterisation, while for chromatography characterisation a set of standard test compounds have been selected.

2.2 Experimental

2.2.1 Reagents and materials

All reagents were of analytical reagent (AR) grade purity unless otherwise stated. Pyrrole (Fluka, LR grade) was distilled before use. Methanol (HPLC grade, BDH), acetonitrile (HPLC grade, Mallinkcrodt) and Milli-Q water were employed to prepare the eluent. Sodium dodecylsulfate (SDS) was purchased from SIGMA and FeCl₃ was from BDH. Solutions of benzene, toluene, phenol, benzoic acid, aniline, N,N-dimethylaniline (DMA), diethylphthalate (DEP), dimethylphthalate (DMP), caffeine or theophylline were prepared in the mobile phase. Naphthalene, anthracene, phenanthrene, pyrene, chrysene, benzantracene, perylene, dibenz(a,c)anthracene, triphenylene, benzo(a)pyrene, and o-terphenyl were dissolved in the mobile phase as well as, in some cases, in pure acetonitrile. The molecular structures and
physical descriptors of these polyaromatic hydrocarbons (PAHs) are presented in Figure 2.1 and Table 2.1 respectively.

Silica (Ultrasphere, Beckman) for stationary phase preparations was used as received. The silica has particle size 10 μm; surface area 220m²/g; and pore size 80Å. Stainless steel columns (4.9 x 50 mm) were purchased from Altech.

The commercial column used was μ-Bondapak C₁₈ (stainless steel, Waters) with dimension 3.9 x 300 mm; particle size 10 μm; carbon loading 10% (w/w); and pore size 50-300Å.

Elemental analysis was carried out at the Analytical Laboratory, Australian National University, Canberra.
Figure 2.1 The structure of PAHs
Table 2.1 The properties of PAHs used in this work

<table>
<thead>
<tr>
<th>PAH</th>
<th>Descriptor *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MW</td>
</tr>
<tr>
<td>naphtalene</td>
<td>128</td>
</tr>
<tr>
<td>phenanthrene</td>
<td>178</td>
</tr>
<tr>
<td>anthracene</td>
<td>178</td>
</tr>
<tr>
<td>pyrene</td>
<td>202</td>
</tr>
<tr>
<td>triphenylene</td>
<td>228</td>
</tr>
<tr>
<td>benzantracene</td>
<td>228</td>
</tr>
<tr>
<td>chrysene</td>
<td>228</td>
</tr>
<tr>
<td>perylene</td>
<td>252</td>
</tr>
<tr>
<td>benzo(a)pyrene</td>
<td>252</td>
</tr>
<tr>
<td>dibenz(a,c)anthracene</td>
<td>272</td>
</tr>
<tr>
<td>( o )-terphenyl</td>
<td>230</td>
</tr>
</tbody>
</table>

* Taken from ref. 125, except \( \partial \) (see Sect. 2.3.7)

Vw = Van der Waals volume

log P = hydrophobicity parameter

\( \partial \) = polarizability
2.2.2 Instrumentation

Chromatographic characterisation and separation were performed using an HPLC system consisting of a Kortec K350 pump (ICI) connected to a Rheodyne 7125 injector with 20 μl sample loop or a Dionex basic module with a built-in 50μl loop injector; an ERC 7210 variable wavelength detector (Erma); and a DP 600 chart recorder (ICI). A column packer unit used to pack stainless steel columns is illustrated in Figure 2.2.

![Figure 2.2 Column packer unit.](image)
2.2.3 Preparation of column packings

A chemical polymerisation technique was employed to prepare polypyrrole chloride and polypyrrole dodecylsulfate coated on the surface of silica particles. Basically the coating procedure was as follows: After being dried at 110 °C overnight, 10 g bare silica was suspended in 50 ml hexane containing 0.3 g pyrrole. This suspension was left in a fume hood to evaporate the solvent until free-flowing pyrrole-coated silica particles were obtained. The silicas were then transferred into water-acetonitrile (9:1) solution containing 0.2 M FeCl$_3$ (and 0.05 M SDS to prepare the DS$^-$ containing material).

In this way pyrroles were polymerised and coated onto the surface of silica particles. The coated particles were then filtered with vacuum, washed with copious amount of water, and finally rinsed with acetone.

2.2.4 Packing procedure

The packing materials were packed into the column by the slurry packing method. A solution of 30-35% MeOH/H$_2$O was used as the slurry solvent, while pure methanol was used as the driving solvent during packing. The packing was carried out at 200 atm for 15 minutes or 1.5 minutes per cm column.

2.2.5 Chromatographic measurements

Columns were flushed with water and methanol before use. During measurement the mobile phase flow rate was adjusted to 1 ml/minute.
eluent output was monitored using a UV-Vis detector ($\lambda = 254$ nm) Retention times were recorded using a stopwatch and the dead-time ($t_0$) was estimated from the retention of water. The use of water to estimate the dead time has been proposed by other workers [126-128]. The mobile phase system used was either a mixture of water-methanol or water-acetonitrile, the composition of which could be varied as required.

2.3 Results and Discussion

2.3.1 Column packings

It was found from elemental analysis that the composition of the polymer layer coated on the surface of silica particles depends on the method of preparation used (Table 2.2). For further discussions, silica coated with polypyrrole doped with chloride ion is designated as PPCl/Si while PPDS/Si refers to silica coated with polypyrrole containing both chloride and dodecylsulfate (DS\textsuperscript{-}) ions. PPCl/Si was found to have lower carbon loading than the PPDS/Si. The higher carbon loading on the latter could be due to the presence of DS\textsuperscript{-} counterions which have long alkyl chain. The data also confirms that DS\textsuperscript{-} counterions were incorporated into the polymer matrix during PPDS/Si preparation. The mole ratio of N:Cl:S is 1.0/0.654/0.156 which suggests there is one Cl\textsuperscript{-} or DS\textsuperscript{-} to every 1 to 2 pyrrole units. From calculation it is found that DS\textsuperscript{-} contributes 31\% (w/w) of the polymer composition. For PPCl/Si the mole ratio of N:Cl is found to be 1.0/0.74 which suggests there is one Cl\textsuperscript{-} every 1 to 2 pyrrole units. Usually monomer/counterion ratios are in the range of 3-4 [74, 129]. The lower than expected monomer/counterion ratio in both cases is
probably due to the fact that \([\text{Fe(Cl)}_4]^-\) was incorporated as a counterion as reported previously [66]. Hence not all chlorides found in elemental analysis are of stand-alone counterions.

Table 2.2 Elemental composition (1) of column packings (%wt).

<table>
<thead>
<tr>
<th>Element</th>
<th>PPCI/Si</th>
<th>PPDS/Si</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2.10</td>
<td>3.07</td>
</tr>
<tr>
<td>H</td>
<td>0.41</td>
<td>0.45</td>
</tr>
<tr>
<td>N</td>
<td>0.57</td>
<td>0.70</td>
</tr>
<tr>
<td>Cl</td>
<td>1.04</td>
<td>1.16</td>
</tr>
<tr>
<td>S</td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>% Polymer</td>
<td>4.12</td>
<td>4.07</td>
</tr>
</tbody>
</table>

(1) Prepared as detailed in Experimental.

2.3.2 Retention of small molecules at fixed eluent composition

The retention behaviour of benzene and derivatives as well as two basic drug compounds is shown in Tables 2.3 and 2.4. In all cases the retention index of the test compounds (vs. benzene) was considered in order to eliminate effects due to variations in other chromatographic parameters. The incorporation of \(\text{DS}^-\) counterions, ie. \(\text{C}_{12}\text{H}_{25}\text{SO}_4^-\), into the polypyrrole matrix was expected to induce more hydrophobic character to the polymer coated silica. As observed previously [66] on bare silica there was no retention for all test compounds considered except aniline.
and DMA. Both aniline and DMA had low capacity factors when 50% methanol was used as the mobile phase. The incorporation of DS− into the polymer matrix significantly increases the interaction of the test solutes with the stationary phase (Table 2.3 and 2.4). Since the polymer loadings are similar in both cases (4.07% w/w for PPDS/Si and 4.12% w/w for PPCl/Si) the most likely reason for these changes is an increase in the hydrophobic character of the stationary phase. Visual observation revealed that PPDS/Si particles were more difficult to wet with water confirming this hypothesis.

Phenol which can interact with the stationary phase through hydrogen-bonding, interacted more strongly with the eluent than the stationary phase, hence the low retention observed on PPCl/Si. Decreasing selectivity relative to benzene (k′benzene/k′phenol) on PPDS/Si is apparently due to the increase in hydrophobic interaction between this solute and the stationary phase. Aniline is an electron-donor compound and is known to be capable of interaction with positive sites in the polymer backbone [66] as well as with free silanol groups on any exposed silica surface. Interaction of this molecule with the PPDS/Si stationary phase was greater than on PPCl/Si. However, the relative retention to benzene is lower on PPDS/Si suggesting that DS− counterions reduce the accessibility of the active sites for the solutes, and that the hydrophobic interactions on this column were more prominent.
Table 2.3. Capacity factors of benzene and derivatives on PPCl/Si and PPDS/Si column with 40% MeOH as the mobile phase.

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>PPCl/Si</th>
<th>PPDS/Si</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k'</td>
<td>RI&lt;sub&gt;b&lt;/sub&gt;</td>
</tr>
<tr>
<td>benzene</td>
<td>0.68</td>
<td>1.0</td>
</tr>
<tr>
<td>toluene</td>
<td>1.38</td>
<td>2.04</td>
</tr>
<tr>
<td>phenol</td>
<td>0.32</td>
<td>0.48</td>
</tr>
<tr>
<td>aniline</td>
<td>1.06</td>
<td>1.58</td>
</tr>
<tr>
<td>DMP</td>
<td>1.47</td>
<td>2.17</td>
</tr>
<tr>
<td>DEP</td>
<td>1.63</td>
<td>2.42</td>
</tr>
<tr>
<td>benzoic acid</td>
<td>∞</td>
<td>∞</td>
</tr>
</tbody>
</table>

RI<sub>b</sub> : retention index (retention relative to benzene).

DMP and DEP are polar compounds. They should be retained longer in a column that has weaker interactions with non-polar compounds. In other words they should interact more strongly on PPCl/Si than on PPDS/Si. However, the reverse seems to be true. Both were retained longer on PPDS/Si suggesting that non-polar interactions were more pronounced. Hydrophobic selectivity (α<sub>DEP/DMP</sub>) between these two compounds (DEP has two excess methylene groups) was higher on PPDS/Si than that on PPCl/Si (2.0 and 1.1 respectively).

Anion-exchange properties were evident on both columns since without salt in the eluent benzoic acid was irreversibly retained.
Table 2.4. Capacity factors of basic compounds and/or drugs on PPCl/Si and PPDS/Si column with 50% MeOH as the mobile phase.

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>PPCl/Si</th>
<th>PPDS/Si</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k'$</td>
<td>$R_I_{b}$</td>
</tr>
<tr>
<td>benzene</td>
<td>0.20</td>
<td>1.0</td>
</tr>
<tr>
<td>aniline</td>
<td>0.50</td>
<td>2.51</td>
</tr>
<tr>
<td>DMA</td>
<td>2.08</td>
<td>16.4</td>
</tr>
<tr>
<td>theophylline</td>
<td>2.02</td>
<td>10.1</td>
</tr>
<tr>
<td>caffeine</td>
<td>2.66</td>
<td>13.3</td>
</tr>
</tbody>
</table>

$R_I_{an}$: retention index (retention relative to aniline)

The chromatographic behaviour of both PPDS/Si and PPCl/Si toward basic solutes is summarised in Table 2.4. Again it is demonstrated that the incorporation of $DS^-$ counterions increased the degree of interaction between the solutes and the stationary phase. DMA like aniline is known to interact with free silanol groups as well as with positive sites in the polymer backbone [66]. The relative retention of these compounds (to benzene) were lower on PPDS/Si, confirming further that the incorporation of $DS^-$ counterions results in an increased hydrophobic interaction capability of the column. The separation selectivity among the basic solutes was observed to be significantly better on PPDS/Si as revealed by the distribution of their relative retention to aniline.
2.3.3 Reversed-phase behaviour

In reversed-phase chromatography the retention of the solute is dependent on the composition of the mobile phase in such a way that the retention decreases with increasing organic modifier content. Correlation of the capacity factors of benzene and benzene derivatives with the eluent composition is shown in Figure 2.3 and 2.4. In all instances the $k'$ values of individual samples decrease with increasing methanol content in the mobile phase. These results thus indicate that both columns investigated are capable of performing as reversed-phase columns. The effect, however, is more significant for PPDS/Si.

Figure 2.3 The dependence of retention on mobile phase composition on PPCl/Si.

(1) benzene, (2) toluene, (3) phenol
(4) DMP.
Figure 2.4 The dependence of retention on mobile phase composition on PPDS/Si.

(1) benzene, (2) toluene, (3) phenol,
(4) DMP

The retention of solute under reversed-phase conditions can be expressed as a function of the mobile phase composition [130-132]:

$$\log k' = \log k_w - S\phi$$  \hspace{1cm} (2.1)

where $\phi$ is the volume fraction of the organic modifier in of the eluent, $S$ is the slope of the $\log k'$ - $\phi$ plot and $\log k_w$ is the intercept obtained by extrapolating $k'$ to pure water mobile phase.

The relationships between $\log k'$ and organic fraction in the eluent are shown in Figures 2.5 and 2.6 for PPCI/Si and PPDS/Si respectively. It is shown that good linear relationships are demonstrated by all substances
considered, with all showing typical behaviour of reversed-phase columns. As can be seen, however, the regression line of aniline crosses the other lines due to its lower slope. This may indicate that the aniline undergoes specific interactions with the stationary phases (see Section 2.3.6).

**Figure 2.5** The dependence of log \( k' \) on mobile phase composition on PPCl/Si.

(1) benzene, (2) toluene, (3) phenol, (4) aniline, (5) DMP, (6) DEP.
Figure 2.6 The dependence of log $k'$ on mobile phase composition on PPDS/Si.

(1) benzene, (2) toluene, (3) phenol
(4) aniline, (5) DMP, (6) DEP.
2.3.4. Column performance for small molecules.

The column performance was evaluated on the basis of peak asymmetry factor \((A_s)\), column efficiency \((N)\), and chromatographic separation. The peak asymmetry factors for PPCl/Si and PPDS/Si are presented in Table 2.5. As can be seen, the asymmetry factors for PPDS/Si are lower than that for PPCl/Si especially for those of non-basic compounds, indicating that the peaks obtained on PPDS/Si are more symmetrical. Presumably, this is because of the reduction of the intensity of polar interactions between the solutes and the stationary phase due to the incorporation of DS\(^-\) counterions.

<table>
<thead>
<tr>
<th>Compound</th>
<th>As PCI/Si</th>
<th>As PPDS/Si</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzene</td>
<td>2.7</td>
<td>1.3</td>
</tr>
<tr>
<td>toluene</td>
<td>3.4</td>
<td>1.2</td>
</tr>
<tr>
<td>aniline</td>
<td>2.2</td>
<td>1.6</td>
</tr>
<tr>
<td>DMA</td>
<td>2.5</td>
<td>1.8</td>
</tr>
<tr>
<td>DMP</td>
<td>3.4</td>
<td>1.6</td>
</tr>
</tbody>
</table>
The column efficiency in terms of plate number, $N$, for PPCl/Si and PPDS/Si is given in Table 2.6. It was found that the efficiency of the columns was not high. Both stationary phases were made from aromatic polymers which were capable of undergoing $\pi-\pi$ interaction with the solutes. In addition the polymers also contained polar groups. These would result in slow mass transfer from the stationary phase to the mobile phase which lead to band broadening and tailing, hence low column efficiency. The apparent low efficiency could also arise from the existence of a small-pore structure in the polymer system itself as observed for other polymeric stationary phases [133]. Another possibility is that the packing technique employed to pack the columns was not ideal.

Table 2.6. Column efficiency of PPCl/Si and PPDS/Si

<table>
<thead>
<tr>
<th>Compound</th>
<th>N*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPCl/Si</td>
</tr>
<tr>
<td>toluene</td>
<td>205</td>
</tr>
<tr>
<td>DMP</td>
<td>90</td>
</tr>
<tr>
<td>DMA</td>
<td>100</td>
</tr>
</tbody>
</table>

Calculated by : $N = 5.54 \left( \frac{t_R}{W_{1/2}} \right)^2$

The examples of chromatographic separation are given in Figure 2.7 and Figure 2.8. It is shown that PPDS/Si produces better separation compared with PPCl/Si. Higher efficiency and better peak shape (lower As) for PPDS/Si should contribute to this phenomenon.
Figure 2.7. Chromatographic separation of small molecules on PPCI/Si
(1) benzene, (2) aniline, (3) toluene, (4) DEP.
Mobile phase: 35 % MeOH/H₂O, detection: λ = 254 nm

Figure 2.8. Chromatographic separation of small molecules on PPDS/Si
(1) benzene, (2) aniline, (3) toluene, (4) DEP,
Mobile phase: 50 % MeOH/H₂O, detection: λ = 254 nm
2.3.5 Hydrophobicity parameter determinations.

As has been shown previously there is a linear relationship between log $k'$ and the organic fraction as expressed in equation 2.1. It has been shown [134, 135] that the log $k_W$ value is closely related to log $P$ the logarithm of the partition coefficient which expresses the equilibrium of a solute between an organic (n-octanol) and an aqueous (water) phases. This parameter has been widely accepted as a measure of a solute hydrophobicity [130, 136]. On the other hand, the value of log $k_W$ is a measure of the retention of a solute if pure water is used as the mobile phase at which condition the hydrophobic interaction is dominant. Many workers have investigated the usefulness of the technique and the applicability of various columns to predict solute hydrophobicity in efforts to replace the shake-flask method (for log $P$ measurement) which is tedious and time consuming [130, 137, 138]. A reasonable success for octadecylsilane (ODS) columns for this purpose could be attributed to the similarity in the interphase system existing in these columns, if methanol-water mobile phase system is used, and that of octanol-water system employed for log $P$ measurement [134]. According to Chen et al [139] the intercept of the log $k'$ - $\phi$ plots, i.e log $k_W$, is determined mainly by the properties of the stationary phase. Relating these two parameters to each other, therefore, may reveal how a stationary phase interacts with a solute under specified conditions, which, in turn reflects the properties of the phase and allows prediction of the solute hydrophobicity.

In other occasions Chen et al [140] found that the slopes of the plot, $S$, for a particular solute using the same mobile phase system were almost constant for similar columns, in this case $C_{18}$ columns. They further
proposed that any variation in S reflected the difference in retention mechanisms between the solutes and the stationary phases which could be due to mixed-mode interactions such as the involvement of silanophilic interactions. Based on the points just discussed it is then reasonable to consider that if two columns packed with very different packing materials are compared with each other, their S values should accordingly not be the same if measured with the same solute and the same mobile phase system. In the following discussion a column packed with polypyrrole chloride-coated silica (PPCl/Si) is compared with one packed with polypyrrole dodecylsulfate-modified silica (PPDS/Si).

### Table 2.7 The values of correlation coefficient for individual solutes for PPCI/Si and PPDS/Si

<table>
<thead>
<tr>
<th>Test compound</th>
<th>r</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPCI/Si</td>
<td>PPDS/Si</td>
<td></td>
</tr>
<tr>
<td>benzene</td>
<td>0.995</td>
<td>0.997</td>
<td></td>
</tr>
<tr>
<td>toluene</td>
<td>0.998</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>phenol</td>
<td>0.997</td>
<td>0.990</td>
<td></td>
</tr>
<tr>
<td>aniline</td>
<td>0.997</td>
<td>0.994</td>
<td></td>
</tr>
<tr>
<td>DMA</td>
<td>0.996</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>DMP</td>
<td>0.992</td>
<td>0.999</td>
<td></td>
</tr>
<tr>
<td>DEP</td>
<td>0.997</td>
<td>0.999</td>
<td></td>
</tr>
</tbody>
</table>

In Table 2.7 the values of the correlation coefficients (r) calculated for each test solute for PPCI/Si and PPDS/Si are presented. As can be seen, very good correlation (r > 0.99) for the plots of log k' against φ are
obtained in all cases indicating that extrapolation to pure water to measure log $k_w$ is reasonable.

The log $k_w$ values for PPCI/Si and PPDS/Si are presented in Table 2.8 together with the ones for a Lichrosorb RP-C$_{18}$ obtained from the literature [137]. The standard hydrophobicity parameter values, i.e log P, of the compounds are also included for reference.

**Table 2.8** The values of log $k_w$ for PPCI/Si, PPDS/Si and RP-C$_{18}$, and log P

<table>
<thead>
<tr>
<th>Solute</th>
<th>log $k_w$ PPC/Si</th>
<th>log $k_w$ PPDS/Si</th>
<th>log $k_w$ RP-C$_{18}$ a)</th>
<th>log P b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzene</td>
<td>1.09</td>
<td>1.12</td>
<td>2.08</td>
<td>2.13</td>
</tr>
<tr>
<td>toluene</td>
<td>1.57</td>
<td>1.75</td>
<td>2.62</td>
<td>2.73</td>
</tr>
<tr>
<td>phenol</td>
<td>1.01</td>
<td>1.00</td>
<td>1.23</td>
<td>1.46</td>
</tr>
<tr>
<td>aniline</td>
<td>0.72</td>
<td>0.74</td>
<td>1.13</td>
<td>0.90</td>
</tr>
<tr>
<td>DMA</td>
<td>2.02</td>
<td>2.08</td>
<td>2.36</td>
<td>2.31</td>
</tr>
<tr>
<td>DMP</td>
<td>1.50</td>
<td>2.15</td>
<td>n.a</td>
<td>n.a</td>
</tr>
<tr>
<td>DEP</td>
<td>1.67</td>
<td>2.80</td>
<td>n.a</td>
<td>n.a</td>
</tr>
</tbody>
</table>

a) from ref. 137

b) from ref. 137, 141

It can be seen that the orders of log $k_w$ values for RP-C$_{18}$ and log P from benzene to DMA are the same, i.e in the series toluene > DMA > benzene > phenol > aniline, indicating they are closely related to each other. This
suggests that the interactions or partition processes between two phases in each system have similarities. For the same series of solutes the orders of log $k_w$ values for PPCl/Si and PPDS/Si are the same, i.e in series DMA > toluene > benzene > phenol > aniline. They are however different from both the series in log P and log $k_w$ values of RP-C$_{18}$, indicating that there are differences in separation mechanisms or solute interactions with these new stationary phase systems. Between PPCl/Si and PPDS/Si themselves an important difference is also noticeable. The log $k_w$ values for DMP and DEP are significantly lower on PPCl/Si compared with that obtained on PPDS/Si. Furthermore, these values on PPCl/Si are similar to those obtained for toluene, while on PPDS/Si they are significantly higher. DMP and DEP have carbonyl groups which are polar and act as hydrogen bond acceptors. Water, on the other hand, is a strong hydrogen-bond donor. The interactions between these opposite groups might reduce the intensity of the hydrophobic interactions between DEP or DMP with the stationary phase. In the pure water environment (log $k_w$), the hydrophobic effect was the main driving force for the interactions to occur between the solutes and the stationary phase. The fact that the influence of the polar groups in DMP and DEP in decreasing the retention was more pronounced on PPCl/Si confirms that this column was less hydrophobic than PPDS/Si. It is also shown that log $k_w$ values for benzene and toluene, both hydrophobic compounds, as measured by extrapolation, are significantly lower on PPCl/Si and PPDS/Si compared with that obtained on RP-C$_{18}$. This suggests further that both polymeric columns are less hydrophobic than RP-C$_{18}$, at least in the theoretically pure water system. In Table 2.9 the values of S, i.e the slope of log $k$ - $\phi$ plots, for PPCl/Si and PPDS/Si are presented together with that for C$_{18}$ columns from the literature for comparison.
Table 2.9 The values of $S$ for PPCl/Si, PPDS/Si, and various C18 columns.

<table>
<thead>
<tr>
<th>Solute</th>
<th>S</th>
<th></th>
<th>Average</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPCl/Si</td>
<td>PPDS/Si</td>
<td>C18 a)</td>
<td></td>
</tr>
<tr>
<td>benzene</td>
<td>3.27</td>
<td>2.55</td>
<td>2.76</td>
<td>2.86±0.37</td>
</tr>
<tr>
<td>toluene</td>
<td>3.67</td>
<td>3.21</td>
<td>3.28</td>
<td>3.39±0.25</td>
</tr>
<tr>
<td>phenol</td>
<td>3.83</td>
<td>2.89</td>
<td>2.29</td>
<td>3.00±0.78</td>
</tr>
<tr>
<td>aniline</td>
<td>2.05</td>
<td>1.48</td>
<td>2.13</td>
<td>1.89±0.35</td>
</tr>
<tr>
<td>DMA</td>
<td>3.41</td>
<td>2.85</td>
<td>3.09 b)</td>
<td>3.12±0.28</td>
</tr>
<tr>
<td>DMP</td>
<td>3.34</td>
<td>3.73</td>
<td>n.a</td>
<td>3.54±0.20</td>
</tr>
<tr>
<td>DEP</td>
<td>3.62</td>
<td>4.60</td>
<td>n.a</td>
<td>4.11±0.49</td>
</tr>
</tbody>
</table>

Average: 3.31± (17.8%) 3.04± (31.9%)

a) from ref. 140
b) from ref. 139

It can be seen that the $S$ values vary among the columns considered. The variation in $S$ values suggests that the interaction or separation mechanisms for the solutes are not the same. This reflects differences either in the type of the stationary phase material or in the degree of interactions between the solutes and the underlying silica. Because the slope shows the effect of changes in mobile phase composition on the solute retention, this will also reflect changes in the physical conditions of the stationary phase as a function of the mobile phase. For example it is
known that the structure of the alkyl chains in bonded phases is dependent on mobile phase composition [142, 143].

It is also an interesting point that if one compares the average value of $S$ and its variation for the whole set of solutes for PPCl/Si to that for PPDS/Si, the variation of the average value of $S$ is 17.8 % for PPCl/Si, while it is 31.9 % in the case of PPDS/Si. This significant difference might suggest that the role of solute interactions on the retention was less pronounced on PPCl/Si when compared to that on PPDS/Si. This may further indicate that PPDS/Si could offer better selectivity than PPCl/Si if used as chromatographic stationary phase. This may also suggest that PPDS/Si is more multifunctional.

It has been proposed previously that relating the values of the hydrophobicity parameter measured in one system to that from other system can provide information on the similarity or differences in the interaction or separation mechanism involved in each system [137, 138]. This principle was applied here to compare PPCl/Si with PPDS/Si by plotting log $k_W$ values for PPCl/Si against those for PPDS/Si, The result is presented in Figure 2.9
According to Minick et al [138] and Melander et al [144] equations correlating two sets of hydrophobicity parameter values represent a linear-energy relationship in which the slope is an estimate of how closely the free energies of the processes compare. The slope of the regression equations obtained here is 1.4031 and the correlation coefficient is 0.846. The relatively large deviation from unity in the slope and relatively low correlation coefficient indicate that the physical processes governing the separation mechanisms in each column system were different. Since the mobile phase systems used for log $k_W$ measurement were the same, the
variation in separation mechanism arises from differences in the stationary phases. Both polymeric phases had the same polymer backbone, i.e. polypyrrole. On PPDS/Si, however, the polymer contained long alkyl chains from the surfactant ions of dodecylsulfate. The hydrophilic ends of the chains which bore negative charge were probably oriented towards the polymer chains. The polymer would, therefore, show hydrophobic character. The existence of the long alkyl chain moieties in the polypyrrole matrix would change the interaction behaviour of the parent polymer with the surrounding system.

2.3.6 Effect of eluent composition on selectivity

The selectivity of a chromatographic column is a function of the thermodynamics of the mass-transfer process and this is an important experimental probe in studies of the solute retention process. It can be expressed as:

\[ \log \alpha = \frac{\Delta (\Delta G^0)}{RT} \]  

(2.2)

This reflects the difference between two solutes in the Gibbs free energy of transfer from the mobile phase to the stationary phase. Selectivity can indicate differences in the different stationary phases if the same mobile phase composition is used when comparing different stationary phases [145]. This suggests that the selectivities measured for different stationary phases using a pair of solutes with the same mobile phase composition should be very similar [146,147]. In the following discussion five pairs of
test samples were used to study the selectivity behaviour of PPCl/Si and PPDS/Si under different mobile phase compositions.

The selectivity variation of the toluene-benzene pair with methanol content in the mobile phase is shown in Figure 2.10. The pair reflects the hydrophobic selectivity available and the variation of this selectivity is more noticeable on PPDS/Si. The results showed that the selectivity tended to increase with increasing water content in the eluent, as is expected if hydrophobic interactions are dominant. This behaviour was more pronounced on PPDS/Si indicating that PPCl/Si was more polar.

![Figure 2.10: Selectivity variation of the pair toluene-benzene with methanol content in the eluent. (1) PPDS/Si, (2) PPCl/Si.](image)
Figure 2.11 presents the selectivity profile for the pair phenol-benzene as the mobile phase composition was varied. This pair reflects selectivity arising from hydrogen-bonding because phenol is capable of being a proton-donor as mentioned previously. As can be seen in the selectivity profile, however, this type of selectivity does not seem to alter with the variation of the mobile phase. Similar behaviour was observed with both polymers. Being a proton-donor compound, phenol could interact with the eluent which was methanol-water system. Both the eluent components were capable of acting as proton-acceptor with water being stronger [141].
The proton donor-acceptor interactions between phenol and the eluent increased as the organic fraction of the eluent decreased, resulting in higher contribution of this interaction in reducing the retention of phenol. This effect was, however, compensated by the increase in hydrophobic effect as the water fraction increased. Thus the retention of phenol on both columns was mainly determined by solvent effect. The fact that the selectivities for both columns toward the pair phenol-benzene were similar and almost constant indicated that there was no specific interaction involving polar groups in phenol with ones in both columns.

![Figure 2.12](image)

**Figure 2.12** Selectivity variation of the pair aniline-phenol with methanol content in the eluent. (1) PPDS/Si, (2) PPCl/Si

The selectivity behaviour for a pair of compounds with very different properties is shown in Figure 2.12. Both compounds are polar, however
the aniline is an electron donor or proton acceptor, whereas phenol is a proton donor. One would expect, therefore, that the pair would show significant selectivity. As can be seen in Figure 2.12, this postulation is verified here with the selectivity increasing with increasing methanol content in the eluent. The trend is shown by both columns but it is more pronounced on PPCl/Si. During polymerisation, electrons were removed from pyrrole system resulting in polymeric structure deficient in electrons, hence generating more polar systems (Lewis acid-like systems which are capable of interacting with electron-donor compounds). In the aqueous-organic mobile phase systems, i.e in the reversed-phase mode, the polar interactions manifested themselves more strongly with the increase in organic fraction. Therefore, aniline, being an electron-donor compound, interacted relatively more strongly in this condition with the polymer. In the water-rich environment, this kind of interaction was negligible because the dominant hydrophobic effects took over the role in the solute-stationary phase interactions. The polar interactions just mentioned were not as strong in the case of PPDS/Si, probably because its higher hydrophobicity due to the incorporation of DS\(^-\) counterions in the polymer matrix and that the presence of the long alkyl chains of DS\(^-\) counterions reduced the chance for the aniline to interact with the polymer backbone. The selectivity behaviour of aniline is further supported by the pair aniline-benzene as seen in Figure 2.13. Here again, the retention of aniline relative to benzene increases with methanol content in the eluent. This argument, however, does not necessarily rule out other possibilities, i.e interactions involving silanophilic groups from the silica support. The fact that silanophilic interactions increase with organic content in the eluent has been reported by other workers [134, 148, 149]. At low organic content the water masks or hydrates the
silanolic sites extensively reducing its chance to interact with solutes. These hydrated sites are demasked at high organic content resulting in the increase in silanophilic interactions. In PPDS/Si the silanol groups are probably more effectively shielded by the presence of dodecylsulfate counterions that have long alkyl chain.

**Figure 2.13** Selectivity variation of the pair aniline-benzene with methanol content in the eluent. (1) PPDS/Si, (2) PPCl/Si.
The variation of the selectivity of the pair DEP-DMP when the mobile phase composition was varied is shown in Figure 2.14. Having carbonyl groups in their structure, both compounds are polar but neutral. The side chains containing these polar groups, as well as the alkyl groups at the outside ends, are positioned meta to each other rendering the polar groups less accessible for interaction with the stationary phase. It is reasonable, therefore, to assume that separation is based on the differences in hydrophobicity due to the difference in the length of alkyl side chains they contain. The selectivity on PPDS/Si increases with increasing water content in the mobile phase, typical of hydrophobic selectivity. On PPCl/Si, however, the selectivity is almost unchanged indicating that separation on the basis of hydrophobicity of the solutes is less favourable.

Figure 2.14 Selectivity variation of the pair DEP-DMP with methanol content in the eluent. (1) PPDS/Si, (2) PPCl/Si.
2.3.7 Chromatographic studies using PAHs

In previous work retention of isomeric PAHs was found to increase with an increase in length-to-breadth (L/B) ratio of the molecule on reversed-phase C₁₈ column[150-152]. It was also found that the chromatographic retention of PAHs increased with increasing molecular weight [153, 154]. In this work, the relationship between molecular weight, planarity, the L/B ratios, polarizability, and hydrophobicity of PAHs and the retention characteristics were considered using PPCl/Si and PPDS/Si columns. Similar work was also performed on a μ-Bondapak C₁₈ column for the purpose of comparison.

Effect of molecular weight on retention

The retention characteristic of eleven PAHs used in this work is summarised in Table 2.10. The C₁₈ column with the highest carbon loading (10% as opposed to 2.10% and 3.67% for PPCl/Si and PPDS/Si respectively) exhibited the lowest degree of retention for PAHs with molecular weights above 230. The incorporation of DS⁻ counterions resulted in a level of interaction much larger than expected if we consider that the DS⁻ ion has only 12 C in its alkyl chain compared to 18 C in the C₁₈ stationary phase. The polymeric structure of the polypyrrole column obviously plays some part in PAH retention. Retention was found to increase with the molecular weight of the PAHs on all columns considered. As illustrated in Figure 2.15 the trend is less pronounced on the C₁₈ column, becomes more significant on PPCl/Si, and is most marked on PPDS/Si. Again the influence of DS⁻ counterions is very significant. By nature the polypyrrole backbone is more polar than the C₁₈ moieties; so it would interact less than C₁₈ as is observed with the low molecular weight PAHs. It is assumed that polypyrrole provides a tape- or...
sheet-like configuration and that PAHs have a molecular configuration which conforms with this. Apart from the ability to interact through hydrophobic interaction, PAHs can also interact with polypyrrole backbone through \( \pi-\pi \) interactions which are maximised if the two species are parallel to each other. This may explain why the addition of one or more aromatic nuclei in PAH molecules results in significant effects on retention with polypyrrole coated-silica. DS\(^-\) counterions, apart from providing more hydrophobic sites, must also induce a polymeric structure that results in stronger retention of the PAH molecules. The net result is PAHs have even stronger retention on PPDS/Si and the increment in aromatic nucleus size results in a more significant effect.

![Figure 2.15](image)

**Figure 2.15** Relationship between \( k' \) and molecular weight of PAHs. (1) C\(_{18}\), (2) PPCI/Si, (3) PPDS/Si.
Table 2.10 k' values of PAHs obtained with 90 % MeCN as the eluent.

<table>
<thead>
<tr>
<th>PAH</th>
<th>C18</th>
<th>PPCI/Si</th>
<th>PPDS/Si</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.193</td>
<td>0.019</td>
<td>0.037</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.304</td>
<td>0.082</td>
<td>0.630</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.326</td>
<td>0.138</td>
<td>0.768</td>
</tr>
<tr>
<td>Pyrene</td>
<td>0.451</td>
<td>0.213</td>
<td>2.52</td>
</tr>
<tr>
<td>Triphenylene</td>
<td>0.482</td>
<td>0.358</td>
<td>4.82</td>
</tr>
<tr>
<td>Benzantracene</td>
<td>0.513</td>
<td>0.463</td>
<td>5.69</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.513</td>
<td>0.452</td>
<td>4.93</td>
</tr>
<tr>
<td>Perylene</td>
<td>0.730</td>
<td>1.59</td>
<td>28.30</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.786</td>
<td>1.98</td>
<td>30.10</td>
</tr>
<tr>
<td>Dibenz(a,c)anthracyene</td>
<td>0.797</td>
<td>1.40</td>
<td>&gt;30</td>
</tr>
<tr>
<td>o-Terphenyl</td>
<td>0.301</td>
<td>-0.04</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Effect of \( L/B \) ratio and molecular planarity

If the above argument holds, polypyrrole columns should be more sensitive to the degree of planarity of PAH molecules, i.e. those which are less planar would elute faster because the contact area for interactions to occur is reduced. The results indicate that this is so. Consider the pair of PAHs, triphenylene and \( o \)-terphenyl, which have similar molecular weight and \( L/B \) ratio. As seen in Figure 2.1 triphenylene has a planar structure. \( o \)-Terphenyl, on the other hand, although it has the same number of carbon atoms and double-bonds, cannot assume a planar structure due to the steric repulsion between the two phenyl rings [133]. On both polypyrrole columns \( o \)-terphenyl eluted essentially at the dead volume suggesting there was little or no interaction. Triphenylene, on the other hand, has significant retention on PPDS/Si column, and on the PPCl/Si column some degree of interactions is noticeable. On the C\(_{18}\) column, however, both PAHs shows some degree of retention, with \( o \)-terphenyl slightly less retained. This indicates that the C\(_{18}\) column is also sensitive to changes in planarity in the PAH molecule, however not as sensitive as the polypyrrole columns.

To evaluate the effect of \( L/B \) ratios on selectivity three PAHs with the same molecular weight (MW=228) were considered, i.e. chrysene (\( L/B=1.72 \)), benzanthracene (\( L/B=1.58 \)), and triphenylene (\( L/B=1.12 \)). A noticeable effect was observed with benzanthracene and triphenylene. Benzanthracene, with higher \( L/B \) ratio, tended to elute more slowly than triphenylene. This trend seems to occur on all columns to different extents. Chrysene with the highest \( L/B \) ratio is expected to elute last, however, this was not the case. It eluted before benzanthracene on PPDS/Si column and tended to coelute on both PPCl/Si and C\(_{18}\) column. It has been
suggested [153] that since chrysene has two pairs of interfering hydrogen atoms compared to one in benzanthracene, chrysene has higher intramolecular steric strain which would reduce the planarity resulting in faster elution.

An interesting phenomenon was also observed with the pair of perylene (MW=252, L/B=1.27) and dibenz(a,c)anthracene (MW=272, L/B=1.24). Since they have similar length-to-breadth ratios, their elution order should be governed by the difference in molecular weight, i.e dibenz(a,c)anthracene should elute after perylene. On PPCl/Si the pair eluted in the opposite order. Following the previous argument, dibenz(a,c) anthracene which has one pair of interfering hydrogen atoms more than perylene, would have reduced planarity and hence elute more quickly.

Correlation between PAH retention and its hydrophobicity and polarizability.

As the mobile phase in reversed-phase chromatography is usually polar, the measure of the hydrophobicity of the solute, log P, is an important factor governing solute retention. Therefore solutes with higher hydrophobicity will be retained longer in the non-polar stationary phases resulting in longer retention times. According to Hanai [155] log P has a linear relationship with log k' measured in reversed-phase liquid chromatography:

\[ \log k' = y \log P + m \]  

(2.3)

where y and m are constant in a given system.
PAHs are relatively nonpolar compounds and the predominant interactions should be inductive and dispersive [156]. The molecular polarizability ($\partial$), therefore, should be the important factor that governs the retention order of PAHs [156]. Molecular polarizability can be calculated by the formula proposed by Miller and Savchik [157]:

$$\partial = \frac{4}{N} \left( \sum r_A \right)^2 (\text{Å}^3)$$

(2.4)

where $N$ is the number of electrons in the molecule and $r_A$ is the atomic hybrid component for each atom in a particular hybrid configuration.

In this study the retention of PAHs has been correlated with their log P and $\partial$ parameters (Table 2.1). The relationships between these parameters and log $k'$ are illustrated in Figures 2.16 and 2.17. The results of regression analysis with linear approximation are summarised in Table 2.11.

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>S</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C\textsubscript{18}</td>
<td>PPCl/Si</td>
</tr>
<tr>
<td>$\partial$</td>
<td>0.0631</td>
<td>0.2059</td>
</tr>
<tr>
<td>log P</td>
<td>0.4981</td>
<td>1.6109</td>
</tr>
</tbody>
</table>

**Table 2.11.** Correlation coefficient ($r$) between log $k'$ and descriptors, and slopes of the regression line (S).
Figure 2.16 Relationship between log $k'$ and log $P$ obtained by linear approximation. (1) C$_{18}$, (2) PPCl/Si, (3) PPDS/Si.

Figure 2.17 Relationship between log $k'$ and and $\partial$ of PAHs obtained by linear approximation. (1) C$_{18}$, (2) PPCl/Si, (3) PPDS/Si.
Good correlations exist \((r > 0.9)\) in all instances between both \(\log k'\) and polarizability, and \(\log k'\) and hydrophobicity of the PAH solutes. The polarizability values seem to be better correlated with \(\log k'\) values than do the hydrophobicity values. It also appears that the correlation between these two parameters with \(\log k'\) is lower on PPCl/Si, while similar magnitudes are shown on \(C_{18}\) and PPDS/Si. The slope values of the regression line indicate that PPDS/Si is the most sensitive to the changes in the solute properties, followed by PPCl/Si, with \(C_{18}\) is the least affected.

This may indicate that the microstructures of the area where the interactions took place were different. In \(C_{18}\) phase it could be discrete, while in polymeric phases it was probably a continuous system. This continuous system was probably more prevalent in PPDS than in PPCl as indicated by the fact that the retention increase with the addition of one aromatic ring in PAH structures was the largest on PPDS/Si.

### 2.4 Conclusion

It has been demonstrated that the incorporation of DS\(^-\) counterions into t polypyrrole stationary phases resulted in stronger hydrophobic interactions and improved hydrophobic selectivity. The selectivity towards basic compounds was improved by the introduction of DS\(^-\) counterions.

From the study of solute retentions under different mobile phase compositions several points can be observed:
(i) polar interaction became more dominant at higher methanol concentrations in the eluent (> 40 %),
(ii) at the lower methanol concentration hydrophobic interaction became more pronounced,
(iii) selectivity toward basic compounds increased with increasing methanol concentration.

The increase in polar selectivity at higher methanol concentrations was probably due to the silanol groups becoming more accessible as the stationary phase became more wetted. The increase in polar selectivity with increasing methanol content, however, could also be due to the genuine specific interactions of the solutes and the stationary phase itself. The changes from polar-interaction-dominated range to hydrophobic-interaction-dominated range was slower on PPDS/Si which resulted in smaller S value.

The incorporation of DS− also had a marked effect on the molecular structure of the stationary phase as demonstrated by the PAH studies. The selectivity to PAHs on the basis of molecular weight is found to be in the order:

\[
\text{PPDS/Si} > \text{PPCl/Si} > \text{C}_{18}.
\]

Both polypyrrole coated-silica columns were capable of distinguishing compounds on the basis of planarity. The ability to discriminate PAH isomers on the basis of length-to-breadth ratio was also demonstrated by both polypyrrole columns.
The chromatographic retention sensitivity to the incremental changes of solute physical parameters, as represented by log P and ð of PAHs, was more pronounced on polypyrrole-based columns than that observed on C$_{18}$ column with PPDS/Si being the highest.
Chapter 3

Polyaniline-coated Silica
3.1 Introduction

In the following discussion the use of polyaniline as a chromatographic stationary phase is examined. Such studies reveal information on the practical utility as well as the molecular interactions that take place on such materials. Polyaniline has a very different molecular structure compared to that of polypyrrole which vary from one oxidation state to the other. Based on this point of view it is expected that if polyaniline is employed as a chromatographic stationary phase it would show some differences in chromatographic properties when compared to a polypyrrole-based stationary phase.

In the course of this work the column packing material was prepared by coating silica particles with polyaniline chloride. The aniline monomer was polymerised directly on the surface of the silica particles using a chemical method and was characterised using elemental analysis. A series of standard test compounds similar to those used in the previous study were employed for chromatographic characterisation.
3.2 Experimental

3.2.1 Reagents and materials

Methanol and acetonitrile (HPLC grade) were obtained from BDH. Aniline (AR) for column packing preparation was supplied by BDH and was distilled before use. Water was distilled and purified using the Milli-Q water system from Millipore. Benzene, toluene, phenol, diethylphthalate (DEP), and caffeine were all purchased from Ajax. Dimethylphthalate (DMP), theophylline and aniline were obtained from BDH, while N,N-dimethylaniline (DMA) was from May and Baker. All of these test samples, from benzene to DMA, were dissolved in methanol, and diluted with the mobile phase. The solutions of polycyclic aromatic hydrocarbon samples (PAH), ie. naphthalene, anthracene, phenanthrene, pyrene, chrysene, benzanthracene, perylene, dibenz(a,c)anthracene, triphenylene, benzo(a)pyrene, and o-terphenyl, were prepared in the mobile phase as well as, in some cases, in pure acetonitrile. The chemical structures of these PAHs are presented in Figure 2.1. The following chemicals were of analytical reagent grade purity, and were obtained from BDH: citric acid, Na$_2$HPO$_4$, NaCl, NaNO$_3$, HCl, KH$_2$PO$_4$, H$_3$PO$_4$, NaNO$_2$, sodium oxalate (Na$_2$Ox), and KCN. General purpose reagent grade K$_2$Cr$_2$O$_7$ for aniline polymerisation was purchased from BDH. Silica (Ultrasphere, Beckman) was used as received, with specifications: pore size 300 Å; surface area 90 m$^2$/g; particle diameter 10 μm. The stainless steel chromatographic column (4.9 x 10 cm) was supplied by Altech.
3.2.2 Preparation of column packing

A chemical polymerisation technique was employed to coat polyaniline chloride coated onto the surface of silica supports. Polyaniline chloride-coated packings were prepared as follows: 10 g oven dried silica was added into 50 ml n-hexane containing 1.5 g distilled aniline. The mixture was stirred for 15 minutes and then was left in a fume hood to evaporate the solvent. Free-flowing aniline-coated particles were obtained. Potassium dichromate (1.325 g) was dissolved in 200 ml 1.5 M HCl into which the silica coated with aniline was added. The polymerisation reaction was allowed to proceed for 3 hours. The coated particles were vacuum-filtered and washed with 0.1 M HCl until the filtrate was colourless. It was then equilibrated in 1.3 M HCl for 1 hour before being vacuum-filtered and dried in an oven at 60 °C for one hour.

The packing was then slurry-packed into a stainless steel column with methanol as the packing solvent. The column was washed with methanol for 4 hours, then for 2 hours with acetonitrile, and finally with water to remove any residual reagents and soluble materials such as low molecular weight fractions before use.

3.2.3 Chromatographic measurements

A methanol-water system was used as the mobile phase for the elution of small molecules including benzene and its functionalised derivatives plus caffeine and theophylline. The mobile phase used for the elution of PAHs was acetonitrile-water. Buffer solutions were used as the mobile phases for anion-exchange experiments. Buffer 1 was prepared as follows:
A solution of citric acid (0.1 M) was mixed with a solution of Na$_2$HPO$_4$ in a ratio of 81 : 19 to make a buffer solution of pH 3. The ionic strength was varied by the addition of sodium chloride. Buffer solutions (phosphate buffers) with different pH but the same ionic strength were prepared according to Christian and Purdy [158]. The flow rate was adjusted to be 1 ml/min. A UV-Vis detector, $\lambda = 254$ nm was employed. The dead time ($t_o$) was determined from the retention of water as described previously.

### 3.2.4 Instrumentation

All chromatographic work was carried out using a Dionex LC system consisting of an LCM-3 module coupled with an Advanced Gradient Pump AGP-1. A Beckman 165 UV-Vis variable wavelength detector, and a DP 600 chart recorder (ICI) were employed. The injector had a capacity of 25 $\mu$l.

### 3.3 Results and Discussion

#### 3.3.1 Chemical composition of column packing

The elemental composition of the polymer layer coated on the surface of silica particles is presented in Table 3.1. The chemical structure of polyaniline in its most conductive form (Figure 3.1) suggests that the mole ratio of N : Cl is 2 : 1. Elemental analysis found that it was 2.02 : 1.00 which is in good agreement with the expected value. The general chemical formula of the polymer as revealed from the elemental
composition is $C_{23.9}H_{18.5}N_{4.0} \cdot 2.02$ HCl which is quite close to the theoretical one, i.e. $C_{24}H_{18}N_{4.2}$HCl as denoted in the chemical structure in Figure 3.1. For further discussion, the column packed with this packing material is designated as PAnCl/Si. It was also found that the carbon loading of this packing material was higher than that of PPCI/Si and PPDS/Si described previously. This suggests that the silica support was better coated in this instance.

![Chemical structure of polyaniline chloride.](image)

**Figure 3.1** Chemical structure of polyaniline chloride.

**Table 3.1** Elemental composition of polyaniline chloride-coated silica packing material.

<table>
<thead>
<tr>
<th>Element</th>
<th>% wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5.12</td>
</tr>
<tr>
<td>H</td>
<td>0.43</td>
</tr>
<tr>
<td>N</td>
<td>1.00</td>
</tr>
<tr>
<td>Cl</td>
<td>1.25</td>
</tr>
<tr>
<td>N/Cl*</td>
<td>2.02</td>
</tr>
<tr>
<td>% Polymer</td>
<td>6.48</td>
</tr>
</tbody>
</table>

* mole ratio
3.3.2 Retention of small molecules at fixed eluent composition

The chromatographic properties under reversed-phase conditions were investigated using a series of small molecules including benzene and some functionalised benzene derivatives. In addition theophylline and caffeine were employed as molecular probes. It has been reported previously that a column packed with polypyrrole dodecylsulfate-modified silica (PPDS/Si) behaved as a reversed-phase column (Section 2.3.3). In this study the properties of PAnCl/Si were investigated and a comparison with the interactions observed on PPDS/Si is discussed. The retention data for benzene and some derivatives as well as theophylline and caffeine are summarised in Table 3.2 and 3.3. To eliminate effects due to variations in other chromatographic parameters, the retention index (vs. benzene) is considered.

Benzene and toluene are non-polar compounds, hence their retention on chromatographic phases is determined by hydrophobic interactions. The retention indices of toluene on both columns are similar suggesting that PAnCl/Si has comparable hydrophobic selectivity (selectivity towards the addition of hydrophobic groups) to PPDS/Si. This similarity is further confirmed by the selectivity factors observed for DEP/DMP. The \( \alpha_{DEP/DMP} \), was 1.99 on PPDS/Si and 1.89 on PAnCl/Si. DEP has two excess methylene groups compared to that of DMP. Aniline is an electron-donor compound, its retention index on PAnCl/Si is lower than on PPDS/Si. The introduction of -NH2 which is an electron-donor group, to the benzene structure decreased the retention value observed relative to benzene. As can be seen in the chemical structure in Figure 3.1,
polyaniline is rich in electron-donor groups which may explain why aniline had low retention on PAnCl/Si. Phenol is a proton donor compound and is capable of interacting through hydrogen bonding interactions. The mobile phase used here was methanol-water which could interact with phenol through hydrogen-bonding interactions producing its lower retention compared to the parent compound, benzene. Its retention index on PAnCl/Si, however, is slightly higher than that obtained on PPDS/Si. This is probably because polyaniline is rich in basic groups. DMP and DEP are polar but neutral compounds. Their retention indices are low on PAnCl/Si which does not possess alkyl groups as found on PPDS/Si. The absence of these groups on PAnCl/Si makes the stationary phase and the solutes less structurally similar leading to less intense hydrophobic interactions and hence low retention.

Table 3.2 Retention index (RIₜ) of benzene and derivatives on PAnCl/Si and PPDS/Si with 40% MeOH as the mobile phase.

<table>
<thead>
<tr>
<th>Test compound</th>
<th>PAnCl/Si</th>
<th>PPDS/Si *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.21</td>
<td>2.23</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.83</td>
<td>0.56</td>
</tr>
<tr>
<td>Aniline</td>
<td>0.46</td>
<td>1.09</td>
</tr>
<tr>
<td>DMP</td>
<td>1.47</td>
<td>3.28</td>
</tr>
<tr>
<td>DEP</td>
<td>2.78</td>
<td>6.52</td>
</tr>
</tbody>
</table>

* Data taken from Section 2.3.2
As can be seen in Table 3.3, all basic solutes, including theophylline and caffeine, have much lower retention indices on PAnCl/Si. This again, might be due to the basicity of the stationary phase. DMA with two ethyl groups has a relatively high retention index, probably because these groups induce higher hydrophobic selectivity.

### Table 3.3 Retention Index (RIₜ) of basic compounds on PAnCl/Si and PPDS/Si with 50% MeOH as the mobile phase.

<table>
<thead>
<tr>
<th>Test compound</th>
<th>PAnCl/Si</th>
<th>PPDS/Si *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Aniline</td>
<td>0.52</td>
<td>1.42</td>
</tr>
<tr>
<td>DMA</td>
<td>2.50</td>
<td>6.84</td>
</tr>
<tr>
<td>Theophylline</td>
<td>0.87</td>
<td>9.81</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.68</td>
<td>17.70</td>
</tr>
</tbody>
</table>

* Data taken from section 2.3.4

#### 3.3.3 Column performance for small molecules

The peak asymmetry factor (As) and the plate number (N) indicating the chromatographic performance of the column are given in Table 3.4. As can be seen, the asymmetry factors are reasonably good suggesting that the peak shapes are relatively more symmetrical than those observed on
PPCl/Si and comparable or even slightly better than those obtained on PPDS/Si, as described previously. The efficiency, however, is relatively low as indicated by the low N numbers for the compounds considered. As has been described previously, this could be due, at least in part, to the intrinsic properties of the polymer itself. The polymer consists of extensive conjugated double bonds that are surely capable of undergoing π–π interactions which would result in band broadening, hence producing low efficiency.

The example of chromatographic separation is given in Figure 3.2. The profile of the chromatogram suggests that, at the specified condition, the column had a good selectivity for the compounds considered. Its low efficiency, however, prevented the column from producing better separation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>As</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniline</td>
<td>1.6</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>Toluene</td>
<td>1.2</td>
<td>250</td>
</tr>
<tr>
<td>DMP</td>
<td>1.5</td>
<td>124</td>
</tr>
<tr>
<td>DMA</td>
<td>1.2</td>
<td>192</td>
</tr>
</tbody>
</table>
Figure 3.2 Separation of aniline (1), phenol (2), DMP (3), toluene (4), and DMA (5) on PAnCl/Si.

Mobile phase : 40% methanol
Detection : \( \lambda = 254 \text{ nm} \)
Flow rate : 1.5 ml/min.
### 3.3.4 Reversed-phase behaviour

It has been shown in the previous chapter that PPCl/Si and PPDS/Si are capable of working as reversed-phase columns as indicated by the decrease of the retention of several small molecules with increasing organic fraction in the eluent. As shown in Figure 3.3, similar behaviour was also observed for PAnCl/Si for benzene, toluene, phenol and aniline which indicates that this column can be used for reversed-phase chromatographic separations.

**Figure 3.3** Relationship between k' value and MeOH fraction in the eluent obtained on PAnCl/Si.

(1) toluene, (2) benzene, (3) phenol, (4) aniline.
It has been mentioned previously that the retention of a solute under reversed-phase conditions can be expressed as a function of the eluent composition (Section 2.3.3). The relationship between log $k'$ and the organic fraction in the eluent is shown in Figure 3.4. It can be seen that good linear relationships exist for all substances considered which is typical behaviour of a reversed-phase column.

**Figure 3.4** Relationship between log $k'$ and MeOH fraction in the eluent obtained on PANCl/Si. (1) toluene, (2) benzene, (3) phenol, (4) aniline.
3.3.5 Effect of eluent composition on selectivity

PPCl/Si was compared with PPDS/Si in terms of variation in selectivity when the mobile phase composition was changed (Section 2.3.6). Some differences were observed in this respect. In this section a similar study was carried out using PAnCl/Si and PPDS/Si.

![Graph showing the relationship between \( \alpha_{\text{tol/benz}} \) and MeOH content obtained on PPDS/Si (1) and PAnCl/Si (2).]

The relationship between the selectivity of the pair toluene-benzene and methanol content in the eluent is presented in Figure 3.5. As has been mentioned previously, this pair of compounds reveals the hydrophobic selectivity of the column. The result indicates that with both columns the retention mechanisms are similar. The selectivity tends to increase with decreasing methanol concentration in the eluent.
Figure 3.6 shows the selectivity variation of the pair aniline-benzene. On PPDS/Si the selectivity increases with methanol content in the eluent. As stated previously, this might be due to either specific interactions between aniline and silanol groups or intrinsic polar interactions between aniline and the polypyrrole backbone which are encouraged as the organic content of the eluent increases. On the other hand, the selectivity is almost constant on PAnCl/Si suggesting that the specific interaction is negligible. Polyaniline is rich in amine nitrogens which behave as proton acceptors or electron donors.

![Figure 3.6](image)

**Figure 3.6** Relationship between $\alpha_{\text{an/benz}}$ and MeOH content in the eluent obtained on PPDS/Si (1) and PAnCl/Si (2).
Silanols as proton donors would interact with the immobilised amine nitrogens through hydrogen-bonding interaction. In this case, the polymer seemed to act as a silanol masking agent, like amines that are normally added to the eluent to suppress silanol specific interaction with solutes. Thus the polyaniline film on the silica surface not only shields the silanols physically, but also chemically and suppresses specific interaction with a solute even more effectively than a PPDS layer. This rationalisation may explain the selectivity behaviour of PAnCl/Si toward the pair aniline-benzene which is different from that observed on PPDS/Si. A similar situation is observed for the pair aniline-phenol (Figure 3.7). As the methanol content in the eluent increases, the selectivity is also increased on PPDS/Si, but remains almost constant on PAnCl/Si.

![Figure 3.7](image)

**Figure 3.7** Relationship between $\alpha_{\text{an/phe}}$ and MeOH content obtained on PPDS/Si (1) and PAnCl/Si (2).
Figure 3.8 shows the selectivity behaviour of the pair phenol-benzene, where both columns demonstrate similar selectivity profiles. The selectivity is slightly higher on PAnCl/Si which might be due to the fact that polyaniline has higher proton-accepting capabilities.

![Graph](image)

**Figure 3.8** Relationship between $\alpha_{\text{phe/benz}}$ and MeOH content in the eluent obtained on PPDS/Si (1) and PAnCl/Si (2).

The selectivity variation of the pair DEP-DMP when the methanol content in the eluent is varied, is shown in Figure 3.9. It can be seen that the selectivity increases on both columns with increasing water content. As stated previously, this pair of compounds have similar structures and undergo predominantly hydrophobic interactions on the column. The selectivity variation with mobile phase composition was similar for both polymers indicating that the mechanism of retention for these compounds is similar.
3.3.6 Hydrophobicity parameter determination.

As has been explained previously (Section 2.3.5), $\log k_w$, is a measure of solute hydrophobicity. In Table 3.5 the values of the correlation coefficient ($r$) of the individual solute for PAnCl/Si are presented. As can be seen, a very good correlation ($r > 0.99$) between $\log k'$ and $\phi$ (organic fraction in the eluent) is obtained in all instances indicating that extrapolation to pure water is acceptable to measure $\log k_w$. 

**Figure 3.9** Relationship between $\alpha_{\text{dep/dmp}}$ and MeOH content in the eluent obtained on PPDS/Si (1) and PAnCl/Si (2).
Table 3.5 The coefficient of correlation for individual test compounds as calculated for PAnCl/Si.

<table>
<thead>
<tr>
<th>Test compound</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzene</td>
<td>0.999</td>
</tr>
<tr>
<td>toluene</td>
<td>0.999</td>
</tr>
<tr>
<td>phenol</td>
<td>0.998</td>
</tr>
<tr>
<td>aniline</td>
<td>0.999</td>
</tr>
<tr>
<td>DMA</td>
<td>0.999</td>
</tr>
<tr>
<td>DMP</td>
<td>0.994</td>
</tr>
<tr>
<td>DEP</td>
<td>0.9935</td>
</tr>
</tbody>
</table>

Nong Chen et al (140) found that the S (slope of the regression line of log k' vs. φ) values for the same solute were almost constant for various C₁₈ columns. This indicated that the interaction mechanisms between the particular solute with the stationary phase on various columns was the same or similar suggesting that the S index was mainly determined by the interaction between the solute and the mobile phase. They postulated further that the reproducibility of the S index for a certain solute can serve as a useful parameter for comparison of the energetic homogeneity between different C₁₈ bonded phases. In the following discussion this principle is extended to compare polyaniline and polypyrrole-based phases and C₁₈ columns.
The values of $S$ for PANCl/Si and PPDS/Si are presented in Table 3.6 together with those for C$_{18}$ phases from the literature. It can be seen that the $S$ values vary among the columns considered. The variation seems to be larger for polar solutes and it is largest for aniline. The variation in $S$ indicates that the interaction mechanism between each solute with each type of the column is not identical.

**Table 3.6** The values of $S$ for PANCl/Si, PPDS/Si, and various C$_{18}$ (average values).

<table>
<thead>
<tr>
<th>Test compound</th>
<th>$S$</th>
<th>Average</th>
<th>RSD(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PANCl/Si</td>
<td>PPDS/Si</td>
<td>C$_{18}$$^a$</td>
</tr>
<tr>
<td>benzene</td>
<td>3.03</td>
<td>2.55</td>
<td>2.76</td>
</tr>
<tr>
<td>toluene</td>
<td>3.65</td>
<td>3.21</td>
<td>3.28</td>
</tr>
<tr>
<td>phenol</td>
<td>2.92</td>
<td>2.89</td>
<td>2.29</td>
</tr>
<tr>
<td>aniline</td>
<td>2.52</td>
<td>1.48</td>
<td>2.13</td>
</tr>
<tr>
<td>DMA</td>
<td>3.69</td>
<td>2.85</td>
<td>3.09</td>
</tr>
<tr>
<td>DMP</td>
<td>4.43</td>
<td>3.73</td>
<td>n.a</td>
</tr>
<tr>
<td>DEP</td>
<td>5.55</td>
<td>4.60</td>
<td>n.a</td>
</tr>
</tbody>
</table>

$^a$ from ref. 139 and 140.
$^c$ deviation from the average value.

The hydrophobicity parameter values ($\log k_w$) for PANCl/Si, PPDS/Si and a Lichrosorb RP-C$_{18}$ are presented in Table 3.7 to explore further the chromatographic properties of the polymeric phases compared to the standard RP-C$_{18}$ phase. The standard hydrophobicity parameter values ($\log P$) are also included for reference.
Table 3.7 The values of log $k_w$ for PAnCl/Si, PPDS/Si and RP-C$_{18}$, and log P.

<table>
<thead>
<tr>
<th>Test compound</th>
<th>log $k_w$</th>
<th>log P$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAnCl/Si</td>
<td>PPDS/Si</td>
</tr>
<tr>
<td>benzene</td>
<td>1.46</td>
<td>1.12</td>
</tr>
<tr>
<td>toluene</td>
<td>2.05</td>
<td>1.75</td>
</tr>
<tr>
<td>phenol</td>
<td>1.34</td>
<td>1.00</td>
</tr>
<tr>
<td>aniline</td>
<td>0.91</td>
<td>0.74</td>
</tr>
<tr>
<td>DMA</td>
<td>2.28</td>
<td>2.08</td>
</tr>
<tr>
<td>DMP</td>
<td>2.21</td>
<td>2.15</td>
</tr>
<tr>
<td>DEP</td>
<td>2.94</td>
<td>2.80</td>
</tr>
</tbody>
</table>

a) from ref. 137
b) from ref. 137, 141.

The variation of the hydrophobicity parameter values for each solute is presented in Table 3.8. As can be seen, values of the hydrophobicity parameter for a particular solute varies significantly with all methods of measurement. This indicates that the interaction mechanism between the solutes and the stationary phase in each method was different. If the distribution profile of the variation (in reference to log P value) within each column group is compared to each other, one can see that the profile of distribution is different for each group. On RP-C$_{18}$ the variations seem to be distributed randomly. A certain trend, however, can be observed for both PAnCl/Si and PPDS/Si. The deviation for hydrophobic solutes (i.e. benzene and toluene) are significantly higher than that for the rest,
i.e. phenol, aniline, and DMA which are polar solutes. This may further indicate that the underlying interaction between the solutes and stationary phase in PAnCl/Si and PPDS/Si were different to those encountered with RP-C_{18}. Between the polymeric phases themselves, differences in the variation are also noticeable. The log $k_w$ values of hydrophobic compounds benzene and toluene, for PAnCl/Si are significantly higher than that for PPDS/Si (Table 3.7). This suggests that with the pure water environment, PAnCl/Si is more hydrophobic than PPDS/Si, but still more polar than RP-C_{18}.

**Table 3.8** The variation of the hydrophobicity parameter values measured on PAnCl/Si, PPDS/Si, Lichrosorb RP-C_{18}, and by shake-flask method.

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Average value ± (% deviation)</th>
<th>log $k_w$/log P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All systems</td>
<td>PAnCl/Si</td>
</tr>
<tr>
<td>benzene</td>
<td>1.55 ± (32.6)</td>
<td>1.80 ± (18.7)</td>
</tr>
<tr>
<td>toluene</td>
<td>2.14 ± (20.6)</td>
<td>2.39 ± (14.2)</td>
</tr>
<tr>
<td>phenol</td>
<td>1.19 ± (14.3)</td>
<td>1.40 ± (4.28)</td>
</tr>
<tr>
<td>aniline</td>
<td>0.927 ± (21.1)</td>
<td>0.90 ± (0.55)</td>
</tr>
<tr>
<td>DMA</td>
<td>2.24 ± (6.25)</td>
<td>2.30 ± (0.65)</td>
</tr>
</tbody>
</table>
In order to directly compare PANCl/Si to PPDS/Si to each other, their corresponding log \( k_w \) values are plotted against each other as illustrated in Figure 3.10. Their relationship is quantified by regression analysis with linear approximation. A good linear relationship is obtained as indicated by the high value of its coefficient correlation \( r = 0.9915 \). The magnitude of the regression slope reflects the similarity in physical processes involved in solute retention on each column [138]. The closer the slope value to unity, the higher the degree of similarity between the kind of processes governing the retention. The slope value obtained in this regression analysis is 0.9185 which is close enough to unity to suggest that there is a high degree of similarity in retention or separation mechanisms in the columns considered but that they are still not identical. Because the mobile phase systems used were the same, differences in the retention or
separation mechanisms should come from the variation of the stationary phase systems.

3.3.7 Chromatographic studies using PAHs

*Effect of MW, L/B, and molecular planarity on PAH retention.* Polyaromatic hydrocarbons have been commonly used as molecular probes to characterise the performance of chromatographic columns [150-154, 159, 160]. In this work, the dependence of retention of PAHs eluted on PAN/Cl/Si on such solute parameters as molecular weight, planarity, length-to-breadth ratio (L/B), was investigated. The $k'$ values for PAHs measured on this column are presented in Table 3.9, together with those measured on PPDS/Si for comparison. As expected, the retention in general increased with increasing molecular weight of PAHs. This is because larger molecules provide more contact area between solutes and packings, hence longer elution time. The relationship between molecular weight of PAHs and their retention is illustrated in Figure 3.11. It is also shown that the effect of molecular size on retention was much more pronounced on PPDS/Si than on PANCl/Si which might be the result of differences in morphological structure.

The effect of L/B ratio on the retention can be traced from the elution order of chrysene, benzantracene, and triphenylene which have the same molecular weight (MW=228). The trend in the elution time was chrysene ($L/B = 1.72$) > benzantracene ($L/B = 1.58$) > triphenylene ($L/B = 1.12$) following the order of their L/B ratio. This trend is also demonstrated by the pair perylene-benzo(a)pyrene, each of which has molecular weight of 252. Perylene with lower L/B ratio (1.27) eluted earlier than
benzo(a)pyrene (L/B=1.50). This phenomenon might suggest that the "slot model" proposed by Wise and Sander [116] as a schematic representation for bonded phase, is applicable for this polymeric stationary phase. The dependence of PAH retention on the L/B ratio could then be explained by an "inclusion" mechanism. The solute with higher L/B ratio, which means it is "slimmer", can penetrate into the "slot" and interact more intensively which results in longer retention time.

Table 3.9 k' values of PAHs on PAnCl/Si and PPDS/Si.

<table>
<thead>
<tr>
<th>PAH</th>
<th>PAnCl/Si</th>
<th>PPDS/Si</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k'80</td>
<td>k'90</td>
</tr>
<tr>
<td>naphthalene</td>
<td>0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>phenanthrene</td>
<td>0.29</td>
<td>0.12</td>
</tr>
<tr>
<td>anthracene</td>
<td>0.44</td>
<td>0.29</td>
</tr>
<tr>
<td>pyrene</td>
<td>0.51</td>
<td>0.33</td>
</tr>
<tr>
<td>triphenylene</td>
<td>0.63</td>
<td>0.35</td>
</tr>
<tr>
<td>benzantracene</td>
<td>0.86</td>
<td>0.54</td>
</tr>
<tr>
<td>chrysene</td>
<td>0.99</td>
<td>0.67</td>
</tr>
<tr>
<td>perylene</td>
<td>1.77</td>
<td>1.14</td>
</tr>
<tr>
<td>benzo(a)pyrene</td>
<td>2.03</td>
<td>1.26</td>
</tr>
<tr>
<td>dibenz(a,c)anthracene</td>
<td>1.46</td>
<td>1.07</td>
</tr>
<tr>
<td>o-terphenyl</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

# from section 2.3.7
k'80: k' values obtained with 80 % MeCN/H2O
k'90: k' values obtained with 90 % MeCN/H2O
In order to study the planarity recognition capability of the column, the retention behaviour of two PAHs with very similar L/B value and molecular weight are examined. They are triphenylene (L/B = 1.12, MW = 228), and o-terphenyl (L/B =1.11, MW = 230). This pair has been used by other workers for the same purpose [117, 161]. As can be seen in Table 3.9, the non-planar solute o-terphenyl, elutes at dead volume indicating there is no or very little solute-packing interaction. On the other hand, triphenylene which is a planar solute, has a low, but relatively significant retention compared to that of o-terphenyl. The result indicates that PAnCl/Si is capable of discriminating solutes with different planarity. The phenomenon described here may support the proposed existence of a slot-like structure in the polymeric phase. The slot description was
normally referred to the alkyl chain structures on the C\textsubscript{18}-bonded phases. On polymeric phases, such as in this case polyaniline, micropore structure may be more appropriate for the term. The shape of this structure should be very thin - thinner than the three-dimensional structure of o-terphenyl - so that this molecule could not penetrate resulting in little interactions, hence no retention.

The retention behaviour of the pair perylene-dibenz(a,c)anthracene is also interesting to evaluate. The pair have similar L/B values but different molecular weights, i.e. 252 and 278 respectively leading to the prediction that perylene elutes faster than its counter part. The reality, however, was the reverse. This indicates that the retention could not be explained by the molecular size alone and that other factor(s) may also contribute. For instance, dibenz(a,c)anthracene has three pairs of interfering hydrogen atoms which produce intramolecular steric strain, leading to a reduction in the molecules' planarity [153]. If the above "slot model" argument holds, the molecular configuration of the pair would suggest that perylene would penetrate into the slot more favourably, resulting in stronger interactions. If this rationalisation is correct, the elution behaviour of the pair perylene-dibenz(a,c)anthracene is further evidence of the capability of PAnCl/Si to recognise solute planarity.

*Examples of PAHs separation.*

An example of the separation of PAHs on PAnCl/Si is given in Figure 3.12 and for comparison, the chromatographic separation of PAHs obtained on PPDS/Si is presented in Figure 3.13. It can be observed that under the same conditions, the separation is significantly better on PPDS/Si than on PAnCl/Si. Anthracene and pyrene were coeluted on
PA\text{ncI}/Si, but were well resolved on PPDS/Si. As has been described previously, the effect of PAH molecular size on the retention was much more pronounced on PPDS/Si than on PAnCl/Si, which resulted in better selectivity for PPDS/Si.

\textbf{Figure 3.12} Separation of PAHs on PAnCl/Si(1) naphthalene, (2) anthracene (3) pyrene, (4) chrysene, (5) benzo(a)pyrene.

Mobile phase : 90\% MeCN/H\textsubscript{2}O. Detection : $\lambda = 254$ nm

Flow rate : 1 ml/min.
Figure 3.13 Separation of PAHs on PPDS/Si

(1) naphthalene, (2) anthracene,
(3) pyrene, (4) chrysene.

Mobile phase: 90% MeCN/H$_2$O
Detection: $\lambda = 254$ nm
Flow rate: 1 ml/min.
Effect of hydrophobicity (log P) and polarizability (δ) of PAH on its retention.

Hydrophobic interactions between the solute molecules and the stationary phase is very important in determining reversed-phase column selectivity and for non-polar solutes, the hydrophobicity is the most important selectivity parameter [162]. A solute with greater hydrophobicity will be retained longer in the relatively non-polar stationary phase [152]. In this discussion the effect of solute hydrophobicity of PAHs as indicated by log P values (Table 2.1) on the retention on PAnCl/Si is examined. The relationship between log k' and log P is illustrated in Figure 3.14. As can be seen, the retention indeed increases with solute hydrophobicity as expected in reversed-phase elution.

![Graph](image-url)

**Figure 3.14** Relationship between log k' and log P on PAnCl/Si.
For non-polar molecules which are not ionised, inductive and dispersive interactions become important between molecules interactions which in turn can determine the retention profile of a solute. Under these circumstances the molecular polarizability ($\partial$) of the solute seems to be a very important factor because the energy of inductive and/or dispersive interactions is dependent on the molecular polarizability. Accordingly, if the molecular polarizability is higher, the molecular interactions increase with the consequence of a longer retention time for the solute. In this section, the correlation between the molecular polarizability of PAHs (Table 2.1) and its retention is examined. The relationship between these two parameters is illustrated in Figure 3.15. There seems to be a linear relationship between solute retention and its molecular polarizability where the former increases with the increase of the latter. This phenomenon is in accord with expected observation.

![Image](image.png)

**Figure 3.15** Relationship between log $k'$ and polarizability ($\partial$) on PAnCl/Si.
Molecular polarizability is one of additive bulk parameters. Like molecular mass or molecular volume, the magnitude of molecular polarizability increases with molecular size particularly for the compounds of the same class [163]. Hydrophobic effect is also size dependent, as its magnitude increases with molecular surface area [134]. Therefore, it is not surprising that the retention of the PAHs considered increases with the increase of the values of these parameters as a larger contact area can be provided by the solute with larger molecular size.

The results of regression analysis on log $k'$ vs. $\varrho$ and log $k'$ vs. log $P$ for this column were compared to those obtained on PPCl/Si and PPDS/Si and are summarised in Table 3.10, presenting the value of the slope ($S$) and correlation coefficient ($r$) of the regression obtained by linear approximation.

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>s</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPCl/Si</td>
<td>PPDS/Si</td>
</tr>
<tr>
<td>log $k'$ vs.$\varrho$</td>
<td>0.2059</td>
<td>0.3041</td>
</tr>
<tr>
<td>log $k'$ vs. log $P$</td>
<td>1.6109</td>
<td>2.3177</td>
</tr>
</tbody>
</table>

These results suggest that the bulkiness or molecular size of the solutes is the important factor having a significant effect in the retention of PAHs. The effect, however, varied depending on the type of column. Among the polymeric phases, the effect was most pronounced on PPDS/Si, and least on PAnCl/Si. The effect on PPCl/Si was somewhere between these two.
Polyaniline is a polyaromatic polymer, thus it should show a high degree of \( \pi-\pi \) interaction with PAHs which would be proportional to the PAH size. The results, however, showed that the effect was not pronounced. The mobile phase system used in this study was an acetonitrile-water system. The acetonitrile molecules could interact with the stationary phase through its \( \pi \) system (available on its cyano groups). Such an interaction lowers that of the solute with the stationary phase. Furthermore, the solutes, which were aromatics, could also interact with acetonitrile molecules through the same mechanism, i.e \( \pi-\pi \) interactions. Consequently, the solutes interacted less strongly with the stationary phase resulting in low retention. The same effect probably worked on PPCI/Si also, but apparently to a lower degree. The fact that the molecular size had a large positive effect on the retention of PAHs on PPDS/Si might suggest the influence of a large steric effect, i.e the polymeric layer provided a large contact area for interaction with planar or nearly planar solute molecules. This might be the result of the incorporation of DS\(^-\) counterions that induced a more orderly microstructure to the polymer.

### 3.3.9 Ion-exchange studies

The chemical structure of polyaniline presented in Figure 3.1 shows that this polymer has localized positive charges at the protonated imine groups. To maintain its electroneutrality, the charges are balanced by negative charges from counteranions. Thus this part of the polymer backbone provides anion-exchange sites which would induce the polymer to behave as an anion-exchanger. In this work the capability of PAnCl/Si to act as an anion-exchange chromatographic column for HPLC was investigated.
Effect of ionic strength and pH.

Figure 3.16 shows the retention behaviour of $\text{NO}_2^-$, $\text{NO}_3^-$ and oxalate anion as a function of the ionic strength (as represented by NaCl concentration) of the buffer solution (phosphate/citric acid, pH 3) which was used as the mobile phase. For the anions considered, the retention was found to decrease with increasing ionic strength. This behaviour is typical of conventional ion-exchange resins.

![Graph showing the effect of NaCl concentration on $k'$ measured on PANc/Sl. Eluent: citric acid/phosphate buffer, pH 3.0. (1) $\text{NO}_3^-$, (2) oxalate, (3) $\text{NO}_2^-$.](image)

It is well known that the level of polyaniline protonation is highly dependent on the pH of the environment. Accordingly, the anion-exchange capability of polyaniline would be affected by pH variation. Figure 3.17
shows how the capacity factor of the anions examined decrease as the eluent pH increases as expected, due to deprotonation process occuring at the anion-exchange sites.

Figure 3.17  Effect of eluent pH on $k'$ measured on PAnCl/Si. (1) NO$_3^-$, (2) oxalate. Eluent : phosphate buffer.

*Stability and reversibility of the exchange capacity.*

To verify that the protonation-deprotonation process affected the ion-exchange capacity of PAnCl/Si, the following experiment was performed. The retention of the anions was measured with pure water and/or 0.15 M NaCl. The column was then flushed with water for 40 hrs at 2 ml/min. after which the retentions were remeasured with 0.15 M NaCl as the
mobile phase. The column was then flushed with 0.05 M HCl for 3 hours at 0.2 ml/min. before the retention of the test anion was again remeasured with 0.15 M NaCl as the mobile phase. The results are summarised in Table 3.11

Table 3.11 The retention of anions on PAnCl/Si with water and 0.15 M NaCl as the mobile phases.

<table>
<thead>
<tr>
<th>Anion</th>
<th>$k'$</th>
<th>H$_2$O</th>
<th>0.15 M NaCl</th>
<th>0.15 M NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NO$_2^-$</td>
<td>$\infty$</td>
<td>0.24</td>
<td>0.10</td>
<td>0.43</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>$\infty$</td>
<td>0.72</td>
<td>0.31</td>
<td>1.27</td>
</tr>
<tr>
<td>O$_x^-$</td>
<td>$\infty$</td>
<td>0.65</td>
<td>0.23</td>
<td>1.06</td>
</tr>
<tr>
<td>SCN$^-$</td>
<td>$\infty$</td>
<td>7.67</td>
<td>4.11</td>
<td>8.20</td>
</tr>
</tbody>
</table>

1. after 40 hrs flush with water
2. after 3 hrs flush with 0.05 HCl

The results confirm that polyaniline has a capacity as an anion exchanger and that this capacity is preserved at neutral pH. It is shown that the capacity seems to decrease with time as represented by the results obtained after the column was flushed with water. However, the capacity recovered after the column was treated with hydrochloric acid which indicates strongly that the ion-exchange capacity was controlled by the degree of polymer protonation.
Selectivity series.

It was found that the selectivity series of the anion observed on this column was different from that of conventional quaternary ammonium based anion exchangers. On conventional anion exchangers the sequence is [16,19, 164]:

\[
\text{oxalate} > \text{nitrate} > \text{thiocyanate} \quad (1)
\]

The sequence observed on PAnCl/Si column is

\[
\text{thiocyanate} > \text{nitrate} > \text{oxalate} \quad (2)
\]

It is believed that for conventional ion-exchangers, the retention increases with the valency and the size of the ion as is demonstrated by the first retention sequence. Oxalate ion is the largest with double charges, whereas the other two ions possess single charge, hence the oxalate ion is retained longer. On the other hand, on PAnCl/Si, oxalate ion was eluted the earliest which might indicate that although the ion is doubly charged, the exchange sites were less accessible to this large ion. This suggests further that most of the sites were not located on the surface of the polymeric phase.

3.4 Conclusion

A column packed with polyaniline-coated silica has been prepared and its chromatographic properties have been examined with emphasis on retention mechanisms and intermolecular interactions have been examined. From the study of retention behaviour under fixed mobile phase composition, it is found that the relative retentions of basic
compounds is low on PAnCl/Si when compared with those observed on PPDS/Si. This might be due to the fact that silanol groups are better shielded and that the polyaniline back-bone is rich in amine groups. The better selectivity towards basic compounds exhibited by PPDS/Si might also be due to the intrinsic polar interactions or specific interactions between the polymer itself and the solutes. However, the hydrophobic selectivities for both of the columns investigated are comparable, which may be because PAnCl/Si has a higher carbon loading.

The chromatographic retention of several solutes examined decreases with increasing organic fraction in the eluent. This indicates that PAnCl/Si behaves as a typical reversed-phase column. This conclusion is supported by the fact that \( \log k' \) values are linearly correlated with organic fraction in the mobile phase.

An examination of the feasibility of the use of PAnCl/Si to assess solute hydrophobicity was conducted. There is some evidence to support that the column can be employed for such a purpose by extrapolating \( \log k' \) values obtained at different eluent composition to the one at zero organic fraction from which \( \log k_w \) values (which are proportional to \( \log P \) values) can be obtained. This potential needs to be explored further by incorporating a wider range of test compounds.

The plot of \( \log k_w \) values obtained on PAnCl/Si compared with PPDS/Si indicates that there is some similarity in physical processes governing the retention of solutes in the columns.
The results obtained from the study of PAH retention suggest that PAnCl/Si is capable of discriminating compounds on the basis of planarity. The selectivity on the basis of length-to-breadth ratio was also examined. In general the retention of PAHs on PAnCl/Si is dependent upon molecular-size. This is indicated by retention times which increase with increasing molecular weight, hydrophobicity and polarizability of the compounds. The difference in dependency of retention on molecular size may reflect the difference in molecular structure of the polymer network.

Anion-exchange properties of PAnCl/Si have been verified by examination of the retention behaviour of simple anions upon variation of ionic strength and pH of the eluent, as normally observed on conventional anion-exchangers.
Chapter 4

Chromatographic Study on the Interactions between Proteins and Polypyrrole and Polyaniline.
4.1 Introduction

In the previous chapters the properties of polypyrrole chloride, polypyrrole dodecylsulfate and polyaniline chloride which were prepared by chemical polymerisation have been investigated. For this purpose a series of sample compounds with small molecular weight were used as the probes. From the interactions between the probes and the polymers measured chromatographically the specific properties of the polymers could be inferred.

In the following discussion further examination of the properties of the polymers is described. As in the previous studies a series of standard compounds and chromatographic methods have been employed. Instead of using simple compounds as the molecular probes, however, a set of proteins have been selected for the purpose. Proteins are large and complex molecules consisting of amino acids as the fundamental units or building blocks. Proteins have a large number of charged groups as well as numerous hydrocarbonaceous chains which could result in a variety of interactions with other systems. Conductive polymers are also a complex system; they could be neutral or charged and also have long hydrocarbonaceous chains. The examination of the interaction between these two complex systems, therefore, could be of interest from which further insight on the properties of conductive polymers could be gained.
Previous studies showed that polypyrrole chloride, polypyrrole dodecylsulfate, and polyaniline chloride behaved as reversed-phase systems when used as chromatographic stationary phases. In this study similar chromatographic systems modified to suit the elution of proteins have been employed to measure the interactions between the polymers and the proteins. The properties of the polymers are then deduced from the results of chromatographic measurements.

4.2 Experimental

4.2.1 Reagents and materials

Myoglobin, α-lactalbumin, ovalbumin, bovine serum albumin (BSA), human serum albumin (HSA), and transferrin were purchased from SIGMA and were used as received. Acetonitrile (MeCN) of HPLC grade purity was supplied by BDH, while water was purified by a Milli-Q water system from Millipore. Aniline from Ajax and pyrrole from Fluka were distilled and stored in a refrigerator before use. Spectrograde trifluoroacetic acid (TFA) was used as the eluent additive. Ammonium sulfate (AR) was purchased from BDH. All other reagents were used as obtained. Silica (Ultrasphere, Beckman) for column packing preparation was used as received. The silica has particle size: 10 μm, surface area: 90 m²/g; and pore size: 300 Å. Stainless steel columns (4.9 x 50 or 4.9 x 250 mm) were obtained from Altech.
4.2.2 Preparation of column packings

The chemical polymerisation technique was employed to prepare polypyrrole chloride, polypyrrole dodecylsulfate, and polyaniline chloride coated onto the surface of silica supports.

**Preparation of polypyrrole chloride-coated silica (PPCl/Si).**

Polypyrrole chloride-coated silica was prepared as follows. 10 g silica was dried overnight in an oven at 100 °C before use. The dried silica was then suspended in 40 ml n-hexane containing 1 g distilled pyrrole. After being stirred for 15 minutes, the suspension was placed in a fume hood to evaporate the solvent until free-flowing silica particles were obtained. The particles were now coated with pyrrole. A solution mixture consisting of 30 ml 1 M FeCl₃, 30 ml acetonitrile, and 120 ml water was made up (total volume was 180 ml) into which pyrrole-coated silica was transferred to undergo polymerisation. The reaction was allowed to proceed for 40 minutes. The polymer-coated particles were collected using a Büchner filter and washed with water and acetone.

**Preparation of polypyrrole dodecylsulfate packings (PPDS/Si).**

The preparation of this packing material was carried out in a similar way as that of PPCI/Si. The solution mixture was, however, also containing sodium dodecylsulfate (0.05 M).

**Preparation of polyaniline chloride packings (PAnCl/Si).**

Polyaniline-coated silica used in the present study was from the same batch used in the previous work (Sec. 3.2.2).
The polymer-coated silica packings so produced were then slurry-packed into stainless steel columns using methanol as the driving liquid as described previously. The columns were then flushed (1 ml/minute) with methanol for 4 hours and with acetonitrile for 2 hours before use. This removed low molecular weight materials.

4.2.3 Chromatographic measurement

The chromatographic measurements were carried out under reversed-phase conditions with acetonitrile-water-0.1% TFA system as the mobile phase. This mobile phase system offers several advantages [165, 166]:

* The pH is lower than 2.5. At this pH the proteins are fully protonated and the dissociation of surface silanols is reduced. The interactions between the proteins and silanols are, consequently, minimised avoiding mixed-mode effects in chromatographic measurements.

* The use of TFA increases the solubility of proteins.

* Precipitation of protein is less likely with acetonitrile than with methanol.

To investigate the effect of salt in the mobile phase ammonium sulfate was also added to modify the eluent properties. Isocratic or linear gradient elution were employed, and the effluent was monitored at 280 nm. The flow rate was 1 ml/minute. Retention times were recorded using a stopwatch or automatically by the recorder. The dead-time ($t_0$) was estimated from the retention of water. Protein samples were dissolved in water at 250 µg/ml ($\equiv 0.025\%$ m/v) before injection. Protein recoveries (%) were calculated from the peak area obtained with the column in place.
relative to the one obtained when the column was replaced with an empty 0.05 x 80 cm plastic tube.

4.2.4 Instrumentation

All chromatographic work was carried out on the HPLC system consisting of a Dionex LC unit (LCM-3 module coupled with Advanced Gradient Pump AGP-1), a UV-VIS variable wavelength detector either a Beckman 165 or a Linear 200, and a Shimadzu C-R5A chromatopac integrator. The injector capacity was 25 μl. The results of elemental analysis were obtained from the Analytical Laboratory, Australian National University.

Table 4.1. The properties of proteins used in this study.

<table>
<thead>
<tr>
<th>Protein</th>
<th>MW</th>
<th>pI</th>
<th>Mol % hydrophobicity *</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-lactalbumin</td>
<td>14,000</td>
<td>5.12</td>
<td>35.8</td>
</tr>
<tr>
<td>myoglobin</td>
<td>17,200</td>
<td>7.0</td>
<td>31.4</td>
</tr>
<tr>
<td>ovalbumin</td>
<td>45,000</td>
<td>4.7</td>
<td>n.a</td>
</tr>
<tr>
<td>BSA</td>
<td>68,000</td>
<td>4.9</td>
<td>32.0</td>
</tr>
<tr>
<td>HSA</td>
<td>66,500</td>
<td>4.7</td>
<td>28.4</td>
</tr>
<tr>
<td>transferrin</td>
<td>78,500</td>
<td>5.5-6</td>
<td>n.a</td>
</tr>
</tbody>
</table>

* Calculated by mole % of strongly hydrophobic residues, such as tryptophan, phenylalanine, and tyrosine [167, 168].
4.3 Results and Discussion

4.3.1. Chemical composition of column packings

The elemental compositions of the polymer layer coated on the surface of silica particles for the three column packings of interest are shown in Table 4.2. The mole ratio of N:Cl in PPCI/Si is 2.1 : 1.0 which suggests that one Cl counterion is associated with 2.1 monomer unit. This result is lower than the expected value which is normally in the range 3-4 [129, 74]. It has been reported previously, that this probably is due to the fact that [Fe(Cl)₄⁻] was incorporated as a counterion [65,66]. During PPyDS/Si preparation, both chloride and dodecylsulfate (DS⁻) counterions were incorporated into the polymer matrix. The mole ratio of N : (Cl + DS) is 2.04 : 1.0 which indicates that every 2 pyrrole monomer units were associated with either one Cl⁻ or DS⁻ counterion. The nature of polyaniline chloride has been discussed previously (Section 3.3.1).

<table>
<thead>
<tr>
<th>Packings</th>
<th>% wt</th>
<th>Mole ratio</th>
<th>Polymer % wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C  H  N  Cl</td>
<td>N/Cl</td>
<td></td>
</tr>
<tr>
<td>PPCI/Si</td>
<td>4.20  0.31 1.12 1.4</td>
<td>2.10</td>
<td>5.63</td>
</tr>
<tr>
<td>PPDS/Si</td>
<td>7.31  0.83 1.17 0.71 0.67</td>
<td>4.17 4.00</td>
<td>5.77</td>
</tr>
<tr>
<td>PAnCl/Si</td>
<td>5.12  0.43 1.00 1.25</td>
<td>2.02</td>
<td>6.48</td>
</tr>
</tbody>
</table>
4.3.2. Isocratic elution

The retention behaviours of α-lactalbumin, myoglobin, ovalbumin, BSA, HSA, and transferrin on PPCI/Si, PPDS/Si, and PAnCl/Si column under reversed-phase condition at 1 ml/minute are presented in Figures 4.1 - 6. It is shown that in general the elution patterns of the proteins on the three columns are similar. The retentions observed for all proteins were very sensitive to the concentration of organic modifier in the mobile phase over a very narrow range. This phenomenon is typical for protein elution on reversed-phase columns [128, 169, 170]. Although they showed similar elution behaviour, still there is a difference between these columns. There is a clear tendency on PPCI/Si column that the retention increases gradually as the acetonitrile concentration decreases, especially for ovalbumin, BSA, HSA and transferrin. It can be observed on PPDS/Si column that the retention times are relatively constant when the concentration of acetonitrile in the mobile phase decreases from 50 % to 35 % and increases slightly where the organic modifier decreases further to 30%. When the organic modifier concentration is reduced to 25%, the retention times sharply increases to infinity. On both polypyrrole-based stationary phases all proteins eluted after the dead volume.

On the other hand, all proteins eluted before or at the dead volume on the PAnCl/Si column when the concentration of acetonitrile was in the range of 50-20%. On going from 20 to 15% of organic modifier in the mobile phase, α-lactalbumin, ovalbumin, and transferrin were irreversibly adsorbed, while the retention of BSA, HSA and myoglobin increased resulting in irreversible adsorption when the concentration of acetonitrile was decreased further to 10%. In this experiment the mobile phase system
used was acetonitrile-water with 0.1% TFA, the pH of which was less than 2.5.

It is well known that polypyrrole and polyaniline are positively charged when in the conductive state. This should favour anion-exchange interactions. At the pH lower than 2.5, however, all protein samples have a net positive charge, hence electrostatic attractions between the stationary phases and the proteins were unlikely. Hydrophobic interactions should predominate. The difference in acetonitrile concentration at which the proteins start to be retained irreversibly reflected the difference in hydrophobicity of the column. Table 4.3 presents the apparent critical concentration of acetonitrile in the eluent at which the proteins could still elute with low retention (k'<5). For comparison data from a literature [128] which were obtained on a column packed with poly(styrene-methylvinyl)-modified-silica, designated as PSM/Si, are included.
Figure 4.1 The retention of $\alpha$-lactalbumin on PAnCl/Si (1), PPcI/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.

Figure 4.2 The retention of myoglobin on PAnCl/Si (1), PPcI/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.
Figure 4.3 The retention of ovalbumin on PANCl/Si (1), PPCl/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.

Figure 4.4 The retention of BSA on PANCl/Si (1), PPCl/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.
**Figure 4.5** The retention of HSA on PAnC/Si (1), PPCI/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.

**Figure 4.6** The retention of transferrin on PAnCl/Si (1), PPCI/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.
In most instances the proteins seem to be more retarded on PSM/Si than on the column packed with conductive-polymer-modified silica. This tendency is "surprising" considering that the carbon content of PSM/Si is much lower in comparison with that of the other columns. The apparently higher hydrophobicity of PSM/Si, as indicated by higher protein retention on this column, could be due to the lack of electrostatic repulsion effect between the proteins and the stationary phase which might be the case with the other three columns. It seems that the "net hydrophobicity" of the column is in the following order:

PPDS/Si > PPCl/Si > PAnCl/Si.
4.3.3 Protein recoveries on isocratic elution

To gain more insight on the elution behaviour of the proteins on the columns the recoveries of the proteins upon isocratic elution were calculated (Figures 4.7-12). It has been suggested that proteins are denatured upon contact with the surface of reversed-phase columns [171]. The longer the contact time between the proteins and the stationary phase, the lower the recoveries [172]. The intensity of hydrophobic interactions is also expected to increase with the density and size of the hydrophobic bonding sites at the surface of the stationary phase [14], and the degree of protein denaturation increases with hydrophobicity of the stationary-phase matrix [51]. In general the recovery profiles decreased as the acetonitrile concentration in the eluent approached the critical concentration at which the proteins start to be irreversibly retained. The decrease in recovery indicates that the proteins interacted strongly with the stationary phase and bind more strongly as the concentration of the organic modifier was decreased. This behaviour is not unexpected in the elution of proteins under reversed-phase conditions where hydrophobic interactions become more significant at lower concentration of organic modifier. The stronger interaction could also lead to on-column denaturation within the pores of the packing. According to Nugent et al [172], the various groups in the proteins during denaturation are free to rearrange themselves and to interact with the corresponding groups on adjacent protein molecules which leads to aggregation and precipitation of the proteins.
Figure 4.7 The recovery of α-lactalbumin on PANCl/Si (1), PPCl/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.

Figure 4.8 The recovery of ovalbumin on PANCl/Si (1), PPCl/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.
Figure 4.9 The recovery of myoglobin on PAnCl/Si (1), PPCl/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.

Figure 4.10 The recovery of BSA on PAnCl/Si (1), PPCl/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.
Figure 4.11 The recovery of HSA on PAnCl/Si (1), PPCl/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.

Figure 4.12 The recovery of transferrin on PAnCl/Si (1), PPCl/Si (2), and PPDS/Si (3) as a function of acetonitrile concentration in the mobile phase.
Although the three columns behaved similarly with respect to the protein recoveries and organic modifier concentration, there is still a difference in the recovery profiles with respect to the molecular size of the proteins. For the large proteins, i.e BSA, HSA, and trasferrin, the recoveries were always in the order PAnCl/Si > PPCl/Si > PPDS/Si. This order was not strictly followed by the smaller proteins. Only when acetonitrile concentrations were 30% or lower, the similar order was shown, above which was not. This might indicate that the hydrophobic binding sites on the stationary phase were more available if the conditions of the environment were favourable for hydrophobic interactions to occur. The overall results, however, indicated that the average protein recoveries were the highest on PAnCl/Si, medium on PPCl/Si, and the lowest on PPDS/Si. If the previous arguments hold, the results on recoveries seemed to suggest, again, that PPDS/Si column was the most hydrophobic followed by PPCl/Si column, whereas PAnCl/Si column was the least hydrophobic.

The higher charge density (positive sites) on polyanilline, as suggested by its structure, may contribute to lower hydrophobicity of PAnCl/Si compared to polypyrrole columns.

The decrease in the recovery of ovalbumin on PAnCl/Si was more gradual compared to those observed on polypyrrole columns. This might be due to the fact that commercial ovalbumin contains at least eight forms [173], and that each form could have different sorptive properties. The effect was not pronounced on PPDS/Si which could probably be due to the stronger interactions on this column.
The recovery profile of HSA on PPCl/Si appears to be different from those of other proteins. In reversed-phase elution, the hydrophobic interaction between the stationary phase and the protein is disrupted by the presence of organic modifiers, such as acetonitrile, in the eluent. Stronger disruption is expected with higher organic content resulting in less protein retardation. However, the solubility of protein decreases at higher organic content that would result in lower recovery. The fact that this effect was more pronounced for HSA might be due to its lower hydrophobicity compared with the other proteins (Table 4.1).

4.3.4 Gradient elution

It has been previously demonstrated that there might be a critical concentration of acetonitrile in the mobile phase at which the proteins started to adsorb or desorb from the stationary phase which occurred on all columns considered. In this section the retention behaviour of the proteins under gradient elution where the composition of the mobile phase was changing gradually is discussed. The gradient steepness, defined as the increase in acetonitrile concentration in the eluent per unit time, was the same for all experiments, i.e. 2 %/minute. The gradient time and gradient volume were also the same, i.e. 20 minutes and 20 ml respectively. The composition of the primary solvent, eluent A, was different for each column. This corresponded to the critical acetonitrile concentration as indicated by the isocratic elution experiments. The retention behaviour of individual proteins under these conditions are illustrated in Figures 4.13 - 15.
Figure 4.13 The retention of $\alpha$-lactalbumin (lact), myoglobin (myo), ovalbumin (ova), BSA, HSA, and transferrin (trans) obtained by gradient elution on PPCI/Si.

A: 20% MeCN-0.1% TFA, B: 60% MeCN-0.1% TFA.

A to B: 20 minutes. Flow rate: 1 ml/min.
Figure 4.14 The retention of α-lactalbumin (lact), myoglobin (myo), ovalbumin (ova), BSA, HSA, and transferrin (trans) obtained by gradient elution on PPDS/Si.

A: 25 % MeCN-0.1 % TFA, B: 65 % MeCN-0.1 % TFA.

A to B: 20 minutes. Flow rate: 1 ml/min.
Figure 4.15 The retention of α-lactalbumin (lact), myoglobin (myo), ovalbumin (ova), BSA, HSA, and transferrin (trans) obtained by gradient elution on PAnCl/Si.

A : 10% MeCN-0.1% TFA,  B : 50% MeCN-0.1% TFA.

A to B : 20 minutes. Flow rate : 1 ml / min.

As can be seen, each column interacted with the same set of proteins in a different manner. On PPDS/Si (Figure 4.14) the size of the protein seemed to be an important factor in determining the intensity of the interactions between the proteins and the stationary phase where those with higher molecular weight interacted more strongly as demonstrated by the retention of BSA, HSA and transferrin. Similar elution pattern, with a lesser degree, is also shown on PPCl/Si (Figure 4.13) which might be due to the similarity of the polymer backbone, i.e polypyrrole.
Comparing the retention behaviour of ovalbumin with that of BSA and the results from isocratic elution indicate that ovalbumin interacted more strongly with the stationary phase than BSA which seemed to correspond with the hydrophobicity of the proteins. On gradient elution, however, the reverse seems to be the case, i.e. BSA interacted with the stationary phase more intensely as indicated by the longer retention time. This phenomenon suggested that under dynamic condition there might be a critical molecular size of the protein which should be large enough to give more chance to interact intensively with the stationary phase. This unique behaviour of PPDS/Si might also be due to the incorporation of DS\textsuperscript{−} counterions, which have long alkyl chain into the polypyrrole backbone, which resulted in unique surface structure of the stationary phase [66]. Changing eluent composition during the gradient course could result in changing polymer and protein properties the kinetic of which may be important in determining the interaction between the protein and the stationary phase. In the case of PPCl/Si the reversal behaviour of ovalbumin was not evident.

The elution behaviour of the proteins on PAnCl/Si is shown in Figure 4.15. It can be seen that the elution profile on this polyaniline column is different from that shown on the polypyrrole columns. Apparently the retention of the proteins on PAnCl/Si was not size dependent, or at least the protein molecular size did not play a significant role in their retention as clearly indicated, for example, by the retention of α-lactalbumin and transferrin. The molecular weight of α-lactalbumin is less than one-fifth of that of transferrin. They eluted, however, at almost the same time. This may indicate that the contact areas between the proteins and the stationary phases were small suggesting that the proteins still maintained the compact
structures. α-Lactalbumin with a hydrophobicity higher than BSA was retained longer on this column. This might be due to the much lower molecular size which rendered the hydrophobic sites more accessible for interaction with the stationary phase. The hydrophobicity of ovalbumin is 30% higher than that of BSA [174]. As can be observed, its retention is higher than BSA with higher molecular weight as well as than α-lactalbumin which has lower molecular weight. This trend of retention indicates that hydrophobic interactions played a significant role during the elution of the protein on PAnCl/Si under the specified conditions. However, it was found that transferrin, which is less hydrophobic than BSA [43], was retained longer than BSA but still eluted earlier than ovalbumin. This suggests that another mechanism was involved, probably the molecular size showed its effect.

The elution order of the proteins on PPCl/Si, PPDS/Si, and PAnCl/Si is summarised in Table 4.4. The elution order for some of the proteins on C₈ and PSDVB columns obtained under similar conditions are also included for comparison. It is expected that retention of the proteins on these columns is predominantly by hydrophobic interaction. This is apparently verified by the fact that the elution order of the proteins follows the order of their hydrophobicity. The elution order for ovalbumin, BSA, and transferrin on PPCl/Si and PPDS/Si are the reverse of that on C₈ and PSDVB column. The retention order on PPDS/Si is especially interesting, it is even the reverse of that suggested by the results from isocratic elution (Table 4.3). This phenomenon reflects the dynamic properties of PPDS/Si during the course of gradient elution. Under isocratic elution protein retention was predominantly by hydrophobic
interaction. Under gradient elution molecular size effect apparently showed up.

The elution order on PAnCl/Si appears to be different from either PSDVB and C₈ or polypyrrole columns and seems to be consistent with the elution profile observed in isocratic elution. The results suggest that protein retention on this column was determined by hydrophobic interaction and, to some extent, by molecular size.

Table 4.4. The elution order of proteins observed on PPCl/Si, PPDS/Si, PAnCl/Si, C₈, and PSDVB column.

<table>
<thead>
<tr>
<th>Column</th>
<th>Elution order</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPCl/Si</td>
<td>transferrin &gt; BSA &gt; ovalbumin</td>
</tr>
<tr>
<td>PPDS/Si</td>
<td>transferrin &gt; BSA &gt; ovalbumin</td>
</tr>
<tr>
<td>PAnCl/Si</td>
<td>ovalbumin &gt; transferrin &gt; BSA</td>
</tr>
<tr>
<td>C₈ (1)</td>
<td>ovalbumin &gt; BSA &gt; transferrin</td>
</tr>
<tr>
<td>PSDVB (2)</td>
<td>ovalbumin &gt; BSA &gt; transferrin</td>
</tr>
</tbody>
</table>

PSDVB = poly (styrene-divinilbenzene)

(1) from ref. 42, (2) from ref. 43
The organic content in the eluent when the proteins eluted from the column is given in Table 4.5. As can be seen, the results indicate that the eluent strength needed to elute the proteins from the column is in the following order: PPDS/Si > PPCl/Si > PAnCl/Si. This is consistent with the results obtained in isocratic elution.

**Table 4.5 % MeCN at elution**

<table>
<thead>
<tr>
<th>Protein</th>
<th>% MeCN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPCl/Si</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>24.7</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>23.4</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>22.7</td>
</tr>
<tr>
<td>BSA</td>
<td>26.0</td>
</tr>
<tr>
<td>HSA</td>
<td>25.9</td>
</tr>
<tr>
<td>Transferrin</td>
<td>26.5</td>
</tr>
</tbody>
</table>

The protein recoveries measured on gradient elution are given in Table 4.6. As can be seen, recoveries obtained on polypyrrole columns are generally lower than those measured on polyaniline column. This is especially true for the large proteins. Interesting behaviour is shown by ovalbumin, the most hydrophobic protein used in this study. The recovery of this protein is the highest on PPDS/Si which is the most hydrophobic column. This might be due to the dynamic properties of the polymer under continuously changing eluent composition as described previously.
### Table 4.6 Protein recoveries measured under gradient elution.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Recovery (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPCl/Si</td>
<td>PPDS/Si</td>
<td>PAnCl/Si</td>
<td></td>
</tr>
<tr>
<td>(\alpha)-Lactalbumin</td>
<td>62</td>
<td>65</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>Myoglobin</td>
<td>30</td>
<td>33</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>22</td>
<td>41</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>BSA</td>
<td>45</td>
<td>37</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>HSA</td>
<td>44</td>
<td>32</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Transferrin</td>
<td>55</td>
<td>50</td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>

#### 4.3.5 Effect of salt

The columns used in this study were made by coating silica particles with polymers. Thus there was always possibility that some silanol groups underwent electrostatic interaction with proteins, which in combination with hydrophobic interaction could lead to poor peak shapes and recoveries. Normally, it is assumed that the ionisation of silanol groups can be suppressed by using mobile system with low pH (<3) as used in this study. However this is not always the case [60,166]. To suppress undesirable electrostatic interactions between solutes and the stationary phase, salts such as ammonium sulfate and sodium chloride can be added into the mobile phase [14]. However, it is well known that the salt such as ammonium sulfate is widely used in hydrophobic interaction chromatography due to its capability of, among other things, inducing
hydrophobic interactions [15,33,175,176] at relatively high concentration. In this study the effect of ammonium sulfate at low concentrations on the retention behaviour of α-lactalbumin and transferrin chromatographed isocratically was investigated. The results on PAnCl/Si column are illustrated in Figure 4.16. Without the salt in the mobile phase both proteins eluted before the dead volume. Their retention behaviour, however, changed dramatically with the addition of ammonium sulfate into the mobile phase. They became infinitely retained with addition of a low level of salt. This indicates that the electrostatic solute-packing interactions were negligible, or obscured by relatively strong hydrophobic-type interactions. The results obtained with two other proteins were similar. The behaviour observed for myoglobin was identical to that for α-lactalbumin while the retention behaviour for HSA was as observed for transferrin.

The effect of salt on protein elution from PAnCl/Si was further investigated using gradient elution with different salt concentration at the start of gradient. The concentration of the salt in the gradient mixture decreased with elution time, a similar gradient profile to that normally used in hydrophobic interaction chromatography (HIC). The proteins were injected individually. The results are summarised in Table 4.7.
Figure 4.16  The effect of salt on the retention of transferrin (1) and α-lactalbumin (2) on PAnCl/Si.

Mobile phase: 40 % MeCN / H₂O - 0.1 % TFA.

Flow rate : 1 ml / min.
Table 4.7 The effect of (NH₄)₂SO₄ concentration in the eluent at the start of linear gradient on the retention of proteins on PAnCl/Si.

<table>
<thead>
<tr>
<th>Protein</th>
<th>tᵣ, minute</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grad 1</td>
</tr>
<tr>
<td>α-lactalbumin</td>
<td>1.42</td>
</tr>
<tr>
<td>myoglobin</td>
<td>1.31</td>
</tr>
<tr>
<td>HSA</td>
<td>2.30</td>
</tr>
<tr>
<td>transferrin</td>
<td>2.42</td>
</tr>
</tbody>
</table>

A: 40 % MeCN - 0.1 % TFA - (NH₄)₂SO₄
B: 40 % MeCN - 0.1 % TFA
A to B: 5 minutes, followed by 15 minutes hold on B
Flow rate: 1 ml/min.
[Salt]: 0.01, 0.02, and 0.03 M in Grad 1, Grad 2, and Grad 3 respectively.

Here again, the results demonstrated that the elution behaviour of the proteins on this particular column was very sensitive to the composition of the mobile phase especially for the large proteins. This high sensitivity to the mobile phase composition may reflect the presence of multiple binding-sites or cooperative interactions due to the complexity of the protein structure as well as, probably, the heterogeneity of the polymeric stationary phase itself.
A closer look, however, reveals that something more fundamental might be occurring during the elution process. With isocratic elution (40% acetonitrile-0.1% TFA) without salt, HSA and transferrin eluted very fast, but were retained irreversibly when the salt was added into the mobile phase. In normal HIC as the ionic strength of the mobile phase decreases, hydrophobic interactions decrease, thus allowing the protein to be eluted from the column. The results from gradient elution, however, showed that once the proteins were adsorbed on these polymers it was very difficult to desorb them back into the eluent. This phenomenon suggested that in the chromatographic condition employed the proteins were unfolded to some extent providing much more binding sites, presumably mostly hydrophobic ones, which resulted in much stronger solute-packing interactions. In the folded conformations most of the hydrophobic amino acid side chains (e.g., alanine, isoleucine, leucine, phenylalanine tryptophan, tyrosine, and valine) are buried in the interior of the molecule [175]. When the proteins unfold, these groups become exposed. It is known that in neutral aqueous solutions at relatively high concentrations, (NH₄)₂SO₄ stabilises the protein structure [177]. In reversed-phase chromatographic systems, however, with the eluent and the packings are known to have a tendency to denature protein, the salt probably induces the reverse effect. This proposition is suggested by the fact that the peak areas of protein chromatograms, measured without the column in place and using the eluent which contained the salt, were much higher than those measured using the eluent without the salt. This indicates that the proteins, in the presence of the salt, were unfolded to a greater extent and exposing more uv-absorbing residues. The possibility that the salt, due to its salting out effect, could decrease the solubility of
the protein was unlikely. In fact, at low concentration range, protein solubility increases with salt concentration [176, 178].

At low pH the proteins had a net positive charge and the polymeric stationary phase itself was positively charged. This would result in repulsive interaction between the proteins and the stationary phase. The presence of a salt possibly reduced this repulsive effect which would result in stronger attractive interactions. This mechanism probably contributed, to some extent, to the dramatically changed retention behaviour of the proteins.

The same study was also carried out on PPCI/Si and PPDS/Si column. The effect of (NH$_4$)$_2$SO$_4$ in the mobile phase on the isocratic elution behaviour of proteins is presented in Figures 4.17 - 4.18, while the results obtained by gradient elution with decreasing salt concentration is summarised in Tables 4.8 - 4.9. It can be seen that similar phenomena as observed on PAnCl/Si column were also appearing on PPCI/Si and PPDS/Si column which suggests that the same process was also occurring on this column.
Figure 4.17 The effect of salt on the retention of transferrin (1) and α-lactalbumin (2) on PPCI/Si. Mobile phase: 40% MeCN/H₂O - 0.1% TFA. Flow rate: 1 ml/min.

Figure 4.18 The effect of salt on the retention of transferrin (1) and α-lactalbumin (2) on PPDS/Si. Mobile phase: 40% MeCN/H₂O - 0.1% TFA. Flow rate: 1 ml/min.
Table 4.8. The effect of (NH$_4$)$_2$SO$_4$ concentration in the eluent at the start of linear gradient on the retention of proteins on PPCl/Si.

<table>
<thead>
<tr>
<th>Protein</th>
<th>$t_R$, minute</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grad 1</td>
</tr>
<tr>
<td>$\alpha$-lactalbumin</td>
<td>1.40</td>
</tr>
<tr>
<td>myoglobin</td>
<td>1.22</td>
</tr>
<tr>
<td>HSA</td>
<td>2.15</td>
</tr>
<tr>
<td>transferrin</td>
<td>2.20</td>
</tr>
</tbody>
</table>

Gradient condition as described in the legend of Table 4.7.

Table 4.9. The effect of (NH$_4$)$_2$SO$_4$ concentration in the eluent at the start of linear gradient on the retention of proteins on PPDS/Si.

<table>
<thead>
<tr>
<th>Protein</th>
<th>$t_R$, minute</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grad 1</td>
</tr>
<tr>
<td>$\alpha$-lactalbumin</td>
<td>1.42</td>
</tr>
<tr>
<td>myoglobin</td>
<td>1.31</td>
</tr>
<tr>
<td>HSA</td>
<td>2.30</td>
</tr>
<tr>
<td>transferrin</td>
<td>2.42</td>
</tr>
</tbody>
</table>

Gradient condition as described in the legend of Table 4.7.
To investigate further the effect of salt on the retention of proteins, the elution of the proteins under hydrophobic interaction chromatography conditions were carried out using PPCI/Si. The mobile phase was 50 mM sodium phosphate buffer pH 6.8 with (NH₄)₂SO₄ in the concentration of 0, 0.05, 0.15, 0.30 and 0.50 M. Isocratic elutions were employed and the same set of protein samples were used. It was found that there was no single protein could be eluted from the column under the specified conditions. The measurements were repeated using sodium chloride in place of ammonium sulfate. According to Melander and Hovarth [179], the effect of salt type on protein retention can be related to the molal surface-tension increment (σ) of the salt, i.e. salts with higher σ produce increased retention at equal molal salt concentrations. In this case, the elution with the mobile phases which contain NaCl should produce lower retention because this salt has σ lower than that of (NH₄)₂SO₄ (1.64 vs. 2.16) [34, 179]. It was observed, however, that again no protein could be eluted from the column. The results from these observations might suggest two things: that under the specified conditions, the hydrophobic interactions between the proteins and the stationary phase were relatively strong; and that ion-exchange interactions were not evident.

It has been observed previously that the retention of the proteins was very sensitive to the salt concentration in the mobile phase. This phenomenon was clearly evident under gradient elution. In the following discussion the effect of organic fraction of the eluent when it reached the column inlet (together with a protein solute in it) on the retention of the protein during gradient elution was examined using PPCI/Si. The gradient condition was as follows. Solvent A = 30% MeCN/H₂O-0.1%TFA -0.05 M NaCl; B = 60% MeCN/H₂O-0.1%TFA-0.05 M NaCl; A to B = 30 minutes; and
flowrate = 1 ml/minute. It was observed that the elapsed time between the start of gradient and the time it reached the injector was approximately 2 minutes. Based on this information the injection time was varied at 1 minute increment. The results are summarised in Table 4.10.

**Table 4.10** Effect of delayed injection on the retention of proteins on PPCI/Si.

<table>
<thead>
<tr>
<th>Protein</th>
<th>t_R (minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Delay time (minutes)</td>
</tr>
<tr>
<td>a-lactalbumin</td>
<td>10.05</td>
</tr>
<tr>
<td>myoglobin</td>
<td>4.25</td>
</tr>
<tr>
<td>BSA</td>
<td>oo</td>
</tr>
<tr>
<td>transferrin</td>
<td>oo</td>
</tr>
<tr>
<td>MeCN, % v/v *</td>
<td>30</td>
</tr>
</tbody>
</table>

*) the corresponding organic fraction at the delay time when the protein sample was injected.

It was found that the retention of small proteins, i.e a-lactalbumin and myoglobin, varied with the injection time. BSA eluted from the column only when it was injected after an 8 minute delay time which
corresponded to 36% organic fraction in the injector at the time of injection; and it was eluted very close to the dead time. It did not elute when it was injected within 7 minute delay time. One minute difference in delay time corresponded to 1% difference in organic fraction when it reached the injector. In the case of transferrin, it could be eluted only if it was injected at 10 minutes delay time which corresponded to 38% organic fraction of the eluent when it reached the injector. Again, the protein eluted from the column close to the dead time. The results also suggested that although sodium chloride is normally considered as relatively poor at driving hydrophobic interactions [175], it induced relatively stronger interaction between the proteins and the polymeric stationary phase compared to that if the salt was absent from the eluent.

The phenomena described here, i.e the high sensitivity of protein retention to the mobile phase composition, strongly indicate the occurrence of interactions involving multiple binding sites that were more pronounced for large proteins. Planar structure of the polymer chain may have significant role. The polymer chains also have an extensive π-system which could result in relatively intense π–π interactions with π-systems within the proteins. The contribution of these interactions decreased with increasing acetonitrile concentration in the eluent since it also has π system which can interact with ones in the polymer chains.

4.4. Conclusion

The interaction between polypyrrole- and polyaniline-based conductive polymers which were prepared by chemical polymerisation and protein
has been investigated by employing HPLC method under reversed-phase condition.

From the study of protein elution under isocratic mode there are two points that can be drawn:

(1) In general the intensity of interaction between the conductive polymers investigated and the proteins are in the following order: PPDS > PPCl > PAnCl.

(2) The intensity of interaction between the conductive polymers and the proteins seems to be less strongly when compared to that which occurred between poly ( methyl vinyl - styrene) and the proteins.

The results from gradient elution study reveal that:

(1) There is an indication that the interactions between polypyrrole-based polymers and the proteins were protein-size dependent. This dependency was more clearly shown by polypyrrole dodecylsulfate.

(2) The dependency on the molecular size of the proteins does not seem to exist during polymer-protein interaction in the case of polyaniline polymer. There is an indication, instead, that the hydrophobicity of the proteins was a significant factor in the polymer-protein interaction, i.e. proteins with higher hydrophobicity retained longer regardless of its molecular weight.

(3) The selectivities toward some of the proteins are compared with those obtained on a conventional C8 column as well as with PSDVB column. It is found that the elution order of the specified proteins on polyaniline column is not the same with that on C8 and PSDVB columns. The order is the reverse in the case of both polypyrrole columns.
The interaction between conductive polymers and the proteins in the presence of ammonium sulfate and sodium chloride in the eluent has also been examined. The results reveal that the polymer-protein interactions were enhanced in the presence of the salt at low concentration. This effect was more prominent for the proteins with high molecular weight.
Chapter 5

Chromatographic Studies on the Effect of Exposure to Redox Reagents on the Properties of Polypyrrole Chloride and Polypyrrole Dodecylsulfate.
5.1 Introduction

Perhaps the most interesting property of conductive polymers is that their physicochemical properties can be modified reversibly by switching the oxidation state either electrochemically or chemically. Several workers have made use of this property to design special liquid chromatographic columns [107-109] with conductive packing materials. It has been demonstrated that the retention of analytes can be controlled by application of an electrical potential to the stationary phase.

As has mentioned above the properties of conductive polymers can also be modified by exposure to chemical oxidants/reductants [180-182]. To date, however, there has been no intensive investigations on the effect of chemical oxidation/reduction of conductive polymers on the chromatographic properties. In this work the effect of polymer exposure to redox reagent solutions has been considered. Two polypyrrole-based conductive polymers, i.e polypyrrole chloride and polypyrrole dodecylsulfate, were investigated. The redox reagents employed were ferric chloride and sodium sulfite.

For the purpose of the study the polymers were chemically polymerised directly on the surface of silica particles which were packed later into chromatographic columns. A series of small molecules, polyaromatic hydrocarbons, basic drugs, and amino acids were used as the test
compounds. The columns were chemically treated by injecting either ferric chloride or sodium sulfite solution.

These redox reagents were not included in the eluent to avoid any changes in the solute properties. For example, aniline is oxidised and polymerised in ferric chloride solution. Other basic compounds could be protonated in acidic solution resulting in different chromatographic behaviour. The possibility that biological compounds, such as amino acids, are oxidised or degraded in ferric chloride solution was also taken into account. Furthermore, it has been reported that prolonged exposure of polypyrrole to ferric nitrate solution degraded the polymer [183], which may happen also with ferric chloride solution.

The effects of this treatment were examined through the elution behaviour of the test compounds under specified conditions.

The effect of chemical exposure on the polymer properties was also examined by potentiometric measurement. The effect was studied by measuring the electrode potential of the polymer film coated onto a platinum electrode before and after chemical redox exposure.

5.2 Experimental

5.2.1. Reagents and Materials

All reagents were of analytical reagent (AR) grade unless otherwise stated. Pyrrole (Fluka, LR grade) was distilled before use. HPLC grade methanol and acetonitrile were obtained from Mallinkrodt, while the
water was purified using a Milli-Q water system from Millipore. Benzene and toluene were purchased from Ajax. Aniline and theophylline were commercially supplied by BDH, while N,N-dimethylaniline (DMA) was from May and Baker. Sodium sulfite, ferric chloride, sodium acetate (NaAc), and sodium chloride were from BDH, while sodium dodecylsulfate (SDS) and Tris (hydroxy-methyl amino methane) was purchased from SIGMA. Hydrochloric acid and glacial acetic acid (HAc) were obtained from Ajax. L-Tryptophan (Trp) and L-Tyrosine (Tyr) were also obtained from SIGMA.

The acetate buffer solution of pH 3.8 was prepared from a mixture consisting of 440 ml 0.2 M HAc, 60 ml 0.2 M NaAc and 500 ml water. The buffer solution of pH 7.4 was prepared by mixing 420 ml 0.1 M HCl with 500 ml 0.1M Tris and 80 ml water. Packing materials were prepared as described previously (Sec. 4.2). Silica (Ultrasphere, Beckman) was used as received. The silica has particle size = 10 μm; surface area = 220 m²/g; and pore size = 80 Å. Stainless steel columns (4.9 x 50 mm) were purchased from Altech. These columns were then packed with either polypyrrole chloride or polypyrrole dodecylsulfate-coated silica using a column slurry packer, with methanol as the driving liquid.

The test samples were dissolved in pure solvent of methanol or acetonitrile and diluted with the mobile phases if required. Trp and Tyr were dissolved in water either individually or as a mixture.
5.2.2 Instrumentation

All chromatographic work was performed on an HPLC system which consisted of a Kortec K35D HPLC pump (ICI), a Rheodyne 7125 injector with 20 µl sample loop (Altech), a variable wavelength UV-Vis detector (ICI) and a Kupp and Zonen BD41 strip chart recorder. An electrochemical cell system consisting of Pt disc electrode (as working electrode), Ag/AgCl (as reference electrode), and a piece of RVC (as counter electrode) that was connected to a galvanostat (home-made) was used to prepare polypyrrole chloride-coated Pt electrode. A pH meter Orion SA 520 (Linbrook) was used for electrode potential measurement. Electrochemical redox manipulation was accomplished with a home-made potentiostat.

5.2.3 Chromatographic measurements

Columns were flushed with water and methanol before use. The mobile phase flow rate was adjusted to 1 ml/min. throughout the experiment. The eluent output was monitored at 254 nm. Retention times were recorded with a stopwatch and the dead time (t₀) was estimated from the retention of water. The mobile phase system used was a mixture of water-methanol or water-acetonitrile, the composition of which could be varied as required. The elution of amino acids was carried out with either buffer-methanol or buffer-acetonitrile eluent depending on which column was being used.

The chromatographic properties of the column were manipulated by treating it with either 0.1 M Na₂SO₃ or 0.1 M FeCl₃. The reductant or the oxidant solution was injected into the column through the injector several
times while water was passing through the column at 0.3 ml/min. After each run of several injections the column was flushed with 50-100 column volume of water before the mobile phase to be used was employed.

Ferric chloride (E° = +0.771V) was chosen here as the oxidant for the following reasons. Firstly, it was used in the preparation of the stationary phase thus avoiding any complication which could be brought in if using other chemicals. For example, if Fe(NO₃)₃ is used as the oxidant, NO₃⁻ would be incorporated into the polymer instead of Cl⁻ and this could change the properties of the film. Secondly, it has been reported that neutral polypyrrole films can be oxidised chemically by various metals such as Fe³⁺ [184]. The resulting oxidised films became more conductive although less conductive than the electrochemically oxidised one [178]. Sodium sulfite is a common reductant (E° = -0.93) and because its reaction products are soluble in water they are easily removed from the column.

5.2.4 Potentiometric measurements

Polypyrrole chloride film was grown on Pt electrodes in an aqueous solution of 0.1M pyrrole. The solution also contained 0.1M NaCl and the pH was adjusted to 2.2 with HCl. The film was grown galvanostatically for 5 minutes with a current density of 2 mA/cm². Carbon (RVC) and Ag/AgCl were used as the counter and reference electrodes respectively. The polymer-coated platinum so produced was later designated as PPCl/Pt.

The freshly prepared PPCl/Pt was rinsed carefully with 0.1M NaCl solution and placed in a solution of 0.1M NaCl. The potential was
measured against Ag/AgCl. The potential as a function of time was monitored for 2 hours. The electrode was then removed from the pH meter and connected to a potentiostat. A potential of -0.5 V (against Ag/AgCl) was applied to the electrode for 5 minutes to reduce the polymer film (still in 0.1M NaCl solution) after which the electrode was reconnected to the pH meter and the potential against Ag/AgCl was again measured for 2 hours. After this the electrode was again connected to the potentiostat; at this time a positive potential of +0.4 V (against Ag/AgCl) was applied to the electrode for 5 minutes to reoxidise the film. After this the electrode potential was measured as before.

To measure the effect of the exposure of the polypyrrole film to redox reagents, a fresh PPCI/Pt electrode was prepared in the same way. A similar sequence of measurements was carried out except that this time the electrode was dipped in 0.1M Na₂SO₃ and 0.1M FeCl₃ solution for 5 minutes to reduce and to reoxidise the film respectively. After exposure to each redox solution, the electrode was rinsed with 0.1M NaCl solution before it was put back into a solution of 0.1M NaCl to measure the potential.

During the course of potential measurements the solution was always purged with nitrogen gas to eliminate the effect of oxygen.
5.3 Results and Discussion

5.3.1 Redox switching on conductive polymers

Conductive polymer films, such as polypyrrole, can be switched from conducting to non-conducting by reducing the polymer films, and made conductive again by reoxidizing the film. The counterions are expelled during reduction and incorporated during oxidation according to:

\[
\begin{align*}
(PPy)^+_n A^-_p & \quad +e \\
\leftrightarrow & \\
(PPy)^0_n & \quad + A^-_s
\end{align*}
\]  

(5.1)

where \(A^-_p\) is the counterion in the polymer and \(A^-_s\) is the counterion in solution. This mechanism is applicable especially for small hydrophilic counterions. For large counterions, such as dodecylsulfate (DS\(^-\)), the situation could be different. According to Panero et al [185] and Martinez et al [186] DS\(^-\) counterions are not easily released after the incorporation of the ions into the polymer matrix. This is not just because of their size which is large, but also because of the presence of the polar end and the long alkyl chain in the molecules, the former being compatible with the charged form of the polymer and the latter with the neutral polymer backbone. The counterion movement in the polymer containing such large counterions can be illustrated according to:

\[
\begin{align*}
(PPy)^+_n A^-_p & \quad + C^+_s \\
\leftrightarrow & \\
(PPy)^0_n A^-_p & \quad C^+_p
\end{align*}
\]  

(5.2)
where $C^+_{(s)}$ is the counter-cation in solution and $C^+_{(p)}$ is the counter-cation in the polymer. During polymer reduction, because the counter-anions are not easy to expel, the counter-cations are incorporated into the polymer to preserve the charge neutrality.

Typical cyclic voltammograms of polypyrrole doped with conventional anion and dodecylsulfate ion are given in Figure 5.1 [187].

**Figure 5.1** Typical cyclic voltammograms of polypyrrole prepared electrochemically with conventional anion (1) and dodecylsulfate ion (2) deposited on gold electrodes [187].
There have been some reports, however, that such large incorporated amphiphilic surfactant anions are released on the reduction of the polymer [187-189]. The lost could be up to approximately 50%. The reduction of the polymer could also lead to some decomposition of the anion resulting in a cleavage of the sulfate group to form a carbocation which may rearrange to produce a dodecene, e.g. \( \text{CH}_3(\text{CH}_2)_8\text{CH} = \text{CHCH}_3 \) [189].

There is some indication that redox process in polypyrrole film involves the formation and deformation of quinoid structure in the polymer backbone [189] according to

![Figure 5.2 Interconversion between aromatic and quinoid structure in polypyrrole upon redox process.](image)

Quinoid structure is found when the polymer is in oxidised state or in the as-prepared polypyrrole.

The redox properties of polypyrrole can be modified by treating the polymer with a base and or an acid [190-192, 193]. Treatment of polypyrrole with strong alkali decreases the conductivity that can be restored by treatment with strong protonic acids. According to Inganas et al [193] the effect of chemical modification on the properties of the
Conductive polypyrrole is due to protonation and deprotonation reactions occurring at the nitrogen atom in the polymer as illustrated in Figure 5.3. On immersion of the polymer in basic solution the proton on the nitrogen is removed and forms water with a hydroxyl ion. The counterion $A^-_{(p)}$ is released during this reaction. On treatment with acid the nitrogen is reprotonated and the anion from the acid is incorporated to balance the charge.

It has been reported that treatment of the base-treated polypyrrole with a Lewis $\text{FeCl}_3$ solution in water increased the conductivity of the polymer [193]. The experiment, however, did not distinguish between the influence of the Lewis and Bronsted acid.
Pei and Qian [190] reported that acid-base treatment on polypyrrole films doped with the dodecylsulfate ion (DS\textsuperscript{−}) resulted in effects similar to those observed on polypyrrole films doped with Cl\textsuperscript{−}, NO\textsubscript{3}\textsuperscript{−}, and ClO\textsubscript{4}\textsuperscript{−}. They also reported that, from elemental analysis, a third of the DS\textsuperscript{−} originally incorporated into the films had been expelled upon treatment with 0.2 M NaOH. The incorporation of sodium into the polymer was also detected. Two-thirds of the incorporated sodium was expelled upon subsequent retreatment of the polymer film with 0.2 HCl and the incorporation of a small amount of Cl\textsuperscript{−} into the film was observed.

In the study described hereafter FeCl\textsubscript{3} and Na\textsubscript{2}SO\textsubscript{3} were used as redox reagents to manipulate the oxidation state of the stationary phases prepared from polypyrrole doped with Cl\textsuperscript{−} and DS\textsuperscript{−}. In oxidation-reduction reaction the reagents work normally through electron transfer processes according to:

\[ \text{Fe}^{3+} + e \rightleftharpoons \text{Fe}^{2+} \quad (5.3) \]

\[ \text{SO}_4^{2−} + \text{H}_2\text{O} + 2e \rightleftharpoons \text{SO}_3^{2−} + 2\text{OH}^- \quad (5.4) \]

However, it was observed that 0.1 M FeCl\textsubscript{3} solution was acidic (pH<2.5), while a fresh solution of 0.1 M Na\textsubscript{2}SO\textsubscript{3} was basic (pH ≈ 10). This could bring about a complex situation. As described previously, the polymer can undergo protonation in acidic media, and deprotonation in basic media. In either way, i.e either through protonation-deprotonation or normal redox reactions, the reductions of the polymer would result in the expulsion of the incorporated counter anion accompanied by the decrease in polymer conductivity. In the case of the polymer containing DS\textsuperscript{−} ions, some of
these ions would be released from the polymer, and some sodium ions are incorporated into the polymer.

In the following discussion a chromatographic examination of the effect of exposure of polypyrrole to the redox reagents is described.

5.3.2 Potentiometric measurement

In this experiment the effect of chemical treatment with redox reagents on polypyrrole chloride films was examined potentiometrically. The physical property which was measured was the electrode potential versus Ag/AgCl. The advantage of this approach was that because there was no potential applied to the electrode, any redox effect imposed chemically to the polymeric film on the surface of the electrode substrate was not overrun, hence the effect can be measured independently.

Figure 5.4 shows the electrode potential of polypyrrole chloride-coated platinum (PPCl/Pt) before and after electrochemical treatment. As can be seen the electrode potential of PPCl/Pt after it was reduced at -0.5 V (against Ag/AgCl) was lower compared to the untreated one. After PPCl/Pt was reoxidised by exposing it to +0.4 V (against Ag/AgCl), and its potential was remeasured, the potential approached the original value.

The effect of chemical manipulation with redox reagent on the potential of PPCl/Pt electrode is illustrated in Figure 5.5. Similar profiles to those observed in Figure 5.4 were obtained. After the electrode was exposed to the reducing agent, the potential decreased to well below the original value only to go up again after exposure to the oxidising agent. It was unlikely that this behaviour resulted from the anion-exchange process.
between $\text{SO}_3^-$ and $\text{Cl}^-$ anions. If this were the case, the potential of the electrode after being exposed to the oxidant following the treatment with the reductant would not have gone much beyond the original value. Based on this consideration and the similar potential profiles of the electrode in response to electrochemical and chemical treatment it is reasonable to consider that redox processes did occur during the exposure of PPCI/Pt electrode to the redox reagents. It should be noted, however, that the chemical treatment imposed on the electrode may have, in fact, produced protonation-deprotonation reactions.

![Graph](image)

**Figure 5.4** The effect of electrochemical reduction-oxidation treatment on the electrode potential of PPCI/Pt as a function of time.  
1. no potential was applied to the electrode  
2. after -0.5 V was applied to the electrode  
3. after +0.4 V was applied to the electrode
Figure 5.5 The effect of the exposure to redox reagents on the electrode potential of PPCl/Pt as a function time.

1. no treatment to the electrode
2. after the electrode was in contact with 0.1M Na2SO3
3. after the electrode was in contact with 0.1M FeCl3
5.3.3 Chemical composition of column packings

The elemental composition of the polymer layer coated on the surface of silica particles for polypyrrole chloride (PPCl/Si) and polypyrrole dodecylsulfate (PPDS/Si) is presented in Table 5.1. The packing materials used in this study came from the same batches as used in the work described in Chapter 4. For further discussion, therefore, the readers are referred to section 4.3.1.

<table>
<thead>
<tr>
<th>Packings</th>
<th>% wt</th>
<th>Mole ratio</th>
<th>Polymer % wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>H</td>
<td>N</td>
</tr>
<tr>
<td>PPCI/Si</td>
<td>4.20</td>
<td>0.31</td>
<td>1.12</td>
</tr>
<tr>
<td>PPDS/Si</td>
<td>7.31</td>
<td>0.83</td>
<td>1.17</td>
</tr>
</tbody>
</table>

5.3.4 Retention behaviour of benzene and derivatives

The effect of chemical manipulation on PPCI/Si with the redox reagents upon the retention behaviour of benzene and toluene is presented in Figure 5.6. It can be seen that this manipulation did not affect the retention of the solutes. Benzene and toluene are considered to be non-polar compounds. Their separation in liquid chromatography is therefore mainly determined by hydrophobic interaction. In addition, benzene as an aromatic compound is an electron donor through the π system. So also is toluene which is even more hydrophobic due to the presence of the methyl group. Their retention on the column remained essentially unaffected by
this redox manipulation. The effect of the treatment of PPDS/Si upon the
elution of benzene and toluene is presented in Figure 5.7. It is shown here
that the retention of both analytes decreased monotonously. It seems that
redox manipulation did not affect the interaction mechanisms between the
solutes and the stationary phase. It has been proposed that the
incorporation of DS\(^{-}\) ions into the polymer backbone improved the
hydrophobic selectivity of the phase [66]. The results here seemed to
suggest that this selectivity \((k_{\text{toluene}}/k_{\text{benzene}})\) was not affected by
reduction-oxidation processes until the second reoxidation treatment by
which the selectivity decreased slightly. The fact that the interaction
between the analytes and the stationary phase decreased with time suggests
that one aspect of the polymer properties was irreversibly changed;
probably the DS\(^{-}\) counterions were leaching out slowly and gradually.

As has discussed previously, some DS\(^{-}\) counterions might be expelled
upon reduction of the polymer while most of them remained in the
polymer matrix. To maintain charge neutrality cations were incorporated.
In another study Zhou et al [195] observed that p-toluenesulfonate
counterions (OTs\(^{-}\)) incorporated in polypyrrole remained in the polymer
matrix upon reduction and that the polymer attracted cations to balance
the charge. However, they also found that when this reduced polymer was
left in contact with a solvent, eg. water, for extended time (typically 30
minutes), the film became virtually free of electrolyte. In the present
study the column was flushed with water after each run of reduction.
Therefore, it was likely that a similar phenomenon occurred here, at least
to some extent.
Figure 5.6 The effect of redox reagent treatment of PPCl/Si on the retention of benzene (2) and toluene (1). Mobile phase: 40% MeOH/H2O at 1 ml/min.
Figure 5.7 The effect of redox reagent treatment of PPDS/Si on the retention of benzene (1) and toluene (2).
Mobile phase: 40% MeOH/H₂O at 1 ml/min.
A different situation was observed with aniline and DMA. The effect of redox reagent treatment of PPCl/Si on retention behaviour is presented in Figure 5.8. The retention of these compounds decreased after the column was treated with the reductant and then increased even beyond the original retention values after the column was in contact with the oxidant. Aniline and DMA are amines which have a tendency to share their unpaired electrons, hence they are electron donors through their lone-paired electrons. The retention behaviour of aniline and DMA after the column was treated with the reagents indicated that such treatment changed the ability of the column to undergo electron donor-acceptor (EDA) interactions through the unshared paired electrons. The increased retention after reoxidation suggests that the polymer was then in a higher oxidation state than the rest potential that resulted in the initial retention values.

The possibility that DS\(^-\) counterions were expelled upon reduction and gradually leached out from the PPDS/Si column during the washing and contact with the mobile phase was indicated by the retention behaviour of aniline on this column. After the second run of column treatment with the reductant, the peak intensity of this analyte was lower and needed more injections to obtain a steady peak height. Although it eluted faster, it was clear that some part of the aniline was irreversibly adsorbed which suggested that some reactive sites on the surface of silica had became more accessible. This might also be due to irreversible changes in the polymer during treatment. Attempts were made to elute aniline after the column was reoxidised. After several injections only very small peaks with irregular shapes were observed. This made it difficult to measure the retention times.
Figure 5.8 The effect of redox reagent treatment of PPCl/Si on the retention of aniline (1) and DMA (2).

Mobile phase: 40% MeOH/H2O for aniline and 60% MeOH/H2O for DMA at 1 ml/min.
The retention behaviour of DMA on PPDS/Si is presented in Figure 5.9. It has been observed previously that DS⁻ counterions intercalated in the polypyrrole stationary phase enhanced the capability of the column for hydrophobic interaction with DMA as well as improving the selectivity [66]. As shown in Figure 5.9, interaction reversibility as observed on polypyrrole chloride column is not shown here. Instead, the retention tended to decrease constantly as the column was subjected to reduction-oxidation treatments. This phenomenon seemed to suggest that, again, DS⁻ counterions were leaching out slowly during the course of
chromatographic measurements. This process probably compensated or offset any effect from redox manipulation.

The effect of chemical manipulation on the chromatographic properties of the polymers was also tested using phenol as the test compound. Because phenol is a proton donor compound the effect of the treatment on the proton-accepting ability of the polymers could be examined (Figure 5.10 and 5.11). As can be seen the retention of phenol on both columns was essentially unaffected by the exposure of the coated polymers to the redox reagents which indicated that its proton-accepting ability was not disturbed.

![Figure 5.10](image)

**Figure 5.10** The effect of redox reagent treatment of PPCl/Si on the retention of phenol. Mobile phase: 40% MeOH/H₂O at 1ml/min.
5.3.5 Retention behaviour of PAHs

Experiments were then carried out using two polyaromatic hydrocarbons (PAHs), i.e. triphenylene and benzanthracene. Both have four aromatic rings that are condensed in different configurations. These PAHs were expected to induce stronger π-π interaction with the polymeric phase. Their retentions as a function of the amount of the redox reagents injected into the columns are illustrated in Figure 5.12 and 5.13 for PPCl/Si and
PPDS/Si respectively. Again there is no clear evidence to suggest that the treatment affected the retention of the test compounds. The results seemed to suggest that redox manipulation of the stationary phase did not affect either hydrophobic or π-π solute-packing interaction.

**Figure 5.12** The effect of redox reagent treatment of PPCl/Si on the retention of triphenylene (1) and benzanthracene (2).

Mobile phase: 75% MeCN/H₂O at 1 ml/min.
Figure 5.13 The effect of redox reagent treatment of PPDS/Si on the retention of triphenylene (1) and benzantracene (2).
Mobile phase: 90% MeCN/H₂O at 1 ml/min.
5.3.6 Retention behaviour of basic drugs

The effectiveness of the chemical manipulation on PPCl/Si was also tested with the elution of theophylline and caffeine (Figure 5.14). It is shown that this redox manipulation affected theophylline more than caffeine. These compounds have very similar molecular structure (Figure 5.15), yet they have quite different basicity which would reflect their capability of interacting through EDA interactions. Theophylline is a stronger base ($pK_a = 3.5$) than is caffeine ($pK_a = 0.6$).

![Graph](image)

**Figure 5.14** The effect of redox reagent treatment of PPCl/Si on the retention of caffeine (1) and theophylline (2)

Mobile phase: 60% MeOH/H$_2$O at 1 ml/min.
This suggests that theophylline tends to donate its unshared paired electrons more readily, and is hence more sensitive to the changes in the EDA interactions capability of the column. If this argument holds true, it confirms further that the redox treatment did change the properties of the stationary phase to some degree. Upon reduction the retention of theophylline decreased and went to the original value upon reoxidation. The improvement in selectivity of the column upon reduction is more clearly demonstrated in the chromatograms (Figure 5.16).
Figure 5.16  Effect of the injected redox reagents into PPCl/Si column on the chromatographic separation of theophylline (1) and caffeine (2).

Mobile phase : 65 % MeOH / H₂O at 1 ml / min.

A. before the reagents were injected
B. after 1.0 ml 0.1 M Na₂SO₃ was injected
C. after 0.8 ml 0.1 M FeCl₃ was injected

(following step B).
The prediction that the presence of DS⁻ counterions would induce different kinds of interactions with the analytes was obvious in experiments involving theophylline and caffeine (Figure 5.17). From the retention point of view, both analytes responded to redox manipulation in a parallel way; there was no essential improvement in selectivity. The retention behaviour of theophylline on the PPDS/Si column was different to that observed with the PPCl/Si column (Figure 5.18). As can be seen, column reoxidation did not restore the separation profile. All the results obtained from this column seemed to suggest that the use of redox manipulation to modify the properties of the stationary phase was not effective. Perhaps it changed to some degree, but then it was offset by the possible irreversible change in the polymer backbone on exposure to high pH. The possibility that Na⁺ ions were incorporated due to the treatment of the column with the reductant should also be taken into account. The presence of these cations might induce different behaviour of the polymer when interacting with the test compounds.
Figure 5.17 The effect of redox reagent treatment of PPDS/Si on the retention of theophylline (1) and caffeine (2). Mobile phase: 60% MeOH/H2O at 1 ml/min.
Figure 5.18 Effect of the injected redox reagents into PPDS/Si column on the chromatographic separation of theophylline (1) and caffeine (2).

Mobile phase: 60 % MeOH/H₂O at 1 ml/min.

A. before the reagents were injected
B. after 1.2 ml 0.1 M Na₂SO₃ was injected
C. after 1.0 ml 0.1 M FeCl₃ was injected

(following step B).
5.3.7 Retention behaviour of amino acids

In this study two amino acids, i.e L-tyrosine (Tyr) and L-tryptophan (Trp) were used as the probes for the following reasons:

(1) their isoelectric points (pI) are similar i.e. 5.67 and 5.88 for Tyr and Trp respectively which makes it easier to adjust the pH.

(2) both have aromatic rings which makes it easy to detect by UV detector without derivatisation.

(3) their hydrophobicity is quite different from each other, with Tyr being less hydrophobic than Trp [194], which would result in different degrees of interaction with the polymeric phase.

Depending on the pH of the solution the charges on the amino acids can be manipulated as illustrated in Figure 5.19. In this work the pH of the buffer components in the eluent were 3.8 and 7.4 which were well apart from the pI value of the amino acids.

![Figure 5.19 General illustration of the charge distribution in an amino acid as a function of the pH of solution](image-url)
The effect of the injection of the Na$_2$SO$_3$ solution into the PPCL/Si column on the retention behaviour of Tyr and Trp is illustrated by the chromatograms in Figure 5.20 and 5.21. At pH 3.8 the amino acids are positively charged. The side chains of the amino acids here are hydrophobic in character. The chromatograms obtained suggest that after the exposure of the polymeric phase to the reductant solution the interaction between the amino acids and the phase became stronger due to increased hydrophobic interactions. The rationalization of the phenomenon is that after the polymeric phase was exposed to the reagent, it was reduced so that its positive charges diminished which resulted in the decrease of the repulsion effect between the polymeric phase and the amino acids.

At higher buffer pH (7.4) the amino acids should have net negative charges which could enhance the electrostatic attraction between these and the polymeric phase. The electrostatic interaction in combination with hydrophobic interactions resulted in longer retention times. A noticeable effect can be observed after the introduction of Na$_2$SO$_3$ solution where the retention of the amino acids decreased. This effect was more marked for Trp which is more hydrophobic than Tyr. The decrease in retention of the amino acids is attributed to reduction of the polymeric phase, removing the positive charges on the polymer backbone.
Figure 5.20 Chromatographic separation of tyrosine (1) and tryptophan (2) on PPCl/Si before (A) and after (B) the introduction of 1.0 ml 0.1 M Na₂SO₃ into the column.

Mobile phase: 30 % MeOH / acetate buffer pH 3.8 at 1 ml/min.
Figure 5.21 Chromatographic separation of tyrosine (1) and tryptophan (2) on PPCI/Si before (A) and after (B) the introduction of 1.0 ml Na$_2$SO$_3$ into the column.

Mobile phase: 30 % MeOH/Tris-HCl pH 7.4 at 1 ml/min.
The effects of chemical manipulation on the chromatographic behaviour of the amino acids using PPDS/Si are illustrated by the chromatograms in Figures 5.22 and 5.23. As can be seen in Figure 5.22 the introduction of \( \text{Na}_2\text{SO}_3 \) solution into the column before the injection of the test compounds resulted in dramatic changes to the retention profile of the amino acids at low pH. Similar behaviour as observed on PPCl/Si occurred on this column. On the original column where the polymeric phase was positively charged the electrostatic repulsion between this phase and the amino acids took effect, lowering the retention time. Upon reduction with \( \text{Na}_2\text{SO}_3 \) solution, the positive charges on the polymeric phase are reduced. Because DS\(^-\) counterions had low mobility which rendered them hard to expel on reduction [185-187] the Na\(^+\) cations could well be incorporated to compensate the negative charges of the counterions. When the amino acids were passing through the chemically treated column in low pH buffer, two different interactions could take place, i.e cation-exchange and or hydrophobic interactions. The positively charged amino acids exchanged with sodium ions, a process that would be accompanied by hydrophobic interactions. As can be seen in Figure 5.22B, the amino acids were then more strongly retained.

In Figure 5.23 the effect of chemical manipulation of PPDS/Si on the retention of the amino acids at neutral pH is shown. Under these conditions the amino acids have a negative net charge encouraging stronger interactions with the untreated positively charged polymeric phase (Figure 5.23A). After the column was treated with \( \text{Na}_2\text{SO}_3 \) solution it was expected that the interaction between the polymeric phase and the amino acids would decrease. However, this was not observed (Figure 5.23B). Instead of becoming weaker, the interactions were even stronger.
The reason behind this phenomenon was not immediately obvious; the possibility of the inclusion of Na\(^+\) was probably responsible for this behaviour. Perhaps the inclusion of these positively charged ions into the polymer increased the attractive interactions between the polymer and the negatively charged amino acids.

**Figure 5.22** Chromatographic separation of tyrosine (1) and tryptophan (2) on PPDS/Si before (A) and after (B) the introduction of 1.2 ml 0.1 M Na\(_2\)SO\(_3\) into the column.

Mobile phase: 30% MeCN / acetate buffer pH 3.8 at 1 ml/min.
Figure 5.23 Chromatographic separation of tyrosine (1) and tryptophan (2) on PPDS/Si before (A) and after (B) the introduction of 1.2 ml 0.1 M Na₂SO₃ into the column.

Mobile phase: 30 % MeCN / Tris-HCl pH 7.4 at 1 ml / min.
5.3.8 Reversibility of the effect of the chemical treatment

It has been demonstrated previously that chemical treatment of the polypyrrole chloride column with redox reagents alters the properties of the polymeric phase as revealed by the elution behaviour of theophylline and caffeine. The reversibility of this treatment was then considered. It is commonly believed that the oxidation state of conductive polymers, such as polypyrrole, can be reversibly altered by reduction-oxidation processes either chemically or electrochemically. In the following discussion the reversibility was tested chromatographically and determined by the variation in the capacity factors observed for theophylline and caffeine.

Figure 5.24 shows the result of reversibility test on PPCl/Si. The retention of theophylline and caffeine was first measured on the fresh column and remeasured after the column was alternately treated by the injection of Na$_2$SO$_3$ and FeCl$_3$ solution. As can be seen the reversibility of the effect of the chemical manipulation was verified. The retention of theophylline decreased to almost zero upon treatment with the reductant and back to almost normal following the treatment of the column with the oxidant. An opposite trend was observed for caffeine.

The reversible behaviour observed with PPCl/Si was not observed with PPDS/Si (Figure 5.25). This does not necessarily mean, however, that oxidation-reduction processes did not occur. As revealed by the retention behaviour of Tyr and Trp in the previous discussion, the lack of the fluctuation in retention on this column for the test compounds might be due to the different mechanism in the reduction-oxidation processes.
Figure 5.24 The retention of caffeine (1) and theophylline (2) on PPCI/Si as a function of the treatment of the column with redox reagent.

Mobile phase: 60% MeOH/H$_2$O at 1 ml/min.

Frc: fresh column

red: after the column was treated with 0.1M Na$_2$SO$_3$

oxd: after the column was treated with 0.1M FeCl$_3$. 
Figure 5.25 The retention of caffeine (1) and theophylline (2) on PPDS/Si as a function of the treatment of the column with redox reagent.

Mobile phase : 60% MeOH/H₂O at 1ml/min.

Frc : fresh column,
red : after the column was treated with 0.1M Na₂SO₃
oxd : after the column was treated with 0.1M FeCl₃
In this study it was unclear which mechanism was more dominant. It could be through either an electron transfer or a protonation-deprotonation process. Both of these processes could lead to an increase or decrease of the conductivity of the polymer, which in turn would lead to interaction with certain test compounds in different ways. Furthermore, the occurrence of irreversible changes was also possible.

5.4 Conclusion

An examination of the effect of the exposure of polypyrrole chloride and polypyrrole dodecylsulfate to redox reagents has been carried out. It was found that the exposure brought changes in the properties of the polymers investigated as indicated by the chromatographic elution behaviour of the test compounds in the columns packed with the polymer-coated packing materials.

The results of this chromatographic study indicate that:

(1) on polypyrrole chloride

* the exposure of the polymer to redox reagents changed the ability of the polymer to undergo interaction through charge-transfer processes, but not through $\pi-\pi$ interactions.
* the exposure altered the ability of the polymer to interact electrostatically.
* the capability of undergoing hydrophobic interactions was not affected by the exposure to redox reagents, provided that there were no other kind of interactions involved.
* the changes seem to be reversible.
on polypyrrole dodecylsulfate

* the capacity for electrostatic interactions might be affected by exposure to the redox reagents.
* the low mobility of DS\(^-\) counterions and the suspected inclusion of sodium ions complicated the interaction of the polymer with electron-donor compounds.
* neither the hydrophobic nor \(\pi-\pi\) interaction capacities were affected by the exposure of the polymer to the redox reagents.

The similar variation in electrode potential measured on polypyrrole chloride-coated platinum that resulted from the exposure of the electrode to electrochemical and chemical redox manipulation might suggest that reduction-oxidation process did occur during chemical exposure. Further investigation, however, might still be needed to be certain that other processes such as protonation-deprotonation were not involved.

The results of the study also suggest that if the polypyrrole chloride polymer is used as a stationary phase for chromatography, the treatment with redox reagents would improve the column selectivity by exploiting the differences in the solutes’ capability for producing electrostatic or charge-transfer interactions.
Chapter 6

General Conclusions
The physicochemical properties of conducting polymers have been studied in this work with the use of high performance liquid chromatography. The practical utility of the polymers as stationary phases in liquid chromatography have also been examined. The conducting polymers that have been the object of study were polypyrrole and polyaniline.

Polypyrrole and polyaniline were chemically synthesized and coated onto silica particles and packed into stainless steel chromatographic columns. Reversed-phase mode was mostly employed for chromatographic characterisation. For anion-exchange studies aqueous mobile phase systems were used.

For general basic properties of conductive polymers a series of simple compounds were used as molecular probes. The results indicated that all the polymers could be useful for chromatographic applications as stationary phases. Mixed mode separation mechanisms were demonstrated particularly by polypyrrole-based stationary phase. Dual function capability, i.e as an ion-exchanger and reversed phase was shown by all of the polymers investigated. The incorporation of dodecylsulfate counterions enhanced the separation selectivity of polypyrrole. This indicated that the interaction of polypyrrole with other substances can be manipulated by changing the counterions. This potential might be useful for specific separations and merit further studies.

The interaction of polypyrrole and polyaniline with planar molecules have been investigated with the use of a set of polyaromatic hydrocarbons. This series of aromatic compounds are useful to elucidate not only the chemical properties of the polymer but also the physical structure of the
polymer network. It has been found that all the polymers behaved in different way toward non-planar and planar PAHs. The polymers did not interact with non-planar solute but did readily with the planar ones. It was observed further that the degree of interaction was dependent on the molecular size of PAH being most pronounced on polypyrrole dodecylsulfate. The two dimensional structure of PAHs was also found to be an important factor governing the interaction of the polymers with the compounds. All these results suggested that there was an order and continuous structure in the polymer micronetworks. This order seemed to be most pronounced in polypyrrole dodecylsulfate.

The interaction of the polymers with large biomolecules has also been investigated. Proteins with different molecular size and hydrophobicity have been used for this purpose. Reversed-phase chromatography at low pH was employed for the study. It was observed that the separation mechanism in polypyrrole was different from that shown by polyaniline, at least in part. The molecular size of the samples seemed, again, to be an important separation factor for polypyrrole phases, while on polyaniline chemical properties, such as hydrophobicity, seemed to be the determinant. Preliminary investigation on the effect of salt on the retention of proteins has been carried out. The results showed that the interaction between protein and the polymeric phases was greatly affected by the salt. This may indicate that multiple binding effects were operating, as well as suggesting the existence of a continuous system in the polymer structure.

Chromatographic studies on the effect of the exposure of polypyrrole to redox reagents has been carried out. The results indicated that the
exposure affected the capability of the polymer to undergo specific interactions through electron-donor-acceptor processes. These specific properties might be useful for chromatographic applications because the separation selectivity can be manipulated.

Based on the information gained from the above study, the following aspects can be considered:

(1) The effect of other counterions with different size, hydrophobicity, and three dimensional structure on the micro-structure of conductive polymers should be investigated. By selective choice of the counterions the steric or shape selectivity of the polymer might be improved or changed as required. This might be useful for separations by membrane technology such as in membrane chromatography.

(2) The potential of the polymers, if used as stationary phases, to separate polyaromatic hydrocarbons on the basis of molecular size should be investigated further. The study might involve different monomers and different counterions. This could be useful for purification or in preparative chromatography.

(3) Specific interactions on polyaniline column seemed to be negligible or very low. Therefore it has potential to be used as a chromatographic tool to measure the hydrophobicity of compounds. Further study is needed to exploit this potential.

(4) Proteins were adsorbed or bound very strongly to the polymer phases as a consequence of salt effects. This phenomenon might be interesting
to investigate further. It could be useful in areas such as chiral separations.

In this study the polymers were surface-deposited on bare silica particles. The physical structure of the particles can affect the physical structure of the polymer resulting in the apparent low chromatographic efficiency. Using silica as the support could also bring complications due to the possibility for silanophilic interactions to occur that would affect the overall chromatographic performance of the polymer. Therefore it may be worth investigating the application of other support materials in studying the chromatographic properties of conductive polymers in order to gain genuine information on the properties of the polymers themselves.
References


109. Deinhammer, R. S., Shimazu, K., and Porter, M. D.,


111. Gonnet, C., Bory, C., and Lachatre, G., *Chromatographia*,


114. Wise, S. A., Bonnet, W. J., Guenther, F. R., and May, W. E.,


118. Jinno, K., Yamamoto, K., Nagashima, H., Ueda, T., and Itoh, K.,


## List of Figures

| Figure 1.1 | Block diagram of liquid chromatography | 3 |
| Figure 1.2 | The measurement of retention | 6 |
| Figure 1.3 | Determination of peak asymmetry | 9 |
| Figure 1.4 | Effect of discrete adsorption sites on the selectivity | 16 |
| Figure 1.5 | Surface silanol groups on silica free silanol, geminal silanols, hydrogen-bonded silanols, siloxane | 22 |
| Figure 1.6 | The mechanism of polypyrrole formation | 29 |
| Figure 1.7 | Polypyrrole | 29 |
| Figure 1.8 | Formation of charge carrier in polypyrrole | 31 |
| Figure 1.9 | The chemical structure of polyaniline | 33 |
| Figure 1.10 | Substructure that can affect the degree of PAH planarity | 39 |
| Figure 2.1 | The structure of PAHs | 46 |
| Figure 2.2 | Column packer unit | 48 |
| Figure 2.3 | The dependence of retention on mobile phase composition on PPCl/Si | 55 |
| Figure 2.4 | The dependence of retention on mobile phase composition on PPDS/Si | 56 |
| Figure 2.5 | The dependence of log k' on mobile phase composition on PPCl/Si | 57 |
| Figure 2.6 | The dependence of log k' on mobile phase composition on PPDS/Si | 58 |
| Figure 2.7 | Chromatographic separation of small molecules on PPCl/Si | 61 |
Figure 2.8. Chromatographic separation of small molecules on PPDS/Si .................................................................61

Figure 2.9 The relationship between log kw values for PPCl/Si and PPDS/Si obtained by linear approximation ...................68

Figure 2.10 Selectivity variation of the pair toluene-benzene with methanol content in the eluent on PPDS/Si and PPCl/Si .................................................................70

Figure 2.11 Selectivity variation of the pair phenol-benzene with methanol content in the eluent on PPDS/Si and PPCl/Si .................................................................71

Figure 2.12 Selectivity variation of the pair aniline-phenol with methanol content in the eluent on PPDS/Si and PPCl/Si .................................................................72

Figure 2.13 Selectivity variation of the pair aniline-benzene with methanol content in the eluent on PPDS/Si and PPCl/Si .................................................................74

Figure 2.14 Selectivity variation of the pair DEP-DMP with methanol content in the eluent on PPDS/Si and PPCl/Si ...............75

Figure 2.15 Relationship between k’ and molecular weight of PAHs on C18, PPCl/Si, and PPDS/Si ..............................................77

Figure 2.16 Relationship between log k’ and log P obtained by linear approximation on C18, PPCl/Si, and PPDS/Si ............82

Figure 2.17 Relationship between log k’ and and d of PAHs obtained by linear approximation on C18, PPCl/Si, and PPDS/Si ........................................................................82

Figure 3.1 Chemical structure of polyaniline chloride ...............91

Figure 3.2 Separation of aniline, phenol, DMP, toluene, and DMA on PAnCl/Si .................................................................96
Figure 3.3 Relationship between k' value and MeOH fraction in the eluent obtained on PANCl/Si

Figure 3.4 Relationship between log k' and MeOH fraction in the eluent obtained on PANCl/Si

Figure 3.5 Relationship between atol/benz and MeOH content obtained on PPDS/Si and PANCl/Si

Figure 3.6 Relationship between αan/benz and MeOH content in the eluent obtained on PPDS/Si and PANCl/Si

Figure 3.7 Relationship between αan/phe and MeOH content obtained on PPDS/Si and PANCl/Si

Figure 3.8 Relationship between αphe/benz and MeOH content in the eluent obtained on PPDS/Si and PANCl/Si

Figure 3.9 Relationship between αdep/dmp and MeOH content in the eluent obtained on PPDS/Si and PANCl/Si

Figure 3.10 The relationship between log kw of PANCl/Si and log kw of PPDS/Si obtained by linear approximation

Figure 3.11 The relationship between k' values and MW for PAHs observed on PANCl/Si and PPDS/Si

Figure 3.12 Separation of PAHs on PANCl/Si

Figure 3.13 Separation of PAHs on PPDS/Si

Figure 3.14 Relationship between log k' and log P on PANCl/Si

Figure 3.15 Relationship between log k' and polarizability (δ) on PANCl/Si

Figure 3.16 Effect of NaCl concentration in the eluent on k'

Figure 3.17 Effect of eluent pH on k' measured

Figure 4.1 The retention of α-lactalbumin on PANCl/Si, PPCl/Si, and PPDS/Si
Figure 4.2  The retention of myoglobin on PAnCl/Si, PPCl/Si, and PPDS/Si ................................................................. 134
Figure 4.3  The retention of ovalbumin on PAnCl/Si, PPCl/Si, and PPDS/Si ................................................................. 135
Figure 4.4  The retention of BSA on PAnCl/Si, PPCl/Si, and PPDS/Si ................................................................. 135
Figure 4.5  The retention of HSA on PAnC/Si, PPCl/Si, and PPDS/Si ................................................................. 136
Figure 4.6  The retention of transferrin on PAnCl/Si, PPCl/Si, and PPDS/Si ................................................................. 136
Figure 4.7  The recovery of a-lactalbumin on PAnCl/Si, PPCl/Si, and PPDS/Si ................................................................. 139
Figure 4.8  The recovery of ovalbumin on PAnCl/Si, PPCl/Si, and PPDS/Si ................................................................. 139
Figure 4.9  The recovery of myoglobin on PAnCl/Si, PPCl/Si, and PPDS/Si ................................................................. 140
Figure 4.10  The recovery of BSA on PAnCl/Si, PPCl/Si, and PPDS/Si ................................................................. 140
Figure 4.11  The recovery of HSA on PAnCl/Si, PPCl/Si, and PPDS/Si ................................................................. 141
Figure 4.12  The recovery of transferrin on PAnCl/Si, PPCl/Si, and PPDS/Si ................................................................. 141
Figure 4.13  The retention of proteins obtained by gradient elution on PPCl/Si ................................................................. 144
Figure 4.14  The retention of proteins obtained by gradient elution on PPDS/Si ................................................................. 145
Figure 4.15 The retention of proteins obtained by gradient elution on PAnCl/Si ........................................ 146

Figure 4.16 The effect of salt on the retention of transferrin and \( \alpha \)-lactalbumin on PAnCl/Si ........................................ 153

Figure 4.17 The effect of salt on the retention of transferrin and \( \alpha \)-lactalbumin on PPCI/Si ........................................ 157

Figure 4.18 The effect of salt on the retention of transferrin and \( \alpha \)-lactalbumin on PPDS/Si ........................................ 157

Figure 5.1 Typical cyclic voltammograms of polypyrrole prepared electrochemically ........................................ 172

Figure 5.2 Interconversion between aromatic and quinoid structure in polypyrrole upon redox process .............. 173

Figure 5.3 Protonation and deprotonation of the polypyrrole upon acid-base treatment ........................................ 174

Figure 5.4 The effect of electrochemical reduction-oxidation treatment on the electrode potential of PPCI/Pt ........ 177

Figure 5.5 The effect of the exposure to redox reagents on the electrode potential of PPCI/Pt ............................ 178

Figure 5.6 The effect of redox reagent treatment of PPCI/Si on the retention of benzene and toluene .................... 181

Figure 5.7 The effect of redox reagent treatment of PPDS/Si on the retention of benzene and toluene .................... 182

Figure 5.8 The effect of redox reagent treatment of PPCI/Si on the retention of aniline and DMA ....................... 184

Figure 5.9 The effect of redox reagent treatment of PPDS/Si on the retention of DMA ....................................... 185

Figure 5.10 The effect of redox reagent treatment of PPCI/Si on the retention of phenol ....................................... 186
Figure 5.11  The effect of redox reagent treatment of PPDS/Si on the retention on phenol .................................187

Figure 5.12  The effect of redox reagent treatment of PPCl/Si on the retention of triphenylene and benzantracene ....188

Figure 5.13  The effect of redox reagent treatment of PPDS/Si on the retention of triphenylene and benzantracene ....189

Figure 5.14  The effect of redox reagent treatment of PPCl/Si on the retention of caffeine and theophylline ..........190

Figure 5.15  Molecular structure of caffeine and theophylline .........191

Figure 5.16  Effect of the injected redox reagents into PPCl/Si column on the chromatographic separation of theophylline and caffeine...............................................192

Figure 5.17  The effect of redox reagent treatment to PPDS/Si on the retention of theophylline and caffeine ............194

Figure 5.18  Effect of the injected redox reagents into PPDS/Si column on the chromatographic separation of theophylline and caffeine ...............................................195

Figure 5.19  General illustration of the charge distribution in an amino acid as a function of the pH of solution .......196

Figure 5.20  Chromatographic separation of tyrosine and tryptophan on PPCl/Si at pH 3.8 before and after column reduction .................................................................198

Figure 5.21  Chromatographic separation of tyrosine and tryptophan on PPCl/Si at pH 7.4 before and after column reduction .................................................................199

Figure 5.22  Chromatographic separation of tyrosine and tryptophan on PPDS/Si at pH 3.8 before and after column reduction .................................................................201
Figure 5.23  Chromatographic separation of tyrosine and tryptophan on PPDS/Si at pH 7.4 before and after column reduction .................................................................202

Figure 5.24  The retention of caffeine and theophylline on PPCl/Si as a function of the treatment of the column with redox reagent ..............................................................................204

Figure 5.25  The retention of caffeine and theophylline on PPDS/Si as a function of the treatment of the column with redox reagent ..............................................................................205
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1</td>
<td>Molecular interactions</td>
<td>14</td>
</tr>
<tr>
<td>Table 2.1</td>
<td>The properties of PAHs</td>
<td>47</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Elemental composition of column packings</td>
<td>51</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Capacity factors of benzene and derivatives on PPCI/Si and PPDS/Si</td>
<td>53</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Capacity factors of basic compounds and/or drugs on PPCI/Si and PPDS/Si</td>
<td>54</td>
</tr>
<tr>
<td>Table 2.5</td>
<td>Peak asymmetry factor for PPCI/Si and PPDS/Si</td>
<td>59</td>
</tr>
<tr>
<td>Table 2.6</td>
<td>Column efficiency of PPCI/Si and PPDS/Si</td>
<td>60</td>
</tr>
<tr>
<td>Table 2.7</td>
<td>Correlation coefficient for PPCI/Si and PPDS/Si</td>
<td>63</td>
</tr>
<tr>
<td>Table 2.8</td>
<td>Log $k_w$ for PPCI/Si, PPDS/Si, RP-C$_{18}$, and log P</td>
<td>64</td>
</tr>
<tr>
<td>Table 2.9</td>
<td>Slope for PPCI/Si, PPDS/Si and C$_{18}$ columns</td>
<td>66</td>
</tr>
<tr>
<td>Table 2.10</td>
<td>$k'$ values of PAHs on C$_{18}$, PPCI/Si, and PPDS/Si</td>
<td>78</td>
</tr>
<tr>
<td>Table 2.11</td>
<td>Correlation coefficient between log $k'$ and descriptors, and slopes of the regression line</td>
<td>81</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Elemental composition of PAnCl/Si</td>
<td>91</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>Retention index on PAnCl/Si and PPDS/Si</td>
<td>93</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>Retention Index of basic compounds on PAnCl/Si and PPDS/Si</td>
<td>94</td>
</tr>
<tr>
<td>Table 3.4</td>
<td>Peak asymmetry factor for PAnCl/Si</td>
<td>95</td>
</tr>
<tr>
<td>Table 3.5</td>
<td>The coefficient of correlation for individual test compounds as calculated for PAnCl/Si</td>
<td>104</td>
</tr>
<tr>
<td>Table 3.6</td>
<td>The values of S for PAnCl/Si, PPDS/Si, and various C$_{18}$ columns</td>
<td>105</td>
</tr>
</tbody>
</table>
Table 3.7 The values of log $k_w$ for PAnCl/Si, PPDS/Si and RP-C$_{18}$, and log $P$ .......................................... 106
Table 3.8 The variation of the hydrophobicity parameter values measured on PAnCl/Si, and PPDS/Si ......................... 107
Table 3.9 $k'$ values of PAHs on PAnCl/Si and PPDS/Si .......... 110
Table 3.10 Correlation between log $k'$ and molecular descriptor
of PAH .................................................................. 117
Table 3.11 The retention of anions on PAnCl/Si ..................... 121
Table 4.1 The properties of proteins ............................................... 130
Table 4.2 Elemental composition of PPCI/Si, PPDS/Si and PAnCl/Si ................................................................. 131
Table 4.3 The critical concentration of acetonitrile for
the elution of proteins .................................................. 137
Table 4.4 The elution order of proteins ........................................ 149
Table 4.5 % MeCN at elution ................................................. 150
Table 4.6 Protein recoveries under gradient elution .................. 151
Table 4.7 The effect of (NH$_4$)$_2$SO$_4$ concentration in
the eluent at the start of linear gradient on the retention of proteins on PAnCl/Si .............................................. 154
Table 4.8 The effect of (NH$_4$)$_2$SO$_4$ concentration in the eluent
at the start of linear gradient on the retention
of proteins on PPCI/Si .................................................. 158
Table 4.9 The effect of (NH$_4$)$_2$SO$_4$ concentration in the eluent
at the start of linear gradient on the retention of
proteins on PPDS/Si ...................................................... 158
Table 4.10 Effect of delayed injection on the retention of
proteins on PPCI/Si ...................................................... 160
Table 5.1  Elemental composition of column packings ............... 179