1990

Use of conducting polymers for chromatography

Hailin Ge

University of Wollongong

Recommended Citation
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USE OF CONDUCTING POLYMERS FOR CHROMATOGRAPHY

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

DOCTOR OF PHILOSOPHY

from

THE UNIVERSITY OF WOLLONGONG

by

Hailin Ge, M. Sc.

Chemistry Department

1990
Acknowledgements

Sincere gratitude is extended to Professor G. G. Wallace for his enthusiastic supervision and continuous encouragement throughout this work.

I would like to thank research fellows, Norman Barisci, Mark Imisides, Richard John, Peter Riley, Melinda Spencer, Peter Teasdale and Chee On Too. Their assistance in preparation and proof reading of my thesis is greatly appreciated.

The editing of the final draft of this thesis by Helen Williams is gratefully acknowledged.

Friendly support from Jeannie and Terry Picktt and Patricia and Peter Pirnke, is highly valued and appreciated.

I would like to thank my wife, Wei and my daughter, Weizheng for their overwhelming support and encouragement. Wei’s patience and persistence in typing this thesis is especially appreciated.

I gratefully acknowledge the assistance of a scholarship from the University of Wollongong during the course of this work.
Abstract

Although conducting polymers have been studied for more than ten years, their use in liquid chromatography is still new. This thesis studies the chemical and electrochemical properties of conducting polymers for use in liquid chromatography. The use of these polymers both as novel stationary phases and as electrochemical detection systems was explored.

Polypyrrole was chosen as an example throughout this study. The polymer was either electrochemically or chemically synthesized on various substrates. The properties of polypyrrole were characterized using chronoamperometry, chronopotentiometry, elemental analysis, cyclic voltammetry, electrogravimetry, scanning electron microscopy (SEM), energy-dispersive x-ray (EDX) analysis, and fast atomic bombardment mass spectrometry and the results indicated that the polymer was suitable for chromatographic studies. Specifically, it was found that polypyrrole had unique properties in anion exchange. It was also found that the polymer was thermally-sensitive.

Hardware and systems for synthesis and characterization of the stationary phases were developed in the course of this work. Polypyrrole incorporated with chloride and dodecyl-sulfate was deposited on reticulated vitreous carbon (RVC) particles and silica based particles for use as liquid chromatographic stationary phases. The properties of the polypyrrole stationary phases were determined by elemental analysis, surface area analysis, SEM and EDX analysis. Studies using polypyrrole stationary phases in
chromatography and electrochemically controlled liquid chromatography were characterized using a selection of test compounds. Both reversed-phase and anion exchange chromatographic behavior was found on the polypyrrole stationary phases, and separations using these mechanisms were achieved. Electrochemical control was found to be effective on RVC based polypyrrole stationary phases.

A polypyrrole dispersed-mercury modified electrode was also developed in the course of this work. Its chemical and physical stability, as well as electrochemical sensitivity and selectivity were investigated. It was demonstrated that the electrode could be used in amperometric detection following HPLC separation.

This study has indicated that it is possible for conducting polymers to be used in chromatographic separation and detection systems.
### Publications


7 Ge Hailin and G. G. Wallace, "Development of a Polymer Dispersed-Mercury Modified Electrode".  

8 Ge Hailin and G. G. Wallace, "Determination of Trace Chloramines Using High Performance Liquid Chromatographic Separation Followed with Amperometric Detection".  

9 Ge Hailin and G. G. Wallace, "Electrochemical Controlled Liquid Chromatography Using Conducting Polymer Stationary Phases".  
*J. Liquid Chromatography*, In press.

*J. Chromatography*, submitted.

11 Roger O'Halloran, Paul Spizziri and Ge Hailin, "Development of a Practical Laboratory Method to Determine Chloramine Species in Potable Waters".  
Melbourne and Metropolitan Board of Works report.

12 Australian Patent, "Chromatographic Polymers",  
PCT/AU89/00039
13 Australian Patent, "Corrosion Inhibitors".

PCT/AU89/00075
Chapter 1
General Introduction

1.1 Development of High Performance Liquid Chromatography

1.1.1 Definition of parameters

1.1.2 Mechanisms of retention

1.1.3 New separation techniques

1.1.3.1 Separation using new stationary phases

1.1.3.2 Separation using electrochemically controlled liquid chromatographic techniques

1.1.4 Electrochemical Detection

1.2 Development of Conducting Polymers

1.3 Aim of the Project and Structure of the Thesis

Chapter 2
Synthesis and Characterization of Polypyrrole
Chapter 3
Ion Exchange Properties of Polypyrrole...

3.1 Introduction
3.2 Experimental
3.2.1 Reagents and materials
3.2.2 Instrumentation
3.2.3 Procedures
3.2.3.1 Preparation of samples
3.2.3.2 Ion exchange
3.2.3.3 Determination of counterions
3.3 Results and Discussion
3.3.1 Ion exchange rate
3.3.2 Ion exchange selectivity series
3.3.3 Effect of the ionic strength on ion exchange
3.3.4 Effect of the pH on the ion exchange process
3.3.5 Effect of application of a potential on the ion exchange process
3.3.6 Reversibility of the ion exchange process
3.4 Conclusion

Chapter 4
Thermal Studies of Polypyrrole...

4.1 Introduction
4.2 Experimental
4.2.1 Reagents
4.2.2 Instrumentation
4.2.3 Procedures
4.3 Results and Discussion
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3.1</td>
<td>Thermal stability of polypyrrole</td>
<td>95</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Thermal treatment and morphological properties</td>
<td>102</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Thermal treatment and electrochemical properties</td>
<td>102</td>
</tr>
<tr>
<td>4.3.4</td>
<td>Thermal treatment and composition changes</td>
<td>108</td>
</tr>
<tr>
<td>4.3.5</td>
<td>Thermal treatment and gas chromatographic behavior</td>
<td>108</td>
</tr>
<tr>
<td>4.4</td>
<td>Conclusion</td>
<td>112</td>
</tr>
</tbody>
</table>

### Chapter 5

#### Development of Hardware for the Study of Polypyrrole Stationary Phases for Use in HPLC

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Introduction</td>
<td>115</td>
</tr>
<tr>
<td>5.2</td>
<td>Experimental</td>
<td>118</td>
</tr>
<tr>
<td>5.2.1</td>
<td>Materials and reagents</td>
<td>118</td>
</tr>
<tr>
<td>5.2.2</td>
<td>Instrumentation</td>
<td>120</td>
</tr>
<tr>
<td>5.2.3</td>
<td>Design and preparation of hardware</td>
<td>121</td>
</tr>
<tr>
<td>5.2.3.1</td>
<td>Electrochemical cells for synthesis of polypyrrole stationary phases</td>
<td>121</td>
</tr>
<tr>
<td>5.2.3.1.1</td>
<td>Packed particle cell</td>
<td>121</td>
</tr>
<tr>
<td>5.2.3.1.2</td>
<td>Slurry cell</td>
<td>124</td>
</tr>
<tr>
<td>5.2.3.1.3</td>
<td>Plate cell</td>
<td>125</td>
</tr>
<tr>
<td>5.2.3.2</td>
<td>Columns for characterization of polypyrrole stationary phases</td>
<td>129</td>
</tr>
<tr>
<td>5.2.3.2.1</td>
<td>Self-compressed column</td>
<td>129</td>
</tr>
<tr>
<td>5.2.3.2.2</td>
<td>ECLC column</td>
<td>132</td>
</tr>
<tr>
<td>5.2.4</td>
<td>Test procedures</td>
<td>137</td>
</tr>
<tr>
<td>5.2.4.1</td>
<td>Procedure for testing new electropolymerization cells</td>
<td>137</td>
</tr>
<tr>
<td>5.2.4.2</td>
<td>Procedure for testing the columns</td>
<td>137</td>
</tr>
<tr>
<td>5.3</td>
<td>Results and Discussion</td>
<td>138</td>
</tr>
<tr>
<td>5.3.1</td>
<td>Electropolymerization cell evaluation</td>
<td>138</td>
</tr>
<tr>
<td>5.3.1.1</td>
<td>Performance of the packed particle cell</td>
<td>138</td>
</tr>
<tr>
<td>5.3.1.2</td>
<td>Performance of the Slurry cell</td>
<td>139</td>
</tr>
<tr>
<td>5.3.1.3</td>
<td>Performance of the plate cell</td>
<td>140</td>
</tr>
<tr>
<td>5.3.2</td>
<td>Column evaluation</td>
<td>141</td>
</tr>
<tr>
<td>5.3.2.1.</td>
<td>Performance of the self-compressed</td>
<td></td>
</tr>
</tbody>
</table>
5.3.2.1.1 Effect of self-compression ........................................ 142
5.3.2.1.2 Pressure-flow rate relationships ............................. 143
5.3.2.1.3 Column efficiency and chromatography on the SCC ........ 144
5.3.2.2 Performance of the ECLC column .............................. 149
5.4 Conclusion .................................................................. 154

Chapter 6
Chromatography on RVC Based Polypyrrole Stationary Phases ........ 156
6.1 Introduction .................................................................. 157
6.2 Experimental .................................................................. 159
6.2.1 Reagents and materials .............................................. 159
6.2.2 Instrumentation .......................................................... 160
6.2.3 Preparation of packings .............................................. 160
6.2.4 Procedures ............................................................... 162
6.3 Results and Discussion .................................................. 162
6.3.1 Surface characterization of the stationary phase .............. 162
6.3.2 Test compounds .......................................................... 165
6.3.3 Reversed-phase chromatography .................................. 168
6.3.3.1 Polypyrrole chloride stationary phase ....................... 168
6.3.3.2 Polypyrrole dodecyl-sulfate stationary phase ............... 171
6.3.3.3 Stability of stationary phases: reversed-phase chromatography ........................................................................... 176
6.3.4 Anion exchange chromatography .................................. 178
6.3.4.1 Polypyrrole chloride stationary phase ....................... 178
6.3.4.2 Polypyrrole dodecyl-sulfate stationary phase ............... 182
6.3.4.3 Stability of stationary phases: anion exchange chromatography ........................................................................... 184
6.3.5 Electrochemically controlled chromatography ................. 184
6.3.5.1 Electrochemical effect on hydrophobicity .................... 195
6.3.5.2 Electrochemical effect on polarity .............................. 197
6.3.5.3 Electrochemical effect on proton acceptor
Chapter 7
Chromatography on Silica-Based Polypyrrole
Stationary Phases

7.1 Introduction.................................................................215
7.2 Experimental............................................................216
7.2.1 Reagents and materials............................................216
7.2.2 Instrumental............................................................216
7.2.3 Columns.................................................................217
7.2.3.1 Electrochemical polymerization.................................217
7.2.3.2 Chemical polymerization........................................217
7.2.3.3 Preparation of columns..........................................218
7.2.4 Procedure..............................................................218
7.3 Results and Discussion..............................................221
7.3.1 Surface characterization...........................................221
7.3.1.1 Elemental analysis of packings................................221
7.3.1.2 Surface area analysis of packings..............................222
7.3.1.3 SEM of packings.....................................................222
7.3.1.4 EDX spectrum of packings.......................................223
7.3.2 Reversed-phase chromatographic characterization.............223
7.3.2.1 Polypyrrole modified C18 stationary phases...............223
7.3.2.2 Polypyrrole modified silica stationary phases...............229
7.3.3 Anion exchange chromatographic characterization...............233
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>amps</td>
</tr>
<tr>
<td>A.C.</td>
<td>alternating current</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>C₆₋₁₁</td>
<td>normal alkanes</td>
</tr>
<tr>
<td>C</td>
<td>coulomb</td>
</tr>
<tr>
<td>C</td>
<td>counter ion in the polymer</td>
</tr>
<tr>
<td>C₈</td>
<td>octyl</td>
</tr>
<tr>
<td>C₁₈</td>
<td>octadecyl</td>
</tr>
<tr>
<td>°C</td>
<td>centigrade</td>
</tr>
<tr>
<td>CE</td>
<td>current efficiency</td>
</tr>
<tr>
<td>Cₗₘ</td>
<td>concentration of a component in the mobile phase</td>
</tr>
<tr>
<td>cm</td>
<td>centimeter</td>
</tr>
<tr>
<td>Cₛ</td>
<td>concentration of a component in the stationary phase</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammetry</td>
</tr>
<tr>
<td>d</td>
<td>differentiate</td>
</tr>
<tr>
<td>D.C.</td>
<td>direct current</td>
</tr>
<tr>
<td>DEP</td>
<td>diethylphthalate</td>
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<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>
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<td>DPV</td>
<td>differential pulse voltammetry</td>
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<tr>
<td>DS</td>
<td>dodecyl-sulfate</td>
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<tr>
<td>DTA</td>
<td>differential thermal analysis</td>
</tr>
<tr>
<td>e</td>
<td>electron</td>
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<td>E</td>
<td>potential</td>
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<tr>
<td>Eₘ₈</td>
<td>applied potential</td>
</tr>
<tr>
<td>EC</td>
<td>electrochemical</td>
</tr>
</tbody>
</table>
ECLC  electrochemically controlled liquid chromatography
EDX  energy-dispersive X-ray analysis
EGA  electrogravimetric analysis
EPMA  electron-probe micro analysis
ESR  electron spin resonance spectrometry
F  Faraday constant
FAB/MS  fast atomic bombardment mass spectrometry
FTIR  Fourier transfer infra-red spectrometry
GC  gas chromatography or glassy carbon electrode
g  gram
h  peak height
h/2  half peak height
HETP (or H)  height equivalent to a theoretical plate
HPLC  high performance liquid chromatography
HSA  human serum albumin
Hz  hertz
I  current
I.D.  inner diameter
IR  infra-red
K  distribution coefficient
k'  capacity factor
Kev  kilo-electron-volt
KV  kilovolts
L  column length
m  meter
M  metal
Molecular weight of the counterion
mc  molecular weight of the counterion
mg  milligram(s)
min  minute(s)
ml  milliliter(s)
mm  millimeter(s)
Mp  molecular weight of pyrrole
ms  milliseconds
mV  millivolt(s)
MΩ  megaohm
n  electron transfer number
N  theoretical plates
nm  nanometer(s)
nm  moles of a component in the mobile phase
NMR  nuclear magnetic resonance spectrometry
n_s  moles of a component in the stationary phase
OD  outside diameter
Ox  oxalate
PDMME  polymer dispersed-mercury modified electrode
PP  polypyrrole
PPb  parts per billion
PP/Cl  polypyrrole chloride
PP/DS  polypyrrole dodecyl-sulfate
ppm  parts per million
PPP  poly-(p-phenylene)
PSD  poly-(styrene-divinylbenzene)
Q  charge
R-  alkyl
R  resistance
Rs  resolution
RVC  reticulated vitreous carbon
SCC  self-compressed column
SDS  sodium dodecyl-sulfate
sec  second(s)
SEM  scanning electron microscopy
t  time
TG  thermal gravimetry
tm  dead time
TMFE  thin mercury film electrode
\( t_R \)  retention time
UV  ultra violet
V  volt(s)
\( V_m \)  volume of the mobile phase
\( V_r \)  retention volume
\( V_s \)  volume of the stationary phase
w  peak width
W  weight
\( w_{1/2} \)  half peak width
x  ratio of counterion to pyrrole units
XPS  x-ray photoemission spectroscopy
\( \alpha \)  separation factor (relative retention)
\( \AA \)  Ångstrom(=10^{-10} m)
\( \mu \)  micro (prefix)
\( \delta \)  partially differentiate
\( \Delta \)  difference
\( \sigma \)  standard deviation
Chapter 1

General Introduction
This work investigates the chemical and electrochemical properties of conducting polymers for use in liquid chromatography. The use of these polymers both as new stationary phase materials and as the basis of electrochemical detection systems has been explored. This chapter reviews developments in each of these areas of liquid chromatography and conducting polymers.

1.1 Development of High Performance Liquid Chromatography

The discovery of chromatography is generally ascribed to Tswett (1) who showed how compounds could be separated on a column containing an adsorbent. Kuhn and Lederer (2, 3) used Tswett's method to separate plant pigments but there was little advance in either the theory or practice of chromatography until 1941 when Martin and Synge (4) published their paper describing the invention of liquid-liquid partition chromatography for the separation of acetylated amino acids. Martin and Synge developed the concept of height equivalent to a theoretical plate (HETP) in their paper, which has been universally accepted as the measure of the efficiency of a chromatographic column.
The emergence of high performance liquid chromatography (HPLC) has been attributed to Huber and Hulsman (5) in 1967. The advantages of HPLC over classical liquid chromatography are:

1. high resolution;
2. high speed;
3. high potential for automation and quantitation;
4. high reproducibility, and
5. ease of operation.

This section reviews definitions of parameters encountered in HPLC, mechanisms of retention, techniques used to induce separations and techniques employed in electrochemical detection in HPLC.

1.1.1 Definition of parameters

Chromatographic retention and separation are based on the differences in the equilibrium distribution coefficient (K) of the sample components between the stationary phase and the mobile phase,

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

(1.1)

where \( C_s \) and \( C_m \) are the concentrations of a component in the stationary and mobile phases respectively. The higher the K value the longer the component is retained in the column. Differences in K values lead to the separation of the components. The K values of compounds are mainly dependent on the properties of the stationary phase, the mobile phase, and the column temperature as well as the compounds themselves.
Figure 1.1  Chromatogram

$t_{R1}, t_{R2}$: retention times of compounds 1 and 2 respectively;

$t_m$: dead time;

$w_1, w_2$: peak width of the compounds 1 and 2 respectively;

$w_{1/2}$: half peak width;

$h$: peak height;

$h/2$: half peak height.
With on-line detection of species eluted from the column a chromatogram as depicted in Figure 1.1 is obtained. The theoretical relationship between the distribution coefficient $K$ and the retention volume $V_R$ (volume of mobile phase required to elute species) in partition chromatography can be expressed as:

$$V_R = V_m + KV_s$$  \hspace{1cm} (1.2)

where $V_m$ and $V_s$ are the volume of the stationary and mobile phases respectively. Another fundamental parameter in chromatography is the capacity factor $k'$ which is defined as:

$$k' = \frac{n_s}{n_m}$$  \hspace{1cm} (1.3)

where $n_s$ and $n_m$ are moles of species in the stationary phase and mobile phase respectively. Therefore $k'$ can be written:

$$k' = K(V_s/V_m)$$  \hspace{1cm} (1.4)

A combination of equation 1.2 and 1.4 gives:

$$V_R = V_m(1+k')$$  \hspace{1cm} (1.5)

or $t_R = t_m (1+k')$  \hspace{1cm} (1.6)

or $k' = (t_R-t_m)/t_m$  \hspace{1cm} (1.7)

Chromatographic selectivity can be expressed usually in terms of relative retention $\alpha$ (also known as separation factor):

$$\alpha = t'_{R_2}/t'_{R_1} = k'_2/k'_1 = K_2/K_1$$  \hspace{1cm} (1.8)

where $t_{R_1}$ and $t_{R_2}$ are defined as in Figure 1.1.

The use of a retention index system in HPLC has been investigated by various workers. The use of analogous series of alkyl aryl
ketones (6), 2-keto alkanes (7) and polyaromatic hydrocarbons (8) has been explored, but none has been accepted universally.

Chromatographic column efficiency is controlled by kinetic processes. As a sample solute band passes through a column, the width of the band increases and the solute is diluted by the mobile phase due to eddy diffusion, molecular diffusion and mass transfer in the column (9). Such band broadening effects have been studied in many different ways (10-12). The band broadening is usually described in terms of the column efficiency or number of theoretical plates \( N \) and defined as:

\[
N = 16 \left( \frac{t_R}{w} \right)^2 \\
\text{or } N = 5.54 \left( \frac{t_R}{w_{1/2}} \right)^2
\]

(1.9)

where \( t_R \) is retention time, and \( w \) and \( w_{1/2} \) are peak width and half peak width respectively. The other useful measure of the column kinetic performance is the height equivalent to a theoretical plate or plate value (HETP or \( H \)),

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

(1.10)

where \( L \) is length of the column. Small \( H \) values, i.e. large \( N \) values, mean more efficient columns. The column efficiency in HPLC generally depends on the size, shape and size distribution of column packing materials (13), the packing techniques (14, 15), the thickness and pore structure of stationary phases (16), column design (17), extra column connections (18) and the viscosity and flow rate of mobile phases (11).
Chromatographic resolution is a parameter which is a combination of the retention, the selectivity and the column efficiency. Resolution \( R_s \) is usually defined by the following equation:

\[
R_s = \frac{2(t_2 - t_1)}{w_1 + w_2} \quad (1.11)
\]

An \( R_s = 1.5 \) value is taken to represent baseline separation in practice. Purnell (19) demonstrated that for column chromatography \( R_s \) can be divided into contributions from retention \([k'_2/(1+k'_2)]\), selectivity \([(\alpha - 1)/\alpha]\) and efficiency \((N^{1/2})\):

\[
R_s = \frac{1}{4}[(\alpha - 1)/\alpha] \frac{[k'_2/(1+k'_2)]N^{1/2}}{1} \quad (1.12)
\]

High \( \alpha \) values, \( k' \) ranging from 1 to 10 and high an \( N \) value are preferable to obtain optimal resolution (19).

1.1.2 Mechanisms of retention

One of the major advantages of liquid chromatography over other separation techniques is the achievement of separation using several different mechanisms of retention. These mechanisms make it possible to separate a wide range of compounds by liquid chromatography. There are four mechanisms available to liquid chromatography: partition, adsorption, ion exchange, and size exclusion.

Liquid partition chromatography involves the use of a liquid stationary phase on a solid support. In early work, the liquid stationary phase was physically coated on the support. This approach suffered from the stripping off of the coating material by either mechanical shearing forces or chemical dissolution effects. The development of chemically-available bonded stationary phases
by Halasz and Sebastian (20) eliminated this problem and promoted the application of HPLC. The majority of commercial bonded phases is prepared from silica by reaction of the surface silanol groups with an organochlorosilane or alkoxyisilane to give the active groups as follows,

\[ \text{Si} - \text{O} - \text{Si} - \text{R} \]

where R can be a hydrocarbon such as C8 or C18, or a polar group such as R-CN or R-NH2.

Two separation methods can be operated using partition chromatography. When the stationary phase is more polar than the mobile phase, this is termed normal phase liquid chromatography and is usually used to separate polar compounds. If the stationary phase is less polar than the mobile phase, the process is referred to as reversed-phase liquid chromatography. Reversed-phase HPLC is the most widely used mode in HPLC (21). When the reversed-phase liquid chromatography is used for separation of ionic or ionizable compounds, ion pair chromatography can be used. A hydrocarbon bonded stationary phase and a mobile phase with a suitable counterion to the sample ions which have lipophilic characteristics, are employed.

Adsorption chromatography is based on the adsorption processes between species and the stationary phase. Similar to partition chromatography, if a polar stationary phase and a nonpolar mobile phase are used the process is cited as normal phase adsorption chromatography, and when a nonpolar stationary phase and a polar mobile phase are employed the mode is known as reversed-phase...
adsorption. The common stationary phases are silica gels, alumina gels and polymer beads such as those made from polyamide.

Ion exchange chromatography involves a mass transfer process. The stationary phase carries a net charge which is neutralized by a counterion from the mobile phase. When sample ions pass through the column, they may then exchange with the counterions. If the stationary phase is positively charged, the process can be expressed in terms of the equilibrium:

\[ R^+Y^- + X^- \rightarrow R^+X^- + Y^- \]  \hspace{1cm} (1.13)

where \( R^+ \) is an ion-exchange site on the surface of the stationary phase and \( X^- \) is a sample ion. This is known as anion exchange. Thus cation exchange can be represented by:

\[ R^-Y^+ + X^+ \rightarrow R^-X^+ + Y^+ \]  \hspace{1cm} (1.14)

Materials such as styrene-divinylbenzene copolymers with functional groups have been employed as stationary phases in ion exchange chromatography. Since these polymer resins may not be operated at high pressure, only moderate column efficiencies are obtained. Consequently, the use of chemically bonded exchangers which consist of a non-porous silica matrix with bonded functional groups has become popular. However, these exchangers can be used only over a limited pH range due to the limited chemical stability of the silica matrix.

Size exclusion chromatography separates compounds according to their molecular size. Small molecules that can freely enter the pores of the stationary phase are said to have a distribution
coefficient $K=1$; large molecules which are completely excluded from all pores have a distribution coefficient $K=0$; and intermediate size molecules will have a $K$ value between 0 and 1. Therefore, larger molecules move through the stationary phase faster than small molecules. Usually solvent molecules are the smallest and are eluted last. The mechanism of separation is complicated but seems to be due to steric exclusion (22). The stationary phases in modern exclusion chromatography include [1] the aerogels (porous glass or silica); [2] the xerogels, e.g. cross-liked dextran and polyacrylamide; and [3] xerogel-areogels, e.g. cross-linked agarose, polystyrene and polyvinylacetate.

1.1.3 New separation techniques

A number of techniques, e.g. modification of stationary phases and variation of mobile phases, have been used to effect HPLC separations (9-11). In this thesis the aspects of novel stationary phases and electrochemically controlled liquid chromatography (ECLC) will be of special concern, and the development of these methods is reviewed in the following sections.

1.1.3.1 Separation using new stationary phases

The development of new stationary phases is of interest to those involved in chromatographic research because the stationary phase plays a major role in determining separations. In order to be suitable for use with HPLC, the stationary phases must meet certain physical and chemical requirements (9-11). Essentially, these materials should be able to produce high chromatographic efficiency and to provide desirable retention and selectivity characteristics. The packings should be physically stable at high pressures and
chemically stable over a wide range of given conditions. All of these general requirements should be taken into account when developing new stationary phases.

The ideal physical form of modern HPLC packings is small-diameter porous particles (9-11) (down to 3 μm). Generally, the two types of small regular packing materials used as stationary phases in HPLC today are microporous particles and porous layer beads.

Usually microporous particles are 3-10 μm in size with various pore size, pore volume and surface areas. They are totally porous and can be prepared from silica, alumina or ion-exchange resins. Compared with microporous particles, porous layer beads (pellicular beads) have large diameters (20-40 μm) and low surface areas and consist of a solid, non-porous core (usually silica) with a thin porous outer shell which can be silica, alumina or an ion exchange resin. Microporous particles dominate HPLC packings because porous layer beads have low capacities and low efficiencies. However, porous layer beads can be easily packed, which makes them suitable for preliminary investigations of new stationary phases.

The chemical forms of modern HPLC packings include inorganic polymers, mainly based on silica gels, and organic polymers. Silica gels are preferable because of their available size, shape, pore size, pore volume and chemically active surface on which modifiers can be applied while organic polymers are important because they are more chemically stable, especially to a wide range of pH values.
Unmodified silica has been used to solve some specific applications, e.g. the separations of basic drugs (23-25). However the selectivity available on unmodified silica is very limited. Consequently, dynamically modified silica on which a modifier from the mobile phase is dynamically grafted (26) has become a new area of interest. Reverse-phase (27-29), ion exchange (30) and mixed-mode (26) mechanisms have been achieved on dynamically modified silica stationary phases.

Chemical modification of silica is a more important approach to changing the selectivities. Functional groups such as alkyl, phenyl, amino and cyano groups have been bonded to silica. Chromatographic selectivity has also been modified by bonding other compounds on to silica. Such modifiers have included polar compounds such as safrole (31), electron donor-acceptors (32-34), hydrogen bonding ligands (35), metallic compounds such as copper (II) (36) and zirconium oxide (37), compounds for protein separations such as melittin (38), concanavalin A (39) and N-hydroxysuccinimide ester (40), and compounds for chiral separations such as L-proline (41), acetylquinine (42) and cellulose (43).

Organic polymers have been used predominantly in ion exchange chromatography (44). However, they have also been used in other chromatographic modes. Poly(styrene-divinylbenzene) (PSD) has been particularly popular. It has been employed not only for high performance ion exchange separation (45, 46) but also for reverse-phase separation (47-50). More selective interactions have been induced by chemical modification of this polymer with functional
groups such as \(-\text{C}_{18}\) (51, 52), amines (53) and formyl (54). However some PSD based polymers have been found to be too soft to be used in high pressure chromatographic systems (55). Consequently, rigid vinyl alcohol copolymer gels have been introduced for use in HPLC (56, 57). Reverse-phase (58-60), and micelle exclusion (61) as well as multimode chromatography (57) have been achieved on this polymer. Other polymers have been also employed in HPLC. For example, polysiloxane (62), glycidyl methacrylate-ethylene dimethacrylate copolymer (63), N-methylpyridinium polymer (64), polyetheneimine (65) and other rigid polymers (66) have been used for reverse-phase (62, 66), ion exchange (65, 66), size exclusion (63, 66) and affinity (64) chromatography.

More recently, polymer-coated silica gels have become popular since they combine the characteristics of both silica and polymers. Poly(alkyl aspartamide) (67), alkyl polysiloxanes (68), polyvinylpyrrolidone (69), poly (2-sulfoethyl aspartamide) (70), polyethyleneimine (71, 72), polyamine (73), poly(butadiene-maleicacid) (74) and polyvinylimidazole (75) have been coated on silica gels. These stationary phases have been used for anion exchange (71-73), cation exchange (70, 74), adsorption (69) and partition (67, 68) chromatography. More complicated polymer-coated silica stationary phases have also been synthesized. Polymer-diol-silica (76) and \(\text{C}_{18}\)-polymer-silica (77) stationary phases have been employed. The polymer coating not only improves the selectivity but also the stability of the stationary phases.
Other novel stationary phases have also been developed recently, especially for biochemical separations. Non-porous packings (78, 79) have been developed to separate proteins, and showed better efficiencies for the separation of large molecules. Internal surface reversed-phases which have a nonpolar surface inside silica pores but a polar surface outside (80-83), have been introduced in order to minimize the interferences from large molecules, such as proteins existing in biochemical samples. Chiral stationary phases developed to separate optical isomers have been reviewed by many workers (84-89).

Preparation of the stationary phases usually involves synthesis of basic supporting materials and modification of these supports. The synthesis and modification of silica has been summarized in various books and reviews (9-11, 21). More specific preparation methods can be found from the references.

Although a great number of separation problems have been solved using present HPLC stationary phases (21), there are still considerable difficulties encountered in contemporary HPLC practice. Some materials such as silica gel have good chromatographic performance but very limited chemical stability (58). On the other hand, many organic polymers can tolerate pH changes but have low pressure resistance (59), which restricts their use in high pressure and high efficiency chromatographic systems. High operation costs may result from the short life time of columns and additional pre-separation may become necessary to compensate for the lack of selectivity. Another problem with HPLC separations is that the prediction of retention times is very difficult compared
with GC. For example, retention time of the same compound on C\textsubscript{18} columns from various manufacturers can be very different (90), since the packings can not be made reproducibly. Therefore, in order to minimize or solve these problems, there is a great need to develop new stationary phase materials and improve manufacturing processes.

1.1.3.2 Separation using electrochemically controlled liquid chromatographic techniques

Electrochemically controlled liquid chromatography (ECLC) is a separation technique in which an electrical potential is applied on a specially designed chromatographic column.

In 1963, Fujinaga et al. (91, 92) used a silver grain stationary phase to separate copper, lead and cadmium. In this work, the column was divided into several sections and different potentials were applied to each section. They termed the method electrolytic chromatography. In 1964, Fujinaga et al. (93) developed a column where continuous potentials against a reference electrode were applied and they investigated effects of experimental conditions on analytical performance. These conditions included types of packing material, grain size of the packing, species and flow rate of eluent, material of the semipermeable membrane between the working and auxiliary electrodes, and composition of the counter electrode. In the same year Blaedel and Strohl (94) used graphite grains (100-200 mesh) as packing materials, designed a column with a three electrode system, and employed these materials in the oxidation of cerous ions [Ce(III)], selective reduction of Fe(III)- U(VI) mixtures, and separation of Cu, Pb, Cd and Zn mixtures. They named this
process "continuous quantitative electrolysis". They then discussed the relationship between the electrode potential and the retention time at a column with amalgamated platinum grains for the deposition of thallium, lead, tin and indium (95).

The mechanism involved in these early experiments is based on electrolytic reactions in a flowing system, in which the column acts as an electrochemical reactor as well as a separator (M1). The chromatographic retention in such a system can be controlled by a potential as described by Blaedel and Strohl (95). When metal ions $M^{n+}$ pass through a column, they may be deposited as free metal on the stationary phase due to the potential. The distribution coefficient $K$ is

$$K = \frac{[M]}{[M^{n+}]} \quad (1.15)$$

If the system is in equilibrium, $K$ is related to the applied potential $E$ through the Nernst equation (at 25°C).

$$E = E^0 + \frac{0.059}{n} \log \left(\frac{[M^{n+}]}{[M]}\right) \quad (1.16)$$

$$\log K = \frac{n}{0.059}(E^0 - E) \quad (1.17)$$

The equation 1.17 indicates that chromatographic retention as well as selectivity depends on the potential applied to the stationary phase. This mechanism is limited to the analytes which undergo reversible electrochemical reactions, such as the metal ions.

A mechanism involving a changing mobile phase composition through an electrochemical reaction on the stationary phase was investigated by Hern and Strohl (M2) (96). Instead of pure graphite, they modified granular graphite with complexing agents
such as chrome Black T or dibenzo-18-crown-6. This modification gave the packing certain chelating properties. The process is such that when a metal ion is passed through the column it will be retained by forming a complex with the stationary phase, and then it can be eluted because H⁺ ions are produced when a potential is applied to the column. A separation of Fe³⁺ from a mixture of Ni²⁺, Co²⁺, and Ca²⁺ was achieved. However, the mechanism is still limited to few applications.

In 1972, Strohl and Dunlop (97) employed a column of graphite particles to separate quinones through an electrosorption process in which a mechanism involving changing stationary and mobile interfaces was introduced (M3). In their experiments, they found that it was possible to adsorb quinone samples on graphite packings by applying a potential. With the proper choice of the potential, selective adsorption and desorption was achieved. This leads to chromatographic separation of quinone mixtures. During this process, analytes and mobile phases did not undergo electrochemical reactions, and the separation mode became more general. This mechanism was also used in trimethylchlorosilane modified carbonaceous material by Antrim et al. (98). It was found that application of potentials can significantly affect capacity factors for toluene, phenol and pyridine. Later, they (99) found that supporting electrolytes could produce a significant change in retention and it was considered that such effects resulted from the electroadsoption of mobile phase molecules. This caused a change of the orientation of the mobile phase at the surface of the stationary phase, thus affecting the partition.
More recently, electroactive materials were used in ECLC by Ghatak-Roy and Martin (100) and a mechanism which involved changing the chemical form of the stationary phases was realized (M4). Using a vinylferrocene/maleic anhydride copolymer modified carbon particles as the stationary phase, the retention of methylviologen ($\text{MV}^{2+}$) can be varied by the application of a potential. The polymer is in a reduced form when the potential is applied and can be represented as follows:

$$
\begin{align*}
\text{CH}_2\text{CH} & \quad \text{CH-CH} \\
\text{Fc} & \quad \text{O=O} \quad \text{C=O} \\
\text{O} & \quad \text{Na}^+ \quad \text{Na}^+
\end{align*}
$$

$\text{MV}^{2+}$ can then be retained due to ion exchange interaction. When the ferrocene (Fc) sites in the polymer are oxidized by the application of a +0.7 V (vs. Ag) potential on the stationary phase the polymer can be represented by:

$$
\begin{align*}
\text{CH}_2\text{CH} & \quad \text{CH-CH} \\
\text{Fc} & \quad \text{O=O} \quad \text{C=O} \\
\text{O} & \quad \text{O} \quad \text{O}
\end{align*}
$$

Now $\text{MV}^{2+}$ is not retained on the column. This experiment showed that the ion exchange behaviour of the stationary phase is modulated by electrochemical control, and it was described as electromodulated ion exchange chromatography.

The stationary phase material in ECLC must satisfy the requirements for conventional liquid chromatography: it should be
chemically inert, mechanically strong, have a high surface-to-volume ratio and dimensionally thin phases. Usually porous, evenly-sized particles are preferable. In addition, the stationary phase must fulfill the electrochemical requirements of possessing good electrical conductivity and be stable over a wide potential range. However, if the stationary phase undergoes an electron transfer reaction that changes its chemical form, but does not decompose it, then an attractive means of affecting chromatographic retention has become available. At the early stage, many inorganic materials have been used as column packings for bulk electrolysis in a flowing system or for separation of compounds through controlled potential electrolysis. These materials included amalgamated nickel powder (101), silver grains (102), tantalum gauze (103), platinum gauze (104), platinum coil (105), glassy carbon grains (106, 107), and graphite granules (108). More examples of stationary phases used in ECLC are summarized in Table 1.1.

The selectivity of stationary phases is, however, still limited in ECLC. A major limitation of ECLC is that most column designs can only work at low pressure, so that the column efficiency is very low. Some columns were designed for high pressure chromatography (97, 98), but the column efficiency was still poor and the theoretical plates were about 2-10 (98), so no separations were achieved.
<table>
<thead>
<tr>
<th><strong>Stationary</strong> phase</th>
<th><strong>Size (μm)</strong></th>
<th><strong>Separation mechanism</strong></th>
<th><strong>References</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver grains</td>
<td>850-1770</td>
<td>M1</td>
<td>91</td>
</tr>
<tr>
<td>Graphite grains</td>
<td>74-150</td>
<td>M1</td>
<td>94</td>
</tr>
<tr>
<td>Amalgamated nickel particles</td>
<td>100</td>
<td>M1</td>
<td>101</td>
</tr>
<tr>
<td>Mercury-coated platinum</td>
<td>380-1780</td>
<td>M1</td>
<td>95</td>
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<tr>
<td>Graphite particles</td>
<td>150-250</td>
<td>M3</td>
<td>97</td>
</tr>
<tr>
<td>Trimethyl-chlorosilane on carbon</td>
<td>105-147</td>
<td>M3</td>
<td>98</td>
</tr>
<tr>
<td>Ion-exchanger modified graphite</td>
<td>105-150</td>
<td>M2</td>
<td>96</td>
</tr>
<tr>
<td>Electroactive copolymer on carbon</td>
<td>40</td>
<td>M4</td>
<td>100</td>
</tr>
</tbody>
</table>
1.1.4 Electrochemical Detection

Electrochemical detection in chromatography has been found to have a number of advantages. Primarily, these are sensitivity, selectivity and economy (109, 110). Electrochemical detection is based on various electrochemical methods, including amperometry (111), coulometry (112), polarography (113) and conductivity (114). However, most reports on electrochemical detection in HPLC have dealt with amperometry because of its ease of operation and the extremely low detection limits which can be achieved (115).

Conventional amperometric detectors consist of a three electrode measuring cell containing a working electrode, a reference electrode and a counter electrode. The potential required for oxidation or reduction of the species being analyzed is applied between the working electrode and the reference electrode, usually Ag/AgCl. The counter electrode sometimes consists of stainless steel parts in the flow line and serves to maintain the potential during operation. The counter electrode also eliminates the flow of current at the reference electrode which would result in large potential shifts. If an electrochemically active sample flows through the detector cell, it becomes partially oxidized or reduced. This process leads to an anodic or cathodic current respectively that is proportional to the concentration of the species being analyzed over a limited concentration range. This signal can, therefore, be converted into a chromatographic peak.

Since the signal is generated on the working electrode, during amperometric detection, the materials for the working electrode will determine the performance of the amperometric detector.
Criteria for selection of electrode materials are many. Basically, chemical, electrochemical and mechanical stabilities are critical. Solid metal electrodes such as silver (116), platinum (117, 118), gold (119), metallic compounds (120), and glassy carbon electrodes (121-125) are very popular due to their mechanical stability and simple construction. Other than solid materials, many other materials such as carbon paste (126), and mercury (127) have been used as electrode materials.

In recent years in order to introduce more electrochemical selectivity, chemically modified electrodes have been employed. A number of modifiers have been attached to conventional electrode surfaces resulting in various chemically modified electrodes such as poly (N-vinylpyrrolidone) on graphite (128), carbon-polymer composite (129), poly (4-vinylpyridine)-coated glassy carbon (130), carbon-polyethylene tubular (131), cobalt phthalocyanine carbon paste (132), polyaniline on glassy carbon (133), and gold chloride on gold (134) for flow through detectors. These electrodes have shown high selectivities.

Limitations of present electrochemical detection in HPLC have been reviewed by many workers (135-138). Primarily, it has been difficult to isolate an electrode which satisfies all the criteria set out above. For example, glassy carbon electrodes are mechanically-stable and chemically-inert under most HPLC conditions, but they provide limited selectivity. Application of electrochemical detection in HPLC has also been reviewed (135-138).
1.2 Development of Conducting Polymers

This section reviews the synthesis, and modification of properties of conducting polymers.

Conducting polymers were discovered in the early 1970s in Shirakawa's laboratory at the Tokyo Institute of Technology (139). However, not until 1977 did MacDiarmid et al. (140) first demonstrate the semiconducting and metallic properties of an organic polymer, polyacetylene. This was followed by a surge of activity in the synthesis and characterization of members of this new class of conducting materials.

A key step in making a polymer conduct electricity is to incorporate certain chemicals into the polymer by a process called "doping". For example, the standard process for making polyacetylene is to synthesize the polymer first and then to dope the polymer using chemicals such as iodine (141). The procedure for doping polymers is much simpler than the one used to dope classical semiconductors such as silicon. Many polymers such as poly(p-phenylene) semiconductors were synthesized more than 30 years ago (142) but it was not until the discovery of the doping technique that these polymers could be classified as conductors (143).

Although it is a technique which is successful in practice, the precise electronic and physical changes that accompany doping are still unclear, i.e. the exact conducting mechanism of conducting polymers is still under investigation. Some aspects of the mechanism are, however, known.
Synthesis of conducting polymers can be carried out chemically or electrochemically. The polymerization process can be either the condensation or addition methods. For example, polyacetylene is formed through the following addition polymerization,

\[ \text{H} \quad \text{C} = \text{C} \quad \text{H} \quad \text{H} \quad \text{C} = \text{C} \quad \text{H} \quad \text{H} \]

while polypyrrole is formed through the following condensation polymerization:

\[ \begin{align*} 
\text{n} & \quad \text{H} \quad \text{C} = \text{C} \quad \text{H} \\
\text{N} & \quad \text{H} \quad \text{C} = \text{C} \quad \text{H} \\
\end{align*} \]

\[ \begin{align*} 
\text{n} & \quad \text{H} \quad \text{C} = \text{C} \quad \text{H} \\
\text{N} & \quad \text{H} \quad \text{C} = \text{C} \quad \text{H} \\
\end{align*} \]

The electrochemical preparation of conducting polymers dates back to early work by Dall'Olio and coworkers (144), who obtained "pyrrole black" on a platinum electrode. However, it was not until subsequent experiments (145) in 1979 that the electrochemical approach to the synthesis of conducting polymers was established. Monomers successfully used in electrochemical polymerization are aromatic and can be oxidized at relatively low anodic potentials, usually below 2.1 V.
Electropolymerization is an electrophilic substitution reaction, but the mechanism is very complex. The general reaction steps have been outlined by previous workers (146).

The overall loss of electrons during polymerization includes two per monomer for the formation of the polymer chain plus the positive charge associated with the oxidation of the polymer, which can be described as follows:

\[
\text{H} \quad + \text{nC}^{-} \quad \rightarrow \quad \left[ \text{H} \quad + \quad \text{nC}^{+} \quad \right] + \quad 2\text{nH}^{+} + (2x+1)\text{ne}^{-}
\]

(1.20)

where \(\text{C}^{-}\) is a counterion to be doped or incorporated into the polymer during the process in order to balance the charge. Usually \(\text{C}^{-}\) is the anion of the supporting electrolyte.

Substrates which do not oxidize concurrently with the aromatic monomer are preferred for use in electrochemical synthesis of conducting polymers. These include noble metals such as platinum and gold, metal oxides such as tin oxide and carbon-based materials such as glassy carbon and graphite.

The solvent and the electrolyte which can be used in electropolymerization usually are not nucleophilic since the polymerization reaction proceeds via radical cation intermediates and will be sensitive to the nucleophilicity of the environment near
the electrode. For this reason, many syntheses have been carried out in aprotic solvents such as acetonitrile. Sometimes nucleophilic aprotic solvents can also be used to prepare good films if the nucleophilicity of the solution is reduced by protic acids. Besides the nucleophilicity, the stability of solvents and electrolytes should be considered when choosing solvents and electrolytes.

The chemical polymerization process involves mixing a monomer with a polymerization agent, usually an oxidant. It is simpler compared to the electrochemical polymerization method since no electrochemical instruments and controls are required. There are two ways to synthesize conducting polymers chemically. One method includes separate polymerization and doping processes, whereas the other combines both steps.

Non-conducting or semiconducting polymers can be first synthesized and then doped to become conducting polymers. For example, poly-(p-phenylene) (PPP) can be synthesized according to the following reaction:

\[
\begin{align*}
\text{benzene} \quad \text{CuCl}_2/\text{AlCl}_3 \quad \text{(1.21)}
\end{align*}
\]

Here benzene is polymerized by a Lewis acid catalyst (such as AlCl₃, AlBr₃ or FeCl₃) and an oxidant (CuCl₂, MnO₂, MoCl₅, FeCl₃, etc). The polymer is then doped either chemically or electrochemically to form a conducting polymer. \(n\)-Type and \(p\)-type doping can be performed by AsF₅ and Na treatment of the polymer respectively.
Some conducting polymers, for example polypyrrole, can be synthesized and doped simultaneously. Polypyrrole can be synthesized according to the following reaction:

\[
\text{FeCl}_3 \rightarrow [\text{H}^+ \text{N}_x^+ \text{Cl}^-]_n
\]

Fe$^{3+}$ has received much attention as an oxidant (147-152). However, other oxidants can also be used for the polymerization. For example polypyrrole can be synthesized using other transition metal ions such as Cu$^{2+}$ (149, 150), Ag$^+$, as well as Cl$_2$ (153).

Modification of the properties of conducting polymers can be carried out in the following ways:
1. using different monomers or their derivatives;
2. incorporating different counterions;
3. forming copolymers or polymer composites; and
4. derivatizing conducting polymers.

Conducting polymer properties can be easily changed through various monomers or their derivatives. There are many monomers which are suitable for forming conducting polymers. Polyacetylene (140), polypyrrole (145, 154, 155) polythiophene (156) poly(p-phenylene) (143) and polyaniline (157) have received a great deal of attention. With all these polymers, the polymer chain is formed by alternating single and double bonds along the polymer backbone.
This conjugated configuration is typical of conducting polymers. Although they are similar in structure, polymers can have greatly differing properties such as thermal stability, air stability, conductivity and other chemical and physical properties. These properties vary depending on the type of polymer and the method used to synthesize it. Examples of other monomers include indole (158, 159), furan (159), azulene (159, 160), pyrene (160), naphthylene (161), 2,5-thienylenevinylene (162), 2,5-pyridinediyl (163) and diphenylamine (164, 165). Derivatives of some monomers such as indole (158), thiophene (166, 167) and pyrrole (168-172) have been also used to form conducting polymers. Therefore, a customized conducting polymer may be synthesized from a selected derivative of a monomer. For example, some chiral conducting polymers have been synthesized using pyrrole or thiophene chiral derivatives as monomers (173), and redox polymers have been produced using pyrrole derivatives (174-175).

Some conducting polymers can have no counterions, since compensation of positive charge sites on the polymer matrix is made by a negatively charged functional group attached to the monomer (176-178). These self-doped conducting polymers may be more stable in structure because no counterion degradation or exchange can occur.

The chemical and physical properties of the conducting polymer depend upon the nature of the counterion. Therefore conducting polymer properties can be modified by the incorporation of various counterions during polymerization. For instance, catalysts (179, 180) and proteins (181) have been incorporated into polypyrrole for specific applications.
Copolymerization and formation of polymer composites are other ways to modify the polymer properties. For example, the mechanical properties of polypyrrole can be improved by copolymerization with N-(4-ferrocenylbutyl-)pyrrole (182) and N-substituted monomers (183), or the formation of composites with polyvinyl alcohol (184) and poly(p-phenylene terephthalamide) (185).

Some functions can also be added into conducting polymers via derivatization, such as implantation of ions (186) and immobilization of functional groups (187).

The attraction of conducting polymers is mainly due to their unique features. For example:

Physically,

(1) the polymers are conductive;
(2) some conducting polymers are anisotropic;
(3) some conducting polymers are thermally stable; and
(4) most conducting polymers are dark and some change color in different media.

Chemically,

(1) the polymers have charged backbones which are neutralized by various counterions;
(2) most conducting polymers are insoluble in common solvents;
(3) some conducting polymers have high pH tolerance;
(4) the polymer composition can be varied by changing monomers, derivatives, and counterions, and by using copolymerization and derivatization; and
(5) some counterions can be released or exchanged through electrochemical or chemical reactions.

Although many conducting polymers have been synthesized, the types of conducting polymers suitable for liquid chromatography are limited by the demanding criteria imposed by chromatographic conditions. For example, the polymer should:

(1) be stable in a range of eluents;
(2) be easily coated on substrates such as silica or be amenable to the formation of polymer beads;
(3) be able to obtain reproducible retentions;
(4) be stable at high pressures; and
(5) be able to provide predetermined molecular interactions.

The features of conducting polymers suggest a great potential in the use of conducting polymers for HPLC separations and detection.

1.3 Aim of the Project and Structure of the Thesis

Although conducting polymers have been studied and applied in many areas, their applications in HPLC have been limited. Therefore, studies using conducting polymers for HPLC separations and detection are of interest in present research and development.

The general aim of the project is to study the feasibility of employing conducting polymers in chromatographic separations and
detection. Polypyrrole (PP) (188-191) was chosen for this study due to the following reasons:

(1) it can be synthesized easily using either chemical or electrochemical methods;
(2) it is very stable in common solvents;
(3) it has been extensively studied and a lot of background information is available;
(4) a number of substrates can be used for the polymer growth; and
(5) a wide variety of counterions can be incorporated into the polymer.

The work described has been split into three parts:

(1) Synthesis and characterization of polypyrrole;
(2) Development of a polypyrrole stationary phase for HPLC and ECLC separation; and
(3) Development of a polypyrrole electrode for HPLC detection.

The aims and approaches of each part will be now be addressed

Part 1: The aim of this part is to study basic aspects of synthesis and characterization of polypyrrole to be used in chromatographic applications. The synthesis and characterization of polypyrrole has been investigated by various workers, but only some aspects relevant to the use of this polymer in chromatography have been studied. In this work, the synthesis and characterization of polypyrrole on various substrates have been investigated. Some chemical and physical properties of these polymers have been studied. This part includes Chapters 2, 3 and 4.
Part 2: This part aims to investigate the feasibility of using conducting polypyrrole as an HPLC stationary phase. Such an investigation is of interest due to the features of conducting polymers which cannot be provided by present chromatographic packings, such as silica gels and polymer beads. It is possible to introduce new selectivity and stability to HPLC through such a new material.

Synthesis and characterization of conducting polypyrrole stationary phases will be studied in this part. Hardware and systems for synthesis and characterization of the stationary phases have been designed and tested, chromatographic characterization of polypyrrole on RVC packings has been carried out, and chromatographic characterization of polypyrrole on silica-based packings has been investigated. This part includes Chapters 5, 6 and 7.

Part 3: The aim of this part is to develop and apply polypyrrole modified electrodes in amperometric detection for HPLC. The development of a conducting polypyrrole dispersed-mercury electrode and the subsequent application of this electrode in the determination of metal ions and chloramines following HPLC separation have been investigated. This part includes Chapter 8.
Chapter 2
Synthesis and Characterization of Polypyrrole
2.1 Introduction

As mentioned in Chapter 1, the synthesis of conducting polymers has been extensively investigated previously. However, many properties of conducting polymers, especially those associated with chromatography, are still not well understood. It is essential, therefore, to establish routine synthetic and characterization techniques to elucidate these properties.

Characterization of polypyrrole has been carried out using various methods. The conducting mechanism has been investigated by energy spectroscopic methods such as high resolution electron energy-loss spectroscopy (192) and in situ ESR-electrochemical experiments (193) in which the presence of polarons, bipolarons and solitons (188, 192, 193) partly explained the conduction mechanism. Conductivity has been determined by the four point probe technique (194-196). Structural information has been obtained using FTIR (197), NMR (198), ESR (193), IR (197, 199, 200), Raman spectroscopy (195, 196, 201), Mossbauer spectroscopy (202) and spectroscopic ellipsometry (203). Polymer composition has been determined using elemental analysis (195) and X-ray analysis methods such as XPS (204-206), EDX (207), and EPMA (194). The morphology of electrodeposited materials has been studied by SEM (195, 196). Thermal stability of polypyrrole has been tested using TG and DTA (199, 208, 209). Other techniques
such as electrical measurements (210) and dynamic mechanical analysis (211) have been used to obtain information on the electronic and mechanical properties of conducting polymers. The electrochemical properties of these conducting polymers have been evaluated using various methods including chronoamperometry (212), chronopotentiometry (213), impedance measurement (214) and cyclic voltammetry (215, 216).

In this work, polypyrrole (PP) has been synthesized electrochemically (217, 218) and chemically (147-153) on a variety of substrates including reticulated vitreous carbon (RVC), stainless steel, gold or tin oxide coated plastic film, carbon fibre, carbon cloth, filter paper and silica gels. Some of these substrates have already been employed for chromatographic separation or detection, e.g. filter paper and silica gel are commonly used as chromatographic stationary phases (9-11, 219), while RVC has been used in flow through amperometric detectors (220). Characterization of polypyrrole has been carried out using the methods listed in Table 2.1.
<table>
<thead>
<tr>
<th>Technique</th>
<th>Reference</th>
<th>Information obtained</th>
</tr>
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<tbody>
<tr>
<td>Chronopotentiometry</td>
<td>213</td>
<td>Polymerization mechanism, conductivity, capacitance</td>
</tr>
<tr>
<td>Chronoamperometry</td>
<td>212</td>
<td>Polymerization mechanism, conductivity, capacitance</td>
</tr>
<tr>
<td>Elemental analysis</td>
<td>195</td>
<td>Polymer composition</td>
</tr>
<tr>
<td>Electrogravimetric analysis (EGA)</td>
<td>221</td>
<td>Polymer composition, current efficiency during electrodeposition</td>
</tr>
<tr>
<td>Scanning electron microscopy (SEM)</td>
<td>195, 196</td>
<td>Polymer morphology</td>
</tr>
<tr>
<td>Cyclic voltammetry</td>
<td>215, 216</td>
<td>Electrochemical properties</td>
</tr>
<tr>
<td>Fast atomic bombardment mass spectrometry (FAB/MS)</td>
<td>222, 223</td>
<td>Counterion identification</td>
</tr>
<tr>
<td>Energy dispersive x-ray analysis (EDX)</td>
<td>207</td>
<td>Counterion semiquantitative analysis</td>
</tr>
<tr>
<td>Thermal gravimetry and differential thermal analysis (TG/DTA)</td>
<td>224, 225</td>
<td>Thermal properties</td>
</tr>
</tbody>
</table>
2.2 Experimental

2.2.1 Reagents and materials

All reagents were of analytical reagent (AR) grade purity unless otherwise stated. Pyrrole was distilled before use. Platinum electrodes were made from 0.635 mm diameter platinum wire of 10 mm length. The electrodes were pretreated with chromic-sulfuric acid and rinsed with water. Reticulated vitreous carbon was obtained from Energy Research Generation, Inc. Electrical contact to the RVC cartridges (18 mm x 3 mm diameter) was made via a wire. The cartridges were rinsed with methanol, pretreated with 6 M HCl solution for 3 hours and then washed with water prior to use. Tantalum wires (0.25 mm diameter) and the stainless steel plates (316, BHP) were polished with a piece of fine glasspaper and steel wool, cleaned with a tissue and washed with water. Tin oxide coated plastic film (Intrex K, <100 L/square inch, Sierracin/Intrex), gold coated plastic film (Intrex G, 1.5 L/square inch, Sierracin/Intrex), carbon fibre (TCM128, Le Carbone-Lorraine), and carbon cloth (Celion GY70, Celanese, Ltd) were rinsed with methanol and then with water before the polymeric coating step. Platinum gauze was used as a counter electrode and a Ag/AgCl (3 M NaCl) reference electrode was employed. Filter paper (Whatman) and silica gel (BDH) were used for coating polypyrrole chemically.

A 0.20 M pyrrole and 0.20 M KCl solution was used to synthesize polypyrrole chloride (PP/Cl) and a 0.10 M pyrrole and 0.10 M sodium dodecyl-sulfate (SDS) solution was used to synthesize polypyrrole dodecyl-sulfate (PP/DS). 1.5 M FeCl₃ was employed as
an oxidant for the chemical synthesis of polypyrrole. Conductive carbon paint was obtained from Probing and Structure.

2.2.2 Instrumentation

Electrochemical polymerization was carried out using a Princeton Applied Research (PAR) Model 173 potentiostat/galvanostat in conjunction with a Model 179 digital coulometer. Cyclic voltammograms were obtained using a Model 175 potential controller (PAR) and Model 173 potentiostat/galvanostat. Alternatively, a CV-27 voltammograph was employed. Chronopotentiograms and chronoamperograms were recorded using an HP-3390A integrator (Hewlett-Packard) which was connected to a 173 potentiostat/galvanostat. Polymer micrographs and EDX spectra were taken by a Hitachi S-450 scanning electron microscope connected to a TN-2000 Microtrace detector (United Science). Fast atom bombardment mass spectra were obtained using a VG analytical model MM 12-12 mass spectrometer.

2.2.3 Procedures

2.2.3.1 Electropolymerization of pyrrole

Electrodeposition of polypyrrole on a platinum electrode was carried out galvanostatically in a 0.20 M pyrrole and 0.20 M KCl solution at 0.20 mA for 200 seconds. Synthesis of polypyrrole on the novel substrates was carried out in a 0.10 M pyrrole and 0.10 M SDS solution. The experimental conditions are summarized in Table 2.2. Chronopotentiograms or chronoamperograms were recorded during polymerization.
Table 2.2  Synthesis of polypyrrole on novel substrates

<table>
<thead>
<tr>
<th>Working electrode</th>
<th>Surface area (cm²)</th>
<th>Current (mA)</th>
<th>Potential (V)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVC</td>
<td>8.4</td>
<td>2.0</td>
<td>--</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>8.4</td>
<td>- -</td>
<td>+0.8</td>
<td>6.6</td>
</tr>
<tr>
<td>Tantalum wire</td>
<td>0.15</td>
<td>0.3</td>
<td>--</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>- -</td>
<td>+0.8</td>
<td>11.7</td>
</tr>
<tr>
<td>Stainless steel</td>
<td>2.0</td>
<td>1.0</td>
<td>--</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>- -</td>
<td>+0.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Carbon fiber</td>
<td>unknown</td>
<td>1.0</td>
<td>--</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>unknown</td>
<td>- -</td>
<td>+0.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Carbon cloth</td>
<td>unknown</td>
<td>2.0</td>
<td>--</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>unknown</td>
<td>- -</td>
<td>+0.8</td>
<td>8.9</td>
</tr>
<tr>
<td>Gold film</td>
<td>1.0</td>
<td>0.5</td>
<td>--</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>- -</td>
<td>0.6</td>
<td>6.3</td>
</tr>
<tr>
<td>Tin oxide film</td>
<td>1.0</td>
<td>0.5</td>
<td>--</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>- -</td>
<td>+0.8</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Counter electrode: platinum
Reference electrode: Ag/AgCl
Monomer solution: 0.10 M pyrrole and 0.10 M SDS
2.2.3.2 Chemical polymerization of pyrrole

Chemical deposition of polypyrrole on filter paper was carried out as follows:

(1) Pure pyrrole was deposited by a pipette on to a piece of Whatman filter paper (7 mg pyrrole/cm\(^2\) paper) and allowed to soak in for 10 minutes;

(2) The paper was dipped into a 1.5 M FeCl\(_3\) solution at room temperature for 30 minutes; and

(3) The polymer-coated paper was rinsed with distilled water.

Chemical deposition of polypyrrole on particles was carried out using the following procedure:

(1) Pure pyrrole was adsorbed on to silica particles (silica gel 60, Merck) (140 mg pyrrole/g silica);

(2) The particles were stirred in a 0.1 M counterion solution in order to form a suspension;

(3) 0.015 M FeCl\(_3\) or 0.03 M K\(_2\)Cr\(_2\)O\(_7\) and 0.01 M HCl were added in the solution immediately after step 2;

(4) The suspension was stirred for 30 minutes; and

(5) The polymer-coated particles were filtered and washed with distilled water using a Buchner funnel.

2.2.3.3 Characterization of polypyrrole

After polymerization the polymer-coated electrodes were electrochemically characterized using cyclic voltammetry in a 0.20 M KCl supporting electrolyte.

EGA samples were prepared by galvanostatic coating of polypyrrole onto gold or tin oxide coated plastic films in 0.10 M pyrrole and
0.10 M counterion solutions at 1 to 2 mA/cm² current density. The films were dried under vacuum at 62°C to remove moisture from the samples and weighed until a constant weight was obtained. Total charge was calculated from the controlled current and the deposition time by \( Q = I t \). The polymer weight was obtained by deducting plastic film weight from total weight.

Using the same procedure as above, samples for elemental analysis were prepared. The elemental analysis was performed by the Analytical Laboratory, Australian National University. Samples synthesized as in section 2.2.3.1 on various substrates were used for SEM as well as EDX characterization. The samples were attached to the sample tray using conductive carbon paint. The electron accelerating voltage was 20 KV. The reflected X-ray signals were accumulated by the EDX detector for 150 seconds, and then transferred to an on-line Apple II computer and stored on a floppy disc. The Cl to P mole ratio was obtained using a computer program (Department of Material Engineering at the University of Wollongong). Samples for FAB/MS were prepared on RVC cartridges (18 mm x 8 mm diameter) by coating PP/DS on RVC using the experimental conditions as in Table 2.2. The samples were then attached to a holder and inserted into the mass spectrometer.
2.3 Results and Discussion

2.3.1 Polymerization of pyrrole

2.3.1.1 Electrochemical polymerization

In order to test the validity of an electrochemical coating approach for use of conducting polymers in chromatography, polymer growth on novel substrates was initially investigated. Substrates investigated were tantalum wire, carbon cloth, carbon fibers, tin oxide coated plastic film, gold coated plastic film, stainless steel and reticulated vitreous carbon cartridge. Both Cl⁻ and DS⁻ were incorporated into polypyrrole during electropolymerization. Using a 0.30 M pyrrole solution containing 0.10 M SDS as the supporting electrolyte, polymers could be grown on all substrates galvanostatically. Polymer formation on all substrates except stainless steel was also possible with 0.20 M KCl as the supporting electrolyte. The anodic potentials required to oxidize the polymer were sufficient to oxidize the stainless steel in the chloride media.

Galvanostatic polymerization processes can be monitored using chronopotentiometry. Chronopotentiograms of polypyrrole growth on various substrates are shown in Figure 2.1. An initial potential peak was observed on all substrates which was followed by a constant potential. A sharp increase in potential at the beginning of the process is presumably due to the charging of the double layer of the electrode surface. Consequently, the resultant peak potential depends on the capacitance of the electrode surface and on the polymer growth rate. The potential was then observed to decrease once polymer growth had started, i.e. a faradaic process occurred. Maintenance of a constant potential indicated that the polymers
were conductive. The difference in peak potentials (Figure 2.1) indicated that the surface chemistry of the electrode influences the electropolymerization processes. The final potential obtained was substrate independent since with large time scales polymer growth occurs on the deposited polymer. As shown in Figure 2.2, a peak potential was generated when the polymerization process was initiated on the platinum electrode during time a-b. However, no peak potential was observed when the electropolymerization process was restarted during time c-d. This indicated that polymer growth was so rapid on the former polymer that the initial charging process could not be observed.

If the polymerization process was potential-controlled then chronoamperometry was employed to monitor the electropolymerization process. Chronoamperograms on various substrates are shown in Figure 2.3. On the tantalum wire and tin oxide foil, the initiation of polymerization process was very slow, so that a current peak due to charging up the double layer of the electrode at the initiation stage appeared. On the other substrates this current peak was overlaid by the faradaic current since the initiation process on these substrates was very fast. During polymer growth a steady current was produced, which indicated that the polymer was conductive and the resistance of the polymer was negligible in this polymerization period, since an increase of the resistance at a constant potential could decrease the current. The difference in current values was presumably due to the difference of the electrode surface area.
Figure 2.1  Chronopotentiograms obtained during electropolymerization on various electrode materials

Conditions: as shown in Table 2.2;

Chronopotentiograms on: a. RVC, b. Ta wire, c. stainless steel, d. carbon fiber e. carbon cloth, f. gold coated plastic film and g. tin oxide coated plastic film.
Figure 2.2 *Chronopotentiogram recorded during the electropolymerization process*

*Electropolymerization solution:* 0.20 M KCl and 0.30 M pyrrole;  
*Working electrode:* RVC cartridge (8 mm diameter x 18 mm);  
*Reference electrode:* Ag/AgCl (3 M NaCl);  
*Auxiliary electrode:* Pt;  
*Galvanostatic control:* 30 mA.
Figure 2.3  Chronoamperograms obtained during electropolymerization on various electrode materials

Conditions: as shown in Table 2.2;

Chronoamperograms on:  a. RVC, b. Ta wire, c. stainless steel, d. carbon fiber e. carbon cloth, f. gold coated plastic film and g. tin oxide coated plastic film.
The mechanism of electropolymerization has been suggested as follows (146):

\[ \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{e}^- \]

\[ 2\text{H}_2\text{N}_2\text{H}^+ \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + 2\text{H}^+ + \text{e}^- \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

\[ \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \rightarrow \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} + \text{H}_2\text{N}_2\text{H}^+ + \text{H}_2\text{N}_2\text{H} \]

After the initial oxidation step, oxidized pyrrole units can couple together and then deprotonate, followed by a one-electron oxidation in order to regenerate the aromatic system. Since the polymerization reaction can only occur when the potential is high enough to oxidize the monomer, the concentration of neutral aromatic species is zero at the electrode and negligible in the region of the electrode. Therefore, in the initial stages of the reaction, the
radical cations first produced may have no opportunity to react with any neutral monomers like electrophiles but undergo radical coupling reactions with other radicals to form dimers. In the polymer growth stages, the coupling reaction must also take place between the radical cations of the oligomers, since the dimer, trimer and polymer are more easily oxidized than the monomer (226). Therefore they will also be in the oxidized state rather than the neutral form during the polymerization.

2.3.1.2 Chemical polymerization
To deposit conducting polymers onto non-conductive substrates, an electropolymerization process may not be suitable. Therefore, chemical polymerization was investigated.

Initially, polypyrrole was synthesized on filter paper in order to test the feasibility of depositing polypyrrole on nonconducting substrates. According to the procedure described in the experimental section, pyrrole was oxidized by Fe$^{3+}$ and a black coating of polypyrrole was deposited on the white paper instantaneously. The polymer coated paper (PP/Cl/Paper) is conductive. This chemical polymerization process has been reported by many workers (147-153). Also polypyrrole powders (147-153) and polypyrrole coated substrates (227, 228) have been produced using this method.

Two steps are involved in this chemical coating procedure. Firstly, pyrrole was absorbed on to the porous filter paper. The
polypyrrole was then formed and deposited on the paper when the paper was soaked in the oxidant solution.

With chemical oxidation counterions incorporated into the polymer are the counterions associated with the oxidant. For example, when FeCl$_3$ is used as an oxidant, the counterions in the polypyrrole are Cl$^-$ ions. Using this approach only a limited range of anions may be incorporated.

The deposition of polypyrrole on silica particles was investigated. When pyrrole-soaked silica particles were stirred in a 0.1 M SDS and 0.015 M FeCl$_3$ solution, PP/DS/Cl was formed on the particles. Elemental analysis indicated that the mole ratio of N:S:Cl was 1.00 : 0.23 : 0.31. These results also indicate that incorporation of counterions other than the anion of the oxidant, during chemical polymerization, is possible.

Oxidants other than Fe$^{3+}$ may also be used. K$_2$Cr$_2$O$_7$ was found to be a useful oxidant for polymerization of pyrrole, since Cr$_2$O$_7^{2-}$ is a very strong anionic oxidant. It was found that when silica particles presoaked with pyrrole were stirred in a 0.01 M HCl, 0.10 M KCl or 0.03 M K$_2$Cr$_2$O$_7$ solution, polypyrrole formed on the particles within a few minutes.

Compared with electropolymerization, the chemical polymerization process is fast so that bulk conducting polymers may be produced in a short period. The method is simple and no special instrumentation is required.
2.3.2 Characterization of polypyrrole

2.3.2.1 Electrochemical characterization of deposited polypyrrole
Polypyrrole chloride (PP/Cl) was electrochemically deposited on a conventional platinum wire electrode as described in the experimental section and then characterized by cyclic voltammetry. Faradaic currents due to the redox reaction of the polymer and charging currents due to change in capacitance of the polymer were detected using cyclic voltammetry (Figure 2.4). A reduction peak (a) at approximately -0.8 V vs. Ag/AgCl in 0.1 M NaN0₃ and an oxidation peak at approximately -0.58 V (a') were observed and are probably due to the following reaction.

$$PP^{+}Cl^{-} + e \rightarrow PP^{0} + Cl^{-}$$ (2.2)

The reduction peak corresponds to release of Cl⁻ according to equation 2.2. The initial scan from positive to negative potentials produced a large reduction current peak and then this reduction current peak decreased with the number of potential scans until equilibrium was reached (Figure 2.4). The reason for this may be that the Cl⁻ concentration in the polymer decreases since NO₃⁻ in the supporting electrolyte will replace Cl⁻ during scanning. This ion exchange process is characterized by a reduction peak (b) at -0.10 V and an oxidation peak (b') at +0.24 V. This process may be explained by the following reactions:

$$PP^{0} + NO_{3}^{-} \rightarrow PP^{+}NO_{3}^{-} + e^{-}$$ (2.3)

$$PP^{+}NO_{3}^{-} + e^{-} \rightarrow PP^{0} + NO_{3}^{-}$$ (2.4)
The reactions 2.2 and 2.3 show an electrochemical ion exchange process, and the reaction 2.4 indicates the redox property of newly formed PP/NO₃ which has different redox potentials from PP/Cl.

Cyclic voltammograms for polypyrrole grown on the various substrates are summarized in Figure 2.5. The cyclic voltammograms on these substrates appear to be less reversible than on the platinum wire due to the use of less conductive substrates and thicker polymers. A thick polymer layer will increase the resistance and capacitance of the electrode, so that a high charging current is observed in the cyclic voltammogram.

Cyclic voltammetry has been widely used for characterizing electroactivities of conducting polymers since it can provide the following information: (1) the reduction potential (2) the oxidation potential, (3) the charging current, (4) the reversibility of the electrochemical reaction, and (5) the counterion exchange process (194, 195, 215-217).

The differences in electrochemical properties of polypyrrole obtained using cyclic voltammetry indicated that the substrate can play an important role in electropolymerization. Such substrate effects were noted previously by other workers (229-231).
Figure 2.4  Cyclic voltammogram of a polypyrrole chloride coated platinum electrode

Working electrode: Pt;
Reference electrode: Ag/AgCl;
Auxiliary electrode: Pt;
Potential range: D.C. between -1.0 V and +0.6 V at scanning rate of 50 mV/sec;
Supporting electrolyte: 0.10 M NaNO₃;
1-3=scan numbers.
Figure 2.5  Cyclic voltammograms of polypyrrole on various substrates

Supporting electrolyte: 0.20 M KCl;

Working electrode: PP/Cl on various substrates

Auxiliary electrode: Pt;

Reference electrode: Ag/AgCl (3 M NaCl);

Potential: D.C. -1.0 - +1.0 V, at scanning rate of 100 mV/s;

2.3.2.2. Elemental analysis

The results of elemental analysis of polypyrrole with different counterions are summarized in Table 2.3. In order to compare with various counterions, other than Cl\(^-\) and DS\(^-\), CIO\(_4\)\(^-\) and mandelate were incorporated in polypyrrole in 0.10 M pyrrole and 0.10 M NaCIO\(_4\) or 0.10 M sodium mandelate solutions. Excess hydrogen was found in all cases except PP/DS. It has been reported by other workers (195) that this is due to side reactions or trapped reagents. Pyrrole unit to counterion ratios were found to range between 2 and 3 which were similar to those reported (188). PP/Cl\(^-\) formed by chemical polymerization showed similar pyrrole unit to counterion ratios to those generated by electropolymerization.

**Table 2.3  Elemental analysis of polypyrrole with various counterions\(^1\)**

<table>
<thead>
<tr>
<th>Polymer</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>C(^-)</th>
<th>pyrrole: C(^-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP/DS(^2)</td>
<td>7.89</td>
<td>11.01</td>
<td>1.00</td>
<td>0.334</td>
<td>2.99</td>
</tr>
<tr>
<td>PP/Cl(^2)</td>
<td>3.97</td>
<td>3.23</td>
<td>1.00</td>
<td>0.385</td>
<td>2.60</td>
</tr>
<tr>
<td>PP/Cl(^3)</td>
<td>4.01</td>
<td>3.82</td>
<td>1.00</td>
<td>0.367</td>
<td>2.72</td>
</tr>
<tr>
<td>PP/Mandelate(^2)</td>
<td>7.62</td>
<td>6.52</td>
<td>1.00</td>
<td>0.466</td>
<td>2.15</td>
</tr>
<tr>
<td>PP/CIO(_4)(^2)</td>
<td>3.97</td>
<td>3.30</td>
<td>1.00</td>
<td>0.476</td>
<td>2.10</td>
</tr>
</tbody>
</table>

1. The analysis was carried out by the Analytical Laboratory, Australian National University.

2. The samples were electrochemically synthesized.

3. The sample was chemically synthesized.
2.3.2.3 Electrogravimetric analysis

2.3.2.3.1 Principle

The electropolymerization can be expressed by equation 1.20. Each pyrrole unit loses two electrons and every \( x \) pyrrole unit loses another electron during the polymerization (146, 188). Given Faraday's law the following equation is derived:

\[
Q = (2x+1) \frac{FW}{xMp+Mc} \tag{2.5}
\]

where, \( Q \) is the total charge, \( F \) is the Faraday constant, \( W \) is the polymer weight, and \( Mp \) and \( Mc \) are the molecular weights of pyrrole and its counterion respectively.

The polymer weight can be divided into two parts, i.e. the weight due to pyrrole (\( Wp \)) and the weight due to its counterion (\( Wc \)). From the structure of polypyrrole:

\[
W = Wp + Wc \tag{2.6}
\]

\[
Wp/Mp = xWc/Mc \tag{2.7}
\]

From equations 2.5, 2.6 and 2.7, an equation relevant to the polymer composition can be derived.

\[
x = \frac{(FW-QMc)}{(QMp-2FW)} \tag{2.8}
\]

Equation 2.8 can be used in electrogravimetry to determine the polymer composition (i.e. pyrrole to counterion ratio), if the current efficiency is 100%.

A further study of equation 2.8 shows that the molecular weight of the counterion can significantly affect results obtained using EGA.
Partially differentiating equation 2.8, the following equations can be obtained.

\[ \frac{\delta x}{x} = \left[ \frac{FQW(M_p - 2M_c)}{(x(QM_p - 2FW)^2)} \right] \frac{\delta W}{W} \quad (2.9) \]
\[ \frac{\delta x}{x} = -\left[ \frac{FQW(M_p - 2M_c)}{x(QM_p - 2FW)^2} \right] \frac{\delta Q}{Q} \quad (2.10) \]

From the above equations, the relative deviation of \( x \) due to weighing the polymer or measuring the charge can be estimated.

Assuming that the total polymer weight \( W \) is 0.100 g and that the measuring precision, \( \Delta W/W \) (approximately equal to \( \delta W/W \)) or \( \Delta Q/Q \) (approximately equal to \( \delta Q/Q \)) is 1% and having \( M_p = 65 \), the analysis error \( \frac{\delta W}{x} \) can be estimated using equations 2.8 and 2.9. The relation of this error to the molecular weight of the counterions can be plotted as Figure 2.6. The result indicates that the measuring error of \( x \) using EGA is dependent on counterion size and consequently a counterion with a small molecular value will introduce a large error in the determination of \( x \), using EGA. For example, determination of the \( x \) value for PP/Cl is not practical since a small measuring error in weight or in the charge can cause a large error in \( x \).
Similarly, given \( M_p = 65 \), the required charge for depositing 0.100 g polymer using different size counterions can be calculated from equation 2.5. The results are shown in Table 2.4. It is evident that for small counterions, for example, \( \text{Cl}^- \) (\( M_c = 35.5 \)), the required charge is not significantly varied by the \( x \) values and consequently a known charge should produce a known amount of polymer independent of \( x \). This conclusion may be used for determining the current efficiency (CE) by assuming an \( x \) value between 2 and 6. The difference between measured and calculated charge from the polymer weight may be substituted in the following equation to give the CE value.

\[
CE(\%) = \frac{(2x+1)FW}{(xM_p+M_c)It} \times 100\% \\
(2.11)
\]
where x can be assumed as 2-6 since it is the suggested range (188) for polypyrrole.

Table 2.4 Required charge for 0.100 g polypyrrole using different size counterions

<table>
<thead>
<tr>
<th>Mc</th>
<th>x=2</th>
<th>x=3</th>
<th>x=4</th>
<th>x=5</th>
<th>x=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>35.5</td>
<td>291.5</td>
<td>293.1</td>
<td>293.9</td>
<td>294.5</td>
<td>294.8</td>
</tr>
<tr>
<td>59</td>
<td>255.3</td>
<td>265.9</td>
<td>272.3</td>
<td>276.4</td>
<td>279.4</td>
</tr>
<tr>
<td>70</td>
<td>241.3</td>
<td>254.9</td>
<td>263.2</td>
<td>268.7</td>
<td>272.7</td>
</tr>
<tr>
<td>99.5</td>
<td>210.2</td>
<td>229.4</td>
<td>241.6</td>
<td>250.1</td>
<td>256.3</td>
</tr>
<tr>
<td>150</td>
<td>172.3</td>
<td>195.8</td>
<td>211.8</td>
<td>223.5</td>
<td>232.3</td>
</tr>
<tr>
<td>265</td>
<td>122.2</td>
<td>146.8</td>
<td>165.4</td>
<td>179.9</td>
<td>191.5</td>
</tr>
</tbody>
</table>

1. Assuming W=0.100 g and giving Mp=65
2. Charge unit is C.

2.3.3.2 EGA results

Table 2.5 summarizes the EGA analysis results. However, compared with elemental analysis (Table 2.3), EGA gives higher x values, particularly in the case of PP/ClO₄. One reason for this positive x value may be that the current efficiency is less than 100%.
Table 2.5  
EGA results of polypyrrole with various counterions

<table>
<thead>
<tr>
<th>Counterion</th>
<th>Mc</th>
<th>x</th>
<th>mean x</th>
<th>σ/x(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS⁻</td>
<td>265</td>
<td>3.13 3.05 3.34</td>
<td>3.15</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.12 3.22 3.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ClO₄⁻</td>
<td>99.5</td>
<td>3.81 3.95</td>
<td>3.88</td>
<td>1.8</td>
</tr>
<tr>
<td>Mandelate</td>
<td>152</td>
<td>2.37 2.32</td>
<td>2.35</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Determination of the current efficiency using Cl⁻ as the counterion during electropolymerization was carried out by EGA. Recording of the current and time employed for the electropolymerization and measuring the resultant polypyrrole weight, and enabled a calculation of the current efficiency, when these values were substituted into equation 2.11. The results further confirmed that the current efficiency is less than 100% (Table 2.6) during electropolymerization. This means that some of the electrical energy has been consumed in side reactions, for example, in formation of the dimer and trimer as reported previously (226), or due to the residual current.
Table 2.6 Determination of current efficiency during electropolymerization

<table>
<thead>
<tr>
<th>Sample No</th>
<th>current density (mA/cm²)</th>
<th>Q_{measured} (C)</th>
<th>W (g)</th>
<th>Q_{calculated} (C)^2</th>
<th>current efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.3</td>
<td>119.28</td>
<td>0.03471</td>
<td>102.02</td>
<td>85.5</td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>153.26</td>
<td>0.04378</td>
<td>128.67</td>
<td>84.0</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>124.11</td>
<td>0.03576</td>
<td>105.10</td>
<td>84.7</td>
</tr>
</tbody>
</table>

1. Monomer solution = 0.25 M NaCl and 0.15 M pyrrole in 25% CH₃CN and 75% H₂O. Substrate = Au coated plastic film.
2. According to equation 2.9 assuming x=4.

2.3.3.3 Scanning electron microscopy

It has been reported by previous workers that the morphology of an electrodeposited conducting polymer can be characterized using SEM (195, 196). In this work morphological information of polypyrrole was obtained on different substrates using SEM (Figure 2.7).
Figure 2.7  Scanning electron micrographs of polypyrrole on various substrates

a. Carbon fiber; b. tin oxide coated plastic film; c. gold coated plastic film and d. stainless steel.
The results indicated that the morphology of the polypyrrole was dependent on the substrates used. The substrate effect may be explained as follows during electrodeposition. (1) During the initial stage of electropolymerization, the polymer cores first form on the sharp edges as shown by Figure 2.8. Those edges were the result of polishing or handling the electrode. (2) After the initiation stage, massive polymers developed from these cores to form a cauliflower-like appearance. For example, the polishing pattern on the stainless steel was observed before polymerization and it was observed after polymerization. Therefore, the substrate effect on the polymer morphology is a function of the nature and type of pretreatment of the material.

![Substrate with polishing traces](image1)  ![Formation of polymer cores](image2)  ![Massive growth of polymers](image3)

**Figure 2.8**  *Electrodeposition process*

2.3.3.4 *Fast atom bombardment mass spectrometry*

Components such as counterions in polypyrrole may be detected using FAB/MS. Usually liquid samples or samples with a liquid carrier, very often glycerol, have been employed in FAB/MS so that a fresh surface can be maintained (232). However, solid samples
such as silica gels (233), polymers (222,) and other materials (223) have also been subjected to FAB/MS.

A typical mass spectrum of negative ions is demonstrated in Figure 2.9. Incorporated DS\textsuperscript{-} as well as its fragments are detected by FAB/MS. FAB/MS therefore can characterize counterions in a conducting polymer. Figure 2.10 shows that the peak intensity decreases promptly with the atom beam scanning. This effect may be due to the fact that secondary ions produced using atomic bombardment could charge up the polymer and consequently reduce the sensitivity (223).

2.3.3.5 Energy-dispersive X-ray analysis
A typical EDX spectrum is depicted in Figure 2.11 with the major peak allocated to the elements. Other than counterion signals, the substrate SnO\textsubscript{2} used for coating the polymer also gave rise to signals. Therefore when using this method of characterization, it is important to select suitable substrates for growing polymers in order to avoid interferences from substrates. It was found that the carbon fibre did not produce EDX responses in the spectral region studied. Tin oxide foil caused background signals in the range between 3.4 to 3.7 KeV. However, signals did not interfere with the signals arising from P, Cl, S, I, Br or Cr. Aluminium from the sample tray gave rise to a response at 1.485 KeV, which interfered with Br. However, this problem may be overcome using an electrode material such as tin oxide foil to shield the aluminium.
Figure 2.9  Fast atom bombardment mass spectrum of PP/DS
Sample: PP/DS/RVC.
Figure 2.10  Deterioration of the mass spectral intensity with atom beam scanning

Sample: PP/DS/RVC.
The precision of EDX was found to be dependent on the experimental conditions employed. It has been reported that 2% precision can be obtained (234). The absolute intensity of EDX signals varied due to factors such as the sampling location, signal accumulation time and the electron accelerating voltage. However, using the relative intensities (i.e. ratio of two element signals) more precise data can be obtained. This procedure minimizes systematic errors. The reproducibility of EDX readings from different sampling points for the same sample obtained for the present method is indicated in Table 2.7.

**Table 2.7**  
*Precision of the EDX method*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ratio of P/Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.26</td>
</tr>
<tr>
<td>2</td>
<td>1.26</td>
</tr>
<tr>
<td>3</td>
<td>1.28</td>
</tr>
<tr>
<td>4</td>
<td>1.22</td>
</tr>
<tr>
<td>Mean value</td>
<td>1.26</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.02</td>
</tr>
<tr>
<td>Precision (%)</td>
<td>2</td>
</tr>
</tbody>
</table>

The result was obtained on different EDX sampling points from the same PP/Cl sample deposited on tin oxide plastic film which was treated in 0.20 M NaH$_2$PO$_4$ for 2 hours.
Figure 2.11 An example of EDX spectrum

sample: PP/Cl/SnO$_2$ film treated in a 0.20 M NaH$_2$PO$_4$

solution.

EDX condition: High voltage=20 KV; accumulation time=200 seconds.
2.4 Conclusion

Electrochemical polymerization has been carried out on various substrates including RVC, gold and tin oxide coated plastic, carbon cloth and fibers, stainless steel and tantalum. Both Cl\(^{-}\) and DS\(^{-}\) have been incorporated into PP. The process has been monitored using either chronopotentiometry or chronoamperometry, and the results indicated that the polymers were conductive. Chemical polymerization of polypyrrole has been carried out on filter paper and silica gels. FeCl\(_3\) can be used for polymerization of pyrrole. Both Cl\(^{-}\) and DS\(^{-}\) have been incorporated into the polymer. However, other than DS\(^{-}\), Cl\(^{-}\) from the oxidant (FeCl\(_3\)), can also be incorporated into polypyrrole, and consequently mixed counterions in polypyrrole have resulted.

Using the characterization methods listed in Table 2.1, some of the properties of polypyrrole have been characterized. The information obtained is summarized as follows.

1. The electroactivity of conducting polymers has been characterized by cyclic voltammetry. The information obtained here is valuable for chromatography, especially for ECLC in which electrochemical processes are involved.

2. The morphology of polypyrrole has been observed using SEM. It has been found that the morphology of polypyrrole is substrate dependent.

3. Polymer composition has been determined using elemental analysis. The pyrrole unit to counterion ratio can be calculated from the analysis.
(4) The pyrrole unit to counterion ratio has also been determined using EGA for PP/DS as well as polypyrrole with some other larger counterions. It has been found that the precision of the method is dependent on the molecular weight of counterions, and consequently the method may only be used for those polymers with large counterions. The current efficiency may be determined when the electropolymerization is carried out using Cl$^-$ which has small molecular weight.

(5) The presence of counterion has been confirmed qualitatively using FAB/MS.

(6) The ratio of two counterions in polypyrrole has been determined quantitatively using EDX.
Chapter 3
Ion Exchange Properties of Polypyrrole
3.1 Introduction

One of the major concerns of this work is to establish mechanisms of molecular interactions which take place on a conducting polymer surface and the investigation of such mechanisms for use in chromatography. With conducting polymers, ion exchange processes are most obvious and have been considered in some detail. In particular, the rate of ion exchange, the selectivity series, the effects of the ionic strength and the solution pH, and the effects of applied potential to the polymer on the ion exchange process are necessary to be studied since these properties may affect selectivities of polymer stationary phases and polymer modified electrodes. Reversibility of the ion exchange process is also of interest as this is essential for ion exchange chromatography. \( \text{Cl}^- \) and \( \text{ClO}_4^- \) have been considered as counterions in polypyrrole, since they are simple, different in size and detectable using EDX.

It is well known that the nature of the anionic species present as a counterion plays an important role in determining the physical properties of conducting polymers. The morphology (195, 217), conductivity (235), structure (236) and mechanical strength (237, 238) of the polymer are all influenced. In addition the chemical (180, 181) and electrochemical properties of conducting polymers (215, 216) are affected by the counterion present. The chemical
properties of polypyrrole, including the ion exchange properties, are not well understood at present.

It has been shown that counterions introduced in the course of the polymerization can be exchanged subsequently without loss of the conductivity (239). Inganas et al. (240) reported reversible ion exchange when polypyrrole hydroxide (PP/OH) was treated with nitric acid and Li et al. (241) also confirmed the occurrence of an ion exchange process using spectroelectrochemical measurements. Munstedt et al. (242) demonstrated that counterion exchange occurred when polypyrrole halide (PP/X) was exposed to HSO₄⁻ in an H₂SO₄ solution. Schenoff and Chien (243) showed anion exchange phenomena on polypyrrole between Cl⁻ and F⁻, and ClO₄⁻. Chao and March (207) showed that chemically synthesized polypyrrole also exhibited ion exchange properties. Although these qualitative studies indicated that ion exchange occurred, limited quantitative ion exchange data are available.

It has already been shown that upon reduction of the polypyrrole the counterion is expelled according to:

\[ \text{PP} + \text{C}^- + \text{e}^- \rightarrow \text{PP}^0 + \text{C}^- \]  

(3.1)

The application of a potential may then be used to influence ion exchange properties of the polymer. This electrochemically controlled ion exchange process has been studied by many workers (244-247), and used in controlled release processes (244, 245).

In this study, the rate and reversibility of the ion exchange process as well as the effects of various solvent properties such as ionic
strength and pH on the ion exchange process have been considered. The ion exchange selectivity series of various anions on conventional anion exchangers has been compared with polypyrrole, and the influence of a potential on the ion exchange rate has been studied.

3.2 Experimental

3.2.1 Reagents and materials
All reagents were analytical grade unless otherwise stated. All solutions were prepared in distilled and deionized water. Pyrrole (Sigma) was freshly distilled before use. Solutions for electropolymerization contained 0.10 M pyrrole and 0.10 M NaCl or NaClO₄ were prepared. Tin oxide coated plastic films were used as substrates for electropolymerization of pyrrole. Conductive carbon paint (Probing and Structure) was used to attach samples to the EDX sample tray.

3.2.2 Instrumentation
Electropolymerization of polypyrrole was carried out with a galvanostat made in the Science Faculty Electronic Workshop at the University of Wollongong. A CV-27 cyclic voltammograph (BAS) was used for electrochemical studies. All EDX data were obtained using a S-450 scanning electron microscope (Hitachi) connected to a TN-2000 Microtrace detector (United Science). A stopwatch was used to monitor time during the test of ion exchange rate.
3.2.3 Procedures

3.2.3.1 Preparation of samples

Tin oxide plastic films (2 cm x 1 cm) were rinsed with acetonitrile and water. A constant current density of 1 mA/cm\(^2\) was then applied for half an hour using various pyrrole/counterion solutions.

After polymerization polypyrrole coated samples were vigorously rinsed with water and acetone or acetonitrile, and then cut into small pieces about 5 mm in length. In order to obtain precise results, every test was conducted on the samples which were cut from the same tin oxide film.

3.2.3.2 Ion exchange

Rates of ion exchange were investigated by soaking tin oxide films coated with polymers in 0.20 M \(\text{NaH}_2\text{PO}_4\) and 0.20 M \(\text{Na}_2\text{HPO}_4\) solutions for a period which was monitored using a stopwatch and then rinsing them with water. The samples were then subjected to EDX analysis of the counterion to P mole ratio. The effect of an applied potential on the ion exchange process was tested by applying -0.35 V and +0.35 V (vs. an Ag/AgCl reference electrode) to the polymer and monitoring time using a stopwatch.

The samples used to test the pH effect on the ion exchange process were soaked for more than 24 hours in phosphate solutions at various pH values, but the same ionic strength. The samples were then rinsed with copious quantities of water before EDX analysis.

The samples used to test the effect of ionic strength on the ion exchange process were soaked in \(\text{NaH}_2\text{PO}_4\) solutions with different
concentration for approximately 24 hours and then rinsed with water. All other ion exchange processes were monitored after the samples were soaked in 0.10 M solutions containing the counterions of interest for more than three hours and then washed with water before EDX analysis.

3.2.3.3 Determination of counterions

Prepared samples were fastened to an aluminum sample tray using conductive carbon paint. The electron accelerating voltage was 20 KV in all experiments unless otherwise stated. The reflected X-ray signals were accumulated by an EDX detector for at least 150 seconds, and then transferred to an on-line Apple II computer and stored in a floppy disc. Semiquantitative measurements were obtained via an Apple IIe computer program (Department of Material Engineering at the University of Wollongong).

3.3 Results and Discussion

Ion exchange on conducting polymers can be represented by the following equation:

$$PP + C^- + A^- \rightarrow PP + A^- + C^-$$  \hspace{1cm} (3.2)

If the original counterion (C\textsuperscript{-}) and the exchanging anion (A\textsuperscript{-}) can be detected in the polymer after the polymer is soaked for an extended period in a solution containing A\textsuperscript{-}, then the ion exchange equilibrium constant can be calculated from the mole ratio of A\textsuperscript{-} to C\textsuperscript{-}. 
3.3.1 Ion exchange rate

Ion exchange processes on conventional resins may be divided into three steps (248, 249):

1. Mass-transfer in the external solution to the resin particles;
2. Diffusion inside the resin phase; and
3. "Chemical" exchange in the vicinity of the exchange groups.

These processes are demonstrated in Figure 3.1.

![Diagram of ion exchange processes]

It has been shown (248, 249) on conventional ion exchange resins that the "Chemical" exchange (step 3) is very rapid, and that the reaction rate is controlled by either the mass-transfer in the
external solution or the diffusion within the resin or possibly a combination of these steps.

It was found that ion exchange on polypyrrole was not rapid under the selected test conditions. The exchange of chloride from polypyrrole with phosphate in solution required 10 minutes to reach equilibrium (Figure 3.2) in 0.20 $\text{M} \ NaH_2PO_4$ (pH=5.5) or 0.20 $\text{M} \ Na_2HPO_4$ (pH=8.5) solutions. Figure 3.3 describes the ion exchange process in the initial 320 seconds. A similar trend of ion exchange rate to that shown in Figure 3.2 was observed in the initial stage. The ion exchange rate can be affected by the physical properties of the polymer, for example the polymer film thickness and porosity since these physical properties can affect diffusion processes. Therefore, it is still difficult to determine the ion exchange kinetics in detail at present.

Higher mole ratios of P to Cl were found in both Figures 3.2 and 3.3, when PP/Cl/SnO$_2$ was soaked in 0.20 $\text{M} \ NaH_2PO_4$ rather than in 0.20 $\text{M} \ Na_2HPO_4$. Presumably, it was due to the pH effect of solution, which will be discussed in detail later.

3.3.2 Ion exchange selectivity series
The retention sequence for various anions on conventional quaternary ammonium based anion exchangers has the following general order (44, 9-10):

Perchlorate>Sulphate>Oxalate>Iodide>Nitrate>Chromate
>Bromide>Thiocyanate>Chloride
**Figure 3.2** Ion exchange rate in polypyrrole

EDX condition: High voltage=20 KV; accumulation time =200 seconds.

Curve 1: PP/Cl/SnO₂ film treated in a 0.20 M NaH₂PO₄ solution.

Curve 2: PP/Cl/SnO₂ film treated in a 0.20 M Na₂HPO₄ solution.
Figure 3.3  *Ion exchange equilibrium in polypyrrole*

EDX condition: High voltage = 20 KV; accumulation time = 200 seconds.

Sample: PP/Cl/SnO$_2$ film treated in a 0.20 M Na$_2$HPO$_4$ solution.
However, this series was not observed with polypyrrole. Table 3.1 indicates the counterion exchange preferences of polypyrrole chloride and polypyrrole perchlorate (PP/ClO$_4$). The mole $\%$Cl$^-$ was used (in Table 3.1) instead of the mole ratio since it was more convenient as a comparison of data.

**Table 3.1 Ion exchange sequence**

<table>
<thead>
<tr>
<th>The counterion on polypyrrole</th>
<th>Ion exchange sequence$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl$^-$</td>
<td>Br$^-$(11.2) &gt; SCN$^-$(23.2) &gt; SO$_4^{2-}$(68.8) &gt; I$^-$(79.2)</td>
</tr>
<tr>
<td></td>
<td>&gt;CrO$_4^{2-}$(93.1)</td>
</tr>
<tr>
<td>ClO$_4^-$</td>
<td>SCN$^-$(2.2) &gt; Br$^-$(3.8) &gt; I$^-$(11.5) &gt; SO$_4^{2-}$(68.8)</td>
</tr>
<tr>
<td></td>
<td>&gt;CrO$_4^{2-}$(86.6)</td>
</tr>
</tbody>
</table>

1. PP/Cl/SnO$_2$ or PP/ClO$_4$/SnO$_2$ was exchanged in 0.1 M counterion solutions for 3 hours and then mole $\%$Cl$^-$ was determined using EDX.

2. Mole $\%$Cl$^-$ (in parentheses) left in polypyrrole.

Incorporated counterions in polypyrrole have been shown to be isolated ions but not polyions, and they are intercalated within the polymer plane. Oyanagi et al. (250) have investigated the bonding states of bromine atoms in conducting polypyrrole bromide by polarized X-ray absorption spectroscopy using synchrotron radiation. This study indicated that bromine atoms were not covalently bonded with the nitrogen or carbon atoms within the polypyrrole chain but were incorporated as isolated ions. This evidence suggested that polypyrrole salts behave like conventional
ion exchange resins. The interplane distance of neutral polypyrrole has been reported to be 3.41 Å (250). If the anions are intercalated between the planes of polypyrrole chains, the interplane spacing should expand according to the size of the anions. This is depicted in Figure 3.4. Thus it appears that a small interplane space is formed when small anions such as Cl⁻ are used as counterions during polymerization. This small inter spacing makes it difficult to replace the anion within the polymer plane with a much larger anion from solution.

![Figure 3.4](image)

**Figure 3.4** Ion exchange mechanism in polypyrrole

The results obtained from this work correlate with those predicted by the postulated hypothesis. The ionic radii of the ions investigated are as follows:

<table>
<thead>
<tr>
<th>Ions</th>
<th>Cl⁻</th>
<th>Br⁻</th>
<th>I⁻</th>
<th>SO₄²⁻</th>
<th>ClO₄⁻</th>
<th>CrO₄²⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radii (Å)</td>
<td>1.81</td>
<td>1.95</td>
<td>2.16</td>
<td>2.30</td>
<td>2.36</td>
<td>2.40</td>
</tr>
</tbody>
</table>

The retention sequence (in Table 3.1) on (PP/ClO₄) follows the above sequence. Therefore, CrO₄²⁻ was found to be more difficult to exchange with ClO₄⁻ compared to the other ions. This is probably
due to its larger size. A similar tendency was noticed on PP/Cl. However, as Cl\(^-\) is smaller than ClO\(_4\)\(^-\), less Cl\(^-\) could be exchanged, especially with the larger anions. The results suggested that the ion exchange process could be affected by the size of counterions incorporated into the polymer during synthesis as well as the size of the exchange ions in solution.

### 3.3.3 Effect of the ionic strength on ion exchange

The effect of ionic strength indicated by molarity of NaH\(_2\)PO\(_4\) on the ion exchange process with the conducting polymer-counterion system was found to be identical to the conventional ion exchange resins (Figure 3.5). As expected, the greater the ionic strength, the greater the rate of ion exchange. The reason is that an increase of ions in the solution leads to stronger competition of these ions for a place within the polymer.

### 3.3.4 Effect of the pH on the ion exchange process

The ion exchange rate at the conducting polymer can be affected by the pH of the solution. As depicted by Figure 3.6, an acidic media is favorable for the ion exchange. The result is the same as that obtained on some conventional anion exchangers (9, 10, 44).

The behavior observed at different pH values is probably due to the change in the ion exchange capacity (44). Since anion exchangers can be protonated at low pH, a higher capacity can be obtained. Such protonation as well as deprotonation processes on polypyrrole have been reported previously (251, 252).
Figure 3.5  Effect of ionic strength on the ion exchange process

EDX condition: high voltage=20 KV; accumulation time=200 seconds.

Sample: PP/Cl/SnO₂ film treated in a 0.20 M NaH₂PO₄ solution.
Figure 3.6  Effect of $pH$ on the ion exchange process

EDX condition: high voltage=20 KV; accumulation time=200 seconds.
Sample: PP/Cl/SnO$_2$ film treated with various phosphate buffers.
3.3.5 Effect of application of a potential on the ion exchange process

At negative potentials, the polymer is less positively charged due to the reduction process described above. This leads to a decrease in the anion exchange capacity. On the other hand, counterions may also be expelled electrochemically when the polymer is fully oxidized (253).

The ion exchange equilibrium was not affected by the application of a positive potential (Figure 3.7a) but the equilibrium was affected when a negative potential was applied (Figure 3.7b). Initially the P to Cl mole ratio increased with time and then decreased with time. This suggested that the ion exchange process reached equilibrium at the initial stage, since it was chemically controlled, and then the process was influenced by negative potentials. Reduction of the polymer released more P which was just exchanged on the surface than Cl which existed in the polymer. Consequently, the P to Cl mole ratio decreased with time. This indicated that the ion exchange process was initially chemically controlled and then electrochemically controlled.

3.3.6 Reversibility of the ion exchange process

The ion exchange process on polypyrrole was found to be reversible (Figure 3.8). This was expected since the total charge and the size of pores in the polymer backbone do not undergo significant changes during this chemical exchange process. Similar behavior has been reported previously (242, 252). The above data (in Figure 3.8) indicated that the polymer may be suitable for use as an ion exchange resin for use in chromatography.
Figure 3.7  Effect of potentials on the ion exchange rate

a. $E_{\text{app}}$ on PP/Cl/SnO$_2$ film: +0.35 V vs Ag/AgCl  
b. $E_{\text{app}}$ on PP/Cl/SnO$_2$ film: -0.35 V vs Ag/AgCl  

EDX condition: high voltage=20 KV; accumulation time=200 seconds.

Curve 1: PP/Cl/SnO$_2$ film treated in a 0.20 M NaH$_2$PO$_4$ solution.  
Curve 2: PP/Cl/SnO$_2$ film treated in a 0.20 M Na$_2$HPO$_4$ solution.
Figure 3.8 Reversibility of the ion exchange process

Exchange times 1 and 3: soaking PP/Cl/SnO₂ in 0.20 M Na₂HPO₄ or 0.20 M Na₂HPO₄ overnight.

Exchange times 2 and 4: soaking above samples in 1 M NaCl overnight.

EDX condition: high voltage=20 KV; accumulation time=200 seconds.
3.4 Conclusion

Ion exchange processes on conducting polypyrrole were found to reach equilibrium within 10 minutes under the given conditions. However, the detailed kinetic processes which occur remains unclear at this stage.

Counterions incorporated in polypyrrole during synthesis and the anions in solutions played an important role in determining the ion exchange behavior of the polymer. The ion exchange selectivity series were found to differ from that of conventional ion exchangers. The selectivity series depends on the size of the originally incorporated anions.

It was found that the ion exchange process could be controlled, by changing the pH and/or ionic strength of a solution. The pH and ionic strength effects are the same as those observed on conventional anion exchangers, i.e. the higher pH or the lower ion strength, the more difficult it is for the ion exchange process to occur. As with the ion exchange process on conventional resins the ion exchange process on polypyrrole was found to be reversible.

Application of a positive potential to the polymer did not change the ion exchange process. However, application of a negative potential was found to inhibit the ion exchange process. It was found that the ion exchange process was chemically controlled initially and then electrochemically influenced.
Chapter 4
Thermal Studies of Polypyrrole
4.1 Introduction

Thermal stability of polypyrrole is required if this material is to be used for chromatographic purposes. For example, it is important to the process of manufacturing polypyrrole since stationary phases are often dried out at high temperatures.

Thermal studies of conducting polymers, in general, are a useful tool, as they serve not only to facilitate a fundamental understanding of polymer properties, but also to assist in the determination of practical applications. Previous reports have indicated that most conducting polymers, especially polypyrrole, are very stable at room temperature (189). At low temperatures, between -267 and 25°C (254, 255), electrical conductivity and other physical properties were altered. At high temperatures, Salmon et al. (256) indicated that polypyrrole doped with perchlorate, tetrafluoroborate and hexafluorophosphate lost conductivity and decomposed at about 150°C in air while toluene-sulfonate-containing films did not decompose until 280°C. Neoh et al. (208) found that the chemically synthesized, conductive polypyrrole-I$_2$ complex was stable up to 184°C.

Investigation of the thermal properties of conducting polymers at high temperature is usually performed with a thermal analyzer (199, 208, 209). Decomposition is determined by weight loss and
differential thermal analysis data during such experiments. Some chemical changes or crystallization processes can also be determined by differential scanning calorimetry. Investigation of the thermal properties of certain materials can also be determined by gas chromatography. When a material is used as a stationary phase, chromatographic changes after temperature conditioning can provide information about the thermal stability of that material. For example, the phase transition temperature and the thermal stability of liquid crystal stationary phases were determined using gas chromatographic methods (257, 258). GC was also used in characterization of conducting polymers (259).

In the course of this work, the thermal stability of conducting polypyrrole has been investigated using both PP/Cl and PP/DS. Polypyrrole was synthesized electrochemically. Morphological, electrochemical and structural changes were characterized using PP/DS by scanning electron microscopy (SEM), cyclic voltammetry (CV) and fast atom bombardment mass spectrometry (FAB/MS), as PP/DS is more easy to prepare and has larger molecular weight than PP/Cl. Changes of polymer properties after thermal treatment were studied with gas chromatography.

4.2 Experimental

4.2.1 Reagents
Pyrrole (Fluka) was distilled before use. The monomer solution for electropolymerization was 0.20 M pyrrole and 0.10 M sodium dodecyl-sulfate (SDS, Sigma) or 0.20 M KCl in water. 1.0 M NaNO₃
was used as supporting electrolyte in the cyclic voltammetric experiments. Normal alkanes from C₆ to C₁₁, benzene, pyridine and 1-butanol were used as chromatographic test compounds. All chemicals were analytical grade unless otherwise stated. Purified water (Milli-Q water system) was employed.

4.2.2 Instrumentation

Thermal treatment, thermal gravimetry and differential thermal analysis (TG/DTA) were carried out using a Rigaku Thermogravimetric Analyzer. Analyses were conducted under nitrogen. Fast atom bombardment mass spectra were obtained using a V.G. Analytical Model MM 12-12 Mass Spectrometer. Cyclic voltammetry was carried out using a Bioanalytical Systems CV-27 Voltammograph. Scanning electron microscopy was performed using a Hitachi S-450 scanning electron microscope. Gas chromatography characterization was performed on a Varian Aerograph Model 1800 instrument. Electropolymerization was carried out using a Princeton Applied Research Model 173 potentiostat/galvanostat and Model 179 digital coulometer.

4.2.3 Procedures

PP/DS and PP/C₁ samples for TG/DTA analysis were electrodeposited galvanostatically on 18 mm x 3 mm diameter RVC cartridges, pretreated as above, to a total charge of 31 C and 33 C respectively. Stand alone PP/DS sample for TG/DTA analysis was coated electrochemically on a gold film (Sierracin/Intrex) at 1.0 mA/cm² current density to more than 10 C charge and then stripped from the film. All samples were rinsed with water and
dried in air after polymerization at room temperature for at least 10 hours.

Samples for SEM, CV and FAB/MS were prepared on 18 mm x 6 mm diameter reticulated vitreous carbon (RVC, Energy Research Generation, Inc) cartridges. The cartridges were pretreated in 6 M HCl for 3 hours and rinsed with water. Polypyrrole dodecyl-sulfate (PP/DS) was grown galvanostatically at 0.75 mA/cm² current density vs. a platinum auxiliary electrode to a total charge of 8.0 C. The samples were then rinsed with water and dried in air at room temperature for at least 10 hours.

Thermal treatment was performed with a TG/DTA analyzer under a nitrogen atmosphere. A temperature programmer was used to increase temperatures from the room temperature to the required temperature at 10°C/min and then to hold at the maximum temperature for about 20 minutes. After this treatment, the sample was divided into sections as required for SEM, CV and FAB/MS analysis.

Chromatographic packings were prepared by electrodeposition of PP/Cl and PP/DS on crushed and acid-washed RVC particles (60-120 mesh) as described later in Chapter 6. The packings were washed with AR grade acetonitrile and distilled water then packed into a 2 m x 3 mm I.D. glass coil column. In total approximately 5 g of PP/Cl/RVC with 71 C charge/g RVC and approximately 4.9 g PP/DS/RVC with 622 C charge/g RVC were employed in each column.
Gas chromatographic characterization was carried out after ten hour conditioning of the column at the required temperature. The dead time was measured by injecting natural gas and the retention time of the test compounds was recorded using a stopwatch. Capacity factors were calculated according to equation 1.4 and retention indices and McReynolds' constants were calculated as has been described previously (260).

4.3 Results and Discussion

It was found that RVC alone lost about 14% of its initial weight when exposed to temperatures between room temperature and 310°C. Over 310°C, the RVC became thermally stable (Figure 4.1B). This weight loss was most likely due to moisture loss and decomposition of functional groups on the RVC. An increase of DTA response over room temperature indicated moisture loss (Figure 4.1C). An increase of DTA response over 170°C indicated that decomposition of RVC occurred.

4.3.1 Thermal stability of polypyrrole

Continuous weight loss was observed from room temperature up to 460°C when a PP/CI/RVC sample was thermally-treated (Figure. 4.2B). About 30% of the polymer initial weight was lost when the polymer was heated to 460°C. The weight loss may have been due to the volatilization of oligomers since polypyrrole has a large molecular weight distribution (188, 189). Alternatively, the weight loss may have been due to the oxidation of polypyrrole which is known to cause release of counterions from the polymer at high
temperatures. The continuous weight loss of PP/Cl may have been due to slow thermal release of the Cl\(^-\) counterion which could be released via thermally-unstable products such as HCl. Decomposition of polypyrrole was indicated by the DTA curve which changed sharply around 170\(^\circ\)C, after a dip at about 100\(^\circ\)C which was due to water loss (Figure 4.2C).

Similar results were obtained from TG/DTA analysis of PP/DS/RVC (Figure 4.3). However, a more dramatic weight loss was observed at temperatures greater than 200\(^\circ\)C which corresponded to the decomposition of SDS itself (as shown in Figure 4.4). The polymer decomposed over 200\(^\circ\)C which resulted in a loss of counterions. DTA data (Figure 4.3C) indicated that at temperatures over 150\(^\circ\)C some changes occurred, but marked changes only occurred at temperatures greater than 210\(^\circ\)C.

TG/DTA analysis of pure PP/DS (Figure 4.5) without the RVC substrate further confirmed that the polymer itself was stable at temperatures below 170\(^\circ\)C and that weight losses (44\%) occurred between 170\(^\circ\)C and 300\(^\circ\)C. The weigh loss was approximately equal to the total weight of DS counterions. At temperatures greater than 300\(^\circ\)C the polymer became thermally stable even up to temperatures as high as 476\(^\circ\)C. Similar behavior was reported previously (209). Thermal stability was shown to be a function of the polypyrrole composition, i.e. the nature of the counterions incorporated during synthesis. However PP/DS was stable until 150\(^\circ\)C and then a sudden weight loss occurred. Unstable polypyrrole has been reported previously (261) when the polymer was in an insulating state.
Figure 4.1 Thermal stability of RVC

Sample: 18 mm x 3 mm diameter RVC cartridge in an aluminium pan;
Reference: aluminium pan;
Responses: A temperature, B. TG and C. DTA.
Figure 4.2  Thermal stability of PP/Cl/RVC

Sample: 18 mm x 3 mm diameter PP/Cl/RVC cartridge in an aluminium pan;
Reference: aluminium pan;
Responses: A temperature, B. TG and C. DTA.
Figure 4.3  Thermal stability of PP/DS/RVC

Sample: 18 mm x 3 mm diameter PP/DS/RVC cartridge in an aluminium pan;

Reference: aluminium pan;

Responses: A, temperature; B, TG and C, DTA.
Figure 4.4  Thermal stability of SDS

Sample: 10.3 mg SDS;

Reference: aluminium pan;

Responses: A temperature, B. TG and C. DTA.
Figure 4.5  *Thermal stability of PP/DS*

sample: 7.1 mg PP/DS;

Reference: aluminium pan;

Responses: A temperature, B. TG and C. DTA.
4.3.2 Thermal treatment and morphological properties

Scanning electron micrographs indicated that after coating with the polymer the smooth surface, initially observed with bare RVC, assumed a rougher appearance, and that after thermal treatment at elevated temperatures the appearance of the surface experienced further changes (Figure 4.6). Specifically, the higher the temperature at which the polymer was treated, the smoother the resulted surface.

This morphological change indicated that structural changes through chemical decomposition occurred during thermal treatment and that the polymer was thermally-sensitive at elevated temperatures.

4.3.3 Thermal treatment and electrochemical properties

Changes in the electrochemical behaviour of polypyrrole after thermal treatment were characterized using cyclic voltammetry. Before treatment cyclic voltammograms as described previously in Chapter 2 were obtained. After treatment at elevated temperatures the redox responses became smaller and eventually disappeared.

Electrochemical changes after thermal treatment further confirmed that PP/DS undergoes chemical changes when treated at elevated temperatures (Figure 4.7). Electrochemical activity, which is usually associated with the polymer conductivity and switching properties, deteriorated gradually after treatment at high temperatures.
Figure 4.6  Morphological changes of PP/DS/RVC after thermal treatment

SEMs: 1. without heating, 2. heated to 173°C, 3. heated to 212°C, 4. heated to 240°C, 5. heated to 263°C, 6. heated to 331°C, 7. heated to 370°C, and 8. RVC substrate;

Heating rate: 10°C/min;

Rest time at maximum temperature: 20 minutes.
Figure 4.7 Electrochemical changes of PP/DS/RVC after thermal treatment

Reference electrode: Ag/AgCl (3 M NaCl);
Auxiliary electrode: RVC;
Scan rate: 50 mV/sec;
Potential range: -1.00 to +0.60 V;
Supporting electrolyte: 0.1 M NaNO₃;
CVs on PP/DS/RVC: 1. without heating;
2. heated to 173°C, 3. heated to 21°C,
4. heated to 240°C, 5. heated to 313°C and
6. heated to 370°C.
Heating rate: 10°C/min
Rest time at maximum temperature about 20 minutes.
4.3.4 Thermal treatment and composition changes

Fast atom bombardment mass spectra were recorded for the PP/DS sample. The DS\textsuperscript- fragment on the FAB/MS disappeared after the polymer was treated at temperatures greater than 180°C. At these temperatures DS\textsuperscript- broke down to form smaller anionic species which were still retained in the polymer (Table 4.1).

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>80 \textsuperscript{(SO\textsubscript{3}\textsuperscript{2-})}</th>
<th>81 \textsuperscript{(HSO\textsubscript{3}\textsuperscript{-})}</th>
<th>96 \textsuperscript{(SO\textsubscript{4}\textsuperscript{2-})}</th>
<th>97 \textsuperscript{(HSO\textsubscript{4}\textsuperscript{-})}</th>
<th>265 \textsuperscript{(DS\textsuperscript{-})}</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>340</td>
<td>0</td>
<td>191</td>
<td>513</td>
<td>322</td>
</tr>
<tr>
<td>173</td>
<td>406</td>
<td>10</td>
<td>187</td>
<td>498</td>
<td>200</td>
</tr>
<tr>
<td>212</td>
<td>301</td>
<td>16</td>
<td>84</td>
<td>295</td>
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</tr>
<tr>
<td>240</td>
<td>141</td>
<td>6</td>
<td>40</td>
<td>115</td>
<td>0</td>
</tr>
<tr>
<td>263</td>
<td>181</td>
<td>6</td>
<td>13</td>
<td>49</td>
<td>0</td>
</tr>
</tbody>
</table>

Heating Rate =10 °C/min;
Time at maximum Temperature =20 minutes.

4.3.5 Thermal treatment and gas chromatographic behavior

Chromatographic data obtained using polypyrrole as the stationary phase in GC by selected test compounds can reflect the chemical changes induced by thermal treatment.

Using RVC particles as the packing material no retention was obtained for all test compounds including normal alkanes (C\textsubscript{6}-C\textsubscript{11}),
benzene and n-butanol at column temperatures up to 120°C. However, retention was obtained after coating the RVC particle with polypyrrole. A chromatogram of normal alkanes is depicted in Figure 4.8. This data indicates that the interactions between polypyrrole and the test compounds considered were significant.

It was also found that the effect of conditioning temperature, i.e. thermal treatment, on the chromatographic retention was significant (Table 4.2). After conditioning columns at elevated temperatures, retention on both PP/Cl/RVC and PP/DS/RVC increased markedly. This was presumably due to changes of the polymer surface condition and chemical composition at high temperatures as shown by the previous data.

**Table 4.2** Effect of conditioning temperature on the chromatographic capacity factors

<table>
<thead>
<tr>
<th>Conditioning temperature (°C)</th>
<th>k' of n-hexane at 180°C on column A</th>
<th>k' of n-hexane at 180°C on column B</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>0.04</td>
<td>0.64</td>
</tr>
<tr>
<td>250</td>
<td>0.63</td>
<td>1.67</td>
</tr>
<tr>
<td>300</td>
<td>2.02</td>
<td>37.9</td>
</tr>
</tbody>
</table>

Column A was 2.5%(w/w) PP/Cl on RVC;
Column B was 40%(w/w) PP/DS on RVC;
Conditioning time: 10 hours.
Carrier gas: N₂ at 30 ml/min;
Detector: FID;
Figure 4.8 Gas chromatogram on polypyrrole stationary phase

Column: 2 m x 3 mm I.D. glass tube packed with PP/DS/RVC particle (60-120 mesh);
Column temperature: 120°C;
Carrier gas: N2 at 30 ml/min;
Detector: FID;
Condition temperature: 150°C.
McReynolds constants for benzene and 1-butanol (Table 4.3) indicated that polypyrrole was a polar stationary phase. Failure to elute another McReynolds test probe, pyridine, again suggested the stationary phase was highly polar. The polarity and selectivity of both stationary phases varied with thermal treatment (Table 4.3). Generally, after treatment at higher temperatures, the stationary phase became less polar, i.e., showed higher selectivity for benzene and low selectivity for 1-butanol.

**Table 4.3** Selectivity change due to thermal treatment of stationary phases

<table>
<thead>
<tr>
<th>Stationary phase</th>
<th>Condition temperature (°C)</th>
<th>2.5% (W/W) PP/Cl</th>
<th>40% (W/W) PP/DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention Index</td>
<td>Benzene</td>
<td>150</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>1-Butanol</td>
<td>864</td>
<td>901</td>
</tr>
</tbody>
</table>

1. Column temperature: 120 °C;
Carrier gas: N2 at 30 ml/min;
Detector: FID;
4.4 Conclusion

PP/Cl and PP/DS decomposed at elevated temperatures. Weight loss of PP/Cl/RVC started at low temperatures and proceeded rapidly at temperatures greater than 170°C. It was found that weight loss of PP/DS started at 150°C. At temperatures greater than 300°C PP/DS became thermally-stable again. It was found that the DS⁻ in the polymer was released and decomposed at these temperatures, and that the polymer backbone was thermally-stable.

Thermal treatment of PP/DS at elevated temperatures changed the morphology of the polymer: the higher the temperature the smoother the surface became. The treatment also changed the electrochemical properties. Cyclic voltammograms indicated that the polymer became less electroactive after treatment at elevated temperatures. The redox properties of the polymer disappeared gradually due to the thermal degradation of the polymer. FAB/MS data further confirmed that at high temperatures DS⁻ was released from the polymer and that this compound decomposed.

Changes due to thermal treatment were also monitored using gas chromatography. The higher the temperature employed to condition the polymer stationary phase, the higher the retention of the test compounds on both PP/Cl and PP/DS columns. This indicated that the polymer is thermally-sensitive and it became less polar after treatment at elevated temperatures.

The results indicate that particular caution is necessary during synthesis and application of polypyrrole stationary phases or
electrodes. For example, the polymer can only be dried at low temperatures since it is thermally-sensitive. On the other hand, it is also possible to change the polymer properties for particular applications by the use of thermal treatment.
Chapter 5

Development of Hardware for the Study of Polypyrrole Stationary Phases for Use in HPLC
5.1 Introduction

Polymeric coatings on particles have usually been deposited by dissolving the stationary phase in a solvent, coating the particles and allowing the solvent to evaporate (9-11). Polymer coatings have also been chemically bonded on particles (20). Conducting polymer particles may be prepared either electrochemically or chemically (Chapter 2). In the former case, an electrode, an electrochemical reaction cell and an electrochemical instrument are required. Electrodeposition of polypyrrole has usually been carried out on conductive plates (229, 231), fibres (195) or discs (194-196). Electrodeposition of polypyrrole on particles for HPLC is more difficult and therefore certain designed hardware is necessary.

In order to characterize polypyrrole stationary phases a suitable column should be chosen. With HPLC, both rigid-walled and flexible-walled columns have been widely used. Comparisons have been made between these two types of columns (262, 263). A distinct characteristic of the flexible-walled column is that the column wall can be deformed by external compression, and as a result, the wall effect (17) can be minimized and the dead volume can be lowered (264, 265). Furthermore, packing a flexible-walled column is easier (266) and additional equipment such as a high pressure slurry packing pump are not required. However, at
present the consumption of packing materials and the cost of column materials for both columns are high. These columns are not desirable for new stationary phase research. Therefore, there is a need to develop an efficient and economic column which is easily prepared in the laboratory.

An ECLC column is an unconventional electrochemical cell. Compared with a conventional analytical cell, the column has following features:

1. an extremely large working electrode surface;
2. a very small cell volume;
3. a very close electrode separation;
4. a flowing electrolyte; and
5. a packing bed as a working electrode.

Consequently, unconventional electrochemical behavior is expected.

To date, most electrochemically controlled chromatography has been performed on low efficiency columns. Attempts to improve column efficiency have been documented by previous workers (98). However, application of such a column under high pressures to obtain high efficiency still presents a problem (99).

In this work, the hardware necessary for the preparation and testing of polypyrrole stationary phases has been developed. This hardware includes cells for electrochemical preparation of polypyrrole stationary phases, and columns for HPLC and ECLC characterization of polypyrrole stationary phases. A packed particle cell, a slurry cell, and a plate cell were designed and their performance was investigated. They were used to coat conducting
polymers on RVC packings electrochemically. A column system developed in the course of this work consists of a flexible-walled column and a stainless steel chamber in which the column is fitted. The column wall can be compressed when mobile phase passes through the chamber. The system is termed the self-compressed column (SCC). The ECLC column designed in this work made use of a Waters radial compression chamber and could be operated at higher column efficiency, higher pressure, and lower dead volume. Three electrodes (i.e. working, reference and auxiliary) were fitted into the column system and a conducting packing, such as a polypyrrole stationary phase, was used as the working electrode.

Electrodeposition of PP/Cl on RVC particles has been used to test the performance of the packed particle cell, while electrodeposition of PP/DS on RVC or silica particles has been used as an example to test the performance of the other cells. The performance of the SCC has been characterized with C_{18} packings. Structure, kinetics and separation performance of the SCC, have been investigated. The performance of the ECLC column has been studied with RVC packings. Electrochemical characteristics of the column have been investigated. The efficiency of applied potential has been tested by injecting an electroactive compound into the column and recording its response variation with the applied potential.
5.2 Experimental

5.2.1 Materials and reagents

The materials required for a packed particle cell are:

1. Stainless steel body and cap (made in the Science Faculty Workshop; the University of Wollongong);
2. Glass wool (10 μm, Ajax);
3. Filter paper (542, Whatman);
4. Silicone rubber (injector septum, Varian);
5. Teflon tubing (0.32 mm I.D., Activon);
6. Tantalum wire (0.25 mm, Goodfellow, UK); and
7. Plastic tubing (FINNTIP 60, Labsystems).

1.9 g of RVC packing was used to test the electrochemical coating process. The RVC was crushed into particles and then sieved into the required size, in the present 45-63 μm. The packings were soaked in 6 M HCl for 12 hours and then washed with distilled water until no Cl⁻ was detected by AgNO₃. Electrosynthesis was carried out using a 0.2 M KCl and 0.2 M pyrrole solution.

The materials required for the slurry cell are described as follows:

1. Porous Teflon tube (made in the laboratory);
2. Rotating electrode (Beckman);
3. Tantalum wire (0.25 mm, Goodfellow);
4. Ni-Cr wire (0.5 mm, Goodfellow); and
5. Filter paper (Whatman).

Both conducting packings, 2 g of RVC particles (<40 μm) and nonconducting packings, 2 g silica gels (BDH, about 50-100 μm diameter) were used to test the electrosynthesis of polypyrrole with this cell. A 0.20 M pyrrole and 0.10 M SDS aqueous solution was
employed to electrosynthesize polypyrrole on the RVC and silica gel respectively.

The materials required for construction of a plate cell are listed as follows:
1. Stainless steel clamp (10 x 60 x 2 mm, made in the laboratory);
2. Glass plate (50 x 50 x 1.5 mm, made in the laboratory);
3. Tantalum foil (50 x 50 x 0.1 mm, Goodfellow);
4. Magnetic stirrer (Labsupplies);
5. Filter paper (542, Whatman);
6. Membrane (Celeanese);
7. Stainless steel plate (50 x 50 x 2 mm, made in the laboratory);
8. Tantalum wire (0.25 mm, Goodfellow).

2 g of RVC particles (45-63 μm), were used to test the performance of the cell. A 0.10 M pyrrole and 0.10 M SDS solution in 50% CH₃CN and 50% H₂O was used as the monomer solution.

The materials for construction of a soft self-compressed column are:
1. Teflon seal ring (made in the laboratory);
2. Stainless steel parts (made in the Science Faculty Workshop; the University of Wollongong);
3. Frit (2μm, Activon); and
4. Fitting (1/4 x 28, Dionex).

ODS-Hypersil (10 μm Shandon) was chosen as the stationary phase for the testing of the column performance. A 65% methanol and 35% water mixture was used as an eluent. All the tests were carried out using dimethylphthalate and benzene as test samples.
Materials for the ECLC column are described as follows:

1. Cartridge holder (RCM 100, Waters);
2. Frit (2 μm, Activon);
3. Tantalum wire (0.25 mm, Goodfellow);
4. Glass wool (10 μm, Ajax);
5. Teflon RCM cartridge and stainless steel fitting (made in the Science Faculty Workshop; the University of Wollongong);
6. Carbon foil (0.1 mm, Goodfellow);
7. Filter paper (542, Whatman);
8. Cross junction (1/4 x 28, Activon); and

RVC packing (5-40 μm) was used as a test stationary phase. The preliminary test of the column was carried out in a 0.1 M NH₄Ac solution in 80% water and 20% CH₃CN.

5.2.2 Instrumentation

A PAR 173 potentiostat/galvanostat with a PAR 179 digital coulometer was used to electrosynthesize polypyrrole. Alternatively, a galvanostat (made in the Science Faculty Workshop; the University of Wollongong) was employed. A Beckman rotating electrode was used to contact the tantalum foil and to provide stirring for the slurry cell.

The HPLC system consisted of a Kortec K350 pump (ICI), a Rheodyne 7125 injector with 20 μl sample loop, a Kortec K95 variable wavelength detector (ICI) and a DP 600 chart recorder (ICI). Retention times were recorded using a stopwatch. A potentiostat (made in the Science Faculty Workshop; the
University of Wollongong) was used as the electrochemical controller during ECLC.

5.2.3 Design and preparation of hardware

5.2.3.1 Electrochemical cells for synthesis of polypyrrole stationary phases

5.2.3.1.1 Packed particle cell

The cell is depicted in Figure 5.1. Electrical contact was made to the conducting particles [5] via a central tantalum wire coil [7] which was supported by a Teflon tube with 0.1 mm pores [6]. A layer of microporous film [3] was used to isolate the packings [5] from the stainless steel tubing [1]. The column was sealed at both ends by silicon rubber [4, 8] and also glass wool [2, 14] to prevent the packing from leaking out of the column. However, the monomer solution was allowed to pass through the space between the rubber [4, 8] and the tubing [1]. A stainless steel cap [10] was screwed on to the outlet of the column. The packing bed was pushed in through plastic tubing [12] and a piece of silicone rubber [13]. The packing was employed as a working electrode and electrically contacted with the tantalum wire coil [7], which was insulated from the stainless steel cap by a layer of plastic [11]. The reference electrode [9] was mounted at the outlet end of the cell, and the stainless steel body [1] was used as an auxiliary electrode. A diagram of the packed particle cell is set out in Figure 5.2. A nitrogen-pressurized bottle [1] was used to pump a monomer solution [2] through the cell. The body of the cell [3], the reference electrode [4] and the tantalum wire [5] were connected to the galvanostat.
Figure 5.1 The packed particle cell for electrodeposition

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<thead>
<tr>
<th>No</th>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Stainless steel body</td>
<td>12 mm I.D., 1/4 x 28 fitting</td>
</tr>
<tr>
<td>2, 14.</td>
<td>Glass wool</td>
<td>10 μm</td>
</tr>
<tr>
<td>3.</td>
<td>Porous film</td>
<td>filter paper 542</td>
</tr>
<tr>
<td>4, 8, 13.</td>
<td>Silicone rubber</td>
<td>injector septum</td>
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<tr>
<td>5.</td>
<td>Conductive particles</td>
<td>RVC</td>
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<td>6.</td>
<td>Teflon tubing</td>
<td>1.6 mm I.D. tubing</td>
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<td>7.</td>
<td>Tantalum wire coil</td>
<td>0.25 mm wire</td>
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<td>9.</td>
<td>Reference electrode</td>
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<td>10.</td>
<td>Stainless steel cap</td>
<td>1/4 x 28, fitting</td>
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</tbody>
</table>
Figure 5.2  Diagram of the packed particle cell for electropolymerization

1. gas pressure pump;
2. monomer solution;
3. body of the cell (auxiliary electrode);
4. reference electrode;
5. working electrode;
6. reagent reservoir.
The following procedure was employed to prepare the packed particle cell (refer to Figure 5.1):

1. All parts the cell were rinsed with CH$_3$CN and H$_2$O.
2. A roll of membrane (12 mm diameter x 100 mm) [3] was inserted into the stainless steel column [1] and attached tightly to the inner wall.
3. A small amount of glass wool (1 g) [2], a silicone septum [4] and the tantalum coil [6, 7] were set in place.
4. The column was packed with RVC using a dry packing method.
5. A silicone septum [8], a small amount of glass wool (1 g) [14] and then another silicone septum [13] were inserted consecutively.
6. The tantalum wire from the coil was allowed to pass through a piece of plastic tubing [12] and an insulating lining [11] on a cap [10], and then the cap was screwed onto the column tightly to compress the packing bed.
7. A reference electrode [9] was fitted into the column.
8. Electrosynthesis of polypyrrole was carried out by drawing a constant current of 20 mA until 190 C total charge had passed.

5.2.3.1.2 Slurry cell

In this cell, a slurry [4] was placed in a porous Teflon tube [2]. A rotating electrode [5], platinum or tantalum foil, was placed in the slurry. A porous membrane [7], usually a piece of filter paper, was used to prevent the packings from leaking through the Teflon tube. A Ni-Cr wire coil (15 mm diameter) [6] around the Teflon tube was employed as an auxiliary electrode. The entire assembly was placed in a beaker [9] with the monomer solution [8]. A reference electrode (Ag/AgCl) [1] was placed in the solution. When a potential
was applied to the rotating electrode, polymer was produced at the electrode surface. This polymer was then adsorbed by the packing material. Electrochemical reactions occur either on the packing or on the electrode once the process is initiated. Since the packing material was mobile in the cell, complete coverage of the particle after an extended period was expected.

The slurry cell can be prepared according to the following procedure:

1. A layer of filter paper [7] was used to cover all the inner surface of a tube [2].
2. The packing was poured into the cell (refer to Figure 5.3).
3. The tubing with the packing was then placed into the monomer solution [8].
4. A rotating electrode [3] was inserted into the packing.
5. The current was set at 10 mA for coating RVC and at 0.2 mA for coating silica at a stirring speed of about 1 cycle per second.

5.2.3.1.3 Plate cell

The plate cell was designed for the electrochemical preparation of conducting polymer stationary phases with particular reference to coating small particles.
Figure 5.3  
*The slurry cell for electrodeposition*

1. reference electrode (Ag/AgCl);
2. porous Teflon tube (15 mm diameter);
3. rotating electrode;
4. packings (RVC particles);
5. tantalum wire coil (0.25 mm);
6. Ni-Cr wire coil (15 mm diameter);
7. porous membrane (Whatman 542);
8. monomer solution;
9. container.
Figure 5.4  The plate cell for electrodeposition
1. stainless steel clamp;
2. glass plate (50 mm x 50 mm x 2 mm);
3. tantalum foil (50 mm x 50 mm x 0.1 mm);
4. packings (RVC particles);
5. tantalum wire (0.25 mm);
6. magnetic stirrer;
7. filter paper;
8. porous membrane;
9. stainless steel sieve (50 mm x 50 mm);
10. stainless steel clamp (made in the Science Faculty Workshop; the University of Wollongong);
11. monomer solution;
12. container;
13. tantalum wire (0.25 mm);
14. galvanostat.
The design of the plate cell was similar to that of the packed particle cell. The conductive packing [4] was wrapped in a filter paper [7] and contacted by a piece of tantalum foil [3]. The whole packing bed acted as a working electrode and was connected to a galvanostat [14] via the tantalum foil [3] and then a tantalum wire [5]. A piece of glass plate [2] and porous stainless steel plate were [9] used to sandwich the packing bed together, and the assembly was then compressed tightly by two stainless steel clamps [1, 10]. The stainless steel plate [9] was also employed as a counter electrode and connected to the galvanostat via the clamp and a tantalum wire [13]. A layer of a porous membrane [8] was placed between the packing bed and the stainless steel plate to prevent conducting particles from contacting the counter electrode. The cell assembly was soaked in a monomer solution (11). A magnetic stirrer [6] was used to provide vigorous agitation.

The plate cell was prepared according to the following procedure (Figure. 5.4):

1. A filter paper (12.5 cm diameter)[7] was folded into a square shape to match the size of plates.
2. The packing was placed on the paper and made into a thin, even layer (50 mm x 50 mm).
3. A tantalum foil [3] was put on top of the packings [4] and then wrapped into the filter paper.
4. A tantalum wire (50 x 50 mm) [5] was placed on top of the tantalum foil and a porous membrane [8] was placed beneath the filter paper.
5. This prepared unit was sandwiched between a glass plate [2] on which another tantalum wire [13] was preset, and a
stainless steel plate (9), and then the unit was clamped tightly by the clamps [1, 10].

6. The system was connected as shown in Figure 5.4 and was ready for coating. A 3 mA constant current was drawn to coat the polymer on the packing material.

5.2.3.2 Columns for characterization of polypyrrole stationary phases

5.2.3.2.1 Self-compressed column

The self-compressed column was designed to enable the characterization of the new stationary phases developed in this work. The design of the SCC system is shown in Figure 5.5. A flexible-walled column was placed in a chamber. When an eluent flows through the system, a high pressure can be generated in the chamber and as a result the column wall will be pressurized in the same way as a radial compressed column system. In order to ensure a higher pressure in the chamber than that on the column inlet, a pressure regulator can be used. The compression force applied to the column wall is equal to the sum of the pressure drop $P$ across the column $\Delta P_2$ and that across the regulator $\Delta P_1$, i.e.

$$P = \Delta P_1 + \Delta P_2 \quad (5.1)$$

The column and the chamber were designed as depicted in Figure 5.6. The column was placed in the chamber and connected to the HPLC system as shown in Figure 5.5.
Figure 5.5  *Diagram of self-compressed column system*

1. solvent reservoir;
2. HPLC pump;
3. regulator;
4. injector;
5. self-compressed column system;
6. detector.
The following procedure was used to prepare the self-compressed columns and to set up the system.

1. A length of Teflon tubing (70 mm) was cut off with a scalpel, two Dionex fittings were inserted in and then both ends of the tubing were flared using a warm Dionex Teflon tubing modifier to form a seal ring.

2. A Teflon tubing with 1.6 mm inner diameter and 0.8 mm wall thickness and another column with 3.2 mm inner diameter and 0.28 mm wall thickness were used in the course of this work. For the latter a piece of Teflon tubing flared at one end (1.6 mm I.D. x 3.2 mm O.D x 4 mm length) was inserted into both ends of the column to support the tube.

3. The column was fitted into a Dionex fitting, in which a stainless steel frit (2 μm) with a Kel-F ring (6.2 mm diameter) was preset.

4. A packing slurry solution was transferred to a slurry packing chamber which was modified from a Waters column with 3.9 mm inner diameter and 30 cm length.

5. The column was fitted in the packing chamber, and the system was connected as shown in Figure 5.8.

6. Immediately after steps [4] and [5], the slurry packing process was started at a preset flow rate to produce a pressure less than the column pressure tolerance (68 atm for the 1.6 mm I.D tubing and 28 atm for the 3.2 mm I.D tubing).

7. The packed column was placed into the column chamber as in Figure 5.6.

8. The column could not be connected with the injector until the air in the column chamber was driven out by flushing the chamber with the eluent.
5.2.3.2.2 ECLC column

A three electrode ECLC column was designed for use with high pressures while producing low dead volume (Figure 5.7). A Waters RCM-100 or RCM 8x10 radial compression cartridge holder was used. The conducting HPLC packing bed [8] was isolated from the counter electrode [6] by a layer of filter paper [7], and isolated from an inlet stainless steel frit [2] and an outlet frit [11] by a layer of glass wool [4, 10]. The counter electrode was connected to an electrochemical controller [17] via a tantalum wire [3], the stainless steel frit [2] and a stainless steel fitting [1]. The working electrode was connected to the electrochemical controller via a tantalum wire [9], the frit [11], a fitting [12] and a cross junction [13]. A reference electrode [16] was inserted in the cross junction. A silicone rubber septum [14] was used to stop the eluent. A frit [15] was used to protect the detector.

The ECLC column was prepared as follows:

1. A teflon column was designed to fit the Waters RCM system. The column's inner diameter and length were 5 and 100 mm respectively.

2. A strip of filter paper (100 mm x 30 mm) and then a strip of carbon foil (80 mm x 15 mm) were evenly rolled onto a metal rod (3 mm diameter).

3. The paper/carbon foil roll was inserted into the column such that they impinged tightly against the column walls with the aid of the metal rod.

5. The end of the tantalum wire was bent and contacted to a stainless steel frit [2] which was placed with the help of a fitting (RCM 100, Waters) [1].

6. The resistance between the fitting and the other end of the carbon foil which was contacted by a thin metal wire, was determined by a multimeter.

7. A piece of glass wool [4] was inserted with the metal rod to cover the entire surface of the frit [2] and the tantalum wire [3].

8. A piece of tantalum wire [9] was inserted into the column to form a connection to the packing.

9. The column was packed with conducting particles by the dry packing method and a piece of glass wool was put on top of the packing bed to prevent it from bleeding.

10. A frit [11] which had a thin hole in the middle to fit the tantalum wire [9] was inserted into the column.

11. A special fitting [12] was mounted and the system was finally connected as in Figure 5.7.
Figure 5.6  The self-compressed column system

1. and 13. sealer (Teflon ring);
2. chamber head (stainless steel);
3 and 11. frits (2 μm);
4 and 10 fitting (Dionex);
5. solvent mixer (sintered stainless steel);
6. separator (stainless steel);
7. chamber chamber end cap (stainless steel);
8. flexible-walled column (Teflon);
9. packing material (C18).
Figure 5.7  The ECLC column system

1. RCM cartridge holder (Waters);
2. frit (2 μm, 6.3 mm, Activon);
3. tantalum wire (0.25 mm);
4 and 10. glass wool (10 μm);
5. RCM cartridge;
6. carbon foil (0.1 mm);
7. membrane (filter paper 542, Whatman);
8. packings;
9. tantalum wire (0.25 mm);
11. frit (Activon, modified);
12. fitting (stainless steel);
13. cross junction (Activon);
14. septum;
15. frit (2 μm Activon);
16. reference electrode (BAS) and
17. potentiostat.
**Figure 5.8**  Diagram of devices used for slurry packing of a self-compressed column

1. HPLC pump;
2. packer (modified from a 30 cm Waters empty column);
3. buffer tubing (3.2 mm O.D x 1.6 mm I.D. Teflon tubing);
4. container;
5 and 7. Dionex female fitting;
6. SCC assembly.
5.2.4 Test procedures

5.2.4.1 Procedure for testing new electropolymerization cells
The resistance between the working and counter electrodes was tested by a multimeter before polymerization. The potential between the working and counter electrodes was measured during galvanostatic polymerization. The packings were checked visibly by a microscope after polymerization.

5.2.4.2 Procedure for testing the columns
The column materials used for the SCC underwent a pressure tolerance test by blocking one end of the tubing and pumping methanol through the other end until a pressure was reached at which the column materials broke down. Dead volume was obtained via injecting water. Column efficiency was calculated from retention time and half peak width of benzene. The column pressure drop was determined by a pressure meter on the HPLC instrument.

The resistance between the working and counter electrodes in the ECLC column was tested by a multimeter. Charging currents were measured by applying potentials on the column and recording current changes with time. Residual currents were obtained at \( E_{\text{app}} = -0.25 \) V with or without deoxygenation of the eluent using nitrogen. Dead volume and efficiency were determined using the same method employed to test SCC. Electrochemical effects on the column were tested by injecting aniline when a potential was applied.
5.3 Results and Discussion

5.3.1 Electropolymerization cell evaluation

5.3.1.1 Performance of the packed particle cell

When the monomer solution was pumped through this cell, electrochemical polymerization occurred and the polymer deposited on the packing. Using a multimeter, it was verified that the working and auxiliary electrodes were insulated from each other. When 10 mA constant current passed through the cell, $E_{app}$ was +0.7 V for steady state polymerization and the potential between the working and auxiliary electrodes was +1.40 V. Tantalum wire was chosen as the contact since it has already been shown that the polymer did not readily deposit on this substrate (Chapter 2). Fresh monomer solution was supplied throughout the polymerization and hydrogen on the inner wall of the body was driven out by the flowing solution. In the present experiment, the flow rate was 1.0 ml/min which ensured adequate monomer supplies and adequate volume to drive out evolved hydrogen. The RVC surface became dull after electropolymerization. The polypyrrole deposit was observed using a microscope, and uneven coverage was observed across the packing bed, i.e. the packing layer closer to the column center was coated thicker than that close to the column wall. Presumably this is due to the uneven current density along the packing bed. Since the outer layer has higher surface area a lower current density than the one in the center is expected. Thus, the reaction occurred more readily on packings closest to the contact.

The advantages of this cell design were that no high pressure pump was required, high current (up to 50 mA) could be applied, a fresh
monomer solution could be readily supplied and a large batch of packing easily prepared. The main disadvantages of this design were the uneven coatings obtained.

5.3.1.2 Performance of the Slurry cell

Using the above set-up the particles contact each other and remain stationary. Therefore the parts of a particle contacting other neighboring particles will not be coated. Alternatively, a slurry cell in which particle containing slurry could be coated was designed (Figure 5.3)

When a 10 mA constant current was passed through the cell in the monomer solution with the RVC packing slurry, Eapp on the rotating electrode was +0.70 V which was the same as that in the packed cell but the potential between the working and auxiliary electrodes was about 4 V. This indicated that the cell resistance was very high and application of large currents caused a high iR drop problem. A 0.25 mA current was drawn when silica was used as a packing slurry. The Eapp was still at 0.70 V but the potential between the working and auxiliary electrodes was 1.00 V. The use of a higher current caused massive polymer growth to occur on the electrode but not on the packing material. Hydrogen evolved during this process at the auxiliary electrode was released into the solution.

Both the RVC and silica became darker after electrodeposition of polypyrrole. Even coverage of the particles was observed with a microscope.
The above results indicated that the slurry cell could be used to coat polypyrrole on conductive packings as well as on non-conductive packings. In the latter case the polymer growth was initiated at the electrode surface and then polymer was transferred on to the non-conductive particle surface physically, presumably via adsorption.

The process took longer in this cell than in the others because a low current could be drawn due to high contact resistance. A disadvantage is that some polymer was deposited on the rotating electrode so that the polymer loading of the packing was more difficult to estimate.

5.3.1.3 Performance of the plate cell

The insulation of the working and auxiliary electrodes was verified using a multimeter. When a 10 mA current flowed through the cell, potential vs. the reference electrode and the potential between the working and auxiliary electrodes were +0.70 V and 1.50 V respectively. Evolved hydrogen was released on the stainless steel plates. The polypyrrole coating was observed after polymerization using a microscope.

A more even coating appearance across the packing bed was found using this cell compared with the packed particle cell. Presumably, a more even current density was achieved on this cell as shown by Figure 5.9. Compared with the other two cells this cell was easy to prepare. The main disadvantage of this cell was the same as the packed particle cell, i.e. the parts of particles which touch other particles were not coated with the polymer. However, due to the irregular shape of these packings, the uncoated surface was only a
very small fraction of the whole particle as verified by SEM in Chapter 6.

![Diagram showing even and uneven current density]

Figure 5.9 Comparison of the packed particle and plate cells

5.3.2 Column evaluation

5.3.2.1. Performance of the self-compressed column

Any flexible tubings can be used for the self-compressed column. However, several criteria must be satisfied. The tubing material should be chemically inert to any possible eluents and the ability to seal the tubing at both ends to withstand high pressures must be feasible. In addition, the tubing should have a certain strength to ensure packing pressures. Teflon tubing was found to be a good candidate for the column material not only because of the mechanical flexibility and chemical inertness but also because of its semitransparent nature which allowed the state of the packing material to be observed. The pressure tolerance was found to be 68 and 28 atm for the 3.2 mm O.D.x 1.6 mm I.D. and 3.75 mm O.D. x 3.2
mm I.D. columns respectively. The packing pressure was 40 and 17 atm respectively for the above columns in this work. A compact packing bed resulted under these pressures.

5.3.2.1.1 Effect of self-compression

The effects of self-compression on the dead volume and efficiencies obtained with this system are shown in Table 5.1.

Table 5.1 Effect of self-compression

<table>
<thead>
<tr>
<th>Flow rate (ml/min)</th>
<th>pressure (atm)</th>
<th>V_m (μl)</th>
<th>N (plate/meter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>0.1</td>
<td>4.5</td>
<td>4.5</td>
<td>0.311</td>
</tr>
<tr>
<td>0.2</td>
<td>8.5</td>
<td>8.5</td>
<td>0.317</td>
</tr>
<tr>
<td>0.3</td>
<td>12.5</td>
<td>12.5</td>
<td>0.311</td>
</tr>
</tbody>
</table>

Test column: 3.75 mm O.D x 3.2 mm I.D x 64 mm 10 μm, ODS-Hypersil
Test sample: benzene
A: no self-compression
B: application of self-compression

The SCC system was evaluated using a 3.75 mm O.D. x 3.2 mm I.D column; there was a ratio of wall thickness to inner diameter of 1:12. The dead volume was determined by injecting water without self-compression. The dead volume decreased by 5% after self-compression was induced. This was due to compression by the external hydraulic pressure. As a result, the column efficiency almost doubled. The column pressure drop was not affected significantly by self-compression.
It was found that self-compression could not be induced using a column with a ratio of wall thickness to inner diameter of 1:2. There was no significant dead volume change on a 3.2 mm O.D x 1.6 mm I.D column with self-compression. It was found that the higher the application of external pressure by a pressure regulator, the lower the column efficiency. The reason for this was that the high ratio of wall thickness to inner diameter made it difficult to compress the column, particularly at the column inlet where the column inner pressure was very high. Due to this difficulty, the high pressure at the column inlet pushed the packing into the column and a void volume was produced. This void volume was observed after the experiment and disconnection of the column. In order to avoid the void volume and minimize the loss of column efficiency, a ratio of wall thickness to inner diameter of 1:12 was used. However, the column walls should be thick enough to enable the withstanding of pressures encountered during packing. The flexibility of the column material also affected the ability to induce self-compression. Therefore, the optimal column diameter was influenced by the flexibility, the pressure tolerance, and the ratio of wall thickness to inner diameter of the column material.

5.3.2.1.2 Pressure-flow rate relationships

The column pressure drop increased with increased flow rate (Figure 5.10). This had been observed previously using stainless steel columns.
5.3.2.1.3 Column efficiency and chromatography on the SCC

The chromatographic kinetics can be described by an HETP-velocity curve (9-11). From a practical point of view this data can be used to compare the column efficiency for the same materials with different methods of column preparation. It was found that using a C18 packing material, the column efficiency obtained with the self-compressed column was similar to that obtained with a stainless steel column which was packed using a slurry packing method at 620 atm (Figure 5.11). No significant decrease in the column efficiency was observed without a pressure regulator, probably since the pressure generated from the column itself was high enough to produce the self-compression effect and excessive pressure had no significant effect. At higher flow rates, the column efficiencies obtained using the SSC system were higher than these obtained using the stainless steel column system.

Extra column effects due to connections and fittings reduced the column efficiency significantly with the present design of the self-compressed column. Table 5.2 shows that the extra column band broadening contributed was 14% (increase in half peak width) due to the additional fittings used in an SCC. This resulted in a 30% loss of the column efficiency.

An example of the chromatography achieved using the SCC is given in Figure 5.12. This further verifies that HPLC on a SCC is feasible.

Some advantages of the self-compressed column (SCC) are that;
(1) the column may be prepared using a normal HPLC pump;
(2) the size of the column may be varied easily.
Table 5.2  Extra column effect on column efficiencies

<table>
<thead>
<tr>
<th>Column system</th>
<th>Stainless steel column 0.39 mm I.D x 150 mm</th>
<th>Addition of 2 frits and 2 fittings (as used in the SCC system)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluent</td>
<td>65:35/MeOH:H₂O</td>
<td>65:35/MeOH:H₂O</td>
</tr>
<tr>
<td>F(ml/min)</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Test sample</td>
<td>Benzene</td>
<td>Benzene</td>
</tr>
<tr>
<td>Capacity factor</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Half peak width (μl)</td>
<td>156</td>
<td>178</td>
</tr>
<tr>
<td>Extra band broadening (μl)</td>
<td>-</td>
<td>22</td>
</tr>
</tbody>
</table>
Figure 5.10  Pressure drop vs. eluent flow rate on a self-compressed column

Eluent: 65% MeOH and 35% H2O;

Column:  3.2 mm I.D. x 3.75 mm O.D. x 64 mm Teflon column packed with 10 μm ODS-Hypersil;

Curves: a. SCC; b. pressure regulator and c. column plus pressure regulator.
Figure 5.11  HETP vs. eluent velocity

Eluent: 65% MeOH and 35% H2O

Column: a. 10 μm ODS-Hypersil in a 3.2 mm I.D. x 64 mm SCC; b. the same column as (a) but with a pressure regulator and c. 10 μm ODS-Hypersil in a 3.9 mm I.D. x 150 mm stainless steel column;

Sample: benzene.
Figure 5.12  *Separation on a self-compressed column*

Column: 3.2 mm I.D. x 64 mm SCC packed with 10 µm ODS-Hypersil;

Eluent: 65% MeOH and 35% H₂O at 0.3 ml/min.;

Detector: UV at 254 nm;

Sample: 20 µl of 1.20 ppm benzoic acid, 2. 15 ppm phenol, 3. 5 ppm aniline, 4. 5 ppm DEP, 5. 5 ppm toluene and 6. 5 ppm DMA in the eluent.
5.3.2.2 *Performance of the ECLC column*

Resistance measurements using a multimeter were carried out after compression in the radial compression module to ensure that there was no short circuit between the working and auxiliary electrodes. When no short circuit was obvious, a resistance value greater than 1 MΩ was obtained, before the eluent, i.e. the supporting electrolyte, was pumped in.

The electrochemical behavior of the packing material in the ECLC column was investigated. The mobile phase was pumped through the column at a flow rate of 0.5 ml/min. and the variation in current with applied potential was recorded (Figure 5.13). With the increase of applied potential negatively or positively, the current increased correspondingly as did the potential between the working and counter electrodes.

With application of a potential, a current decreased with time and then became steady (Figure 5.14). This indicated that a charging process due to the capacitance of the column occurred when a potential was applied. The residual current at the steady state was found to be due to dissolved oxygen in the mobile phase. Deoxygenation of the mobile phase using nitrogen significantly decreased the residual current (Figure 5.15). The pressure drop on this column was about 30 atm using an 80% water and 20% acetonitrile mobile phase with 0.10 M NH₄Ac at a flow rate of 0.5 ml/min. The dead volume determined by injecting water with these conditions was found to be 0.52 ml and the column efficiency
using benzene as a test compound was found to be 400 plates per meter.

The effect of applied potentials was tested by injecting aniline into the column and recording the UV response. It was found that upon application of positive potentials over +0.2 V or negative potentials over -0.25 V the aniline response decreased significantly. This verified that the column was affected by the potentials. At positive and negative potentials, aniline was oxidized on the working and auxiliary electrodes respectively. The in situ electropolymerization of conducting polypyrrole was carried out on this column when a current was drawn on the column and a 0.10 M pyrrole and 0.05 M SDS solution in 1:1/CH₃CN:H₂O was pumped through the column. Deoxygenation using nitrogen was performed in the reagent container. This in situ coating method eliminated a column packing procedure, but a high pressure pump was required.
Figure 5.13  Electrochemical characteristics of the ECLC column

Column: 5 mm I.D. x 100 mm ECLC column packed with <40 μm RVC particles;
Eluent: 80% H₂O and 20% CH₃CN with 0.1 NH₄Ac at 0.5 ml/min.
Figure 5.14  Charging current and residual current on the ECLC column

Column: 5 mm I.D. x 100 mm ECLC column packed with <40 µm RVC particles;
Eluent: 80% H₂O and 20% CH₃CN with 0.1 NH₄Ac at 0.5 ml/min.
Figure 5.15 Effect of deoxygenation on the residual current obtained using the ECLC column

Column: 5 mm I.D. x 100 mm ECLC column packed with <40 μm RVC particles;
Eluent: 80% H₂O and 20% CH₃CN with 0.1 NH₄Ac at 0.5 ml/min;
N₂=with deoxygenation using nitrogen;
AIR=without deoxygenation.
5.4 Conclusion

Three special electrochemical cell designs were developed in the course of this work in order to produce polypyrrole stationary phases. It was verified that polypyrrole could be coated on RVC particles using each cell as well as on silica gels using the slurry cell. The packed particle cell and the plate cell could only be used to coat conducting particles while the slurry cell could be used to coat both conducting and non-conducting particles. The iR drop on the packed particle and plate cells was smaller than that on the slurry cell and as a result, higher currents may pass through these cells. The particles could not be totally covered with polypyrrole using either the packed particle or plate cells since parts of the particle contact each other. The uneven coating along the packing bed in the packed particle cell was found to be due to the uneven current density. These problems were overcome in the plate cell.

Two column designs have been investigated in this study in order to characterize polypyrrole stationary phases. The self-compressed column has been designed to obtain a reasonably efficient column packed within the laboratory, while the ECLC column has been designed to obtain a column to which an electrical potential can be applied. The principle of self-compression was similar to that of a flexible-walled column (262-265) but in the self-compression mode the eluent was also a compression fluid and no additional device was required. The preparation of the SCC with a normal HPLC pump was possible and relatively high performance was achieved. The column dimensions applicable with the SCC were more flexible than
could be employed using stainless steel columns or radial compressed cartridges. Therefore, the SCC was a good candidate for new stationary phase studies in which frequent changing of packing materials is necessary.

The ECLC column developed in the course of this work included three electrodes and it has been developed for use with a Waters RCM cartridge holder. The column efficiency of this ECLC column was higher than that of previous ones (99). The electrochemical behavior was similar to that of conventional electrochemical cells in which a charging current due to the capacitance of the column and a residual current due to dissolved oxygen in the mobile phase were determined. The column packing was affected by the application of potentials, and in situ polymerization of polypyrrole was also possible.
Chapter 6
Chromatography on RVC Based Polypyrrole
Stationary Phases
6.1 Introduction

Polypyrrole was formed on various conducting substrates as described in previous Chapters. However, in order to make substrates suitable for HPLC supports, there are a few specific criteria to be considered. The material should be able to:

1. tolerate high pressure;
2. be chemically stable;
3. be readily made in dimensions suitable for packings;

In addition, the material should have low background HPLC interactions, e.g. adsorption, and be inexpensive.

RVC is an ideal material as it meets all of the above requirements. Also, as RVC is conductive, it can be coated electrochemically and used for ECLC.

A number of counterions have been incorporated into polypyrrole as described in previous Chapters. Chloride and dodecyl-sulfate were selected as the counterions for further investigations in chromatography for the following reasons:

1. Cl\(^-\) is a simple anion whereas DS\(^-\) is a long chain-aliphatic, surfactant anion, and
2. Cl\(^-\) will not contribute to reversed phase chromatographic interactions, whereas DS\(^-\) may affect the reversed-phase chromatographic interactions due to its long chain.
These two counterions are complementary since PP/Cl should predominantly show backbone effects whereas PP/DS is more dependent upon the counterion nature.

Surface characterization of new stationary phases is often carried out using specific surface area analysis (267), scanning electron microscopy (268) and elemental analysis (268). Specific surface area analysis characterizes the surface properties of the packings. SEM shows particle shape, size and size distribution. Elemental analysis provides information of the stationary phase loading.

Chromatographic characterization of new stationary phases is generally carried out by injecting a group of known test compounds under specified conditions (266, 269, 270). The capacity factors and relative retentions of these compounds indicate the properties of the stationary phase. For example, hydrophobicity of stationary phases was determined using nonpolar compounds such as benzene and toluene (90, 271-273). Many other compounds such as naphthalene (90, 272), anthracene (90, 272), dimethylphthalate (90, 270, 274), diethylphthalate (90, 270, 274), aniline (273) and phenol (272), were previously used to characterize reversed-phase chromatography. Both inorganic and organic ions (266) were used as test compounds for ion exchange chromatography. Thus by judicious selection of test compounds, the mechanism of interaction of new stationary phases can be characterized.

Characterization of stationary phases for use in ECLC is more complicated as it is affected by the application of electrochemical
stimuli. The electrochemical characteristics of a polypyrrole column such as the voltammetric behavior as well as the charging and discharging behavior may be determined using methods similar to those used for conventional cells (275). Electrochemical influences on chromatographic interaction may be determined by comparing the retention behavior of a group of test compounds with and without the application of potentials.

In the course of this work bare RVC, PP/Cl/RVC and PP/DS/RVC particles were prepared and used as stationary phases for chromatography and ECLC. SEM was used for surface characterization and a group of test compounds have been chosen for chromatographic characterization. After preparation of the columns, the electrochemical characteristics were determined. Then chromatographic interactions with and without application of potentials were investigated and the efficiencies of ECLC columns were evaluated.

6.2 Experimental

6.2.1 Reagents and materials
All reagents were of analytical reagent (AR) grade purity unless otherwise stated. HPLC grade acetonitrile and distilled, deionized (Milli Q Water System) water were used throughout this project. Acetate buffer (1:1 molar ratio of sodium acetate and acetic acid), and ammonia acetate were used as the electrolyte in the mobile phase as required. Pyrrole (Sigma) was distilled before use and dissolved in 0.10 M NaCl or 0.10 M sodium dodecyl-sulfate
solutions. The solvent is 50% water and 50% acetonitrile unless otherwise stated. RVC packings were obtained using the same method as described in Chapter 5. All samples for injection were diluted in the eluent.

6.2.2 Instrumentation

Electrochemical polymerization was carried out using the same instrumentation as in Chapter 5. All HPLC experiments were carried out with a Kortec K350 HPLC Pump (ICI), an ERC-7210 spectrophotometric detector at 254 nm (ERMA Optical Works) and a Rheodyne 7512 injector with a 20 µl sample loop. Alternatively, a Waters Model M-6000A chromatography pump and a Dionex 4000i chromatography pump with a Waters Model 450 variable wavelength detector or a Waters Model 430 conductivity detector were used. Retention time was recorded with a stopwatch and chromatograms were obtained from an ICI D-600 chart recorder.

6.2.3 Preparation of packings

Packings were prepared according to the procedures described in Chapter 5. All packings were vacuum dried at 62°C to remove moisture before they were packed using a dry-packing method (266). Specifications of the columns are summarized in Table 6.1.
Table 6.1  Columns packed with RVC-based polypyrrole stationary phases

<table>
<thead>
<tr>
<th>Column</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP/Cl</td>
<td>PP/DS</td>
<td>PP/DS</td>
<td>RVC</td>
<td>PP/Cl</td>
<td>PP/Cl</td>
<td>PP/DS</td>
<td>PP/DS</td>
<td></td>
</tr>
<tr>
<td>RVC particle size (μm)</td>
<td>&lt;45</td>
<td>&lt;45</td>
<td>&lt;45</td>
<td>&lt;45</td>
<td>&lt;45</td>
<td>&lt;45</td>
<td>&lt;45</td>
<td></td>
</tr>
<tr>
<td>RVC particle weight (g)</td>
<td>1.5</td>
<td>0.8</td>
<td>2.5</td>
<td>1.04</td>
<td>1.0</td>
<td>1.11</td>
<td>1.24</td>
<td>1.0</td>
</tr>
<tr>
<td>Counterion</td>
<td>Cl</td>
<td>Cl</td>
<td>DS</td>
<td>DS</td>
<td>Cl</td>
<td>Cl</td>
<td>DS</td>
<td>DS</td>
</tr>
<tr>
<td>Polymerization device</td>
<td>P</td>
<td>S</td>
<td>In</td>
<td>-</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Polymerization current (mA)</td>
<td>10</td>
<td>10</td>
<td>40</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total charge (C)</td>
<td>115</td>
<td>152</td>
<td>420</td>
<td>-</td>
<td>90</td>
<td>98</td>
<td>110</td>
<td>89</td>
</tr>
<tr>
<td>Column materialb</td>
<td>SS</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>Column diameter (mm)</td>
<td>3.9</td>
<td>5</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Column length (cm)</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Electropolymerization solutionc</td>
<td>0.50 M</td>
<td>0.10 M</td>
<td>0.10 M</td>
<td>-</td>
<td>0.10 M</td>
<td>0.10 M</td>
<td>0.10 M</td>
<td>0.10 M</td>
</tr>
<tr>
<td>KCl</td>
<td>SDS</td>
<td>SDS</td>
<td>NaCl</td>
<td>NaCl</td>
<td>SDS</td>
<td>SDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.50 M</td>
<td>0.20 M</td>
<td>0.10 M</td>
<td>0.10 M</td>
<td>0.10 M</td>
<td>0.10 M</td>
<td>0.10 M</td>
<td>0.10 M</td>
<td></td>
</tr>
<tr>
<td>Py</td>
<td>Py</td>
<td>Py</td>
<td>Py</td>
<td>Py</td>
<td>Py</td>
<td>Py</td>
<td>Py</td>
<td></td>
</tr>
</tbody>
</table>

a. P=packed particle cell, S=slurry cell, In=in situ coated and P=plate cell.
b. SS=stainless steel and T=Teflon.
c. Py=pyrrole.
6.2.4 Procedures

All samples were injected in triplicate and an average retention time was taken. In ECLC experiments, the baseline was perturbed by application of the external potential. Injections were made after the baseline stabilized. The dead time was measured by injecting water and recording the first solvent dip. Using water to determine the dead time was suggested by previous workers (276, 277). Retention time was recorded using a stopwatch from a chart recorder and peak width was measured from chromatograms. The ECLC experiments were carried out continually in seven days in order to avoid any effects of polymer degradation.

6.3 Results and Discussion

6.3.1 Surface characterization of the stationary phase

Scanning electron micrographs (Figure 6.1) indicated that a layer of PP/Cl formed on the smooth RVC particle surface. The shape of these pellicular particles was irregular. The polymer coating did not completely cover the particle surface due to the polymerization process in which adjacent particles were in contact and thus could not be fully coated.

An EDX spectrum (Figure 6.2) shows that counterions were incorporated in the polymer coating. However, elemental analysis of the stationary phase loading could not be performed as there is a large carbon, as well as nitrogen and hydrogen, background on RVC (220). Determination of the specific surface area was unsuccessful as the surface area (<6 m²) was not large enough to register on the apparatus.
Figure 6.1  Scanning electron micrographs of RVC based polypyrrole packings

SEMs on: a. bare RVC particle, b. PP/Cl/RVC particle.
Figure 6.2  EDX spectrum of RVC based polypyrrole packings

EDX on:  a. PP/Cl/RVC and b. PP/DS/RVC.

EDX condition: High voltage=20 KV; accumulation time =200 seconds.
6.3.2 Test compounds

A selection of test compounds were used for chromatographic characterization. Their properties are summarized in Table 6.2. Two groups of test compounds was used. Group A included eight compounds that were used to characterize the chromatographic interactions and ECLC effects on the polypyrrole stationary phases, while group B compounds were used to obtain additional information of characterization.

In group A, benzene was chosen as the principal unit and functional groups were added in order to determine the role played by specific molecular interactions. For example the methyl group of toluene represented introduction of a hydrophobic interaction which has a non-polar character, while the methyl-ester groups of dimethylphthalate (DMP) and diethylphthalate (DEP) were used as markers for polar interactions. Phenol was used to detect proton donor effects due to the -OH group, while aniline was used to detect electron donor effects due to the non-bonded electrons on the nitrogen. N,N-dimethylaniline (DMA) had similar molecular characteristics to aniline but some workers (266) have used it previously to test for adsorption. Benzoic acid has a pKa value of 4.2 and was thus used to test the anion exchange capability of the stationary phase. All these compounds were easily detected with a conventional UV detector due to the phenyl group.

In order to compare with commercial columns (90) and to introduce an alternative characterization method, group B was used. Nonpolar, polar, acidic and basic interactions were characterized using naphthalene and anthracene, dimethylphthalate and
diethylphthalate, benzoic acid and toluic acid, and caffeine and theophylline respectively. To obtain accurate retention times, the concentrations of the test compounds were kept constant to avoid changes in retention times especially due to tailing peaks.
### Table 6.2A Test compounds

<table>
<thead>
<tr>
<th>Group</th>
<th>Test compounds</th>
<th>M.W</th>
<th>Boiling point (°C)</th>
<th>pH</th>
<th>PKₐ</th>
<th>PKₐ</th>
<th>Solubility in H₂O (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Benzene</td>
<td>78.11</td>
<td>80.1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.7</td>
</tr>
<tr>
<td>A</td>
<td>Toluene</td>
<td>92.13</td>
<td>110.6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>very slightly</td>
</tr>
<tr>
<td>A</td>
<td>DMP</td>
<td>194.19</td>
<td>283.7</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>4.3</td>
</tr>
<tr>
<td>A</td>
<td>DEP</td>
<td>222.24</td>
<td>298</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>A</td>
<td>Phenol</td>
<td>94.11</td>
<td>182</td>
<td>6.0</td>
<td>10.0</td>
<td>--</td>
<td>66.7</td>
</tr>
<tr>
<td>A</td>
<td>Benzoic acid</td>
<td>122.12</td>
<td>249.2</td>
<td>2.8</td>
<td>4.2</td>
<td>--</td>
<td>3.4</td>
</tr>
<tr>
<td>A</td>
<td>Aniline</td>
<td>93.12</td>
<td>184-186</td>
<td>8.1</td>
<td>--</td>
<td>9.3</td>
<td>3.5</td>
</tr>
<tr>
<td>A</td>
<td>DMA</td>
<td>121.18</td>
<td>192-194</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.4</td>
</tr>
</tbody>
</table>

### Table 6.2B Test compounds

<table>
<thead>
<tr>
<th>Group</th>
<th>Test compounds</th>
<th>M.W</th>
<th>Boiling point (°C)</th>
<th>pH</th>
<th>PKₐ</th>
<th>PKₐ</th>
<th>Solubility in H₂O (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Naphthalene</td>
<td>128.16</td>
<td>217.9</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>B</td>
<td>Anthracene</td>
<td>178.22</td>
<td>342</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>B</td>
<td>DMP</td>
<td>194.19</td>
<td>283.7</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>4.3</td>
</tr>
<tr>
<td>B</td>
<td>DEP</td>
<td>222.24</td>
<td>298</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>B</td>
<td>Benzoic acid</td>
<td>122.12</td>
<td>249.2</td>
<td>2.8</td>
<td>4.2</td>
<td>--</td>
<td>3.4</td>
</tr>
<tr>
<td>B</td>
<td>m-Toluic acid</td>
<td>136.4</td>
<td>263</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.85</td>
</tr>
<tr>
<td>B</td>
<td>Caffeine</td>
<td>194.19</td>
<td>--</td>
<td>6.9</td>
<td>14</td>
<td>14.2</td>
<td>21.7</td>
</tr>
<tr>
<td>B</td>
<td>Theophylline</td>
<td>180.17</td>
<td>--</td>
<td>8.8</td>
<td>13.7</td>
<td>8.3</td>
<td>--</td>
</tr>
</tbody>
</table>

6.3.3 Reversed-phase chromatography

Chromatographic characterization without EC control was carried out using RVC (column 4), PP/Cl/RVC (column 5) and PP/DS/RVC (column 7) packed columns. The test compounds in group A were employed. Results are summarized in Table 6.3.

Most of the test compounds exhibited low retentions on the bare RVC column with and without an electrolyte in the eluent. However, toluene and dimethylaniline were strongly retained and the eluted peaks tailed markedly. The retention may be due to two processes, adsorption and hydrophobic interactions. The tailing peaks were indicative of the adsorption process. Adsorption is usually dependent on molecular size and shape (11), and should have resulted in similar retentions for most of the other test compounds. Higher retention of toluene and N,N-dimethylaniline indicated that hydrophobic interactions were caused by other factors. Carbon based stationary phases have been previously reported as being hydrophobic (278, 279).

6.3.3.1 Polypyrrole chloride stationary phase

Retention characteristics of PP/Cl/RVC were compared with those obtained on RVC. The relative retention of toluene vs. benzene decreased on PP/Cl/RVC. This indicated that PP/Cl had less selectivity to hydrophobic groups than bare RVC.
### Table 6.3 Retention on different columns

<table>
<thead>
<tr>
<th>Sample</th>
<th>Packing material</th>
<th>20% CH₃CN+80%H₂O</th>
<th>0.1 M NH₄Ac in 20% CH₃CN+80%H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k'</td>
<td>k'/k' benzene</td>
<td>k'</td>
</tr>
<tr>
<td>Benzene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVC</td>
<td>0.52</td>
<td>1.00</td>
<td>0.58</td>
</tr>
<tr>
<td>PP/Cl/RVC</td>
<td>0.75</td>
<td>1.00</td>
<td>0.78</td>
</tr>
<tr>
<td>PP/DS/RVC</td>
<td>1.39</td>
<td>1.00</td>
<td>1.20</td>
</tr>
<tr>
<td>Toluene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVC</td>
<td>1.69</td>
<td>3.25</td>
<td>1.69</td>
</tr>
<tr>
<td>PP/Cl/RVC</td>
<td>1.66</td>
<td>2.21</td>
<td>1.69</td>
</tr>
<tr>
<td>PP/DS/RVC</td>
<td>2.62</td>
<td>1.88</td>
<td>3.28</td>
</tr>
<tr>
<td>Dimethylphthalate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVC</td>
<td>0.27</td>
<td>0.52</td>
<td>0.57</td>
</tr>
<tr>
<td>PP/Cl/RVC</td>
<td>1.03</td>
<td>1.37</td>
<td>1.17</td>
</tr>
<tr>
<td>PP/DS/RVC</td>
<td>2.36</td>
<td>1.70</td>
<td>2.84</td>
</tr>
<tr>
<td>Phenol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVC</td>
<td>0.35</td>
<td>0.67</td>
<td>0.40</td>
</tr>
<tr>
<td>PP/Cl/RVC</td>
<td>0.66</td>
<td>0.88</td>
<td>0.65</td>
</tr>
<tr>
<td>PP/DS/RVC</td>
<td>0.96</td>
<td>0.69</td>
<td>1.11</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVC</td>
<td>0.35</td>
<td>0.67</td>
<td>0.17</td>
</tr>
<tr>
<td>PP/Cl/RVC</td>
<td>∞</td>
<td>∞</td>
<td>0.65</td>
</tr>
<tr>
<td>PP/DS/RVC</td>
<td>∞</td>
<td>∞</td>
<td>4.04</td>
</tr>
<tr>
<td>Aniline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVC</td>
<td>0.31</td>
<td>0.60</td>
<td>0.32</td>
</tr>
<tr>
<td>PP/Cl/RVC</td>
<td>1.06</td>
<td>1.41</td>
<td>0.59</td>
</tr>
<tr>
<td>PP/DS/RVC</td>
<td>∞</td>
<td>∞</td>
<td>1.02</td>
</tr>
<tr>
<td>N,N-Dimethyl aniline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVC</td>
<td>2.24</td>
<td>4.31</td>
<td>3.56</td>
</tr>
<tr>
<td>PP/Cl/RVC</td>
<td>4.50</td>
<td>6.00</td>
<td>2.69</td>
</tr>
<tr>
<td>PP/DS/RVC</td>
<td>∞</td>
<td>∞</td>
<td>5.89</td>
</tr>
</tbody>
</table>
The increase in the relative retentions of DMP vs. benzene on PP/Cl/RVC indicated that compared with RVC, PP/Cl was polar presumably due to its polar pyrrole units. The capacity factors obtained for phenol were low on both RVC and PP/Cl/RVC, however, a slight increase in the relative retention vs. benzene was observed on PP/Cl/RVC. From this it was concluded that PP/Cl was slightly more selective to the proton donors. An increase in relative retentions of aniline and DMA vs. benzene on PP/Cl/RVC indicates that PP/Cl has stronger selectivities to electron donors than RVC, probably due to its positively charged backbone.

Interactions due to polarity and electron acceptor capability were found using PP/Cl stationary phase, while hydrophobicity and proton acceptor capability on this stationary phase were very low.

Addition of a salt to the mobile phase had no effect on the hydrophobicity, polarity or proton acceptor capability of the stationary phase. The electron acceptor capability did decrease as observed upon addition of the salt by the decrease of the relative retentions of aniline and DMA. This is probably due to the solvation of electron accepting sites in the presence of ionic species (280).

Reversed-phase chromatographic behavior on the PP/Cl/RVC column was further confirmed using naphthalene, isoquinoline, quinoline and quinaldine as samples. The capacity factor was found to be a function of methanol concentration in the eluent (Figure 6.3). A plot of log $k'$ vs. methanol concentration was approximately linear as is typical of that observed on conventional reversed-phase columns (9-11).
6.3.3.2 *Polypyrrole dodecyl-sulfate stationary phase*

The relative retention time of toluene vs. benzene on the PP/DS/RVC column was lower than that on the RVC column. The results indicated that the selectivity for the hydrophobic groups on PP/DS was not as strong as that on bare RVC. With PP/DS packing increased selectivity to polar groups was observed. Electron acceptor capability was affected. However, the selectivity to proton donors increased slightly.

Addition of salt to the mobile phase produced notable changes in the PP/DS/RVC stationary phase. The selectivities to hydrophobic groups, polar groups and proton donors increased due to the effect of the salt. This may be due to changes in the orientation of the long chain surfactant in polypyrrole in the presence of the salt (281-283). However, on the PP/DS/RVC column addition of the salt in the mobile phase markedly decreased the selectivity to electron donors, which may be caused by the same factors as those for the PP/Cl/RVC column.

The reversed-phase chromatographic behavior on the PP/DS/RVC column was further studied using dimethyl- and diethyl-phthalate. A linear plot of log k' vs. methanol concentration was found (Figure 6.4).
Figure 6.3 Capacity factors of quinoline, quinaldine, isoquinoline and naphthalene vs. methanol concentration of the mobile phase;

Column: PP/Cl/RVC in a 3.9 mm I.D. x 150 mm stainless steel column (column 1);

Eluent: methanol and water at 1.0 ml/min.;

Sample: a. 100 ppm quinoline, b. 100 ppm quinaldine, c. 100 ppm isoquinoline and d. 100 ppm naphthalene;

Detector: UV at 217 nm.
Figure 6.4 Capacity factors of phthalates vs. methanol concentration of the mobile phase;

Column: PP/DS/RVC in a 5 mm I.D. x 100 mm Teflon cartridge (column 2);
Eluent: methanol and water at 1.0 ml/min.;
Samples: 30 ppm DMP and 30 ppm DEP;
Detector: UV at 275 nm.
A comparison of the RVC based stationary phases for reversed phase chromatography is summarized in Table 6.4. PP/Cl showed stronger selectivities to hydrophobic groups and proton acceptors than PP/DS, while the salt effect made PP/DS more selective to hydrophobic groups than PP/Cl, which probably resulted from reorientation of surfactant groups in the solution. The surfactant effects on the polypyrrole properties were studied previously (281-283).

Table 6.4 Comparison of the RVC-based stationary phases

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>without a salt effect</td>
</tr>
<tr>
<td>Hydrophobicity</td>
<td>RVC&gt;PP/Cl&gt;PP/DS</td>
</tr>
<tr>
<td>Polarity</td>
<td>PP/DS&gt;PP/Cl&gt;RVC</td>
</tr>
<tr>
<td>Proton acceptor capability</td>
<td>PP/Cl&gt;PP/DS=RVC</td>
</tr>
<tr>
<td>Electron acceptor capability</td>
<td>PP/DS&gt;PP/Cl&gt;RVC</td>
</tr>
</tbody>
</table>

In an attempt to evaluate the chromatographic behavior of the PP/DS stationary phase, both polar and nonpolar, as well as acidic and basic test compounds were evaluated and compared with performance on other commercial packings (90).

For polar and nonpolar test compounds, results are summarized in Table 6.5. The capacity factor obtained upon injection of anthracene was much larger than that obtained on other commercially available
The large degree of band broadening with a reasonable asymmetry factor of 1.5 would suggest the high retention was due to high π-π electronic interaction between polypyrrole and the anthracene. Naphthalene was not retained to the same degree and consequently an extremely large selectivity factor was obtained.

**Table 6.5 Retentivity and selectivity of nonpolar and polar compounds on polypyrrole**

<table>
<thead>
<tr>
<th>Composition of the mobile phase (CH$_3$OH:H$_2$O)</th>
<th>Nonpolar compounds</th>
<th>Polar compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k'(\text{anthracene})$</td>
<td>$\alpha(\text{anthracene/ naphthalene})$</td>
<td>$k'(\text{DEP})$</td>
</tr>
<tr>
<td>65:35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>50:50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>45:55</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>85:15</td>
<td>22.7(0.4-6.4)</td>
<td>15.04(1.0-2.9)</td>
</tr>
<tr>
<td>100:0</td>
<td>9.43</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) Figures in parentheses were obtained from commercial columns (90).

The chromatographic behavior of the polar test compounds (diethylphthalate and dimethylphthalate) was much closer to the expected behavior as observed on commercially available columns. Much higher efficiencies (c.f. anthracene) were obtained with the polar compounds. Good selectivity factors were observed for separation of these closely related phthalates.
For basic and acidic test compounds, the data is summarized in Table 6.6. The retention of the basic test compound, caffeine, was of the same order as the other commercially available columns. However, the retention of the acidic test compound was much higher than that on the other commercial columns, presumably due to ion exchange interactions which will be discussed in the next section.

6.3.3.3 Stability of stationary phases: reversed-phase chromatography

The stability of the capacity factors obtained using reversed-phase chromatography was investigated. Retention data for naphthalene on PP/Cl/RVC was constant after passing 8 liters of 60% MeOH and 40% H₂O as the mobile phase through the column. Stability for reversed-phase chromatographic interactions was found on PP/DS/RVC (Table 6.7). Retention increased after passing 4 liters of the mobile phase through the column probably due to the loss of the DS counterions. These results suggested that the reversed-phase chromatographic interactions occurred primarily on the polymer backbone, which changed little after passing 8 liters of mobile phase through the column (refer to Chapter 4).
Table 6.6  Retentivity and selectivity of basic and acidic compounds on polypyrrole

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>Basic compounds</th>
<th>Acid compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k'(caffeine)</td>
<td>α(caffeine/theophylline)</td>
</tr>
<tr>
<td>CH₃CN:10</td>
<td>6.14 (0.3-7.5)ᵃ</td>
<td>1.61 (1.2-3.4)</td>
</tr>
<tr>
<td>mM NaAc pH 4.5=20:80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH₃CN:0.17 M NaAc pH 4.5=20:80</td>
<td>1.28</td>
<td>1.44</td>
</tr>
<tr>
<td>CH₃CN:0.17 M NaAc pH 4.5=50:50</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

ᵃ The data in parentheses are from most commercial columns (90).
Table 6.7  Stability of \( k' \) on the PP/DS/RVC column

<table>
<thead>
<tr>
<th>Sample</th>
<th>( k_1' )</th>
<th>( k_2' )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>1.02</td>
<td>1.20</td>
</tr>
<tr>
<td>Dimethylphthalate</td>
<td>2.57</td>
<td>2.84</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.97</td>
<td>1.11</td>
</tr>
<tr>
<td>Aniline</td>
<td>0.94</td>
<td>1.02</td>
</tr>
</tbody>
</table>

The experiment was running daily in an eluent of 80% water and 20% CH\(_3\)CN with 0.1 M NH\(_4\)Ac. \( k_1' \) and \( k_2' \) were determined after passing 0.7 liter and 4 liters of the mobile phase through the column respectively.

6.3.4 Anion exchange chromatography

6.3.4.1 Polypyrrole chloride stationary phase

The ability of polypyrrole to undergo anion exchange was discussed in Chapter 3. This process was found to be reversible, and therefore it may be used in chromatography. Initially, simple ions (nitrate, nitrite and dichloroacetate) were used as test compounds for the PP/Cl/RVC column and the chromatographic behavior was studied.

Simple anions were not retained on the column packed with RVC but were retained on a PP/Cl/RVC column. The influence of anion exchange parameters such as solvent strength and pH on capacity factors are shown in Figures 6.5 and 6.6 respectively. As in ion exchange on conventional columns, the higher the ionic strength the lower the capacity factors are. In the pH range investigated, the test anions were ionic. The results obtained indicated that the
lower the pH value the higher the column ion exchange capacity, i.e. the longer the retention.

As shown in Chapter 3, the retention sequence for anions is different from a conventional anion exchange resin. This has been confirmed in the chromatographic studies (Table 6.8). Anions such as sulfate and chromate are usually retained for a long time on conventional resins (44, 9-11). This was not observed on the polypyrrole chloride stationary phase. The mechanism of this effect has already been discussed in Chapter 3. This anion exchange interaction was further confirmed using benzoic acid as a test compound (Table 6.3). Benzoic acid was not eluted until a salt was added to the mobile phase. This is a typical ion exchange process.

Table 6.8  Anion exchange selectivity sequence

<table>
<thead>
<tr>
<th>Sample</th>
<th>k'</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₃⁻</td>
<td>15.1</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>11.7</td>
</tr>
<tr>
<td>SO₃⁻</td>
<td>11.0</td>
</tr>
<tr>
<td>Br⁻</td>
<td>10.4</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Sample concentration: 1.0x10⁻³ M;
Mobile phase: 0.010 M sodium acetate, 1.0 ml/min;
Detector: conductivity.
Figure 6.5  Capacity factors of nitrate, nitrite and dichloroacetate vs. ionic strength of the mobile phase

Column: PP/Cl/RVC in a 3.9 mm I.D. x 150 mm stainless steel column (column 1);
Eluent: NaAc solution at 1.0 ml/min;
Samples: a. $1.0 \times 10^{-3}$ M dichloroacetic acid, b. $1.0 \times 10^{-3}$ M NO$_2^-$ and c. $1.0 \times 10^{-3}$ M NO$_3^-$.
Figure 6.6 Capacity factors of nitrate, nitrite and dichloroacetate vs. pH of the mobile phase

Column: PP/Cl/RVC in a 3.9 mm I.D. x 150 mm stainless steel column (column 1);
Eluent: 0.05 M NaAc solution adjusted pH with HAc at 1.0 ml/min;
Samples: a. $1.0 \times 10^{-3}$ M dichloroacetic acid, b. $10^{-3}$ M NO$_2^-$ and c.$1.0 \times 10^{-3}$ M NO$_3^-$. 
6.3.4.2 Polypyrrole dodecyl-sulfate stationary phase

As occurred with PP/Cl, benzoic acid was well retained on PP/DS (Tables 6.3 and 6.6). The addition of salt decreased these interactions as would be expected for an ion exchange process. An increase of ionic strength in the mobile phase decreased the capacity factors of anionic compounds on the PP/DS/RVC column (Figure 6.7). As the buffer concentration was increased without changing the acetonitrile composition, the capacity factors for the test compounds decreased. In addition the column efficiency increased considerably (Table 6.9). This indicated that the ion exchange process proceeded more quickly at the higher buffer concentration.

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>m-Toluate</th>
<th>Benzoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$CN (%)</td>
<td>NaAc buffer</td>
<td>k'</td>
</tr>
<tr>
<td>pH=4.5 (M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.01</td>
<td>28</td>
</tr>
<tr>
<td>50</td>
<td>0.17</td>
<td>4.7</td>
</tr>
<tr>
<td>50</td>
<td>0.50</td>
<td>1.9</td>
</tr>
</tbody>
</table>

It was found that there was a higher capacity factor for benzoic acid on PP/DS than on PP/Cl (Table 6.3). This is probably due to other interactions from the surfactant retained in the polymer. Such a mixed-mode phenomenon has been reported previously (57).
Figure 6.7 Capacity factors of benzoate and m-toluate vs. the ionic strength of the buffer in the mobile phase

Column: PP/DS/RVC in a 5 mm I.D. x 100 mm Teflon cartridge (column 2);

Eluent: 50% CH$_3$CN and 50% acetate buffer (pH 4.5);

Sample: 30 ppm benzoic acid and 30 ppm m-toluic acid.
6.3.4.3 Stability of stationary phases: anion exchange chromatography

It was found that the anion exchange capacity of PP/Cl decreased with time (Figure 6.8). This capacity loss is probably due to degradation of polypyrrole chloride. It has been reported (189, 284, 285) that degradation occurred in an aqueous solution. It has also been shown that such degradation was due to attack by OH\textsuperscript{-} at the 3-position (189) with some breakdown in conjugation. Such processes led to a loss in the positive charge from the polymer and as a result anion exchange capacity was decreased. This degradation was found to be irreversible (253, 286).

The same decreases in capacity were also found on PP/DS/RVC. The capacity factor for benzoic acid decreased by 40% after passing 4 liters of 80% H\textsubscript{2}O and 20% CH\textsubscript{3}CN through the column.

6.3.5 Electrochemically controlled chromatography

ECLC columns have a considerable capacitance due to their high surface area. Charging (Figure 6.9) and discharging (Figure 6.10) processes were observed after application and removal of potentials. Compared with bare RVC columns, both PP/Cl/RVC and PP/DS/RVC have higher capacitance due to the polymer layer. Therefore a longer time is required to charge the column (Figure 6.9). In addition discharging occurs slowly. For example, discharging of a PP/DS column with 80% 0.10 M NH\textsubscript{4}Ac and 20 CH\textsubscript{3}CN as electrolyte took more than one hour (Figure 6.10).
Figure 6.8  \textit{Stability of RVC based polypyrrole packings}

Column:  PP/Cl/RVC in a 3.9 mm I.D. x 150 mm stainless steel column (column 1);

Eluent:  a. 0.005 M NaAc at 1.0 ml/min.

Samples:  a. 1.0x10^{-3} M dichloroacetic acid, b. 1.0x10^{-3} M NO_{2}^{-} and c.1.0x10^{-3} M NO_{3}^{-};

Detector:  UV at 217 nm.
**Figure 6.9** Charging process on ECLC columns

Column: a. RVC, b. PP/Cl/RVC and c. PP/DS/RVC in 5 mm I.D. x 100 mm Teflon cartridges (column 4, 5 and 7);

Eluent: 80% H$_2$O and 20% CH$_3$CN with 0.1 M NH$_4$Ac at 0.5 ml/min;

E$_{app}$: -0.25 V vs. Ag/AgCl;

Current responses on: a. RVC, b. PP/Cl/RVC and c. PP/DS/RVC.
Figure 6.10 Discharging process on ECLC columns

Column: PP/DS/RVC;
Eluent: 80% H₂O and 20% CH₃CN with 0.1 M NH₄Ac at 0.5 ml/min;
E_app: +0.35 V vs. Ag/AgCl;
Potential response E(V) is between the working and auxiliary electrodes.
With both PP/Cl and PP/DS packings a constant residual current was observed after the application of the potential (Figure 6.9). This residual current was mainly generated from electrochemical reactions due to the presence of electroactive substances in the mobile phase, especially from the reduction of dissolved oxygen. Figure 6.11 shows how residual current changes with flow rate and after deoxygenation on a PP/Cl/RVC packed column. Similar results were obtained on a PP/DS/RVC column. Oxygen was reduced at the working electrode (packings) at more negative potentials and on the counter electrode (carbon foil) at more positive potentials. No pH change due to the reduction of oxygen in the eluent was detected at the outlet of the column.

The current between the working and auxiliary electrodes varied with the applied potential vs. Ag/AgCl reference electrode (Figure 6.12). At positive or negative potentials a current was observed on all three columns but this residual current was higher on polymeric columns. As indicated in Figure 6.11, this residual current results from reduction of dissolved oxygen on packings at negative potentials or on the carbon foil auxiliary at positive potentials.
Figure 6.11  Residual current on an ECLC column

Column:  PP/Cl/RVC;
Eluent:  80% H2O and 20% CH3CN with 0.1 M NH4Ac at 0.5 ml/min;
Eapp:  -0.25 V vs. Ag/AgCl;
Responses due to:  a. discharge current, b. residual current at 0.5 ml/min., c. residual current at 1.0 ml/min., d. residual current at 1.5 ml/min. and e. current after deoxygenation.
**Figure 6.12** *Electrochemical behavior on ECLC columns*

Column: a. RVC, b. PP/Cl/RVC and c. PP/DS/RVC in 5 mm I.D. x 100 mm Teflon cartridges (column 4, 5 and 7);

Eluent: 80% H₂O and 20% CH₃CN with 0.1 M NH₄Ac at 0.5 ml/min;

Current responses on: a. RVC, b. PP/Cl/RVC and c. PP/DS/RVC.
The potential drop between the working and auxiliary electrodes vs. applied potential was approximately linear in all columns (Figure 6.13). This potential is dependent on the cell resistance and the current.

In a preliminary test of ECLC, a D.C. potential was applied across the packing bed and the counter electrode. Upon application of the potential, the capacity factor for basic compounds (e.g. caffeine) increased with a more negative potential while the capacity factor for acidic compounds decreased (Figure 6.14). The capacity factor returned to the original value when the negative potential was removed. Application of a more positive potential (up to +1.00 V) decreased the capacity factor for the acidic compounds while increasing it for the basic compounds. Beyond +1.00 V the capacity factor for all compounds decreased markedly. It is well known that polypyrrole is irreversibly oxidized beyond these potentials. Note that the selectivity factor (a) for caffeine and theophylline increased with the application of a negative potential (Figure 6.15). This indicates that potentials on the packing bed can change not only the retention but also the selectivity of the polypyrrole stationary phase, which is usually more desirable.

The results verified that the retention and separation on a polypyrrole stationary phase can be controlled or modified by the applied potentials. In order to understand the mechanism of such a process further investigations have been carried out.
Figure 6.13 *Potential drop between the working and auxiliary electrodes vs. applied potential*

Column: a. RVC, b. PP/Cl/RVC and c. PP/DS/RVC in 5 mm I.D. x 100 mm Teflon cartridges (columns 4, 5 and 7);
Eluent: 80% H₂O and 20% CH₃CN with 0.1 M NH₄Ac at 0.5 ml/min;
Current responses on: a. RVC, b. PP/Cl/RVC and c. PP/DS/RVC.
Figure 6.14 Capacity factors vs. applied potentials on a PP/DS column

Column: PP/DS/RVC in an 8 mm I.D. x 100 mm Teflon cartridge (column 3);
Eluent: 50% CH$_3$CN and 50% 0.4 M acetate buffer (pH 4.5) at 1.0 ml/min;
Sample: a. 10 ppm theophylline, b. 10 ppm caffeine, c. 30 ppm benzoic acid and d. 30 ppm m-toluic acid;
Detector UV at 254 nm.
Figure 6.15  *Selectivity factors vs. applied potentials on a PP/DS column*

Column: PP/DS/RVC in an 8 mm I.D. x 100 mm Teflon cartridge (column 3);

Eluent: 50% CH$_3$CN and 50% 0.4 M acetate buffer (pH 4.5) at 1.0 ml/min;

Curve: a. $\alpha$=k'caffeine/k'theophylline;
b. $\alpha$=k'benzoic acid/m-toluic acid;

Detector UV at 254 nm.
It was observed from preliminary ECLC results (Figures 6.14 and 6.15) that application of a more positive potential (over +1.0 V which was found to be equivalent to $E_{app} = +0.25$ V) between working and auxiliary electrodes caused irreversible degradation of the polymer, and that application of a more negative potential reduced the polymer (194, 195, 215-217). Therefore a range from -0.35 to +0.25 V vs. Ag/AgCl was chosen in the subsequent investigation, since in this range, polypyrrole is neither irreversibly oxidized nor completely reduced. RVC, PP/Cl/RVC and PP/DS/RVC packing materials were investigated.

6.3.5.1 Electrochemical effect on hydrophobicity

Changes in hydrophobicity were monitored by the relative retention of toluene vs. benzene. Electrochemical control significantly changed the hydrophobicity of the RVC (Figure 6.16). After the application of positive or negative potentials, the RVC surface became less hydrophobic which was indicated by the decrease of the relative retention of toluene vs. benzene. This indicated that the hydrophobic surface of the RVC became polar at positive or negative potentials. Alternative switching of potentials between positive and negative states resulted in the capacity factor changing reversibly. Such changes in the hydrophobicity of RVC have been reported previously (287, 288).
Figure 6.16  *EC effects on hydrophobicity*

Column: a. RVC, b. PP/Cl/RVC and c. PP/DS/RVC in 5 mm I.D. x 100 mm Teflon cartridges (columns 4, 5 and 7);
Eluent: 80% H₂O and 20% CH₃CN with 0.1 M NH₄Ac at 0.5 ml/min
Such effects on hydrophobicity were not observed with PP/Cl/RVC and PP/DS/RVC columns (Figure 6.16). A low relative retention of toluene indicated that in both cases the RVC surface was sufficiently covered by the polypyrrole and hence contributions due to RVC were sufficiently masked. In the potential range investigated no significant change of hydrophobicity of the polymeric materials was observed.

6.3.5.2 Electrochemical effect on polarity
Changes in polarity were monitored by the relative retention of DMP vs. benzene. In this potential range, the relative retention of DMP to benzene slightly increased on RVC at negative potentials but decreased slightly on PP/Cl/RVC and PP/DS/RVC columns (Figure 6.17). This was not significant.

6.3.5.3 Electrochemical effect on proton acceptor capability
Changes in proton acceptor capability were monitored by the relative retention of phenol vs. benzene. It was found that relative retention of phenol increased with the increasing of potentials on all three columns (Figure 6.18). The number of proton accepting sites on the RVC surface increases when the surface is oxidized. It seems that these hydrophobic sites became proton accepting sites. The relative retentions of phenol on both the conducting polymer columns were found to have a similar trend to those on the RVC column, and it is therefore difficult to distinguish the electrochemical effects on the polymers from this tendency.
Figure 6.17  EC effects on polarity

Column: a. RVC, B. PP/Cl/RVC and c. PP/DS/RVC in 5 mm I.D. x 100 mm Teflon cartridges (columns 4, 5 and 7);
Eluent: 80% H₂O and 20% CH₃CN with 0.1 M NH₄Ac at 0.5 ml/min.
Figure 6.18  *EC effects on proton acceptor capability*

Column:  a. RVC, B. PP/Cl/RVC and c. PP/DS/RVC in 5 mm I.D. x 100 mm Teflon cartridges (columns 4, 5 and 7);
Eluent:  80% H₂O and 20% CH₃CN with 0.1 M NH₄Ac at 0.5 ml/min.
6.3.5.4 Electrochemical effect on electron acceptor capability

Changes in electron acceptor capability were determined by the relative retention of aniline vs. benzene.

Only a narrow range of potentials was used to investigate the effect of potential on electron donor properties since the test compound, aniline, was oxidized on the packing bed when the applied potential was greater than +0.20 V and on the counter electrode when E_{app} was less that -0.25 V. DMA was oxidized at even lower potentials.

In contrast to the relative retention due to proton donor effects, the relative retention due to electron donor effects increased with increasing negative potential on the RVC column (Figure 6.19). Apparently hydrophobic sites on the RVC surface became electron accepting sites at more negative potentials.

On conducting polymer stationary phases, similar effects were observed. A similar trend on the PP/Cl/RVC column to that on the RVC column was seen although the degree of the change was small, while on the PP/DS/RVC column a distinct difference was observed with reference to the RVC column. The results suggested that the electron acceptor capability on both PP/Cl/RVC and PP/DS/RVC was affected by applied potentials.

6.3.5.5 Electrochemical effect on anion exchange

Changes in anion exchange capability were monitored by the relative retention of benzoic acid vs. benzene.
Figure 6.19 EC effects on electron acceptor capability

Column: a. RVC, b. PP/Cl/RVC and c. PP/DS/RVC in 5 mm I.D. x 100 mm Teflon cartridges (columns 4, 5 and 7);
Eluent: 80% H₂O and 20% CH₃CN with 0.1 M NH₄Ac at 0.5 ml/min.
Ion exchange interactions were observed on PP/Cl/RVC and PP/DS/RVC but not on RVC. These interactions were dramatically affected by applied potentials. The results further confirmed the previous discovery, and the "umbrella" shape curves were obtained again on both PP/Cl/RVC and PP/DS/RVC (Figure 6.20), i.e. capacity factors were low at both negative and positive potential.

6.3.5.7 Reversibility of effects induced by application of potentials
Within the potential range investigated, reversibility of electrochemical effects on the stationary phase was studied. Using benzoic acid as a test compound (Figure 6.21) it was found that the retention time decreased from level 1 to level 2 due to application of a -0.24 V vs. Ag/AgCl potential on PP/DS/RVC and that it gradually returned to the original value 30 minutes after removal of the potential. This indicated that changes in the polymer induced by applied potential are reversible.

The processes involved in this reversible effect can be explained as discharge of the polymer. It has been verified that a discharge process can take a certain period (Figure 6.10). Therefore, the charged polymer can still influence the retention until the polymer is fully discharged.
Figure 6.20 EC effects on ion exchange capability

Column: a. RVC, B. PP/Cl/RVC and c. PP/DS/RVC in 5 mm I.D. x 100 mm Teflon cartridges (columns 4, 5 and 7);
Eluent: 80% H2O and 20% CH3CN with 0.1 M NH4Ac at 0.5 ml/min.
Figure 6.21  Reversibility of ECLC

Column: PP/DS/RVC in an 8 mm I.D. x 100 mm Teflon cartridge (column 3);

Eluent: 50% CH$_3$CN and 50% 0.5 M acetate buffer (pH 4.5) at 1.0 ml/min;

Sample: 30 ppm benzoic acid;

Detector: UV at 254 nm;

Responses due to: 1. retention without a potential,
2. retention with $E_{app} = -0.24$ V vs Ag/AgCl, 3. retention after release of the potential.
6.3.6 Column performance

6.3.6.1 Chromatographic efficiency

The chromatographic efficiencies attainable with the present packing were not very high (Table 6.10). However, compared with previous ECLC columns, a significant advancement has been made using the present design. The column efficiency did not change with the application of potentials. A plot of HETP vs. mobile phase velocity is shown in Figure 6.22. The curve had a similar shape to that of conventional packings.

Table 6.10 Column efficiency on RVC-base columns

<table>
<thead>
<tr>
<th>Test</th>
<th>Compounds</th>
<th>N (plates/m)²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RVC</td>
<td>PP/Cl/RVC</td>
</tr>
<tr>
<td>Benzene</td>
<td>350</td>
<td>600</td>
</tr>
<tr>
<td>Toluene</td>
<td>80</td>
<td>300</td>
</tr>
<tr>
<td>DMP</td>
<td>150</td>
<td>450</td>
</tr>
<tr>
<td>DEP</td>
<td>150</td>
<td>420</td>
</tr>
<tr>
<td>Phenol</td>
<td>530</td>
<td>620</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>2000</td>
<td>400</td>
</tr>
<tr>
<td>Aniline</td>
<td>680</td>
<td>650</td>
</tr>
<tr>
<td>DMA</td>
<td>1640</td>
<td>160</td>
</tr>
</tbody>
</table>

1. Mobile phase: 20% CH₃CN and 80% H₂O with 0.1 M NH₄Ac.
2. N was calculated according to the references 9-11.
**Figure 6.22** HETP vs. mobile phase velocity on RVC base polypyrrole packings

Column: PP/Cl/RVC in a 3.9 mm I.D. x 150 mm stainless steel column (column 1);

Eluent: 0.05 M NaAc solution adjusted pH with HAc at 6.9;

Samples: a. NO$_2^-$ and b. NO$_3^-$. 
The reason for this low efficiency was clearly due to the nature of the packing used. Large size (up to 45 μm) and wide size distribution (from 1 μm to 45 μm) as well as the very irregular shape of the particle made it impossible to obtain a high column efficiency.

6.3.6.2 Column reproducibility of retention
The reproducibility of retention on both PP/Cl/RVC and PP/DS/RVC for various test compounds was investigated (Table 6.11). The capacity factors agree within a 50% deviation. This indicated that the column preparation procedures were not very reproducible.

6.3.6.3 Separation examples
Although the column efficiency was not very high, separation of some compounds on RVC based polypyrrole columns with polypyrrole stationary phases was still possible. Nonpolar compounds, benzene and toluene, polar compounds, DMP and DEP, and proton acceptors, aniline and N,N-dimethylaniline were separated as the examples (Figure 6.23). Anion exchange separations of simple anions such as nitrate, nitrite and trichloroacetate, as well as m- and p-toluic acid were achieved on polypyrrole stationary phases (Figure 6.24).
Table 6.11 Reproducibility of capacity factors on polypyrrole columns

<table>
<thead>
<tr>
<th>Compounds</th>
<th>PP/Cl/RVC</th>
<th></th>
<th></th>
<th>PP/DS/RVC</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k’ on</td>
<td>k’ on</td>
<td>deviation (%)</td>
<td>k’ on</td>
<td>k’ on</td>
<td>deviation (%)</td>
</tr>
<tr>
<td></td>
<td>Column 5</td>
<td>Column 7</td>
<td></td>
<td>Column 6</td>
<td>Column 8</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>0.78</td>
<td>0.55</td>
<td>17</td>
<td>1.20</td>
<td>1.00</td>
<td>9</td>
</tr>
<tr>
<td>Toluene</td>
<td>1.69</td>
<td>1.05</td>
<td>23</td>
<td>3.28</td>
<td>2.27</td>
<td>18</td>
</tr>
<tr>
<td>DMP</td>
<td>1.17</td>
<td>0.71</td>
<td>24</td>
<td>2.84</td>
<td>2.17</td>
<td>13</td>
</tr>
<tr>
<td>DEP</td>
<td>1.67</td>
<td>0.83</td>
<td>34</td>
<td>4.22</td>
<td>3.72</td>
<td>6</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.65</td>
<td>0.49</td>
<td>14</td>
<td>1.11</td>
<td>0.94</td>
<td>8</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>0.65</td>
<td>0.56</td>
<td>7</td>
<td>1.35</td>
<td>4.40</td>
<td>50</td>
</tr>
<tr>
<td>Aniline</td>
<td>0.59</td>
<td>0.49</td>
<td>9</td>
<td>1.02</td>
<td>0.88</td>
<td>7</td>
</tr>
<tr>
<td>N,N-DMA</td>
<td>2.69</td>
<td>1.82</td>
<td>19</td>
<td>5.89</td>
<td>4.33</td>
<td>15</td>
</tr>
</tbody>
</table>

Mobile phase: 20% CH$_3$CN and 80% H$_2$O with 0.1 M NH$_4$Ac.

The separation of caffeine and theophylline with the application of a potential was achieved. Despite the low column efficiency obtained it was found (Figure 6.25) that the application of an anodic potential can affect a separation using solvent conditions under which such separation is not normally achieved.
Figure 6.23  Reversed-phase chromatographic separations on RVC based polypyrrole stationary phases

a. samples: 1. benzene and 2. toluene  
column: PP/DS/RVC (column 7);  
Eluent: 80% H$_2$O and 20% CH$_3$CN with 0.1 M at 1.0 ml/min;  
Detector: UV at 254 nm.  
b. samples: 1. DMP and 2. DEP;  
Column: PP/DS/RVC (column 2);  
Eluent: 55% H$_2$O and 45% MeOH at 1.0 ml/min.;  
Detector: UV at 275 nm.  
c. samples: 1. aniline and 2. DMA;  
The column, eluent and detector are the same as in (a).
Figure 6.24  Anion exchange chromatographic separations on RVC based

a. Samples: 1. $1.5 \times 10^{-3}$ M dichloroacetate, 2. $10^{-3}$ M NO$_2^-$ and 3. $10^{-3}$ M NO$_3^-$;
Column: PP/Cl/RVC (column 1);
Eluent: 0.05 M NaAc at 0.5 ml/min.;
Detector: UV at 215 nm.

b. Samples: 1. m-toluic acid and 2. p-toluic acid;
Column: PP/DS/RVC (column 2);
Eluent: 50% CH$_3$CN and 50% 0.34 M acetate buffer (pH 4.5) at 1.0 ml/min;
Detector: UV at 226 nm.
Figure 6.25  *Separation of caffeine and theophylline with ECLC*

Column: PP/DS/RVC (column 7);
Eluent: 8% CH$_3$CN and 92% 0.02 M acetate buffer (pH = 4.5) at 1.0 ml/min;
Sample: 1. 50 ppm theophylline and 2. 50 ppm caffeine;
Chromatogram: A. without $E_{app}$, B. $E_{app}$ = -0.35 V vs Ag/AgCl.
6.4 Conclusion

RVC particles were coated electrochemically with polypyrrole. Surface characterization of the stationary phase using SEM confirmed the presence of this coating. Columns were prepared using two polypyrrole stationary phases, PP/Cl and PP/DS. A selection of test compounds was considered to characterize these columns. The test compounds were designed so that both reversed-phase chromatographic interactions could be characterized. Reversed-phase chromatographic interactions were found on polypyrrole based stationary phases. The polarity, proton acceptor capability and electron acceptor capability increased when polypyrrole was coated on to RVC. Retention, due to reversed-phase chromatographic interactions, was stable after passing 8 liters of the mobile phase through the columns.

Anion exchange interactions have also been determined on the polypyrrole stationary phases. The anion exchange process was not the same as a conventional anion exchange resin since there was a different retention preference observed on these new stationary phases. However, the effects of pH and ionic strength in the mobile phase were as expected for ion exchange resins. The anion exchange properties of polypyrrole based stationary phases were not constant after passing 4 liters of the mobile phase through the columns.

Electrochemical behavior of columns designed for ECLC exhibited similar behavior to conventional electrochemical cells. However, prolonged charge and discharge processes due to the increase of
resistance and capacitance, particularly on the polypyrrole columns, were observed. The residual current obtained on these packings with the application of potential was attributed to the reduction of dissolved oxygen in the eluent.

The anion exchange properties of polypyrrole were shown to be affected at more positive or negative potentials. The electrical potential also had a slight influence on the electron acceptor capability, but no significant effects on hydrophobicity, polarity and proton acceptor capability were found. The effect of applied potential on the packing was found to be reversible, i.e. the chromatographic interaction could return to the original level after withdrawal of the applied potential.

ECLC columns developed in this work have proven to exhibit improved performance compared with previous studies (97, 98) However, the columns still had lower efficiency than commercial columns. Some separations have been achieved with and without the aid of electrochemical control. The reproducibility of the column performance was shown to be inadequate at present. Improvement of the column preparation procedure is still necessary in order to achieve high reproducibility of stationary phase performance.
Chapter 7
Chromatography on Silica-Based Polypyrrole Stationary Phases
7.1 Introduction

Silica is the most popular stationary phase support used in liquid chromatography at present (21). It is readily available in appropriate particle sizes, pore sizes, pore volumes and surface areas for HPLC. Furthermore, it can be modified to provide stationary phases with different interaction modes. These attributes make silica a suitable support for polypyrrole stationary phases. As reviewed in Chapter 1, silica still suffers from some shortcomings. These shortcomings may be partly overcome by modification of silica particles.

Coating with polypyrrole will modify the selectivity of silica. The polymer coating may also protect silica from chemical attack. Silica coated with polypyrrole may also be sufficiently conductive to use as a silica based material in ECLC.

In this chapter, bare and C18 bonded silica have been coated with polypyrrole. Chloride and dodecyl sulfate were chosen as counterions in order to compare results with the different substrates used in Chapter 6. Surface characterization of the coated silica was carried out using SEM, EDX, elemental analysis and surface area analysis. Using the self-compressed column system which was developed in Chapter 5, both reversed-phase and anion exchange chromatography were characterized using the same test
compounds as in Chapter 6. The column performance was investigated and separations were carried out using the polypyrrole stationary phase.

7.2 Experimental

7.2.1 Reagents and materials
Silica gels (10 μm Spherisorb, PhaseSep) and C18 bonded silica gels (10 μm ODS-Hypersil, Shandon) were used as substrates for coating polypyrrole. Pyrrole was distilled before use. NaCl (AR) and SDS (Sigma) were used as counterions and a 1.5 M FeCl3 (LR) solution was used as a chemical polymerization agent. Test compounds were as in Chapter 6.

Acetonitrile (AR) and purified water (Millipore) were used as solvents, and ammonium acetate (AR) and the phosphate buffer (mixture of Na2HPO4 and NaH2PO4, AR) were added to the mobile phase as required.

7.2.2 Instrumental
SEM and EDX were carried out using instrumentation described in Chapters 2. Elemental analysis results were obtained from the Analytical Laboratory, Australian National University. Surface areas of stationary phases were measured on Area Metre II (Strohlein Instruments). The HPLC system was the same as that used in Chapter 6.
7.2.3 Columns

7.2.3.1 Electrochemical polymerization

Electrochemical coating of polypyrrole chloride on C18 bonded silica was carried out using the slurry cell as described in Chapter 5. The rotating platinum electrode was pretreated with H2SO4−K2Cr2O7, rinsed with water, dried at 150°C and weighed. Polymerization was carried out in 0.1 M pyrrole and 0.1 M Cl− or DS− in 50%CH3CN and 50% H2O. Packings were prepared as shown in Table 7.1. After polymerization, the rotating electrode was rinsed with water and dried at 62°C in vacuum to remove moisture and weighed again in order to determine the weight of the polymer on the electrode.

7.3.3.2 Chemical polymerization

The following procedure was employed for chemical polymerization,

1) 1 g of silica or C18 bonded silica was placed in a 100 ml beaker;

2) 1 ml pyrrole was added in the beaker, and stirred for 10 minutes with a glass rod;

3) 100 ml of distilled water was placed in the beaker, and 0.01 mole counterions (Cl− or DS−) were added as required. The mixture was stirred with a magnetic stirrer to form a suspension;

4) 1 ml of 1.5 M FeCl3 solution was added and 100 ml of 0.01 M Cl− in distilled water was placed in the beaker. The solution was stirred vigorously for 30 minutes;

5) The coated particles were collected using a Buchner filter and washed with 200 ml of water.

Packings prepared according to this procedure are summarized in Table 7.2.
Table 7.1  Electrochemically prepared polypyrrole on C_{18} bonded silica

<table>
<thead>
<tr>
<th>Column</th>
<th>PP/Cl/C_{18}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of C_{18} packings(g)</td>
<td>0.78</td>
</tr>
<tr>
<td>Polymerization current (mA)</td>
<td>0.25</td>
</tr>
<tr>
<td>Total charge (C)</td>
<td>27.0</td>
</tr>
<tr>
<td>Weight of polymer on packings¹ (g)</td>
<td>1.2x10^{-3}</td>
</tr>
<tr>
<td>Polymer loading (% W/W)</td>
<td>0.15%</td>
</tr>
</tbody>
</table>

¹ The total weight of polypyrrole was calculated according to equation 2.4 assuming x=4 and current efficiency=100%. The weight of polymer on the packings was obtained by subtracting the weight of the polymer on the rotating electrode from the total weight.

7.2.3.3 Preparation of columns
Self-compressed columns were used for all chromatographic characterizations. The packing procedure was described in Chapter 5. All columns were washed by methanol for 8 hours before use. An ECLC column was prepared according to the procedure described in Chapter 5 with 0.80 g of PP/Cl/Silica.

7.2.4 Procedure
SEM and EDX analysis of packings was carried out using the same procedure as described in Chapters 2 and 3. Specific surface area analysis and elemental analysis were carried out using 0.12-0.16 g of each packing. All the samples were dried at 62°C in vacuum for 3 hours before analysis.
Table 7.2  Chemically prepared polypyrrole columns on silica based supports

<table>
<thead>
<tr>
<th>Packing</th>
<th>Substrates</th>
<th>PP/Cl/C18</th>
<th>PP/Cl/DS/C18</th>
<th>Silica</th>
<th>PP/Cl/Silica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymers</td>
<td>C18</td>
<td>C18 bonded silica</td>
<td>C18 bonded silica</td>
<td>silica</td>
<td>silica</td>
</tr>
<tr>
<td>Counterions</td>
<td>Cl-</td>
<td>Cl- + DS-</td>
<td></td>
<td>Cl-</td>
<td></td>
</tr>
<tr>
<td>Packing size</td>
<td>10 µm</td>
<td>10 µm</td>
<td>10 µm</td>
<td>10 µm</td>
<td>10 µm</td>
</tr>
<tr>
<td>Column type</td>
<td>SCC</td>
<td>SCC</td>
<td>SCC</td>
<td>SCC</td>
<td>SCC</td>
</tr>
<tr>
<td>Column size</td>
<td>3.2 mm I.D. x 64 mm</td>
<td>3.2 mm I.D. x 64 mm</td>
<td>1.6 mm I.D. x 64 mm</td>
<td>1.6 mm I.D. x 64 mm</td>
<td></td>
</tr>
</tbody>
</table>

HPLC characterization conditions are summarized in Table 7.3. The HETP vs. mobile phase velocity test was performed on the PP/Cl/C18 column using benzene as a sample in a 65% MeOH and 35% H2O eluent. The effects of pH and ionic strength on chromatographic interaction were determined on the PP/Cl/Silica column in 50% MeOH and 50% phosphate buffer in the pH range of 5.8-7.8.
Table 7.3 HPLC characterization conditions

<table>
<thead>
<tr>
<th>Column</th>
<th>C\textsubscript{18} bonded silica based</th>
<th>Bare silica based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>65% MeOH + 35% H\textsubscript{2}O</td>
<td>50% H\textsubscript{2}O with and without 0.1 M NH\textsubscript{4}Ac</td>
</tr>
<tr>
<td>Flow rate (ml/min)</td>
<td>0.50</td>
<td>0.30</td>
</tr>
<tr>
<td>Test compounds</td>
<td>Benzene 50 ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toluene 50 ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dimethylphthalate 10 ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diethylphthalate 10 ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenol 30 ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Benzoic acid 20 ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aniline 20 ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N,N- Dimethylaniline 20 ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Theophylline 10 ppm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caffeine 10 ppm</td>
<td></td>
</tr>
</tbody>
</table>

ECLC performance was tested on the PP/Cl/Silica column using the same test compounds as in Chapter 6 in a 50% MeOH and 50% H\textsubscript{2}O eluent with 0.1 M NH\textsubscript{4}Ac.

Stability of PP/Cl/C\textsubscript{18} in acidic media was compared to that of C\textsubscript{18} bonded silica by passing HNO\textsubscript{3} at different pH values through the column for 10 minutes. Stability in the basic media was tested by soaking PP/Cl/Silica and silica in 0.5 M NaOH, washing packings
with water and acetone before drying at 62°C in vacuum and recording daily weight loss.

7.3 Results and Discussion

7.3.1 Surface characterization

7.3.1.1 Elemental analysis of packings

All elemental analysis results are summarized in Table 7.4. It was found that on C18 bonded silica only a small amount of polypyrrole was coated. From Table 7.4, it can be seen that using the chemical polymerization method 3.9% of PP/Cl and 4.8% of PP/Cl/DS were coated on C18 bonded silica, and 7.4% of PP/Cl were coated on bare silica.

Table 7.4 Elemental analysis of silica based packings

<table>
<thead>
<tr>
<th>packings</th>
<th>C%</th>
<th>H%</th>
<th>N%</th>
<th>C%</th>
<th>S%</th>
<th>Polymer%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18</td>
<td>9.45</td>
<td>1.80</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PP/Cl/C18</td>
<td>11.82</td>
<td>1.99</td>
<td>0.69</td>
<td>0.64</td>
<td>0.0</td>
<td>3.9</td>
</tr>
<tr>
<td>PP/Cl/DS/C18</td>
<td>12.59</td>
<td>2.10</td>
<td>0.59</td>
<td>0.46</td>
<td>0.31</td>
<td>4.8</td>
</tr>
<tr>
<td>silica</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PP/Cl/silica</td>
<td>4.91</td>
<td>0.33</td>
<td>1.16</td>
<td>0.99</td>
<td>0.0</td>
<td>7.4</td>
</tr>
</tbody>
</table>

1. Polymer% (W/W) was calculated from the weight difference between polypyrrole modified supports and bare supports.

2. Polypyrrole was chemically coated.
7.3.1.2 Surface area analysis of packings

The polymer coating slightly reduced the specific surface area (Table 7.5). As polypyrrole covered the surface of the packing, the surface area of the original packing decreased. However, the changes in surface area were not dramatic, since the average polymer thickness is less than one nanometer, which was almost the same as the width of a pyrrole molecule 0.225 nm (289) i.e. almost a monomolecular layer.

Table 7.5 Surface area analysis of silica based packings

<table>
<thead>
<tr>
<th>Packings</th>
<th>Specific surface area (m²/g)</th>
<th>Estimated¹ polymer thickness (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18</td>
<td>98.5</td>
<td>-</td>
</tr>
<tr>
<td>PP/Cl/C18</td>
<td>96.5</td>
<td>0.26</td>
</tr>
<tr>
<td>PP/Cl/DS/C18</td>
<td>83.4</td>
<td>0.33</td>
</tr>
<tr>
<td>Silica</td>
<td>149</td>
<td>-</td>
</tr>
<tr>
<td>PP/Cl/Silica</td>
<td>137</td>
<td>0.33</td>
</tr>
</tbody>
</table>

1. Assuming polymer density=1.5 g/cm² [reference (188, 217)] polymer thickness = polymer%/((polymer density x specific surface area).

7.3.1.3 SEM of packings

An example of SEMs of silica based packings is shown in Figure 7.1. The other packings have similar SEMs. Although the characteristic black color of the polypyrrole coated particles was visible to the naked eye, no significant difference was observed using SEM. This
indicated that the polypyrrole coating was very thin which further confirms the results from elemental and surface area analysis.

7.3.1.4 **EDX spectrum of packings**

Counterions in polypyrrole coated stationary phases were detected using EDX analysis. Not only counterions but also the silica background signals appeared on all spectra (Figure 7.2). The spectra verified that EDX can be used as a means of characterization to determine elemental composition of the packing. When polymers were chemically synthesized using FeCl₃, Cl⁻ as well as FeCl₄⁻ was incorporated as a counterion which was confirmed by the existence of Fe signals in spectra as well as by the other workers (151). The addition of DS⁻ in the monomer solution resulted in incorporation of DS⁻ as demonstrated in Chapter 2.

7.3.2 **Reversed-phase chromatographic characterization**

7.3.2.1 **Polypyrrole modified C₁₈ stationary phases**

Initially, chromatographic characterization was carried out on an electrochemically coated stationary phase (Table 7.6). The results obtained indicated that there was little difference between the C₁₈ column and the polypyrrole modified C₁₈ column. This was probably due to the low polymer loadings obtained (Table 7.1).
**Figure 7.1** Scanning electron micrograph of silica based polypyrrole packings

Sample: PP/Cl/DS on 10 μm ODS-Hypersil.
Figure 7.2  EDX spectra of silica based polypyrrole packings

Spectrum on:  a. PP/Cl/C18, b. PP/Cl/DS/C18 and c. C18.;


EDX condition: High voltage=20 KV;accumulation time =200 seconds.
The estimated average polymer thickness was approximately 0.01 nm, which is about 1/10 of the width of a pyrrole molecule (289). This indicated that the polymer could not cover the whole C₁₈ particle surface. Therefore interactions on C₁₈ background were much more significant than those on the polymer.

### Table 7.6  Capacity factors of some compounds on electrochemically prepared polypyrrole stationary phases

<table>
<thead>
<tr>
<th>Compounds</th>
<th>k' on C₁₈</th>
<th>k' on PP/Cl/C₁₈</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethylphthalate</td>
<td>0.89</td>
<td>0.89</td>
</tr>
<tr>
<td>Diethylphthalate</td>
<td>2.13</td>
<td>2.01</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.60</td>
<td>0.63</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>0.12</td>
<td>0.10</td>
</tr>
<tr>
<td>Aniline</td>
<td>0.84</td>
<td>0.84</td>
</tr>
<tr>
<td>Theophylline</td>
<td>0.26</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Mobile phase: 65% MeOH and 35% H₂O, 0.5 ml/min.

By using a chemical polymerization process, a higher polymer loading was found with the elemental analysis than that by using the electrochemical polymerization process. Chromatographic interactions were characterized using some test compounds and the results are presented in Table 7.7.

Generally, capacity factors on the polypyrrole modified columns for all the compounds, except in a few circumstances, are very similar to those on the C₁₈ column. Two possible reasons for this are [1] that
the polypyrrole coating is porous and molecules can pass through these pores to interact with the C18 coating; and [2] that the C18 surface is not fully covered. Therefore, polypyrrole coating can not completely alter the original properties of C18 stationary phase but it does contribute to the retention properties.

Table 7.7  Chromatographic interactions on polypyrrole coated C18 bonded silica

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>C18</th>
<th>PP/Cl/C18</th>
<th>PP/Cl/DS/C18</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k'</td>
<td>k'/k'</td>
<td>k'</td>
</tr>
<tr>
<td>Benzene</td>
<td>2.02</td>
<td>1.00</td>
<td>1.86</td>
</tr>
<tr>
<td>Toluene</td>
<td>3.66</td>
<td>1.81</td>
<td>3.33</td>
</tr>
<tr>
<td>Dimethylphthalate</td>
<td>0.89</td>
<td>0.44</td>
<td>1.25</td>
</tr>
<tr>
<td>Diethylphthalate</td>
<td>2.13</td>
<td>1.05</td>
<td>2.71</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.60</td>
<td>0.30</td>
<td>0.70</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>0.12</td>
<td>0.06</td>
<td>∞</td>
</tr>
<tr>
<td>Aniline</td>
<td>0.84</td>
<td>0.42</td>
<td>0.63</td>
</tr>
<tr>
<td>N,N-dimethyl aniline</td>
<td>4.29</td>
<td>2.12</td>
<td>3.19</td>
</tr>
<tr>
<td>Theophylline</td>
<td>0.26</td>
<td>0.13</td>
<td>6.23</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.31</td>
<td>0.15</td>
<td>6.04</td>
</tr>
</tbody>
</table>

Mobile phase: 65% MeOH and 35% H2O, 0.5 ml/min

Capacity factors of benzene and toluene decreased marginally on both polymer stationary phases, but the relative retention of toluene was almost unchanged. This indicated that polypyrrole was
less hydrophobic than the C_{18} packing but it did not change its selectivity for hydrophobic groups.

Capacity factors and the relative retention of DMP and DEP were slightly increased on PP/Cl/C_{18} but remained unchanged on PP/Cl/DS/C_{18}. This indicated that the PP/Cl coating contributed polarity to the stationary phase. However, incorporation of DS\textsuperscript{-} neutralizes this effect. This is probably due to the contribution of the nonpolar surfactant.

Phenol (a proton donor) has low retention on all columns. Only a slight increase of retention and relative retention was observed with polypyrrole packings. The proton acceptor capability of C_{18} and both polypyrrole stationary phases was found to be negligible.

Electron donors, aniline and N,N-dimethylaniline, exhibited high retention and relative retention on the C_{18} column and the peaks tailed, which indicated that an adsorption process was involved. The coating of PP/Cl onto C_{18} reduced this effect but did not eliminate it. This was probably due to the fact that the C_{18} was only partially covered. However, PP/Cl/DS on C_{18} increased the electron acceptor capability of the stationary phase which may have resulted from the polar end of the surfactant. This effect was reported previously (281).

The addition of salt to the eluent was not carried out using C_{18}-based polypyrrole stationary phase because the background effect of the C_{18} stationary phase was very strong.
Contributions of PP/Cl to polarity and contributions of PP/DS to electron acceptor capability are the most notable effects of coating the C_{18} phases. In order to minimize the background effect of C_{18}, investigation of reversed-phase chromatography of polypyrrole may be carried out on other supports such as bare silica gels.

7.3.2.2 Polypyrrole modified silica stationary phases

Using the chemical polymerization method, polypyrrole chloride was coated onto silica packing material. Chromatographic characterization of PP/Cl/Silica was conducted using the same test compounds as used previously (Table 7.8). The results are summarized in Table 7.8.

With a silica stationary phase, retention was observed for all of the test compounds investigated, with the exception of aniline and N,N-dimethylaniline. These compounds are known to interact with the silanol groups on the silica surface (9-11, 21). Compared with C_{18} based supports, bare silica based supports did not contribute significantly to retention.

Retention of all compounds on polypyrrole modified silica was observed, which indicated that a reversed-phase chromatographic interaction was generated by the PP/Cl.

For nonpolar compounds, benzene and toluene, the capacity factors observed were not very high, which indicated that PP/Cl contributed only minor hydrophobic properties.
Table 7.8  Chromatographic interactions on polypyrrole coated silica

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>In 50% MeOH and 50% H₂O</th>
<th>In 50 MeOH and 50% H₂O with 0.1 M NH₄Ac</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k' silica</td>
<td>k' PP/Cl/Silica</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.1</td>
<td>0.41</td>
</tr>
<tr>
<td>Toluene</td>
<td>0</td>
<td>0.67</td>
</tr>
<tr>
<td>DMP</td>
<td>0</td>
<td>0.83</td>
</tr>
<tr>
<td>DEP</td>
<td>0</td>
<td>0.85</td>
</tr>
<tr>
<td>Phenol</td>
<td>0</td>
<td>0.38</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>0</td>
<td>∞</td>
</tr>
<tr>
<td>Aniline</td>
<td>0.12</td>
<td>3.32</td>
</tr>
<tr>
<td>DMA</td>
<td>0.41</td>
<td>9.13</td>
</tr>
</tbody>
</table>

Column: PP/Cl/Silica 1.6 mm I.D x 64 mm

1. k' < 0.1.
2. k' was unstable.

However, the capacity factors and relative retentions were higher for polar compounds, dimethylphthalate and diethylphthalate. As expected, polypyrrole chloride produced a certain degree of polarity on the silica surface, probably from the polar part of the polymer matrix. The low retention values obtained for the proton donor confirmed that PP/Cl had very low proton acceptor capability. High electron acceptor capability was found on PP/Cl/Silica since the high retention and relative retention of aniline and N,N-dimethylaniline.
were obtained. This again verified that polypyrrole has a certain degree of electron acceptor capability. Since electrons are lost during polymerization and positive sites formed on the polymer matrix, the electron donors can access these sites.

The addition of salt in the mobile phase reduced capacity factors for benzene, toluene, DMP, DEP and phenol, but it increased their relative retentions. This salt effect was unclear. Reduction of capacity factors as well as relative retentions of aniline and DMA was found due to addition of the salt. As expected, this is due to solvation of the electron accepting sites on the polymer as indicated previously.

The reversed-phase chromatographic process was further confirmed by the effect of an organic modifier in the aqueous mobile phase. Using benzene and dimethylphthalate as test compounds, it was verified that this reversed-phase chromatography mode is similar to that on conventional reversed-phase stationary phases, i.e. the logarithm of capacity factors is approximately proportional to the concentration of methanol concentration (Figure 7.3).
Figure 7.3 Capacity factors of benzene, toluene, DMP and DEP vs. the methanol concentration in the mobile phase

Column: PP/Cl/Silica in a 1.6 mm I.D. x 64 mm SCC;
Detector: UV at 254 nm;
Sample: a. 50 ppm benzene, b. 50 ppm toluene c. 10 ppm DMP and d. 10 ppm DEP
Compared with C18 based polypyrrole stationary phases, the bare silica based stationary phase provided a blank background. Partition due to hydrophobicity, polarity, proton acceptor capability and electron acceptor capability was found on PP/Cl/Silica. The results have further confirmed the findings on RVC based polypyrrole stationary phases: reversed-phase chromatography on polypyrrole stationary phases, particularly due to polarity and electron acceptor capability, is possible.

7.3.3 Anion exchange chromatographic characterization
Anion exchange interactions were observed using PP/Cl/C18, PP/Cl/DS/C18 and PP/Cl/Silica packing (Table 7.7 and 7.8). Benzoic acid was permanently retained on these columns unless a salt was introduced in the eluent.

The effect of pH on ion exchange was investigated. It was found that the capacity factor of benzoic acid increased with a decrease of the pH of the phosphate buffer with the same solvent strength (Figure 7.4). Such a pH effect had little influence on the retention of aniline and N,N-dimethylaniline. This corresponded with the results obtained on RVC based polypyrrole stationary phases and further confirmed the pH effect on the anion exchange capability of polypyrrole stationary phases.
Figure 7.4  Capacity factors vs. pH of the mobile phase

Column: PP/Cl/Silica in a 1.6 mm I.D. x 64 mm SCC;
Detector: UV at 254 nm;
Sample: a. 20 ppm benzoic acid, b. 20 ppm aniline and c. 20 ppm DMA.
The influence of ionic strength on ion exchange was also determined. The capacity factor of benzoic acid decreased with an increase of ionic strength of the buffer in the eluent but the retention of aniline and N,N-dimethylaniline did not alter significantly (Figure 7.5). This is typical behavior for ion exchange processes. The results corresponded with those obtained on RVC based polypyrrole stationary phases.

7.3.4 ECLC on polypyrrole modified silica

7.3.4.1 Electrochemical characterization

Previous workers converted various nonconductive substrates into conductors by coating polypyrrole chemically (227, 228). It is feasible that silica particles could be made conductive after coating with polypyrrole chloride. The ECLC column employed has been described in Chapter 5.
Figure 7.5 Capacity factors vs. ion strength of the mobile phase

Column: PP/Cl/Silica in a 1.6 mm I.D. x 64 mm SCC;

Detector: UV at 254 nm;

Sample: a. 20 ppm benzoic acid, b. 20 ppm aniline and c. 20 ppm DMA.
Electrochemical characteristics of the ECLC column packed with PP/Cl/Silica are summarized in Figure 7.6. Some differences between RVC based and silica based packings were observed. With silica packings the current flowing in the column and the potential between the working and counter electrodes did not change until the applied potential was -0.60 V. At this point a sudden change of the current and potential occurred. Decomposition of aniline and N,N-dimethylaniline occurred at more negative potentials (E_{app} < -0.6 V) than on RVC based columns (E_{app} < -0.2 V). These effects can be attributed to the more resistive packing bed obtained when using silica. Unlike RVC, silica is an insulator and therefore polypyrrole modified silica is not as conductive as polypyrrole modified RVC.

7.3.4.2 ECLC characterization

The ECLC behavior was characterized by using the same set of test compounds as used previously (Chapter 6). No significant EC influences on the chromatographic interactions were found (Figure 7.7). These results as well as the unique electrochemical behavior found above indicated that the PP/Cl/Silica was not suitable for use in ECLC. The potential drop across the packing bed was very high due to the resistance of the packing. Consequently, only a small part of the packing around the central tantalum wire (see Figure 6.3) was affected by the applied potential.
Figure 7.6  Electrochemical character of an ECLC column with silica based polypyrrole packings

Column: 10 μm PP/Cl/Silica in a 4 mm I.D. x 100 mm ECLC column;
Detector: UV at 254 nm;
Eluent: 20% CH₃CN and 80% H₂O with 0.1 M NH₄Ac at 0.5 ml/min.
Figure 7.7 Electrochemical effect on chromatographic interactions on silica based polypyrrole packings

Column: 10 µm PP/Cl/Silica in a 4 mm I.D. x 100 mm ECLC column;

Detector: UV at 254 nm;

Eluent: 20% CH₃CN and 80% H₂O with 0.1 M NH₄Ac at 0.5 ml/min.

Relative retention: a. benzoic acid, b. DMP, c. toluene, d. phenol and e. aniline.
7.3.5 Chromatographic performance

Although the reproducibility of the stationary phases has not been studied in this section as preparation of polypyrrole coated silica stationary phases is still being optimized, stability of these silica-based stationary phases was examined. The stability and separation performance was also investigated.

7.3.5.1 Column efficiency

Compared with RVC supports, a much improved performance was obtained with silica based supports. It was found that column efficiency on C18 based columns was much higher than bare silica based columns (Table 7.9). Low efficiency on bare silica based columns was expected since there was a high polymer loading on bare silica and as a result a high π-π interaction and a slow mass transfer process, as described by other workers (56, 58). Consequently, a low polymer loading and modified surface is preferable.

Compared with C18 stationary phases, the polypyrrole coated C18 phase had the same column efficiency. This indicated that a high efficiency polypyrrole stationary phase could be produced when using a highly efficient support.

The column efficiency of PP/Cl/C18 differed little from that on C18. A comparison of HETP vs. mobile phase velocity curves (Figure 7.8) shows that a thin polypyrrole coating would not reduce the column efficiency notably.
Table 7.9  Efficiency of silica based polypyrrole stationary phases

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>( N^1 ) (plates /m) on PP/Cl/Silica(^2)</th>
<th>( N ) (plates /m) on PP/Cl/C(_{18})(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>1150</td>
<td>6790</td>
</tr>
<tr>
<td>Toluene</td>
<td>960</td>
<td>7950</td>
</tr>
<tr>
<td>DMP</td>
<td>790</td>
<td>4870</td>
</tr>
<tr>
<td>DEP</td>
<td>710</td>
<td>5630</td>
</tr>
<tr>
<td>Phenol</td>
<td>1330</td>
<td>4670</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>410</td>
<td>--</td>
</tr>
<tr>
<td>Aniline</td>
<td>1150</td>
<td>4030</td>
</tr>
<tr>
<td>N,N-Dimethylaniline</td>
<td>750</td>
<td>6080</td>
</tr>
</tbody>
</table>

1. Measuring precision approximately 30%.
2. Self-compressed column (1.6 mm I.D x 64 mm) using 50% MeOH + 50% H\(_2\)O with 0.1 M NH\(_4\)Ac as the eluent.
3. Self-compressed column (3.2 mm I.D x 64 mm) using 65% MeOH + 35% H\(_2\)O as the eluent.
Figure 7.8  

**HETP vs. mobile phase velocity on silica based packings**

Column A: PP/Cl/C18 in a 3.2 mm I.D. x 64 mm SCC;

Column B: C18 in a 3.2 mm I.D. x 64 mm SCC;

Eluent: 65% MeOH and 35% H2O;

Sample: 50 ppm benzene;

Detector: UV at 254 nm.
7.3.5.2 Stability of polypyrrole modified stationary phases at extreme pH

As described previously, the bonding between silica and a modifier breaks down due to acidic attack, and the silica dissolves in basic media. In order to characterize the role of polypyrrole coating in protecting supports, PP/Cl/C\textsubscript{18} and PP/Cl/Silica were used to test acidic and basic attack respectively. Compared with the C\textsubscript{18} stationary phase, polypyrrole modified C\textsubscript{18} stationary phase resisted acidic attack better, but it still could not fully protect the stationary phase from acidic attack (Figure 7.9). A capacity factor decrease was found on both stationary phases when the stationary phases were treated at pH lower than 2. Modification of polypyrrole did not fully protect the stationary phase since the polypyrrole layer was very thin and porous.

Polypyrrole chloride modified silica, as well as bare silica, was treated by 0.5 M NaOH to determine the basic stability of the polypyrrole modified stationary phase. It was found that both PP/Cl/Silica and bare silica slowly dissolved in 0.5 M NaOH (Figure 7.10). This indicated that polypyrrole could not protect the silica from basic attack under the present conditions.
Figure 7.9  Stability of silica based packings at acidic media

Column A: PP/Cl/C18 in a 3.2 mm I.D. x 64 mm SCC;
Column B: C18 in a 3.2 mm I.D. x 64 mm SCC;
Eluent: 65% MeOH and 35% H2O with a 0.01 M NaH2PO4 and 0.01 M Na2HPO4 buffer (pH = 6.9) at 0.5 ml/min.
Figure 7.10 Stability of silica based packings at basic media

Sample: a. PP/Cl/Silica and b. Silica treated in 0.5 M NaOH solution;
7.3.5.3 *Separation examples*

Chromatographic separations were achieved on polypyrrole modified stationary phases. Figure 7.11 shows a separation of mixtures of test compounds. The polypyrrole modified C₁₈ column displayed different selectivity to the C₁₈ column (Figure 5.12). For example, although benzoic acid appeared first on the C₁₈ column, it did not come out on the PP/Cl/C₁₈ column; phenol and aniline could be separated on the C₁₈ column but not on the PP/Cl/C₁₈ column; benzene and diethylphthalate, and aniline and dimethylphthalate could be separated on the PP/Cl/C₁₈ column but not on the C₁₈ columns.

Basic drugs, caffeine and theophylline, were separated on the PP/Cl/C₁₈ column (Figure 7.11) but they were eluted almost in a void volume under the same conditions on the C₁₈ columns. This further shows the special selectivity of polypyrrole.
Figure 7.11 Separations on silica based polypyrrole stationary phases

Column: PP/Cl/C₁₈ in a 3.2 mm I.D. x 64 mm SCC;

Eluent: 65% MeOH and 35% H₂O at 0.3 ml/min.;

Detector: UV at 254 nm;

Responses due to: a. (1). 5 ppm theophylline and (2). 5 ppm caffeine; b. (1). 15 ppm phenol, (2). 5 ppm aniline, (3). 5 ppm DMP (4). 50 ppm benzene, (5). 5 ppm DEP, (6). 50 ppm toluene and (7). 5 ppm DMA.
7.4 Conclusion

Modification of silica based supports by polypyrrole was carried out either chemically or electrochemically. However, the electrochemical method only produced a low loading of polypyrrole. Using chemical polymerization incorporation of counterions other than those from the polymerization reagent was possible. This was verified by elemental analysis and EDX. PP/Cl/C_{18}, PP/DS/C_{18} and PP/Cl/Silica were produced for chromatographic characterization.

Elemental analysis indicated that the polymer loading was low on the C_{18} based supports but slightly higher than that on the silica packings. Consequently, poor coverage of the supports resulted. Thus, an improved procedure to coat polypyrrole on porous supports is required. The surface area of the original supports was reduced due to the polypyrrole coating. These surface changes, however, were not significant enough to be distinguished by SEM.

No reversed-phase chromatographic interactions due to polypyrrole were found using the electrochemically prepared PP/Cl/C_{18}. Increase in both polarity and electron acceptor capability were found on the C_{18} based columns prepared chemically. Similar contributions were observed on PP/Cl/Silica. Anion exchange behavior was confirmed on all polypyrrole modified stationary phases. The salt and pH effects on the anion exchange process were found with RVC base polypyrrole stationary phases.
Application of a potential produced no effects on chromatographic interactions.
The column efficiencies were dependent on support materials for polypyrrole stationary phases. The column efficiency was high on C\textsubscript{18} based supports but very low on bare silica based supports. Stability of stationary phases at extreme pH was improved by coating polypyrrole on the silica based supports.
Chapter 8
Development of a Polypyrrole Dispersed-Mercury Modified Electrode
8.1 Introduction

Mercury electrodes have been used extensively in voltammetry for monitoring reduction processes. They are particularly suited to these purposes due to the high hydrogen over potential which is available (290), and useful for the determination of metal ions. This is because amalgam formation can be employed to preconcentrate the metal ions using voltammetric stripping methods. However, construction of mercury electrodes for HPLC detectors is more complicated than solid electrodes.

In earlier work, a dropping mercury electrode (DME) was used for HPLC detection (135). The DME provides a constantly renewable electrode surface, essentially eliminating the problem of electrode surface contamination. Unfortunately, the disadvantages of the DME are many, for example, complicated cell design, high noise level, current oscillations and the need for some form of damping. Thin mercury film electrodes (TMFE) were introduced for electrochemical detection when it was realized that increased sensitivity could be obtained due to the improved surface area to volume ratio (291) and the improved mechanical stability for use in flow through cells (292, 293). TMFEs also overcome the range of the problems when using DMEs. These thin film mercury electrodes still suffer from some limitations due to their limited mechanical stability and also limited electrochemical selectivity. These properties are
particularly important when the devices are to be used in a flowing solution, such as in flow injection analysis or HPLC.

Conducting polymers have been used to modify electrodes for amperometric detection. In previous work the polymer coatings have been used to alter the sensitivity and selectivity of the electrodes. In particular, the concept of polymer modified mercury film electrodes (294, 295) was introduced, whereby dispersed mercury electrodes were formed by complexing mercury ions onto an appropriately derivatized surface. Dispersed mercury droplets were then formed by reducing the mercury ions electrochemically. These electrodes, therefore, combine the high sensitivity of the thin mercury film electrode with the high selectivity of the chemically modified electrode.

In the course of this work, a novel approach which enables the preparation of polypyrrole dispersed-mercury modified electrodes (PDMME) was investigated. Electropolymerization of inherently conducting polymers such as pyrrole has been described previously. When this process is induced at a mercury film substrate, the mercury is oxidized at potentials necessary to cause polymerization. However, if the potential is switched rapidly to a negative potential to reduce the mercury before it diffuses from the electrode surface, then the mercury will be redeposited. Simultaneously, a layer of conducting polypyrrole is formed on the electrode surface. The procedure is such that a range of counterions may be incorporated during the polymerization process.
The surface of the PDMME has been studied using SEM in order to employ this electrode in amperometric detection. Voltammetric properties, sensitivity, selectivity and chemical and physical stability have been tested using Cu(II), Pb(II) and Cd(II). Voltammetric responses can characterize the electrochemical performance of the electrode as well as the monitoring of the amperometric detector. Selectivity usually is an important criterion for a chemically modified electrode, and it is critical for selective detection in HPLC, while chemical and physical stabilities can indicate the feasibility of using the electrode in HPLC.

The application of the PDMME in amperometric detection following HPLC separation has been tested. The detection of both metal ions [Cu(II), Pb(II) and Cd(II)] and nonmetallic compounds such as chloramines has been investigated. Control of these trace metal ions and chloramines is of importance in environmental analysis.

The presence of chloramines during the chlorination of water containing ammonia and organic amines may give rise to taste and odor problems in drinking water (296). The main chloramine species are monochloramine (NH₂Cl) and dichloramine (NHCl₂). Amperometric titration, stabilized neutral orthotolidine colorimetry and diethyl-p-phenylene diamine (DPD) titration were used to determine chloramines (297). HPLC using a C₁₈ column with photometric detection of chloramines following HPLC separation has been reported (298).
8.2 Experimental

8.2.1 Reagents and standard solutions
All reagents were AR grade purity unless otherwise stated. A 0.01 M mercury nitrate in 1% HNO₃ aqueous solution was used to plate the mercury film on the glassy carbon electrode (GC). Polypyrrole was coated on to the thin mercury film electrode from a 0.10 M pyrrole (which was distilled before use) and 0.1 M NaNO₃, 0.10 M SDS or 0.10 M sodium oxalate aqueous solution. Standard solutions of Cu(II), Pb(II), and Cd(II), were prepared from their nitrate salts. A solution containing 58.5% NaCl, 17.5% L-ascorbic acid and 3.9% NaOH was used as supporting electrolyte. The eluent for metal ion separations was prepared by dissolving 0.050 M oxalic acid and 0.095 M LiOH in deionized water. Standard chloramine solutions were prepared by mixing 0.10 M NH₄NO₃ and 0.10 M NaClO₄ stock solutions as described by previous workers (298). The eluent for chloramine detection was prepared by dissolving 0.010 M NaH₂PO₄ in distilled water and adjusting pH to 6.9 with 0.010 M Na₂HPO₄.

8.2.2 Instrumentation
Preparation of the electrodes and voltammetric analysis was performed using a BAS-100A Electrochemical Analyzer (BAS). Anodic stripping voltammetry was carried out with a PDV-2000 Portable Digital Voltammeter (Chemtronics). Flow through and HPLC experiments were carried out using a Dionex Series 4000i chromatographic system and a Dionex pulsed amperometric detector. A Chemtronics glassy carbon electrode (diameter 3 mm) was employed in the stationary cell tests and a Dionex flow through cell with a glassy carbon (diameter 3 mm) electrode was used in the
flow through experiments. All reference electrodes used were Ag/AgCl. Platinum auxiliary electrodes were employed throughout this work. Scanning electron micrographs were obtained using a Hitachi S-450 scanning electron microscope. HPLC separation of Cu(II) Pb(II) and Cd(II) was performed on a Dionex HPIC-CS5 column. HPLC separation of chloramines was conducted on a Waters 3.9 mm I.D x 150 mm length stainless steel column packed with 5 μm spherical C18 packings.

### 8.2.3 Preparation of the electrodes

Polypyrrole dispersed-mercury modified electrodes (PDMME) were prepared according to the following procedure. A glassy carbon electrode was polished with 3 μm alumina powders on a polishing cloth, and then rinsed with acetone and distilled water. A thin mercury film was produced by applying a potential at -0.50 V vs. Ag/AgCl to the glassy carbon (GC) electrode in a stirred 0.010 M Hg(NO₃)₂ solution for 60 seconds and then scanning the potential at 100 mV/sec to -0.10 V. Polypyrrole was coated on the mercury film by cycling the potential at 1 V/sec between -0.40 and 0.80 V in various monomer solutions as described in 8.2.1. The prepared electrodes were rinsed with distilled water. Using this procedure polypyrrole nitrate, polypyrrole dodecylsulfate and polypyrrole oxalate dispersed-mercury modified electrodes (PP/NO₃/Hg/GC, PP/DS/Hg/GC and PP/Ox/Hg/GC) were prepared.

### 8.2.4 Evaluation of the electrodes

The performance of the electrodes was characterized voltammetrically using various potential waveforms including cyclic, differential pulse, square and alternating current (A.C.).
Anodic stripping voltammetric analysis of Cu, Pb and Cd was performed using the Chemtronics procedure which involved plating samples for 60 seconds and holding the potential for 15 seconds at -900 mV and then sweeping at 375 mV/sec to 0 mV and holding for 2 seconds.

The chemical stability and selectivity of the electrode were investigated by storing the electrode in distilled, deionized water and by recording differential pulse voltammograms for Cu(II), Pb(II) and Cd(II) daily.

The mechanical stability of the electrode was tested in an amperometric detector with the an eluent flowing at 2.0 ml/min and recording the response from a Cu(II) sample.

A standard solution containing Cu(II), Pb(II) and Cd(II) was used to test the performance of the PDMME for metal ion detection in HPLC, while a solution of NH₂Cl and NHCl₂ was employed to evaluate the performance of the PDMME for non metallic compounds detection.

8.3 Results and Discussion

Using the procedure described in the experimental section, electropolymerization of the pyrrole monomer was carried out on a predeposited mercury layer. This resulted in a suitable polypyrrole dispersed-mercury modified electrode into which dodecylsulfate, nitrate or oxalate could be incorporated as counterions to the
conducting polymer. Both the mercury film and the polymer were observed visibly on the electrode surface. Surface and electrochemical characterization, sensitivity, selectivity and stability test, and chromatographic application were then investigated.

8.3.1 Surface characterization of the electrode
SEMs indicated that before the polymerization step, the electrode was unevenly covered with mercury drops (Figure. 8.1a) and that after the polymerization step, the mercury was redistributed over the electrode in fine, even drops (Figure. 8.1b).

8.3.2 Electrochemical characterization of the PDMME
8.3.2.1 Voltammetry
PP/NO_3/Hg/GC was used as an example to investigate the electrochemical behavior of the polypyrrole dispersed-mercury modified electrodes.

Voltammetric analysis of a Cu(II), Pb(II) and Cd(II) standard solution using different waveforms was carried out on this PDMME. Both anodic and cathodic potential scans were applied and it was found in all waveforms that the anodic process was much more sensitive than the cathodic process (Figure 8.2). Presumably, proconcentration of the metals occurred at more cathodic potentials. The effect was particularly predominant in the A.C. (Figure 8.2c) voltammetry. This effect indicated that stripping voltammetry could be carried out using this electrode as with the thin mercury film electrode.
Figure 8.1  Scanning electron micrographs of a PDMME and a mercury electrode

SEM on: a. normal thin mercury film electrode  b. polypyrrole nitrate dispersed-mercury modified electrode (PP/NO₃/Hg/GC).
Figure 8.2 Voltammetric detection of Cu, Pb, and Cd using various waveforms

Electrodes: working=PP/NO3/Hg/GC, reference=Ag/AgCl;
Waveform: a. cyclic D.C. scan rate = 100 mV/sec;
b. Osteryoung square wave, frequency = 15 HZ;
c. A.C. wave, scan rate = 4 mV/sec, A.C. amplitude = 25 mV, frequency = 50 HZ;
d. differential pulse, scan rate = 4 mV/sec, pulse amplitude = 50 mV, pulse width = 50 ms, pulse period = 1000ms;
arrows indicate the direction of the scan.
8.3.2.2 Stripping voltammetry

Stripping voltammetric analysis was achieved on the PDMME. Compared with a conventional thin mercury film electrode the PDMME has a similar sensitivity for metal detection. Examples of calibration curves are shown in Figure 8.3. The calibration curves for Cu(II), Pb(II) and Cd(II) detection were linear to at least 200 ppb. Low detection levels of 1 ppb were easily obtained on both electrodes. The results further confirm that the PDMME can behave like a conventional TMFE.

8.3.3 Sensitivity of the PDMME

8.3.3.1 Effect of mercury film thickness

Sensitivity of the PDMME was investigated using a PP/NO₃/Hg/GC electrode. The effect of varying the mercury film thickness on the voltammetric responses was studied. The magnitude of the responses observed from differential pulse voltammetric (DPV) analysis of metal ions (Cu, Cd, and Pb at the 300 ppb level) were constant with mercury plating times of 60, 120 and 180 seconds from a 0.010 M Hg(NO₃)₂ solution with an applied potential of -0.50 V. However, the background current did increase with increased mercury plating time and consequently plating times of 60 seconds were employed.
Figure 8.3  Calibration curves of Cu, Pb and Cd detection

Stripping voltammetric conditions: deposition time=60sec, applied potential=-900mV;

Calibration curves: a. on an Hg/GC electrode and b. on a PP/NO₃/Hg/GC electrode;

Peak height=counts.
8.3.3.2 Effect of polymer thickness

The effect of increasing the thickness of the polymer layer was then investigated. DPV responses decreased with the number of potential cycles in the monomer solution (Figure 8.4) which increased the polymer thickness. With thicker polymers, the ratio of mercury to polymer on the electrode surface decreased and the metal ions had less access to the dispersed mercury droplets. Furthermore, the conductivity of the substrate decreased particularly at more cathodic potentials where polypyrrole coatings were inherently less conductive and consequently the current reduced. A procedure involving 50 potential cycles at 100 mV/sec gave rise to adequate sensitivity and improved mechanical stability. With thicker polymer coatings the background current also increased due to the electroactive nature of the polymer. This increase in the background current eventually affected the determination of copper (II) (Figure 8.4), since polypyrrole undergoes an oxidation/reduction process about -0.40 V vs Ag/AgCl. This process was the major factor contributing to the increased background current.
Figure 8.4  Effect of polymerization time on PDMME performance

Differential pulse voltammetric conditions: scan rate = 4 mV/sec, pulse amplitude = 50 mV, pulse width = 50 ms, pulse period = 1000 ms;

Polymerization time:  a. 60 seconds, b. 120 seconds and c. 180 seconds.
8.3.4 Selectivity of the PDMME

One of the most important advantages of conducting polymer modified electrodes is the selectivity (179-181). Such selectivity may be modified by the incorporated anions. Consequently, a simple anion (nitrate), a surfactant anion (dodecyl sulfate) and an organic anion (oxalate) were incorporated into polypyrrole and the selectivity of PDMMEs studied.

The responses for Cu, Pb and Cd were selective on the electrodes incorporated with different counterions after the electrodes were stored in water for more than 10 hours (Figure 8.5). The magnitude of responses changed with the electrodes. The cadmium response was small on the PP/DS/Hg/GC electrode, while the lead response on the PP/Ox/Hg/GC electrode was higher than those of cadmium and copper. Different selectivities were noted for solution responses as well as after preconcentration.

This selectivity was attributed to the contribution of the counterion to the chemical properties of the polymer formed on the surface of the mercury film. The mercury ions generated during polymerization can react with counterions to form complexes which can exist on the mercury electrode surface. Since nitrate can not complex mercury ion to change the surface state of the film, no selective detection of Cu, Pb and Cd was expected. However, both dodecylsulfate and oxalate (299) may react with mercury ions to form complexes and change the surface state of the mercury film and therefore contribute to the selective detection of metal ions.
Figure 8.5  Selectivity of polypyrrole dispersed-mercury modified electrodes

Experimental condition as in Figure 8.4;

Responses due to: a. Cu (II), b. Pb(II) and c. Cd(II) (200 ppb);

Voltammograms on: 1. PP/NO3/Hg/GC,
2. PP/DS/Hg/GC and 3. PP/Ox/Hg/GC.
8.3.5 Stability of the PDMME

8.3.5.1 Chemical stability

The chemical stability of PP/DS/Hg/GC and PP/NO₃/Hg/GC was studied by soaking the electrodes in distilled water and then studying voltammetric responses periodically. It was found that significant changes in metal ion responses occurred with both electrodes over the first 10 hours (Figure 8.6). The responses then remained stable for 72 hours. After this period the response on both electrodes deteriorated, and double peaks for Cu(II) appeared during stripping voltammetry due to the formation of intermetallic compounds (300). The results were compared with a pure mercury film electrode. The similar stable period was observed in metal ion responses except for the changes in the first 10 hours.

On a freshly made PP/DS/Hg/GC electrode, a high background response appeared from the potential range of -0.2 V to -0.5 V. The response disappeared gradually with potential scanning. This background response is probably caused by the large reduction peak on the polymer as shown by the cyclic voltammogram (Chapter 2). An interference with Cu and Pb responses can result from this background response.
Figure 8.6 Chemical stability of polypyrrole dispersed-mercury modified electrodes

Differential pulse voltammetric conditions: scan rate = 4 mV/sec, pulse amplitude = 50 mV, pulse width = 50 ms, pulse period = 1000 ms;

Curve: 1. PP/NO₃/Hg/GC, 2. PP/DS/Hg/GC.
8.3.5.2 Physical stability

The stability of PP/NO₃/Hg/GC and Hg/GC electrodes was evaluated by injecting samples containing 0.6 ppm Cu(II) at flow rates of 1.0 ml/min and recording the peak height (Figure.8.7). The responses decreased with time, probably due to the loss of mercury, especially at flow rates greater than 2 ml/min. The Hg/GC electrode lost substantial amounts of mercury. However, the PP/NO₃/Hg/GC retained mercury drops on the surface. This was evident from visual appearance after use.

The improved stability of the PDMME is probably caused by the polymer layer. The shining glassy carbon surface can physically hold mercury droplets due to adsorption. This adsorption, however, is not strong enough to resist the mechanical shearing force from the flowing eluent and therefore the mercury droplets can be stripped off from the electrode surface. However, when a layer of polypyrrole is formed on the electrode, most likely on the glassy carbon surface, it can support the mercury droplets and prevent them from being stripped off. Therefore, the stability of the electrode is improved.
Figure 8.7  Stability of polypyrrole dispersed-mercury modified electrodes in a flow through cell

Sample: 50 μl of $1 \times 10^{-5}$M Cu; 1. PP/NO$_3$/Hg/GC electrode, 2. Thin mercury film electrode;

Experimental conditions: reference to the experimental section.
8.3.6 Amperometric detection of Cu, Pb and Cd following HPLC separation

The PDMME was used as an amperometric detector following HPLC separation (Figure 8.8). Using reversed pulse detection, i.e. resting at -1.0 V for 360 ms and pulsing to -0.1 V for 60 ms greatly reduced the effect of oxygen and increased the sensitivity. This has been previously reported by other workers (301). Responses due to reduction of Cu(II) and the oxidation of Pb(II) and Cd(II) were obtained using these conditions. Detection limits for those metal ions after HPLC separation are listed in Table 8.1.

Table 8.1 Detection limits for HPLC/EC analysis of metal ions

<table>
<thead>
<tr>
<th>Metal</th>
<th>Detection Limit* (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>0.27</td>
</tr>
<tr>
<td>Pb</td>
<td>0.11</td>
</tr>
<tr>
<td>Cd</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*50 μl injection

Detection limit = 2N[M]/H

[M] = Concentration of the standard sample in ppm;
H = Peak height of the standard sample in nA;
N = Baseline noise level in nA.
**Figure 8.8**  
HPLC separation and detection of metal ions using a PDMME

Column: Dionex HPIC-CS5;

Mobile phase: 0.050 M H$_2$C$_2$O$_4$ + 0.095 M LiOH, pH 4.8, 1.0ml/min;

Pulsed amperometric detector: E$_1$ = -0.1 V, E$_2$ = -1.0 V, t$_1$ = 60ms, t$_2$ = 360ms;

Electrode: PP/NO3/Hg/GC;

Response: 1. H$_2$O, 2. 4.0 x 10$^{-6}$ M Pb, 3. 5.0 x 10$^5$ M Cu and 4. 5.0 x 10$^{-5}$ M Cd.
8.3.7 Detection of chloramines following HPLC separation
In this section application of the PDMME to the amperometric detection of chloramines was investigated. PP/NO₃/Hg/GC has been used as an example of the PDMMEs.

8.3.7.1 Voltammetry

In order to identify the voltammetric response of NH₂Cl and NHCl₂, a glassy carbon electrode and a solution containing 0.01 M NH₄⁺ in 0.01 M phosphate buffer (pH 6.7) were employed. The solution was deoxygenated with nitrogen for five minutes and then a differential pulse voltammogram was recorded (Figure 8.9). Some of the chloramines were lost during deoxygenation due to their volatility. However, this did not affect the identification of the electrochemical responses. NH₂Cl and NHCl₂ responses were observed (Figure 8.9) at -0.11 and -0.34 V vs. Ag/AgCl respectively. A response due to reduction of hypochlorite was observed at -0.24V. This response disappeared after addition of NH₄⁺ and was replaced by the NH₂Cl and NHCl₂ responses. When the ClO⁻ concentration was increased, the chloramine responses became greater as expected. A black buffer solution, 0.01 M ClO⁻ in the buffer and 0.01 M NH₄⁺ in the buffer were checked as the blank solutions.

Attempts to determine chloramines using the PDMME were not successful as a high background current was produced, probably due to the presence of oxygen. Vigorous deoxygenation removed chloramines as well.
Figure 8.9  Differential pulse voltammogram of NH$_2$Cl and NHCl$_2$

Reference electrode: Ag/AgCl;

Experimental conditions: scan rate=10 mV/sec.,
pulse height = 50 mV, duration between pulses = 0.5 sec.;
Samples: 1. 0.01M Na$_2$HPO$_4$ + 0.01 M NaH$_2$PO$_4$ as solvent,
2. 0.01M NH$_4^+$, 3. 0.01 M ClO$_4^-$, 4. 0.01 M ClO$_2^-$ + 0.01M NH$_4^+$,
5. 0.05 M ClO$_2^-$ + 0.01M NH$_4^+$;

Responses were due to reduction of: a. NH$_2$Cl, b. NHCl$_2$, c. ClO$_2^-$.
8.3.7.2 Amperometric detection

Amperometric detection using the PDMME following HPLC separation was investigated. An example of separation and detection of monochloramine and dichloramine is given in Figure 8.10. Compared with a bare glassy carbon electrode, the PDMME can produce much higher responses as well as higher noise levels. In order to increase the sensitivity, it is necessary to minimize the background noise on the PDMME.

Detection limits for monochloramine and dichloramine using amperometric detection with the PDMME were compared with those using UV detection and amperometric detection with the glassy carbon electrode (Table 8.2). The concentration was calibrated by a amperometric titration method. The amperometric titration could only give the results for monochloramine and other chloramines, but not for an individual dichloramine. Therefore, the detection limit for dichloramine was assumed to be less than the value obtained from other chloramines.
Figure 8.10  HPLC separation and detection of NH$_2$Cl and NHCl$_2$ using a PDMME

Mobile phase: 0.01 M phosphate buffer pH 6.9; at 0.8 ml/min.;
Sample: 25 µl of 2.5 x 10$^{-3}$ M NaClO + 1.0 x 10$^{-3}$ M NH$_4$NO$_3$;
Responses: 1. NO$_3^-$, 2. NH$_2$Cl, 3. NHCl$_2$ and 4. O$_2$;
Amperometric detection: $E_{app} = -0.10$ V vs Ag/AgCl, electrode = PP/NO$_3$/Hg/GC.
UV detection: at 221nm.
Table 8.2 Detection limit for monochloramine and dichloramine

<table>
<thead>
<tr>
<th>Detector</th>
<th>UV at 221 nm</th>
<th>Amperometric at -0.40 V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>PDMME</td>
</tr>
<tr>
<td>NH₂Cl (M)</td>
<td>1.0 x 10⁻⁵</td>
<td>2.1 x 10⁻⁶</td>
</tr>
<tr>
<td>NHCl₂ (M)</td>
<td>&lt;2.8 x 10⁻⁶</td>
<td>&lt;2.7 x 10⁻⁷</td>
</tr>
</tbody>
</table>

Sensitivity with the amperometric detector was higher than that with the UV detector. However, there was no significant difference using the PDMME and the glassy carbon electrode, since higher noise levels were obtained on the PDMME.

8.4 Conclusion

A polypyrrole dispersed-mercury electrode was developed in the course of this work. A layer of fine mercury droplets was dispersed in the polypyrrole modifier on the electrode surface. Voltammetric characterization indicated that the electrode has similar characteristics to the conventional thin mercury film electrode and that it can be used in voltammetric analysis with various waveforms as well as in anodic stripping voltammetric analysis of metal ions. The sensitivity of the PDMME is similar to that of the thin mercury film electrode but it is dependent on the thickness of the polymer. Selective responses for metal ions have been achieved by incorporating various counterions into polypyrrole during polymerization. The electrode was chemically stable in 72 hours.
The best performance of the electrodes occurred within 10 to 72 hours after preparation. In a flow through system, the PDMME shows higher mechanical stability than the conventional thin mercury film electrode. This characteristic enables it to be used as an amperometric detector in HPLC analysis.

Application of the PDMME in HPLC detection was achieved. Cu(II) Pb(II) and Cd(II) were detected employing a reversed pulse potential technique following ion exchange separation. Chloramines were detected following reversed phase separation. In both cases, the PDMME showed high sensitivity.

Acknowledgement
Contributions to this work from Mr Zhao Huijun and the Melbourne and Metropolitan Board of Works is greatly appreciated.
Chapter 9
General conclusion
The chemical and electrochemical properties of conducting polymers for use in liquid chromatography have been studied in this work using polypyrrole as an example.

Polypyrrole was electrochemically and chemically synthesized on various substrates. The electrodeposition of polypyrrole was carried out on RVC cartridges, tantalum wires, stainless steel plates, gold or tin oxide coated plastic films, carbon fibres and carbon cloth, while chemical polymerization was studied using filter paper and silica gels. The properties of polypyrrole were characterized using chronoamperometry, chronopotentiometry, elemental analysis, cyclic voltammetry, SEM, EDX, FAB/MS and EGA. The results indicated that the polymer had a porous structure and that the counterions were incorporated into the polymer and consequently, it is suitable for chromatographic studies. In particular, the anion exchange properties of polypyrrole were investigated and it was found that polypyrrole had unique properties in the anion exchange processes which could be used for chromatography. Using TG and DTA it was also shown that the polymer was thermally-sensitive. This indicated that particular caution is necessary during the production of polypyrrole-based materials for the use of chromatography.

Polypyrrole with Cl⁻ and DS⁻ incorporated was used as new stationary phases for chromatographic separations. Hardware and
the systems for synthesis and characterization of the stationary phase were developed in the course of this work. These included cells for electrochemical coating of the polymer stationary phase on packing materials and columns for chromatographic characterization and ECLC studies. These devices were evaluated before they were used for chromatography. Using the hardware, both RVC based and silica based polypyrrole stationary phases were produced. Both reverse phase and anion exchange chromatographic behavior were found on these new stationary phases, which confirmed the feasibility of using this polymer for chromatographic separation. It was also shown that the polymer coated on RVC particles can be used in ECLC. Electrochemical effects on some reverse phase and anion exchange properties were proven. Separation examples demonstrated that the polymer is useful in chromatography.

Polypyrrole modified electrodes were used for HPLC detection. A polypyrrole dispersed-mercury modified electrode was developed during the course of this work. The electrochemical properties including selectivities and sensitivities have been studied and they have proven useful for HPLC detection. It has been observed that the electrode was chemically and physically stable. The application of the electrode in amperometric detection following HPLC separation has been achieved.

Although investigations on conducting polymers for use in liquid chromatography were carried out in this work, some problems were still not overcome and consequently, further research in this area is necessary.
Synthesis of conducting polymers has only been conducted using polypyrrole and a few counterions. and also the properties of these conducting polymers have not been fully characterized. Therefore, the following aspects should be considered in future work.

1. New monomers such as aniline and polypyrrole derivatives should be investigated. They may improve selectivity and the stability of the conducting polymers.

2. New counterions such as complexing agents and large anions should be incorporated into the conducting polymers, which may induce specific characteristics in the polymer. Some of these counterions may not be exchangeable, and therefore, may improve the stability of the polymer for use in chromatography.

3. Ion exchange processes should be studied in detail. The ion exchange kinetic and thermodynamic properties as well as the effects of application of potentials on these properties, should also be investigated.

4. Physical characteristics such as porosity and mechanical properties of conducting polymers should be determined. These are important for the future development of the use of these polymers in chromatography.

Although some success has been achieved, the stability and selectivity of the stationary phases and the column efficiency of the present study were still limited. Future work in chromatography should concentrate on the following areas:

1. Use of a stable conducting polymer for chromatography should be studied. For example, application of a self-doped
polymer or a polymer incorporated with unexchangeable counterions should be considered.

2. Use of commercially available carbon-based packing materials should be investigated in order to improve the column efficiency, especially for ECLC.

3. The procedure and hardware for producing conducting polymer stationary phases should be improved in order to obtain high performance packing materials.

4. The mechanism of retention should involve detailed investigation in order to apply the polymer to chromatographic analysis.

5. Application of the polypyrrole modified C18 stationary phase in HPLC analysis should be studied.

6. Conducting polymer particles should be synthesized for the use of packing materials.

The PDMMEs have demonstrated a high potential for chromatographic detection. However, application of the electrodes in HPLC analysis has only been investigated in limited areas. Expansion of the use of the electrodes in HPLC analysis should be studied.
References


List of Figures

Figure 1.1 Chromatogram ................................................................. 4
Figure 2.1 Chronopotentiograms obtained during electropolymerization on various electrode materials ................................................................. 4 4
Figure 2.2 Chronopotentiogram recorded during the electropolymerization process ................................................................. 4 5
Figure 2.3 Chronoamperograms obtained during electropolymerization on various electrode materials ................................................................. 4 6
Figure 2.4 Cyclic voltammogram of a polypyrrole chloride coated platinum electrode ................................................................. 5 2
Figure 2.5 Cyclic voltammograms of polypyrrole on various substrates ................................................................. 5 3
Figure 2.6 Effect of counterion size on the precision of electrogravimetric analysis ................................................................. 5 7
Figure 2.7 Scanning electron micrographs of polypyrrole on various substrates ................................................................. 6 2
Figure 2.8 Electrodeposition process ................................................................. 6 3
Figure 2.9 Fast atom bombardment mass spectrum of PP/DS ................................................................. 6 4
Figure 2.10 Deterioration of the mass spectral intensity with atom beam scanning ................................................................. 6 6
Figure 2.11 An example of EDX spectrum ................................................................. 6 8
Figure 3.1 Ion exchange processes ................................................................. 7 7
Figure 3.2 Ion exchange rate in polypyrrole ................................................................. 7 9
Figure 3.3  Ion exchange equilibrium in polypyrrole........................................80
Figure 3.4  Ion exchange mechanism in polypyrrole........................................82
Figure 3.5  Effect of ionic strength on the ion exchange process........................................84
Figure 3.6  Effect of pH on the ion exchange process........................................85
Figure 3.7  Effect of potentials on the ion exchange rate........................................87
Figure 3.8  Reversibility of the ion exchange process........................................88
Figure 4.1  Thermal stability of RVC.................................................................97
Figure 4.2  Thermal stability of PP/Cl/RVC.........................................................98
Figure 4.3  Thermal stability of PP/DS/RVC.........................................................99
Figure 4.4  Thermal stability of SDS.................................................................100
Figure 4.5  Thermal stability of PP/DS...............................................................101
Figure 4.6  Morphological changes of PP/DS/RVC after thermal treatment.........................106
Figure 4.7  Electrochemical changes of PP/DS/RVC after thermal treatment.........................107
Figure 4.8  Gas chromatogram on polypyrrole stationary phase..................................110
Figure 5.1  The packed particle cell for electrodeposition.......................................122
Figure 5.2  Diagram of the packed particle cell for electropolymerization ..........................123
Figure 5.3  The slurry cell for electrodeposition...................................................126
Figure 5.4  The plate cell for electrodeposition.....................................................127
Figure 5.5  Diagram of self-compressed column system...........................................130
Figure 5.6  The self-compressed column system....................................................134
Figure 5.7  The ECLC column system...............................................................135
Figure 6.7  Capacity factors of benzoate and m-toluate vs. the ionic strength of the buffer in the mobile phase.................................................................................................................. 183

Figure 6.8  Stability of RVC based polypyrrole packings.............................................................................................................................................................................. 185

Figure 6.9  Charging process on ECLC columns............................................................................................................................ 186

Figure 6.10  Discharging process on ECLC columns....................................................................................................................... 187

Figure 6.11  Residual current on an ECLC column............................................................................................................................ 189

Figure 6.12  Electrochemical behavior on ECLC columns...................................................................................................................... 190

Figure 6.13  Potential drop between the working and auxiliary electrodes vs. applied potential.................................................................................. 192

Figure 6.14  Capacity factors vs. applied potentials on a PP/DS column.............................................................................................. 193

Figure 6.15  Selectivity factors vs. applied potentials on a PP/DS column.............................................................................................. 194

Figure 6.16  EC effects on hydrophobicity................................................................................................................................. 196

Figure 6.17  EC effects on polarity................................................................................................................................................. 198

Figure 6.18  EC effects on proton acceptor capability...................................................................................................................... 199

Figure 6.19  EC effects on electron acceptor capability .................................................................................................................. 201

Figure 6.20  EC effects on ion exchange capability...................................................................................................................... 203

Figure 6.21  Reversibility of ECLC............................................................................................................................................ 204

Figure 6.22  HETP vs. mobile phase velocity on RVC base polypyrrole packings.................................................................................. 206

Figure 6.23  Reversed-phase chromatographic separations on RVC based polypyrrole stationary phases............................................................................. 209

Figure 6.24  Anion exchange chromatographic separations on RVC based......................................................................................... 210
Figure 6.25 Separation of caffeine and theophylline with ECLC ................................................................. 211

Figure 7.1 Scanning electron micrograph of silica based polypyrrole packings .............................................. 224

Figure 7.2 EDX spectra of silica based polypyrrole packings ........................................................................ 225

Figure 7.3 Capacity factors of benzene, toluene, DMP and DEP vs. the methanol concentration in the mobile phase ................................................................. 232

Figure 7.4 Capacity factors vs. pH of the mobile phase .................................................................................. 234

Figure 7.5 Capacity factors vs. ion strength of the mobile phase .................................................................. 236

Figure 7.6 Electrochemical character of an ECLC column with silica based polypyrrole packings .................. 238

Figure 7.7 Electrochemical effect on chromatographic interactions on silica based polypyrrole packings .......... 239

Figure 7.8 HETP vs. mobile phase velocity on silica based packings ............................................................. 242

Figure 7.9 Stability of silica based packings at acidic media ......................................................................... 244

Figure 7.10 Stability of silica based packings at basic media ......................................................................... 245

Figure 7.11 Separations on silica based polypyrrole stationary phases .......................................................... 247

Figure 8.1 Scanning electron micrographs of a PDMME and a mercury electrode ............................................ 258
Figure 8.2 Voltammetric detection of Cu, Pb, and Cd using various waveforms.................................259
Figure 8.3 Calibration curves of Cu, Pb and Cd detection.................................................................261
Figure 8.4 Effect of polymerization time on PDMME performance.........................................................263
Figure 8.5 Selectivity of polypyrrole dispersed-mercury modified electrodes........................................265
Figure 8.6 Chemical stability of polypyrrole dispersed-mercury modified electrodes............................267
Figure 8.7 Stability of polypyrrole dispersed-mercury modified electrodes in a flow through cell..................269
Figure 8.8 HPLC separation and detection of metal ions using a PDMME................................................271
Figure 8.9 Differential pulse voltammogram of NH2Cl and NHCl2.........................................................273
Figure 8.10 HPLC separation and detection of NH2Cl and NHCl2 using a PDMME.................................275
## List of Tables

| Table 1.1 | Some Stationary Phases used in ECLC | 20 |
| Table 2.1 | Characterization techniques used in this work | 36 |
| Table 2.2 | Synthesis of polypyrrole on novel substrates | 39 |
| Table 2.3 | Elemental analysis of polypyrrole with various counterions | 54 |
| Table 2.4 | Required charge for 0.100 g polypyrrole using different size counterions | 58 |
| Table 2.5 | EGA results of polypyrrole with various counterions | 59 |
| Table 2.6 | Determination of current efficiency during electropolymerization | 60 |
| Table 2.7 | Precision of the EDX method | 67 |
| Table 3.1 | Ion exchange sequence | 81 |
| Table 4.1 | The ion intensity of FAB/MS peaks obtained from PP/DS after heating to different temperatures | 108 |
| Table 4.2 | Effect of conditioning temperature on the chromatographic capacity factors | 109 |
| Table 4.3 | Selectivity change due to thermal treatment of stationary phases | 111 |
| Table 5.1 | Effect of self-compression | 142 |
| Table 5.2 | Extra column effect on column efficiencies | 145 |
| Table 6.1 | Columns packed with RVC-based polypyrrole stationary phases | 161 |
| Table 6.2 | Test compounds | 167 |
Table 6.3 Retention on different columns..............................................169
Table 6.4 Comparison of the RVC-based stationary phases...............................................................174
Table 6.5 Retentivity and selectivity of nonpolar and polar compounds on polypyrrole.........................................175
Table 6.6 Retentivity and selectivity of basic and acidic compounds on polypyrrole........................................177
Table 6.7 Stability of $k'$ on the PP/DS/RVC column.............................178
Table 6.8 Anion exchange selectivity sequence..............................179
Table 6.9 Effect of mobile phase on capacity factors and column efficiency of anions........................................182
Table 6.10 Column efficiency on RVC-base columns.................................................205
Table 6.11 Reproducibility of capacity factors on polypyrrole columns................................................208
Table 7.1 Electrochemically prepared polypyrrole on C18 bonded silica....................................................218
Table 7.2 Chemically prepared polypyrrole columns on silica based supports................................................219
Table 7.3 HPLC characterization conditions.............................................220
Table 7.4 Elemental analysis of silica based packings...........................221
Table 7.5 Surface area analysis of silica based packings.....................222
Table 7.6 Capacity factors of some compounds on electrochemically prepared polypyrrole stationary phases................................................226
Table 7.7 Chromatographic interactions on polypyrrole coated C18 bonded silica......................................227
Table 7.8 Chromatographic interactions on polypyrrole coated silica.....................................................230
Table 7.9 Efficiency of silica based polypyrrole stationary phases ............................................................................. 241
Table 8.1 Detection limits for HPLC/EC analysis of metal ions ..................................................................................... 270
Table 8.2 Detection limit for monochloramine and dichloramine ....................................................................................... 276