The determination of probes for the indirect detection of anions in capillary zone electrophoresis using light emitting diode detection

Peter Ashley Balding

Edith Cowan University

Follow this and additional works at: https://ro.ecu.edu.au/theses_hons

Part of the Analytical Chemistry Commons

Recommended Citation


This Thesis is posted at Research Online.
https://ro.ecu.edu.au/theses_hons/1065
Edith Cowan University

Copyright Warning

You may print or download ONE copy of this document for the purpose of your own research or study.

The University does not authorize you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following:

• Copyright owners are entitled to take legal action against persons who infringe their copyright.

• A reproduction of material that is protected by copyright may be a copyright infringement. Where the reproduction of such material is done without attribution of authorship, with false attribution of authorship or the authorship is treated in a derogatory manner, this may be a breach of the author’s moral rights contained in Part IX of the Copyright Act 1968 (Cth).

• Courts have the power to impose a wide range of civil and criminal sanctions for infringement of copyright, infringement of moral rights and other offences under the Copyright Act 1968 (Cth). Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.
THE DETERMINATION OF PROBES FOR THE INDIRECT
DETECTION OF ANIONS IN CAPILLARY ZONE
ELECTROPHORESIS USING LIGHT EMITTING DIODE
DETECTION

PETER ASHLEY BALDING
BSc(Applied and Analytical Chemistry)Hons

Faculty of Computing, Health and Science

EDITH COWAN UNIVERSITY
November 2006
USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.
This thesis is dedicated to my father, Frank Balding (1948-2005), one of life's true gentlemen.
DECLARATION

I certify that this thesis does not, to the best of my knowledge and belief:

(i) incorporate without acknowledgement any material previously submitted for a degree or diploma in any institution of higher education;
(ii) contain any material previously published or written by another person except where due reference is made in the text; or
(iii) contain and defamatory material

Signed

Dated 14/2/07
ACKNOWLEDGEMENTS

In reflection over the year, I have realised how much I have learnt and experienced both academically and personally. In particular, I believe my honours year has given me the experience I require in a research capacity to become a better scientist. To my supervisor, Dr Mary Boyce, your dedication to your work and the enthusiasm you have for chemistry is contagious. I have thoroughly enjoyed working on this project, which is directly attributed to your guidance and commitment to me as your student. I am especially thankful for the encouragement, criticism and motivation that kept me on track to complete the year, which wasn’t without its dramas!

I would also like to acknowledge the input from Dr Michael Breadmore and Dr Mirek Macka. Firstly, Michael as a specialist in inorganic anion analysis and pre-concentration methodologies, your ongoing correspondence was much appreciated. To Mirek, your supply of the LED housing and expertise in the area of LED-indirect detection was also gratefully appreciated.

This project could not have gone ahead without the financial support from the Centre for Ecosystem Management and School of Natural Sciences at Edith Cowan University.

To my extended family and friends your ongoing encouragement and support was a significant motivational tool for me, and for that I thankyou.

Finally, I would like to acknowledge and thank my immediate family. Mum you are an inspiration for me, in everything that you do. Your love and support throughout the last year is so very much appreciated. To Andrew, thanks for being there to have a laugh and enable me to “de-stress” over the year. The support that you have shown for me while completing my final year is gratefully appreciated. To Dad, even though you are no longer with us, your help and guidance in my earlier years has helped me reach this point. You were a true gentleman in every sense of the word and sadly missed.
ABSTRACT

This study investigated the potential of a number of compounds to act as probes for the indirect detection of inorganic and organic anions separated by capillary zone electrophoresis and using light emitting diodes (LED) as the light source. Desirable characteristics of the probe anion included: high molar absorptivity, moderate to high electrophoretic mobility, and having a wavelength of maximum absorbance near the maximum emission wavelength of a LED. A preliminary study led to the evaluation of eleven possible compounds. Two of these, p-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid were selected for further study as they fulfilled the criteria previously mentioned. The optimised BGEs containing p-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid as the visualising agent were used to separate a mixture of 11 anions. Both probes were best suited for the separation and detection of anions of moderate mobility such as phosphate and benzoate. Limits of detection of 0.003 mM were achieved for benzoate using the p-nitrophenol BGE and 0.006 mM for phosphate using the 4-hydroxy-3,5-dinitrobenzoic acid BGE. Multiple co-ionic electrolytes were explored to help increase the ability to efficiently analyse for analytes with a wider range of mobilities. A p-nitrophenol/4-hydroxy-3,5-dinitrobenzoic acid system and a p-nitrophenol/chromate system were investigated. Both systems displayed improvements in peak shape compared to a separation utilising a single probe. The optimised BGEs were then applied to oxalate using samples of interest to the alumina industry. p-nitrophenol displayed potential for the routine analysis of oxalate in high oxalate concentration samples, whereas 4-hydroxy-3,5-dinitrobenzoic acid was the probe of choice for the determination of oxalate in typical Bayer liquor samples.
PUBLICATIONS FROM THIS THESIS

Part of the content presented in this thesis has been presented at a national chemistry conference, Interact 2006 and submitted to Electrophoresis for publication. The details are supplied below.


Balding, P., Boyce, M., Breadmore, M., Macka, M. The determination of probes for the indirect detection of anions in capillary zone electrophoresis using light emitting diode detection. Submitted to Electrophoresis Nov 2006
TABLE OF CONTENTS

DECLARATION i
ACKNOWLEDGEMENTS ii
ABSTRACT iii
PUBLICATIONS FROM THIS THESIS iv
TABLE OF CONTENTS v

Chapter 1
Introduction and Literature Review
1.1 Introduction 1
1.2 Capillary zone electrophoresis (CZE) 2
1.3 Indirect detection 4
1.4 Instrumental strategies to improve detection sensitivity 6
1.4.1 Detection pathlength 6
1.4.2 Baseline noise 8
1.5 Chemical strategies to improve detection sensitivity 9
1.5.1 Probe mobility 9
1.5.2 Molar absorptivity 10
1.6 Other considerations 11
1.6.1 Probe concentration 11
1.6.2 Toxicity 11
1.7 Buffering of BGE 11
1.8 Pre-concentration 13
1.8.1 Field amplified sample stacking (FAAS) 13
1.8.2 Isotachophoresis 15
1.9 Application of CZE to the analysis of relevant alumina industry samples 18
Aims 20

Chapter 2
Experimental
2.1 Instrumentation 21
2.1.1 Capillary electrophoresis instrumentation 21
2.1.2 Spectrophotometer instrumentation 21
2.2 Reagents 22
2.2.1 Probes 22
2.2.2 Buffering reagents 22
2.2.3 Anionic analytes 22
2.2.4 Other chemicals 23
2.3 Preparations 23
2.3.1 Preparation of analytes 23
2.3.2 Preparation of BGE’s 23
2.4 Experimental methods 23
2.4.1 Spectrophotometric measurements 23
2.4.2 Capillary conditioning 24
2.4.3 Mobility measurements 24
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4.4 Probe concentration</td>
<td>24</td>
</tr>
<tr>
<td>2.5 Calculations</td>
<td>25</td>
</tr>
<tr>
<td><strong>Chapter 3</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Characterisation of Probes</strong></td>
<td></td>
</tr>
<tr>
<td>3.1 Choice of Probe and buffering</td>
<td>26</td>
</tr>
<tr>
<td>3.1.1 Probe selection</td>
<td>26</td>
</tr>
<tr>
<td>3.1.2 Buffering and reversal agent</td>
<td>28</td>
</tr>
<tr>
<td>3.2 Experimental for selected probes</td>
<td>28</td>
</tr>
<tr>
<td>3.2.1 Spectrophotometric data</td>
<td>28</td>
</tr>
<tr>
<td>3.2.2 Mobility data</td>
<td>30</td>
</tr>
<tr>
<td><strong>Chapter 4</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Probe Performance</strong></td>
<td></td>
</tr>
<tr>
<td>4.1 Performance of selected probes</td>
<td>36</td>
</tr>
<tr>
<td>4.2 Minimising electro-migrational dispersion</td>
<td>40</td>
</tr>
<tr>
<td>4.3 Maximising detection limits</td>
<td>42</td>
</tr>
<tr>
<td>4.4 Comparison of alternative probes to chromate</td>
<td>44</td>
</tr>
<tr>
<td>4.5 Multiple co-ion electrolytes</td>
<td>48</td>
</tr>
<tr>
<td><strong>Chapter 5</strong></td>
<td></td>
</tr>
<tr>
<td><strong>The Analysis of Oxalate in Relevant Alumina Industry Samples</strong></td>
<td></td>
</tr>
<tr>
<td>5.1 Determination of oxalate in industry relevant samples</td>
<td>52</td>
</tr>
<tr>
<td>5.2 Samples with high oxalate concentration</td>
<td>52</td>
</tr>
<tr>
<td>5.3 Samples with a medium oxalate concentration (Bayer liquor)</td>
<td>54</td>
</tr>
<tr>
<td>5.4 Samples with a low oxalate concentration</td>
<td>57</td>
</tr>
<tr>
<td><strong>Chapter 6</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Conclusions</strong></td>
<td>60</td>
</tr>
<tr>
<td><strong>Chapter 7</strong></td>
<td></td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>61</td>
</tr>
</tbody>
</table>
Chapter 1
Introduction and literature review

1.1 Introduction

Capillary zone electrophoresis (CZE) is a separation technique that is slowly coming to the forefront of ion analysis, providing an alternative method to ion chromatography (IC) and related methods. CE offers significant benefits over IC. It is a micro-separation technique, in that it utilises microlitres of analyte and millilitres of background electrolyte (BGE) compared to millilitres of sample and litres of mobile phase for IC. The capillaries are inexpensive, typically $20-30 dollars each compared to several hundred dollars for an IC column. Most importantly, however, CZE offers significantly higher separation efficiencies when compared to IC. For example, Jones and Jandik [1] illustrated how CZE can resolve 30 ions in less than 3 minutes. A comparable separation by IC takes approximately 20 minutes [2].

CZE enables the separation of both cations and anions in a single run using the same column [1]. Separation conditions can be manipulated to cater for cations or anions individually if required. This results in efficient separations with short runtimes, typically less than 10 minutes. Direct detection is often employed for analyte recognition, however, this detection method limits the compounds that can be analysed to those species that absorb light. In general the majority of inorganic ions are optically transparent in the useful UV-VIS region. To overcome this limitation, indirect detection is employed. Compounds that have been used as probes (absorbing species) have in the past absorbed strongly in the UV (214 nm or 254 nm) or high visible region. The UV region is limited by high levels of noise while the bulky dyes often used in the high visible region can adhere to the capillary wall. Anion analysis has experienced some growth in the number of probes used with indirect detection [3]. However, because there is a lack of available light sources with strong emission in the low visible region, few probes have been identified for use in this part of the spectrum.
Chapter 1 Introduction and literature review

This section provides an overview of the technique of CZE along with a review of indirect detection and parameters essential for a successful system for anion analysis, both instrumental and chemical. Pre-concentration techniques will be briefly discussed in relation to the analysis of real samples and the application of CZE to the analysis of relevant alumina industry samples.

1.2 Capillary zone electrophoresis

The CZE system is a relatively simple system, which is one of its main advantages. A typical instrument consists of a fused silica capillary typically 30-100 cm long. The internal diameter (I.D.) can vary from 25 to 75 µm. Each end of the capillary is dipped in the background electrolyte (BGE). A potential difference is applied across the capillary by immersing an electrode in each BGE reservoir and connecting them to a power pack (0±30 kV), see figure 1-1.

![Figure 1-1- Typical schematic of a capillary electrophoresis instrument](image)

To effect a separation, a voltage is applied across the capillary, which generates electro-osmotic movement of the BGE. This bulk movement of the BGE or electro-osmotic flow (EOF) is due to the interaction of the charged BGE species with the charged silica surface of the capillary wall. The wall is most negatively charged at high BGE pH. Hence, use of basic BGE’s ensures that the effective EOF is maximised. The EOF in a normal system relies on the build up of cationic BGE constituents to the negatively charged silanol sites along the capillary wall. When charge is applied to the system the loosely bound cationic species migrate towards the negative detector end of the capillary as a bulk flow.

As ions are charged they will possess their own intrinsic mobility, which is dependant on their charge to radius ratio. Anions migrate toward the positive
electrode and cations toward the negative electrode. Under normal CZE conditions, the anions will migrate counter to the EOF and away from the detection end. However, at high pH their net mobility will be in the direction of the EOF (see figure 1-2). Cations in the same system migrate with the EOF and exit the capillary much sooner.

![Figure 1-2: Schematic diagram detailing the migration of cations and anions in a capillary filled with a BGE at high pH and with an applied positive voltage.](image)

Fast anion analysis can be achieved, with the anions migrating in the same direction as the EOF and towards the detector end, by reversing the charge on the wall of the capillary. This is brought about by coating the wall with a positively charged surfactant or polymer. Tetradecyltrimethylammonium bromide (TTAB) is a commonly used wall surfactant and is normally utilised as part of the BGE [4]. However, polydiallyldimethylammonium chloride (PDDAC) is a surfactant that has shown to provide a more permanent coating and as a result can be applied via a pre-run flush rather than being added to the BGE [5,6]. The resulting positive wall produces an EOF, which moves in the direction of the positive electrode (similar to the anions). In addition, the potential applied across the capillary is reversed so that the positive electrode is at the detection end where the anions exit the column (figure 1-3).
As with other separation techniques CZE has also been coupled with a variety of detection systems. Fluorescence, mass spectrometry and even electrochemical detection have been used for a variety of applications [7,8,9]. However, due to its simplicity UV-VIS detection is commonly employed. The majority of inorganic and some organic anions don’t absorb strongly in the high UV to visible region, necessitating the use of indirect UV-VIS detection. A major limitation when indirect UV-VIS detection is employed is sensitivity. There have been many ways in which to improve detection sensitivity and these will be discussed in the following sections.

1.3 Indirect detection

Indirect detection (ID) is a common form of detection that is applied to inorganic and organic species that do not absorb light in the useful UV-VIS region. An absorbing species, termed the probe or visualising reagent is added to the BGE. The ID system works on the premise that the capillary, prior to injection, is filled with the highly absorbing BGE, thus creating a background absorbance. The analyte is then injected and a run initiated. As the analytes separate out and move through the capillary they actively displace the probe. This displacement is measured at the detection window resulting in a decrease in the background absorbance and the detection of the analyte, figure 1-4.
Chapter 1  

Introduction and literature review

Most commonly, chromate has been used as a probe for the analysis of fast anions. Chromate has been shown to be relatively versatile in its uses, as methodology has been developed for several applications including the beverage and alumina industries [10,11]. It exhibits a high mobility which is beneficial for the analysis of fast anions such as chloride, bromide, nitrate and sulphate. However, peak shape seems to be only minimally compromised for slower anions such as phosphate and carbonate in a didodecyldimethylammonium bromide (DDAB) system [12]. An optimised chromate system for the trace analysis of inorganic anions has been shown to yield detection limits in the low parts per trillion concentration range [3]. Amongst all these advantages, chromate does have a significant disadvantage. Chromate is classed as an “occupational carcinogen” [13]. Chromate’s mutagenic risks are now well known, therefore it is vital that alternative probes be explored that are not as hazardous but are as effective.

![Figure 1-4- Raised baseline typical for indirect detection (negative peaks represent detected analytes)](image)

As mentioned earlier a significant disadvantage of ID in CZE is sensitivity. The lack of sensitivity has been well documented in the literature [3,12] and can be related to equation 1-1 [14]. It shows mathematically the factors that effect detection sensitivity with respect to indirect detection. To maximise limits of detection (LOD) probe concentration, $C_p$, and baseline noise, $N_{BL}$, should be kept at a minimum and the transfer ratio, $TR$, dynamic reserve, $D_r$, molar absorptivity, $\varepsilon$, and pathlength, $l$, should all be maximised. With respect to ID, there is a direct relationship between probe concentration, $C_p$, and the dynamic reserve, $D_r$. 

5
Decreasing the probe concentration, subsequently decreases the dynamic reserve. This, therefore, offsets any improvement in LOD [3].

\[
C_{\text{lod}} = \frac{C_p}{\text{TR}.D_r} = \frac{N_{\text{BL}}}{\text{TR}.\varepsilon.l} \tag{1-1}
\]

where \( C_p \) is the probe concentration (\text{molL}^{-1})

\( \text{TR} \) is the transfer ratio (number of moles of probe displaced by one mole of analyte)

\( D_r \) is the dynamic reserve (ratio of background absorbance to noise)

\( N_{\text{BL}} \) is the baseline noise

\( \varepsilon \) is the molar absorptivity of the probe

\( l \) is the detection cell pathlength

There have been several avenues taken to improve detection sensitivity based on the limiting factors mentioned above. The factors that have attracted much study include detection pathlength, baseline noise, probe mobility and molar absorptivity. These factors will be discussed further in the following sections.

1.4 Instrumental strategies to improve detection sensitivity

1.4.1 Detection pathlength

One of the avenues to improve sensitivity in indirect CZE systems has been to increase the pathlength. Johns et al [3] noted that the limited path length available from the capillary used was a significant factor in reducing detection sensitivity. This finding can be related to the Lambert-Beer law, equation 1-2.

\[
\text{Absorbance} = \varepsilon.c.l \tag{1-2}
\]

Where \( \varepsilon \) is the molar absorptivity of the probe (Lmol\(^{-1}\)cm\(^{-1}\)), \( c \) is the probe concentration (molL\(^{-1}\)) and \( l \) is the pathlength for detection (cm). Clearly, the pathlength is directly proportional to the obtainable absorbance for a given probe.
Chapter 1

Introduction and literature review

In theory the effective pathlength should be equal to the I.D. of the capillary used, however this is not the case in practice. The effective pathlength is governed by the way in which light passes through the capillary. If light passed directly through the centre of the capillary, then the pathlength and effective pathlength would be equal. However, due to polychromatic emission of the light source and refraction of the light beams through the capillary, the effective pathlength is almost always shorter when experimentally derived [15]. Ma and Zhang [16] explored a limited range of capillaries, typically from 25 μm to 74 μm in I.D. They found that increasing the I.D. did not yield sufficient increases in sensitivity. The subsequent increase in system current which resulted in joule heating was believed to be a significant factor in limiting any increases in LOD.

Bubble cells have been explored to increase the effective pathlength at the point of detection. Therefore, inadvertent increases in joule heating were avoided. Weston et al [17] used a capillary with a separation I.D. of 75 μm but at the point of detection the I.D. increased to 300 μm. This system minimised significant noise resulting from joule heating, however, even though there was almost a 4 fold increase in I.D., they only realised a 2 fold increase in the signal to noise ratio (S/N). Doble et al [18] used a Z cell to increase the pathlength to 3 mm, however like Weston, experienced only minor gains in detection sensitivity compared to the increase in the pathlength. These small gains were a direct result of subsequent increases in baseline noise, N_{BL} [6].

1.4.2 Baseline noise

There are many factors that contribute to baseline noise. These can be divided into two categories, instrumental noise and chemical noise. The instrumental noise consists of factors such as spatial and intensity stability of the light source, output of the light source at a particular wavelength, and other instrumental factors (electronic noise, thermal stability and mechanical rigidity of the optics). The chemical noise is a result of undesirable fluctuations in the probe concentration, leading to changes in the background absorbance of the BGE. [3]
Reductions in baseline noise in CZE have been achieved through the use of LED's as the light source. LED's are inexpensive, small in size, robust and reliable, have long lifetimes \((10^5 \text{ hours})\), low heat production, high linearity of output with current used and have extremely low noise particularly in the visible region \([10]\). Their use as a viable alternate light source to deuterium or mercury lamps in CZE has been explored \([10,19,20]\). Chromate, a commonly used probe for anion analysis, absorbs significantly at two wavelengths, 275 nm and 371 nm. However it is usually monitored at 254 nm, as this is a significant output wavelength for the majority of UV sources. King et al \([10]\) used this configuration at 254 nm and compared it against an LED system emitting at 379 nm. Baseline noise was found to decrease by 70% when using the LED system. With respect to equation 1, a reduction in baseline noise, \(N_{BL}\), will significantly improve the obtainable limits of detection.

In addition, LED's offer the benefit of being able to match or closely match the absorption maximum of the probe to their wavelength output leading to further increases in detection sensitivity. As noted by King et al \([10]\) the absorptivity of chromate was much more intense at 379.5 nm \((\varepsilon = 3.80 \times 10^3)\) than at 254 nm \((\varepsilon = 2.58 \times 10^3)\) further helping to improve sensitivity by 47%. LED's offer a suite of different wavelength outputs, which as technology increases will no doubt grow, hopefully to include more LED's within the UV range for non-coloured probes.

In addition to providing an excellent reduction in baseline noise LED's show a much improved stability. Johns et. al. \([20]\) illustrated with the use of a blue LED and Orange G as the probe, the average baseline drift from the start to the end of a run was 0.024 mAU and an average baseline fluctuation of 0.094 mAU was found. Thus, indicating that the LED light source emission was quite stable and robust.
1.5 Chemical strategies to improve detection sensitivity

1.5.1 Probe mobility

Probe mobility is a significant factor in maximising sensitivity. Johns et al [3] found, using the Kohlrausch regulating function, that the transfer ratio (ratio of the number of moles of probe displaced by the number of moles of analyte) did not occur on an equivalent basis as one would assume. Rather the effective displacement of the probe largely depended on the mobility of the analyte compared to that of the probe. Thus, in order to maximise the transfer ratio and hence LOD (see equation 1-1) the mobility of the analyte and probe must be as closely matched as possible.

Moreover, peak shape is dependant on the mobility matching of the analyte and probe. A zone broadening mechanism known as “electro-migrational dispersion” is significant if the mobilities are mismatched. If the mobility of the probe is faster than the analyte then the analyte band will be tightly packed at the back and diffuse at the front, resulting in a fronting peak. If the mobility of the probe is slower than the analyte then the band will be diffuse at the back and tightly packed at the front, resulting in a tailing peak. If the mobilities of the probe and analyte are matched then a symmetrical peak will result, figure 1-5.

![Figure 1-5- Diagram showing the effects of probe mobility on peak shape: (a) probe mobility < analyte mobility, (b) probe mobility = analyte mobility, (c) probe mobility > analyte mobility](image)

Given the importance of mobility matching to maximise detection limits it is quite reasonable that the combination of multiple probe co-ions will offer further
improvements in LOD. Doble and Haddad [6] discussed the benefit of using a probe that has a mobility closest to the analytes of interest. If a sample contains a variety of anions with different mobility’s they will more likely displace the probe closest to their own mobility. Doble and Haddad also found that the fewer co-ions in the BGE the better with respect to system peaks. They discovered that with n probes in the BGE there were n-1 system peaks. These, system peaks can cause interference with analytes of interest. However, it was also established that there is a linear relationship between the mobility of the system peak and the mole fraction of the fastest probe, therefore, allowing manipulation of the BGE to avoid system peak interferences. The use of multiple probes provides improvements in LOD over a wider mobility range.

1.5.2 Molar absorptivity
The use of highly absorbing probes is another method for increasing sensitivity in CZE. The importance of molar absorptivity has been comprehensively reviewed in the literature [16,21-23]. Increasing the molar absorptivity of a probe will simultaneously reduce the necessary probe concentration, which lowers limits of detection [6]. A 50 fold increase in sensitivity was reported by Foret et al. [24] for the detection of anions when the low absorbing probe, benzoate was replaced by the higher absorbing sorbate.

The use of highly absorbing dyes has also been explored. Doble et al [18] used bromocresol green for the determination of C_2-C_8 alkane sulphonic acids and Johns et al [11] used tartrazine for the detection of inorganic anions. Highly absorbing dyes are particularly useful when coupled with LED detection as most dyes absorb strongly in the visible region and the majority of LED’s manufactured to date emit strongly in this region as well.

There are some problems associated with the use of highly absorbing dyes. Firstly, they require significant dilution to bring the absorbance within linear working range of a UV-VIS detector. Dilution has shown to limit the analyte concentration range that can be used before electro-migrational dispersion occurs
resulting in the broadening of peaks [11]. Secondly, most highly absorbing dyes have been found to absorb onto the silica capillary wall, leading to non-reproducible EOF's and migration times, baseline fluctuations and increased noise [19,25]. Ideally, highly absorbing compounds of a less bulky nature are desirable to maintain good sensitivity without compromising reproducibility and robustness.

1.6 Other considerations

1.6.1 Probe concentration

Probe concentration is an important BGE parameter which must be maximised, as it has significant influence on electro-migrational dispersion. Increasing the probe concentration inadvertently increases LOD (equation 1-1), however, in all buffer systems it must be maximised to reduce band broadening. Electromigrational dispersion can also be minimised by ensuring that the electrolyte concentration is at least two orders of magnitude greater than the sample [26]. Again, this can be achieved by maximising the probe concentration in the BGE.

1.6.2 Toxicity

Chemical safety is of significant concern for organisations around the world. The toxicity of chemicals used in CZE is an area that can be focussed on to reduce hazardous exposure to users of the technique. With the technique moving towards mainstream use, in particular for portable applications, the potential for greater exposure is of concern. Thus, the toxicity of chosen reagents should be of significance when developing a CZE system. The choice of probe and buffering reagent forms a part of this study, with particular focus on toxicity of the probe.

1.7 Buffering of BGE

Buffering of the BGE in CZE has been shown to be a necessity [27]. Voltage is utilised to affect a separation, therefore, the electrolytic liberation of hydrogen and hydroxide ions at the anode and cathode, respectively, must be counteracted. pH can significantly effect the charge on the capillary wall, resulting in changes in the effective EOF. The change in pH from one end of the capillary to the other
can have significant effects on the reproducibility and ruggedness of a system, resulting in changes in selectivity and sensitivity [11].

Buffering of the BGE can be achieved in several ways. The use of a weak acid as a probe such as benzoate [28] provides a small degree of buffering around the pKₐ of the acidic probe. However, effective buffering using this method is limited by the pKₐ, the probe’s concentration and the ability of the probe to be partially ionised. Thus, only anions of moderate to low mobility are suitable for this system.

Another method of buffering is to use a co-ion as the buffering species such as borate [28] and phosphate [29], however, this is not ideal as the introduction of a non-absorbing co-ion results in competitive displacement of the probe, reducing sensitivity significantly (refer to equation 1-1).

The best and probably most common method of BGE buffering involves the use of a counter-ion. By utilising the acidic potential of the probe, a counter-ion is added and titrated against the probe. This method provides suitable buffering without the introduction of a co-ion. Tris(hydroxymethyl)aminomethane Tris [4] and diethanolamine (DEA) [30] have been used successfully for the analysis of inorganic anions.

The last method of buffering is to use an isoelectric ampholyte. This form of buffering only works if the pKₐ of the functional groups attached to the ampholyte is close enough to its isoelectric point, pI (within 2 pH units) [31]. For this reason there are only a limited number of ampholytic species available for suitable buffering.
1.8 Pre-concentration

Indirect UV-VIS detection is not always sensitive enough for the detection of analytes of interest. One avenue to combat this is to use more sensitive detection techniques such as mass spectrometry, however, this involves significant outlay costs. Another common option to improve sensitivity is to concentrate the sample, on-column before detection. This section will discuss the use of two common methods of pre-concentration, field amplified sample stacking and isotachophoresis for the improvement in sensitivity.

1.8.1 Field amplified sample stacking (FASS)

FASS is the simplest and most common form of on-column concentration and usually occurs naturally during a standard CZE separation. It can however, be manipulated to maximise its effect of concentrating the sample within the capillary.

FASS is usually facilitated by ensuring that the electrical conductance of the BGE is at least 10 times that of the sample [32], which is usually achieved by the sample and BGE having different electrolyte concentrations. The intrinsic mobility of the individual ions is directly related to the field strength that each experience. The field strength within the sample plug is generally higher than that in the BGE due to its lower conductivity (simple samples, water matrix). The sample is often hydro-statically or hydro-dynamically injected into the system, creating a discontinuous buffer system. Voltage is then applied and the ions accelerate up to the sample-BGE interface, where they slow rapidly, effectively being concentrated in a tight band (see figure 1-6)
Problems with this method are experienced for real sample analysis when the conductivity of the sample matrix is inadvertently higher relative to that of the BGE and a loss in stacking occurs. Mikkers et al [25] found that the conductivity of the BGE can be manipulated to ensure that its conductivity is at least 10 times greater than the sample matrix by increasing the electrolyte concentration. However, this introduces significant shortfalls as the current within the system increases, resulting in a rise in joule heating within the capillary, leading to decreases in separation efficiency and sample resolution [32]. To counteract joule heating, the field strength used for the separation can be reduced to bring the current within instrumental limits. Reducing the field strength subsequently minimises the EOF resulting in prolonged runtimes. To reduce the effects of high currents and increase sample stacking, simple dilution of the sample can give the necessary reduction in electrolyte concentration, providing the concentration of the analyte of interest is relatively high.

To increase sample stacking, larger injection volumes have been employed in some cases. However, Burgi and Chien [32] found that increasing the sample volume introduces another shortfall, laminar flow. Laminar flow results from a different EOF being generated between the sample plug and BGE and is directly related to variations in their field strength, meaning the sample analytes experience two different EOF’s as they move from the sample plug to the BGE plug. The flow profile arising from the variation in EOF can either be concave or
convex, depending on whether the BGE has a higher or lower EOF [33]. Minimising the EOF to reduce this effect has been explored, however, the downside to this is the longer migration times for the low mobility ions [34].

FASS when optimised can offer significant improvements in sensitivity. 10 fold improvements in limits of detection have been achieved [35].

1.8.2 Isotachophoresis (ITP)

ITP stacking is a useful technique, especially for samples containing a high-ionic strength matrix. This technique involves the injection of the sample between a leading and terminating electrolyte. Variations of this method can utilise the BGE co-ion as a leading or tailing ion and then the process only requires the introduction of one other ion into the sample composition to facilitate effective stacking.

The leading electrolyte contains an ion of high concentration and higher mobility than the sample analyte. The terminating electrolyte contains a co-ion of high concentration and lower mobility than the analytes. When a voltage is applied to the system a potential gradient is created over all the bands. As a result each band migrates with the same velocity [33]. It is important to note that this gradient is formed due to the variation in conductivity of each band. This directly results in different electric field strengths across the bands. If an analyte species slows and merges into a slower band, it will subsequently experience higher field strength and be accelerated until it reaches its own zone again. This process is ongoing until equilibrium is reached and a normal CZE mode is initiated. The electrolyte catches up to the sample zone, removing the field gradient causing the samples to migrate according to their intrinsic mobility [33] (see figure 1-7).

It is possible to conduct ITP by utilising components already present in the background matrix of real samples. A real sample may contain a leading or a terminating electrolyte and as a result may be utilised for stacking. If a leading electrolyte is present, the mobility of the analytes must be less. Alternatively, if a terminating electrolyte is present the mobility must be lower than the samples [36].
Chapter 1

Introduction and literature review

Common improvements in LOD associated with single capillary ITP are in the 400 fold region [36], however, when coupled capillaries are used sensitivity enhancements of greater than 10 000 fold have been reported [38].

1.8.3 Hydrostatic injection vs electrokinetic injection

Hydrostatic injection involves the syphoning of the sample and has been reported to be reproducible with values between 1.3 % and 0.55 % RSD, for migration time [39]. Electrokinetic injection involves the application of a potential (eg. 5 kV) to the system while the injection end of the capillary is submersed in the sample vial. It has the advantage of offering improvements in detection sensitivity as it stacks the analytes during the injection stage. Common, improvements in LOD for electrokinetic injection have been reported to be at least 10 fold over pressure injection [40]. However, electrokinetic injection suffers from poor reproducibility (migration time and peak area) when there are large differences in the sample matrix, particularly with respect to conductivity. Breadmore et al reviewed various stacking methodologies including electrokinetic injection [41]. Both forms of injection will be utilised throughout this work.
Figure 1-7- Schematic diagram of ITP being applied to stack a sample. (a) Sample introduction into the capillary. The sample contains both a leading and terminating co-ion; (b) Potential application, initiating the stacking procedure. Two bands of anions with differing mobility result; (c) Each sample zone migrates individually towards the detector end of the capillary. The stacking procedure is complete and a normal CZE separation results.
1.9 Applications of CZE to the analysis of relevant alumina industry samples

IC has been the technique most commonly used for the analysis of ions of interest in Bayer liquors [41-44]. In addition, gas chromatography (methylation) has been employed for the analysis of oxalate in alumina samples. However, CZE provides a viable alternative method as it is not hampered by many of the interferences encountered by conventional methods. Significant expense is often incurred for IC analysis as the high aluminium content and alkalinity contaminate the suppressors, requiring replacement. Studies have shown that successful Bayer liquor analysis is possible with molybdate and chromate buffered CZE systems [41,43]. This section provides an overview of this work.

Ion chromatography is the method most widely used for the analysis of Bayer liquor samples, particular for their oxalate content. Only a limited number of studies have been conducted with respect to Bayer liquor analysis using CZE and as previously mentioned chromate has been the probe of choice for the majority of work. Jackson [44] provided an excellent comparison between IC and CZE analysis of Bayer liquors. He showed that CZE offered increased separation efficiency with the use of a relatively simple 5 mM chromate, 0.5 mM CIA-Pak OFM anion-BT (pH 8.0) BGE. The concentration of chloride, sulphate and oxalate was determined simultaneously, which was not possible by IC. However, Jackson found that IC permitted better reproducibility with respect to retention time. Due to matrix interferences for IC analysis precision for oxalate content was much improved when using CZE. An average of 1.71% R.S.D. and 2.85% R.S.D. for oxalate content was obtained for CZE and IC, respectively. Chovancek et al. [42] found that a system consisting of 5 mM molybdate buffered with 20 mM diethanolamine (DEA) to a pH 9.2 enabled excellent precision of < 0.07% and < 2.04% for migration time and peak area, respectively. A Bayer liquor sample diluted 1000 fold gave LOD for oxalate of < 0.51 mg/L. However, the authors noted the need to use lower dilutions of the sample (100 fold) for analytes present at low concentrations. However, it was evident for a
100 fold dilution that de-stacking and electro-migrational dispersion was occurring.

An area that has not experienced much growth in the analysis of Bayer liquors by CZE is the application of alternative stacking methodologies. Chovancek et al [42] used FASS to increase sensitivity with respect to a Bayer liquor process sample. However, other stacking methods such as large volume sample stacking (LVSS) and ITP have not yet been sufficiently explored. As has been discussed in sections 1.7.2 ITP has offered further improvements in LOD with a host of different applications and, therefore, should be thoroughly investigated for their applications to alumina industry samples such as Bayer liquors.
Aim

Light emitting diodes offer improvements in sensitivity by reducing baseline noise. There are many probes that have been identified for use with indirect detection, however, many of these are not compatible with LEDs. This project aims to identify probes that can be used with LEDs for the indirect detection of anions. Compounds that absorb within the region of 370-450 nm will be targeted as there are so far no LEDs available below this region and above this, involves the use of bulky dyes which has previously been investigated.
2.1 Instrumentation

2.1.1 Capillary electrophoresis instrumentation
A Waters Quanta 4000 (Milford, MA, USA) CE unit interfaced with Varian Star data acquisition software was used for the study. The instrument was equipped with either a deuterium lamp (214 nm or 254 nm) or LED light sources of varying wavelengths (370, 400 and 428 nm). A run voltage of -25 kV was used for all runs unless otherwise stated, and at room temperature (~22°C).

Fused-silica capillaries (Poly-micro Technologies, Phoenix, AZ, USA) with an internal diameter of either 50 or 75 μm and 60 cm (52 cm effective length) in length were used for this study, unless otherwise stated. Detection windows were made by burning the outer coating of the capillary with a butane torch. The window was subsequently cleaned using a lint free wipe.

2.1.2 Spectrophotometer instrumentation
A Varian Cary UV-VIS instrument (Varian Australia Pty. Ltd.) was used to obtain spectrophotometric data for each probe. This instrument utilised a double beam and 1 cm quartz cell for both sample and reference reservoirs. The spectral band width was set at 2.00 nm. The signal averaging time was 0.100 seconds with a data interval of 1.0 nm, resulting in a scan rate of 600 nm/minute.
2.2 Reagents

All chemicals used in this study were of the highest obtainable grade. All analytes and electrolytes were made up in water from a Millipore (Bedford Massachusetts, USA) Milli-Q water system.

2.2.1 Probes

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Formula</th>
<th>Supplier</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium trioxide</td>
<td>CrO$_3$</td>
<td>Ajax Chemicals</td>
<td>99%</td>
</tr>
<tr>
<td>3,5-dinitrosalicylic acid</td>
<td>C$_7$H$_4$N$_2$O$_7$</td>
<td>BDH Ltd</td>
<td>97%</td>
</tr>
<tr>
<td>5-nitrosalicylic acid</td>
<td>C$_7$H$_5$NO$_5$</td>
<td>Fluka</td>
<td>98%</td>
</tr>
<tr>
<td>3-hydroxy-4-nitrobenzoic acid</td>
<td>C$_7$H$_3$NO$_5$</td>
<td>Fluka</td>
<td>97%</td>
</tr>
<tr>
<td>4-nitrophthalic acid</td>
<td>C$_8$H$_5$NO$_6$</td>
<td>Fluka</td>
<td>97%</td>
</tr>
<tr>
<td>2,4-dinitrobenzoic acid</td>
<td>C$_7$H$_4$N$_2$O$_6$</td>
<td>Aldrich</td>
<td>96%</td>
</tr>
<tr>
<td>3,5-dinitrobenzoic acid</td>
<td>C$_7$H$_4$N$_2$O$_6$</td>
<td>Aldrich</td>
<td>99%</td>
</tr>
<tr>
<td>4-hydroxy-3,5-dinitrobenzoic acid</td>
<td>C$_7$H$_4$N$_2$O$_7$</td>
<td>Aldrich</td>
<td>95%</td>
</tr>
<tr>
<td>4-hydroxy-3-nitrobenzoic acid</td>
<td>C$_7$H$_3$NO$_5$</td>
<td>Aldrich</td>
<td>98%</td>
</tr>
<tr>
<td>2-nitrophenol (o-nitrophenol)</td>
<td>C$_8$H$_5$NO$_3$</td>
<td>Aldrich</td>
<td>98%</td>
</tr>
<tr>
<td>4-nitrophenol (p-nitrophenol)</td>
<td>C$_8$H$_3$NO$_3$</td>
<td>Aldrich</td>
<td>99%</td>
</tr>
<tr>
<td>3-nitrophthalic acid</td>
<td>C$_8$H$_5$NO$_6$</td>
<td>Aldrich</td>
<td>99%</td>
</tr>
</tbody>
</table>

2.2.2 Anionic analytes

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Formula</th>
<th>Supplier</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium formate</td>
<td>HCOONa</td>
<td>Sigma</td>
<td>99.2%</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>NaCl</td>
<td>Scharlau Chemie</td>
<td>99.8%</td>
</tr>
<tr>
<td>Sodium pentanesulphonate</td>
<td>C$<em>3$H$</em>{11}$O$_3$SNa</td>
<td>Fluka</td>
<td>99%</td>
</tr>
<tr>
<td>Sodium octanesulphonate</td>
<td>C$<em>8$H$</em>{17}$O$_3$SNa.H$_2$O</td>
<td>Fluka</td>
<td>99%</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>NaNO$_3$</td>
<td>Ajax Chemicals</td>
<td>99%</td>
</tr>
<tr>
<td>Sodium sulphate</td>
<td>Na$_2$SO$_4$</td>
<td>BDH Ltd</td>
<td>99.5%</td>
</tr>
<tr>
<td>Sodium oxalate</td>
<td>(COONa)$_2$</td>
<td>BDH Ltd</td>
<td>99%</td>
</tr>
<tr>
<td>Sodium benzoate</td>
<td>C$_8$H$_5$COONa</td>
<td>BDH Ltd</td>
<td>99.5%</td>
</tr>
<tr>
<td>Sodium succinate</td>
<td>(CH$_3$COONa)$_2$.6H$_2$O</td>
<td>BDH Ltd</td>
<td>99%</td>
</tr>
<tr>
<td>tri-Sodium orthophosphate</td>
<td>Na$_3$PO$_4$.12H$_2$O</td>
<td>BDH Ltd</td>
<td>98%</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>CH$_3$COONa</td>
<td>BDH Ltd</td>
<td>99.5%</td>
</tr>
</tbody>
</table>
2.2.3 Other chemicals

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Formula</th>
<th>Supplier</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris(hydroxymethyl)aminomethane</td>
<td>C₄H₁₁NO₃</td>
<td>Sigma</td>
<td>99.9%</td>
</tr>
<tr>
<td>Diethanolamine</td>
<td>(HOCH₂CH₂)₂NH</td>
<td>Ajax Chemicals</td>
<td>98.5%</td>
</tr>
<tr>
<td>Tetradecyltrimethylammonium bromide (TTAB)</td>
<td>C₁₇H₃₈NBr</td>
<td>Sigma</td>
<td>99%</td>
</tr>
<tr>
<td>Poly(diallylimethylammonium chloride) (PDDAC)</td>
<td>C₈H₁₆ClN</td>
<td>Aldrich</td>
<td>20% in H₂O</td>
</tr>
<tr>
<td>Mesityl oxide</td>
<td>C₆H₁₀O</td>
<td>Aldrich</td>
<td>90%</td>
</tr>
</tbody>
</table>

2.3 Preparations

2.3.1 Preparation of analytes

Individual standard stock solutions of analytes were prepared by dissolving the required amount of the sodium salt to make a concentration of 100 mM. Two anion mixes were also prepared. Anion mix 1 with chloride, nitrate, sulphate, oxalate, formate, pentanesulphonate and octanesulphonate and anion mix 2 contained chloride, nitrate, sulphate, oxalate, formate, succinate, phosphate, acetate, pentanesulphonate, octanesulphonate and benzoate.

2.3.2 Preparation of BGE’s

The required amount of probe was dissolved in approximately 60 ml milli-Q water at room temperature. A calibrated pH meter was used to monitor the pH of the solution as additions of Tris were made, up to pH 8.1 (pKa = 8.1 @ 22°C). The solution was made up to the required volume. For the BGE’s containing an EOF modifier (TTAB), the required amount was added prior to making up to volume.

2.4 Experimental Methods

2.4.1 Spectrophotometric measurements

Each potential probe was subject to measurements to find their lambda max and corresponding molar absorptivity. These measurements were carried out by dissolving the required amount of probe into Milli-Q water and adjusting the pH to 8.1 with sodium hydroxide. A wavelength scan from 200 nm to 700 nm was recorded.
2.4.2 Capillary conditioning
Two EOF modifiers were utilised throughout this work, TTAB and PDDAC. TTAB was used at a concentration of 0.5 mM and formed part of the BGE. A 3 minute capillary flush with the BGE in between each run was required to condition the capillary. A 0.5% PDDAC solution was applied to the capillary as a pre-run flush following a 1 minute flush of both 0.1 M NaOH and milli-Q water. Due to PDDAC’s strong adherence to the capillary wall it was applied at the beginning of a set of runs, with the system only requiring a 3.0 minute BGE purge between runs. For bare capillary work the system was purged with 0.1 M NaOH and milli-Q water for 1 minute each prior to a set of runs, with the system only requiring a 3.0 minute BGE purge between runs.

2.4.3 Mobility measurements
Each probe was injected onto the column as an analyte and their migration time recorded. For co-EOF work PDDAC or TTAB was used as described in section 2.4.2. A bare capillary was employed for counter-EOF work and was conditioned as per section 2.4.2. Mesityl oxide was used as the EOF marker. Each probe was monitored at 214 nm using a UV source.

2.4.4 Probe concentration
The optical limit for the selected probes was found by making up a range of buffers containing 5 mM to 60 mM of each probe. Each buffer was purged through a 50 μm capillary and using the best suited LED (370, 400 or 428 nm) to the probe, the corresponding absorbance recorded.
2.5 Calculations

Effective mobility’s were calculated based on the following calculation:

\[
\mu_{\text{eff}} = \left( \frac{l_{\text{eff}}}{t_{\text{mig}}} \right) - \left( \frac{l_{\text{eff}}}{t_{\text{eof}}} \right) \times \frac{l_{\text{tot}}}{V}
\]  

(2-1)

where \( \mu_{\text{eff}} \) = effective mobility of analyte (cm\(^2\)/V.s)

\( l_{\text{eff}} \) = length of capillary to detection window (cm)

\( l_{\text{tot}} \) = total length of capillary (cm)

\( t_{\text{mig}} \) = migration time of analyte (seconds)

\( t_{\text{eof}} \) = migration time of EOF (seconds)

\( V \) = applied voltage (V)

Separation efficiencies were based on peak width at half height using the following equation:

\[
N = 5.55 \left( \frac{t_{\text{mig}}}{t_{1/2}} \right)^2
\]

(2-2)

where \( N \) = number of theoretical plates

\( t_{\text{mig}} \) = analyte migration time (seconds)

\( t_{1/2} \) = peak width at half height (seconds)

Noise was calculated based on inspection of a stable part of the baseline. Detection limits were calculated at three times baseline noise.
3.1 Choice of Probe and buffering

3.1.1 Probe selection
The probes (listed in table 3-1) that were chosen for this study were selected based on some preliminary data. Chemical data bases were used to identify a series of compounds that would potentially fulfil the following criteria. Each probe had to absorb in the low LED region (370-450 nm), exhibit a relatively high molar absorptivity (> chromate), have a moderate to high mobility and be inexpensive, pure and relatively non-toxic.

Each probe was required to absorb most strongly between 370 nm and 450 nm. This range was chosen as there are so far no commercially available LED’s below 370 nm and above 450 nm, probes tend to be bulky compounds (dyes). While dyes are highly absorbing, they have been shown to have an affinity for the capillary wall [45], resulting in non-reproducible EOF’s and increased baseline noise. As benzene compounds with a substituted nitro group/s resulted in yellow coloured aqueous solutions and absorbed in the 400 nm region, a number of these were investigated. The location of the functional nitro groups was also a consideration; preliminary work indicated that isomers exhibited very different spectral properties (see section 3.2.1). Therefore, all available isomers were investigated to ensure any variation between isomers was accounted for.

The corresponding molar absorptivity of each probe within the given range was critical in obtaining adequate LOD. Thus, given the common probe, chromate had an absorptivity of 3960 Lmol⁻¹cm⁻¹ at 371 nm [10] it was deemed that each of the alternate probes had to exhibit a comparable if not improved molar absorptivity to match or better the detection limits associated with chromate.
The chosen probes were evaluated for their potential mobility, taking into account their functionality and ability to ionise in a basic matrix. Both singly and doubly charged species were considered to provide a range of mobilities from which to choose from and to increase the likelihood of being able to match the mobilities of the intended analytes. Thus, it was intended that a fast and moderately mobile probe would be chosen for analysis.

The cost and purity of each selected probe enabled the elimination of several probes before any experimental work took place. The compounds in table 3-1 are organised into their isomeric groups. For example, 3,5-dinitrosalicylic acid and its isomer 4-hydroxy-3,5-dinitrobenzoic acid are at the top of the table. While both of these isomers were purchased, only two of three available isomers of 2,4-dinitrobenzoic acid were acquired; 2,5-dinitrobenzoic acid was omitted based on both cost ($136.00/1 g) and impurity (presence of other isomers) and 3,4-dinitrobenzoic acid was omitted based on significant cost ($43.00/250 mg). For similar reasons only three of four isomers of 5-nitrosalicylic acid and two of the three isomers of p-nitrophenol were purchased for evaluation. All prices were current up to 30/10/06 from www.sigma-aldrich.com.

The toxicity of each probe was also considered. Toxicity was explored by studying the MSDS obtained from Sigma-Aldrich and Chemwatch. Chromate’s toxicity was firstly considered to help gauge risks involved for the alternative probes. With respect to Chemwatch, chromate was given a toxicity level of 4 (extreme toxicity) which is representative of significant mutagenic and cumulative effects associated with chromate. All the compounds, including chromate contained general risks such as irritant to the eye’s (R36), irritant to the respiratory tract (R37) and irritant to the skin (R38). Therefore, based on the information obtained at this stage 2,4-dinitrophenol was eliminated from any further work as it possessed a toxicity similar to that of chromate, Chemwatch rating = 4. The highest toxicity rating given to any of the remaining alternative probes was 3 (high toxicity) for 3,5-dinitrosalicylic acid. Thus, the remaining probes (including 3,5-dinitrosalicylic acid) presented risks that are typical of chemicals in common use and therefore were deemed safe for further evaluation.
Key experimental data (spectral and mobility) was collected for the potential probes listed in table 3-1.

3.1.2 Buffering and reversal agent

As discussed in section 1.5.1 counter-ionic buffering provides an adequate method of buffering by utilising the acidic potential of the probe itself and avoids the introduction of co-ions into the electrolyte. It was found that the commonly used counter ion, DEA (diethanolamine) [4,10,30] was troublesome to work with at room temperature and in addition had a Chemwatch rating = 2 (moderate toxicity), therefore, warranting the use of a more user friendly, safer counter ion. Tris(hydroxymethyl)aminomethane was chosen as the counter-ionic buffering component as it was easier to work with, was less toxic than DEA and had shown promise in some previous applications [28,46]. In addition, it had a pKb of 8.1, which was adequate for ionisation of the selected probes. However, a borate buffer (pH 10) was also utilised to determine if an increase in pH impacted significantly on the ionisation (and hence mobility) of the probes.

Commonly, in anion analysis the co-EOF mode is employed to reduce analysis time. Probably the most commonly used reversal agent is tetradecyltrimethylammonium bromide (TTAB) [4]. However, due to its permanency on the capillary (see section 1.2) polydiallyldimethylammonium chloride (PDDAC) was employed throughout this study.

3.2 Experimental data for selected probes

3.2.1 Spectrophotometric data

The UV-VIS spectrum was recorded for each of the eleven probes listed in table 3-1 to determine their molar absorptivity at the LED wavelengths of interest (370, 400, 426 nm). The $\lambda_{\text{max}}$ and strongest absorbing wavelength in the LED region were recorded for each probe, table 3-2.
Two probes did not absorb in this region, 2,4-dinitrobenzoic acid and 3-nitrophthalic acid. However, as technology increases, LED’s emitting significantly in the low UV range are quite possible and may allow strong absorbing probes such as 2,4-dinitrobenzoic acid to be considered. In addition, given that 2,4-dinitrobenzoic acid exhibited a high molar absorptivity of 13904 Lmol\(^{-1}\)cm\(^{-1}\) at 249 nm, it warrants consideration for use with a 254 nm UV source.

The majority of the probes were found to have a strong absorbance (> chromate) close to or within the range of 370-450 nm. Three probes were not investigated further based on their limited absorptivity in this region. These probes included 3,5-dinitrobenzoic acid, \(\alpha\)-nitrophenol and 4-nitrophthalic acid. 3,5-dinitrobenzoic acid exhibited absorbance at two usable wavelengths 370 nm and 400.0 nm, however, absorbances of only 115 Lmol\(^{-1}\)cm\(^{-1}\) and 77 Lmol\(^{-1}\)cm\(^{-1}\) were recorded. \(\alpha\)-nitrophenol absorbed at 426.0 nm, however, at this wavelength the molar absorptivity was only 865 Lmol\(^{-1}\)cm\(^{-1}\). 4-nitrophthalic acid displayed only limited molar absorptivity at 370 nm (20 Lmol\(^{-1}\)cm\(^{-1}\)).

Furthermore, the difference in spectral properties between isomers re-enforced the necessity to explore all available isomers. While the shift in \(\lambda_{\text{max}}\) was somewhat expected, the large difference in molar absorptivity was quite interesting. \(p\)-nitrophenol exhibited a 20 fold increase in molar absorptivity in the LED region of interest compared to \(\alpha\)-nitrophenol. Similarly, 3,5-dinitrosalicylic acid displayed a 2 fold increase in molar absorptivity in the LED region compared to 4-hydroxy-3,5-dinitrobenzoic acid.

The eleven probes were now reduced to six probes based on their spectral data. These included 5-nitrosalicylic acid, 3-hydroxy-4-nitrobenzoic acid, 4-hydroxy-3-nitrobenzoic acid, 3,5-dinitrosalicylic acid, 4-hydroxy-3,5-dinitrobenzoic acid and \(p\)-nitrophenol. The six probes were then evaluated based on their mobility data, which forms the discussion in the following section.
3.2.2 Mobility data

The mobility of each probe was determined in a number of buffer systems. Each probe was injected into each system and detected as an analyte using a 214 nm UV source and filter. Mesityl oxide was used as the EOF marker [47]. As previously discussed, chromate is the most commonly used probe in anion analysis, therefore, its mobility was determined for comparison purposes.

The mobility of all the probes was determined on a PDDAC system using a HCl/Tris buffer system (pH 8.10) and is presented in table 1-3. The order of the probes with respect to their mobility (fastest to slowest) was: 3-hydroxy-4-nitrobenzoic acid, 4-hydroxy-3-nitrobenzoic acid, 3-nitrophthalic acid, 4-nitrophthalic acid, 3,5-dinitrosalicylic acid, 4-hydroxy-3,5-dinitrobenzoic acid, 2,4-dinitrobenzoic acid, 3,5-dinitrobenzoic acid, 5-nitrosalicylic acid, p-nitrophenol and o-nitrophenol. The mobility of the probes ranged from $-45.3 \times 10^{-5}$ cm$^2$/V.s to $-24.3 \times 10^{-5}$ cm$^2$/V.s. Noticeably, the isomers tended to display similar mobility to one another. For example o-nitrophenol and p-nitrophenol displayed a mobility within $3 \times 10^{-5}$ cm$^2$/V.s of each other. Similarly, 3-nitrophthlic acid and 4-nitrophthalic acid were within $2 \times 10^{-5}$ cm$^2$/V.s of each other. All isomers were within $3.0 \times 10^{-5}$ cm$^2$/V.s of each other, with the exception of 5-nitrosalicylic acid which was $15 \times 10^{-5}$ cm$^2$/V.s from 3-hydroxy-4-nitrobenzoic acid. Therefore it can be concluded that the different arrangement of the acidic functional groups only had limited effect on the electrophoretic mobility of the isomers.

All the probes had significantly lower mobilities than chromate. The fastest of the probes 2-hydroxy-4-nitrobenzoic acid, had a mobility $20 \times 10^{-5}$ cm$^2$/V.s less than chromate. The high mobility of chromate is a key factor in its ability to resolve and detect the fast anions (Cl, NO$_3^-$ and SO$_4^{2-}$). Therefore, the lower mobilities exhibited by the probes in this study indicated that they may not be suitable for fast anion analysis.
Chapter 3 Characterisation of probes

The mobility obtained for chromate in the PDDAC system was $64.1 \times 10^{-5}$ cm$^2$/V.s, which was lower than a common mobility quoted for chromate in a wall coated system ($-73.4 \times 10^{-5}$ cm$^2$/V.s, [6]). Therefore, to ensure that the mobility that was being obtained was indeed independent of the EOF modifier, a well used modifier, TTAB was employed (table 1-3). The mobility of each probe did not deviate significantly from those obtained for the PDDAC system. The largest change in mobility was no more than $4.5 \times 10^{-5}$ cm$^2$/V.s which was recorded for 4-nitrophthalic acid. Therefore the choice of wall reversal agent did not significantly alter the mobilities of the probes.

A bare capillary was also explored with a HCl/tris buffer (pH 8.1). The mobility obtained for chromate ($-77.2 \times 10^{-5}$ cm$^2$/V.s) was significantly greater but comparable to that quoted in the literature for a bare capillary system ($-81 \times 10^{-9}$ m$^2$/V.s, [47]). Interestingly, the mobility of the probes in this study increased only slightly with the exception of 4-hydroxy-3,5-dinitrobenzoic acid and 2,4-dinitrobenzoic acid, which decreased, further indicating that the probes under investigation had significantly lower mobilities relative to chromate. Finally, a high pH buffer was employed to determine if increasing the pH would increase the ionisation of the acidic sites and hence mobility of each probe.

A borate/NaOH buffer system was used to determine the mobility of the probes on a bare capillary. The mobility for each probe obtained on this system was compared to those obtained from the bare capillary using the HCl/Tris buffer. p-nitrophenol and o-nitrophenol were the only group of isomers to display higher mobility in this system. The rest of the probes exhibited lower mobility in this system with the exception of 3,5-dinitrosalicylic acid. This could be explained by looking at the structures of each probe. Both p-nitrophenol and o-nitrophenol are singly charged, which may cause them to display a more dramatic mobility change in a higher pH buffer than the doubly charged species. However, from the results in table 3-3 it can be concluded that the selected probes are all significantly slower than chromate and are almost unaffected by the wall coating and pH variations.
Chapter 3 Characterisation of probes

Having evaluated the spectral and mobility data, six probes were selected for further evaluation. However, as 5-nitrosalicylic acid had a similar mobility to \( p \)-nitrophenol but had a molar absorptivity 7 times less, it was omitted from further study. Therefore, the five probes investigated further (chapter 4) were 3,5-dinitrosalicylic acid, \( p \)-nitrophenol, 4-hydroxy-3,5-dinitrobenzoic acid, 4-hydroxy-3-nitrobenzoic acid and 3-hydroxy-4-dinitrobenzoic acid.
<table>
<thead>
<tr>
<th>Probe</th>
<th>Structure</th>
<th>Probe</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,5-dinitrosalicylic acid</td>
<td><img src="image1" alt="Structure" /></td>
<td>4-hydroxy-3,5-dinitrobenzoic acid</td>
<td><img src="image2" alt="Structure" /></td>
</tr>
<tr>
<td>2-nitrophenol (o-nitrophenol)</td>
<td><img src="image3" alt="Structure" /></td>
<td>4-nitrophenol (p-nitrophenol)</td>
<td><img src="image4" alt="Structure" /></td>
</tr>
<tr>
<td>2,4-dinitrobenzoic acid</td>
<td><img src="image5" alt="Structure" /></td>
<td>3,5-dinitrobenzoic acid</td>
<td><img src="image6" alt="Structure" /></td>
</tr>
<tr>
<td>3-nitrophthalic acid</td>
<td><img src="image7" alt="Structure" /></td>
<td>4-nitrophthalic acid</td>
<td><img src="image8" alt="Structure" /></td>
</tr>
<tr>
<td>5-nitrosalicylic acid</td>
<td><img src="image9" alt="Structure" /></td>
<td>4-hydroxy-3-nitrobenzoic acid</td>
<td><img src="image10" alt="Structure" /></td>
</tr>
<tr>
<td>3-hydroxy-4-nitrobenzoic acid</td>
<td><img src="image11" alt="Structure" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 3-2: Molar absorptivity and lambda max data for the eleven probes

<table>
<thead>
<tr>
<th>Probe</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$\varepsilon$ (Lmol$^{-1}$cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-nitrosalicylic acid (2-hydroxy-5-nitrobenzoic acid)</td>
<td>202.0 370.0</td>
<td>15820 2300</td>
</tr>
<tr>
<td>3-hydroxy-4-nitrobenzoic acid</td>
<td>233.0 426.0</td>
<td>14288 4082</td>
</tr>
<tr>
<td>4-hydroxy-3-nitrobenzoic acid</td>
<td>263.0 400.0</td>
<td>19131 3922</td>
</tr>
<tr>
<td>3,5-dintrosalicylic acid (2-hydroxy-3,5-dinitrobenzoic acid)</td>
<td>368.0 370.0</td>
<td>12838 12811</td>
</tr>
<tr>
<td>4-hydroxy-3,5-dinitrobenzoic acid</td>
<td>254.0 426.0</td>
<td>22200 6560</td>
</tr>
<tr>
<td>p-nitrophenol</td>
<td>399.0 400.0</td>
<td>16402 16401</td>
</tr>
<tr>
<td>o-nitrophenol</td>
<td>227.0 426.0</td>
<td>3060 825</td>
</tr>
<tr>
<td>2,4-dinitrobenzoic acid</td>
<td>249.0</td>
<td>13904</td>
</tr>
<tr>
<td>3,5-dinitrobenzoic acid</td>
<td>370.0 400.0</td>
<td>115 77</td>
</tr>
<tr>
<td>3-nitrophthalic acid</td>
<td>205.0</td>
<td>273</td>
</tr>
<tr>
<td>4-nitrophthalic acid</td>
<td>205.0 370.0</td>
<td>14290 20</td>
</tr>
</tbody>
</table>
Table 3-3: Mobility data for the eleven probes compared to chromate

<table>
<thead>
<tr>
<th>Probe</th>
<th>PDDAC $\mu$ep ($10^{-5}$ cm²/V.s) HCl:Tris pH 8.11</th>
<th>TTAB $\mu$ep ($10^{-5}$ cm²/V.s) Tris:HCl pH 8.11</th>
<th>Bare capillary $\mu$ep ($10^{-5}$ cm²/V.s) Tris:HCl pH 8.11</th>
<th>Bare capillary $\mu$ep ($10^{-5}$ cm²/V.s) Borate:NaOH pH 10:00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromate</td>
<td>-64.1</td>
<td>-67.3</td>
<td>-77.2</td>
<td>-</td>
</tr>
<tr>
<td>5-nitrosalicylic acid</td>
<td>-30.3</td>
<td>-</td>
<td>-34.5</td>
<td>-32.9</td>
</tr>
<tr>
<td>3-hydroxy-4-nitrobenzoic acid</td>
<td>-45.3</td>
<td>-</td>
<td>-47.5</td>
<td>-44.7</td>
</tr>
<tr>
<td>4-hydroxy-3-nitrobenzoic acid</td>
<td>-42.8</td>
<td>-41.0</td>
<td>-48.2</td>
<td>-42.7</td>
</tr>
<tr>
<td>3,5-dinitrosalicylic acid</td>
<td>-39.0</td>
<td>-</td>
<td>-44.1</td>
<td>-45.4</td>
</tr>
<tr>
<td>4-hydroxy-3,5-dinitrobenzoic acid</td>
<td>-36.6</td>
<td>-37.0</td>
<td>-35.9</td>
<td>-33.8</td>
</tr>
<tr>
<td>o-nitrophenol</td>
<td>-24.3</td>
<td>-</td>
<td>-26.4</td>
<td>-31.4</td>
</tr>
<tr>
<td>p-nitrophenol</td>
<td>-26.9</td>
<td>-21.1</td>
<td>-29.5</td>
<td>-30.7</td>
</tr>
<tr>
<td>2,4-dinitrobenzoic acid</td>
<td>-33.1</td>
<td>-28.9</td>
<td>-30.1</td>
<td>-28.6</td>
</tr>
<tr>
<td>3,5-dinitrobenzoic acid</td>
<td>-31.6</td>
<td>-28.5</td>
<td>-32.4</td>
<td>-28.1</td>
</tr>
<tr>
<td>3-nitrophthalic acid</td>
<td>-41.8</td>
<td>-46.1</td>
<td>-49.3</td>
<td>-</td>
</tr>
<tr>
<td>4-nitrophthalic acid</td>
<td>-40.0</td>
<td>-44.5</td>
<td>-47.2</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: - denotes no definite peak for probe, hence no data was collected
Chapter 4

Probe performance

4.1 Performance of selected probes

The probes of choice p-nitrophenol, 3,5-dinitrosalicylic acid, 4-hydroxy-3,5-dinitrobenzoic acid, 3-hydroxy-4-nitrobenzoic acid and 4-hydroxy-3-nitrobenzoic acid were evaluated based on their ability to separate an anion mix containing chloride, nitrate, sulphate, oxalate, formate, pentanesulphonate and octanesulphonate (anion mix 1). The BGE's consisted of 20 mM concentrations of each probe, buffered to pH 8.1 with Tris. A 60 cm (52 cm, effective length) x 75 μm (I.D.) fused silica capillary coated with PDDAC was used for this experiment. A 10 second hydrostatic injection at 10 cm was used with a negative run potential of 25 kV. Limits of detection and %RSD for migration time were calculated from the resulting electropherograms (table 4-1).

All seven analytes were baseline resolved in the p-nitrophenol system (figure 4-1a). The migration times were very fast with all seven analytes eluting before 5 minutes, compared to about 7 or 8 minutes for the other probes. The early eluting analytes such as chloride, nitrate and sulphate exhibited some fronting which can be attributed to the large difference in mobility between them and p-nitrophenol. By contrast the sharp, symmetrical peaks for the slower analytes pentanesulphonate (-29.7x10^-5 cm^2/Ns, [48]) and octanesulphonate indicated that these analytes were better matched to the mobility of the probe, (-26.9x10^-5 cm^2/Ns, found in chapter 3). The calculated LOD also highlight the effect of mobility matching and peak sharpness. The LOD for the slower probes pentanesulphonate and octanesulphonate were 0.011 mM for both. The LOD for the faster analytes were, as expected, greater with chloride, nitrate and sulphate exhibiting LOD of 0.050, 0.047 and 0.030 mM, respectively. A baseline noise of 0.04 mV was recorded for the separation and the system showed no sign of baseline drift.

The 4-hydroxy-3,5-dinitrobenzoic acid system provided better resolution of all seven analytes than p-nitrophenol (figure 4-1b). The migration times for all
seven analytes were longer than for the \( p \)-nitrophenol system with all the analytes eluting after 7.5 minutes. Chloride, nitrate and sulphate exhibited better peak shape in this BGE than the \( p \)-nitrophenol BGE. The taller more symmetrical peak profile indicated that these faster analytes were better matched to the faster probe 4-hydroxy-3,5-dinitrobenzoic acid \((-36.6 \times 10^{-5} \text{cm}^2/\text{Vs}, \text{found in chapter 3})\). In contrast to \( p \)-nitrophenol, the peaks for the slower analytes, pentanesulphonate and octanesulphonate exhibited some tailing. The LOD for all seven analytes compared to those obtained for \( p \)-nitrophenol were poorer. This can be attributed to the 2.5 fold decrease in molar absorptivity associated with 4-hydroxy-3,5-dinitrobenzoic acid at the usable LED wavelength of 426 nm \((\varepsilon = 6560 \text{ L.mol}^{-1}\text{cm}^{-1})\) compared to \( p \)-nitrophenol \((400 \text{ nm, } \varepsilon = 16401 \text{ L.mol}^{-1}\text{cm}^{-1})\). 4-hydroxy-3,5-dinitrobenzoic acid displayed the best reproducibility (not optimised) of all the probes with a %RSD for migration time of less than 1 % for the majority of analytes. The baseline noise which was monitored during the separation was 0.03 mV and no significant baseline drift was recorded.

The seven analytes were also fully resolved for the BGE containing 4-hydroxy-3-nitrobenzoic acid. However, the system displayed poor stability with a fluctuating baseline (0.075 mV) during the run and this was evident by the poor LOD obtained for the analytes, \( \geq 0.02 \text{ mM} \text{} \) \( \text{table 4-1} \). Similarly, 3-hydroxy-4-nitrobenzoic acid displayed good resolution of all seven analytes, however, due to a fluctuating baseline of 1.1 mV \( \text{figure 4-1c} \), even higher LOD were recorded, \( \geq 0.5 \text{ mM} \text{} \) \( \text{table 4-1} \). 3,5-dinitrosalicylic acid was the poorest performing probe with some anions not detected and a drifting baseline from 835 – 880 mV over the course of a run (electropherogram not shown). This and difficulty in obtaining reproducible mobility data \( \text{chapter 3} \) suggested that it interacted strongly with the capillary wall. Therefore, based on baseline stability and LOD, \( p \)-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid were chosen for further work.
Figure 4-1 – Electropherograms of the separation of 7 anions (1-chloride, 2-nitrate, 3-sulphate, 4-oxalate, 5-formate, 6-pentanesulphonate, 7-octanesulphonate) in a BGE with different probes (a) p-nitrophenol, (b) 4-hydroxy-3,5-dinitrobenzoic acid, (c) 4-hydroxy-3-nitrobenzoic acid, (d) 3-hydroxy-4-nitrobenzoic acid. Run conditions as described in section 4.1.
Table 4-1: Migration time and LOD data for anions separated using five different probes. Separations completed on a 75 µm capillary with an applied voltage of 25 kV and an injection time of 10 seconds. All probes were present in their buffered form with Tris (pH of 8.10) and at a concentration of 20 mM.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Migration time, s</th>
<th>LOD (mM)</th>
<th>Migration time, s</th>
<th>LOD (mM)</th>
<th>Migration time, s</th>
<th>LOD (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>2.46 (1.32)</td>
<td>0.050</td>
<td>2.81 (1.86)</td>
<td>0.04</td>
<td>2.86 (1.32)</td>
<td>0.04</td>
</tr>
<tr>
<td>Nitrate</td>
<td>2.61 (1.27)</td>
<td>0.047</td>
<td>3.07 (0.85)</td>
<td>0.04</td>
<td>3.00 (2.10)</td>
<td>0.2</td>
</tr>
<tr>
<td>Sulphate</td>
<td>2.70 (1.39)</td>
<td>0.030</td>
<td>3.37 (0.95)</td>
<td>0.04</td>
<td>3.18 (1.59)</td>
<td>0.2</td>
</tr>
<tr>
<td>Oxalate</td>
<td>2.81 (1.34)</td>
<td>0.029</td>
<td>3.42 (0.98)</td>
<td>0.04</td>
<td>3.32 (2.45)</td>
<td>0.2</td>
</tr>
<tr>
<td>Formate</td>
<td>3.07 (1.85)</td>
<td>0.032</td>
<td>3.87 (0.84)</td>
<td>0.05</td>
<td>3.74 (2.97)</td>
<td>0.2</td>
</tr>
<tr>
<td>Pentanesulphonate</td>
<td>4.31 (2.79)</td>
<td>0.011</td>
<td>6.50 (0.89)</td>
<td>0.05</td>
<td>6.00 (6.66)</td>
<td>0.2</td>
</tr>
<tr>
<td>Octanesulphonate</td>
<td>4.72 (3.06)</td>
<td>0.011</td>
<td>7.49 (1.98)</td>
<td>0.07</td>
<td>6.78 (3.72)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

4-Hydroxy-3-nitrobenzoic acid

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Migration time, s</th>
<th>LOD (mM)</th>
<th>Migration time, s</th>
<th>LOD (mM)</th>
<th>Migration time, s</th>
<th>LOD (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>2.89 (0.81)</td>
<td>0.050</td>
<td>3.05 (1.34)</td>
<td>0.4</td>
<td>3.36 (2.15)</td>
<td>1.2</td>
</tr>
<tr>
<td>Nitrate</td>
<td>3.00 (2.10)</td>
<td>0.04</td>
<td>3.05 (1.34)</td>
<td>0.07</td>
<td>3.03 (2.23)</td>
<td>0.4</td>
</tr>
<tr>
<td>Sulphate</td>
<td>3.18 (1.59)</td>
<td>0.2</td>
<td>3.21 (1.43)</td>
<td>0.2</td>
<td>3.89 (2.89)</td>
<td>0.04</td>
</tr>
<tr>
<td>Oxalate</td>
<td>3.32 (2.45)</td>
<td>0.2</td>
<td>3.31 (0.59)</td>
<td>0.5</td>
<td>4.09 (3.17)</td>
<td>0.04</td>
</tr>
<tr>
<td>Formate</td>
<td>3.74 (2.97)</td>
<td>0.2</td>
<td>3.81 (1.84)</td>
<td>0.7</td>
<td>4.81 (3.63)</td>
<td>0.6</td>
</tr>
<tr>
<td>Pentanesulphonate</td>
<td>6.00 (6.66)</td>
<td>0.2</td>
<td>6.15 (3.23)</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Octanesulphonate</td>
<td>6.78 (3.72)</td>
<td>0.3</td>
<td>6.96 (3.72)</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

3,5-Dinitrosalicylic acid

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Migration time, s</th>
<th>LOD (mM)</th>
<th>Migration time, s</th>
<th>LOD (mM)</th>
<th>Migration time, s</th>
<th>LOD (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>2.86 (1.32)</td>
<td>0.050</td>
<td>2.86 (1.32)</td>
<td>0.04</td>
<td>2.86 (1.32)</td>
<td>0.04</td>
</tr>
<tr>
<td>Nitrate</td>
<td>3.00 (2.10)</td>
<td>0.4</td>
<td>3.05 (1.34)</td>
<td>1.0</td>
<td>3.03 (2.23)</td>
<td>0.4</td>
</tr>
<tr>
<td>Sulphate</td>
<td>3.18 (1.59)</td>
<td>0.2</td>
<td>3.21 (1.43)</td>
<td>0.2</td>
<td>3.89 (2.89)</td>
<td>0.04</td>
</tr>
<tr>
<td>Oxalate</td>
<td>3.32 (2.45)</td>
<td>0.2</td>
<td>3.31 (0.59)</td>
<td>0.5</td>
<td>4.09 (3.17)</td>
<td>0.04</td>
</tr>
<tr>
<td>Formate</td>
<td>3.74 (2.97)</td>
<td>0.2</td>
<td>3.81 (1.84)</td>
<td>0.7</td>
<td>4.81 (3.63)</td>
<td>0.6</td>
</tr>
<tr>
<td>Pentanesulphonate</td>
<td>6.00 (6.66)</td>
<td>0.2</td>
<td>6.15 (3.23)</td>
<td>0.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Octanesulphonate</td>
<td>6.78 (3.72)</td>
<td>0.3</td>
<td>6.96 (3.72)</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
4.2 Minimising electro-migrational dispersion

Electro-migrational dispersion was evident for the separation of the fast anions, particularly, when p-nitrophenol was employed as the probe. It compromised resolution even for the relatively simple anion mix (figure 4.1). This section investigated minimising electro-migrational dispersion by exploring two key parameters, column diameter and probe concentration.

The work to date was completed on a 75 μm fused silica capillary. The anion mix was separated on a 50 μm capillary using a BGE containing 20 mM p-nitrophenol buffered with Tris (pH 8.1). The resulting electropherogram was compared with the electropherogram recorded for the same separation but on a 75 μm capillary (figure 4-2). Clearly, there was a reduction in peak height, due to the reduced pathlength, however, due to the reduction in baseline noise, similar LOD were recorded. Furthermore, the reduction in electro-migrational dispersion reported in the literature [49], was clearly demonstrated in this work. All analytes were fully resolved, this was particularly apparent for the nitrate and sulphate peaks, which were just baseline resolved when separated on a 75 μm capillary.

The optimal probe concentration was determined to further minimise electro-migrational dispersion (section, 1.6.1). However, the probe concentration is limited by the upper linear range of the detection system used. This was determined for each probe in the following manner: The separation capillary was filled with BGE containing different concentrations of the probes (5-40 mM). The absorbance was recorded for each concentration. The absorbance vs concentration of probe was plotted (figure 4-3). The optimal concentration for p-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid was 15 mM (400 nm) and 20 mM (428 nm), respectively. At higher probe concentration, detector response deviated from linearity. As expected, p-nitrophenol reached the linearity limit before 4-hydroxy-3,5-dinitrobenzoic acid as it is the higher absorbing probe.
Figure 4-2: Electropherograms showing the comparison between (a) 75 μm I.D. capillary and (b) 50 μm I.D. capillary of anion mix 1. Run conditions and migration order as figure 4-1.
4.3 Maximising detection limits

Having optimised the BGE to minimise electro-migrational dispersion, the LOD were optimised. This was completed by determining the optimal injection volume and current limitation associated with both the p-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid buffer systems.

The optimal injection volume was determined for each probe to maximise LOD. The effect of increasing current (due to increasing probe concentration) on baseline noise was also considered as increases in baseline noise adversely affect LOD.
Anion mix 1 (0.5 mM) was hydrostatically injected onto the capillary for different time periods (10 to 80 seconds). Peak height for chloride vs injection time was plotted for both probes (figure 4-4). *p*-nitrophenol displayed a relatively linear increase ($r^2 = 0.995$) in peak height up to a 30 second hydrostatic injection volume. 4-hydroxy-3,5-dinitrobenzoic acid also displayed a similar increase ($r^2 = 0.991$) up to 30 seconds. Therefore, 30 seconds was taken to be the optimal injection volume with respect to maximising LOD for both *p*-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid.

![Graphs](https://example.com/graphs.png)

**Figure 4-4** - Injection volume linearity curve for (a) *p*-nitrophenol and (b) 4-hydroxy3,5-dinitrobenzoic acid. Both curves used the peak height of chloride associated with a hydrostatic injection volume at 10 cm.
At this point the relationship between system current and baseline noise was explored. Several BGE’s with varying concentrations (5 mM to 60 mM) of 4-hydroxy-3,5-dinitrobenzoic acid were purged through the system. A run was initiated and the current for each concentration measured. Also, the noise for each run was measured and a relationship between probe concentration and both system current and noise plotted (figure 4-5). As expected the system current increased linearly with probe concentration. The baseline noise increased significantly as the concentration of the probe exceeded 40 mM. However, as this increase in baseline noise occurred higher than the optimal probe concentration found optically for 4-hydroxy-3,5-dinitrobenzoic acid, it was not a consideration with this probe. With respect to p-nitrophenol, which is a higher absorbing probe than 4-hydroxy-3,5-dinitrobenzoic acid, $\varepsilon = 16401 \text{ Lmol}^{-1}\text{cm}^{-1}$ compared to $\varepsilon = 6560 \text{ Lmol}^{-1}\text{cm}^{-1}$ (chapter 3), the probe concentration is also limited optically before current negatively impacts on baseline noise.

![Figure 4-5](image)

**Figure 4-5** - A plot of probe concentration vs baseline noise and system current. This plot was recorded over a series of concentrations (5 mM-60 mM) of 4-hydroxy-3,5-dinitrobenzoic acid buffered to pH 8.1

### 4.4 Comparison of alternative probes to chromate

The optimised BGE’s consisting of 15 mM p-nitrophenol and 20 mM 4-hydroxy-3,5-dinitrobenzoic acid both buffered with Tris to pH 8.1, were then evaluated for their ability to separate a more complex anion mix. The mix consisted of chloride, nitrate, sulphate, oxalate, formate, succinate, phosphate, acetate, pentanesulphonate, octanesulphonate and benzoate (anion mix 2). The mix was
Chapter 4  

Probe performance

designed to cover a wide mobility range. The mixture was also separated using a chromate buffer monitored using a 370 nm LED. The resulting electropherograms (figure 4-6) give significant insight into the potential applicability of the identified probes with respect to the mobility of the intended analytes.

As illustrated earlier and highlighted here for anion mix 2, p-nitrophenol is the probe of choice for the slower anions (figure 4-6a). Despite having optimised the probe concentration and reduced the I.D. of the capillary, significant electromigrational dispersion was observed for the faster analytes. This was supported by the number of theoretical plates obtained for the fast anions such as chloride and oxalate, $10.9 \times 10^3$ and $8.3 \times 10^3$, respectively. In contrast the number of theoretical plates for the slower anions of pentanesulphonate, octanesulphonate and benzoate were $73.1 \times 10^3$, $116.1 \times 10^3$ and $88.9 \times 10^3$, respectively. Furthermore, the LOD for the latter analytes were 0.004, 0.003 and 0.003, respectively, which were the highest obtained for any analytes using p-nitrophenol. The contrast in the peak shapes for the faster analytes in the chromate system compared to the p-nitrophenol system can be attributed to the mobility difference between the two probes, where chromate has a higher mobility and is better suited to these faster analytes than p-nitrophenol. Similarly, for the same reason the slower anions are better suited to p-nitrophenol compared to chromate.

4-hydroxy-3,5-dinitrobenzoic acid provided adequate resolution of all analytes, including the faster analytes such as chloride through to formate and the slower anions such as pentanesulphonate, octanesulphonate and benzoate (figure 4-6b). It was clear that phosphate was the analyte in the mix best suited to the probe, given its significantly larger peak size and more symmetrical shape than the other analytes. In support of this observation, phosphate recorded the best efficiency of all the analytes in this system at $280.6 \times 10^3$ theoretical plates (table 4-2). Chromate displayed, more symmetrical peak shapes for the faster analytes than 4-hydroxy-3,5-dinitrobenzoic acid which was seen visually (figure 4-6) and re-enforced by the efficiencies obtained for chloride in the chromate system ($112 \times 10^3$) and the 4-hydroxy-3,5-dinitrobenzoic acid system ($27.2 \times 10^4$).
Figure 4.6 - Electropherograms showing the comparison of the probes (a) p-nitrophenol (b) 4-hydroxy-3,5-dinitrobenzoic acid and (c) 5 mM chromate in a buffered BGE. Run conditions: -25 kV potential, probes buffered to pH 8.1 with TRIS, except chromate, pH 9.2 with DEA. 30 second hydrostatic injection at 10 cm. 15 mM p-nitrophenol, 20 mM 4-hydroxy-3,5-dinitrobenzoic acid. 1- chloride; 2- nitrate; 3- sulphate; 4- oxalate; 5- formate; 6- succinate; 7- phosphate; 8- acetate; 9- penianesulphonate; 10- octanesulphonate; 11- benzoate
### Table 4-2 – Detection limits and efficiency data for optimised $p$-nitrophenol, 4-hydroxy-3,5-dinitrobenzoic acid and chromate (370 nm) systems

<table>
<thead>
<tr>
<th>Analyte</th>
<th>$p$-nitrophenol</th>
<th>4-hydroxy-3,5-dinitrobenzoic acid</th>
<th>Chromate (370 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LOD (mM)</td>
<td>Efficiency (plates x10$^5$)</td>
<td>LOD (mM)</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.020</td>
<td>10.9</td>
<td>0.030</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.020</td>
<td>-</td>
<td>0.030</td>
</tr>
<tr>
<td>Sulphate</td>
<td>0.010</td>
<td>-</td>
<td>0.018</td>
</tr>
<tr>
<td>Oxalate</td>
<td>0.010</td>
<td>8.3</td>
<td>0.017</td>
</tr>
<tr>
<td>Formate</td>
<td>0.010</td>
<td>15.8</td>
<td>0.017</td>
</tr>
<tr>
<td>Succinate</td>
<td>0.010</td>
<td>6.6</td>
<td>0.009</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.006</td>
<td>10.9</td>
<td>0.006</td>
</tr>
<tr>
<td>Acetate</td>
<td>0.005</td>
<td>35.2</td>
<td>0.010</td>
</tr>
<tr>
<td>Pentanesulphonate</td>
<td>0.004</td>
<td>73.3</td>
<td>0.020</td>
</tr>
<tr>
<td>Octanesulphonate</td>
<td>0.003</td>
<td>116.1</td>
<td>0.020</td>
</tr>
<tr>
<td>Benzoate</td>
<td>0.003</td>
<td>88.9</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Note: - denotes no data recorded due to co-migration
In summary, \( p \)-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid have shown to be suitable probes for the analysis of inorganic anions. When used for analytes closest to their mobility, LOD and efficiencies are significantly improved and as a result it was seen that \( p \)-nitrophenol was more suitable for the slower anions and 4-hydroxy-3,5-dinitrobenzoic acid for the moderately mobile anions.

### 4.5 Multiple co-ionic electrolytes

The use of multiple co-ionic electrolytes has been shown to extend the range of analytes that can be sufficiently determined in a single run [50]. Therefore the combination of two probes in a single BGE was explored. Two different systems were explored, namely \( p \)-nitrophenol/4-hydroxy-3,5-dinitrobenzoic acid and \( p \)-nitrophenol/chromate.

A BGE containing 10 mM \( p \)-nitrophenol and 10 mM 4-hydroxy-3,5-dinitrobenzoic acid buffered with Tris to pH 8.1 was prepared. A 426 nm LED was chosen as the light source as 4-hydroxy-3,5-dinitrobenzoic acid (\( \varepsilon = 6560 \text{ Lmol}^{-1}\text{cm}^{-1} @ 426 \text{ nm} \)) absorbed most strongly at this wavelength. \( p \)-nitrophenol had a lambda max at 400 nm (\( \varepsilon = 16402 \text{ Lmol}^{-1}\text{cm}^{-1} \)), however, it also absorbed quite strongly at 428 nm (\( \varepsilon = 10497 \text{ Lmol}^{-1}\text{cm}^{-1} \)), see figure 4-7.

![Wavelength scan for 4-hydroxy-3,5-dinitrobenzoic acid, chromate and \( p \)-nitrophenol](image)

The analytes were all resolved and a stable baseline was recorded (figure 4-8). A small system peak, typical of a multiple co-ion system, was recorded at 6.2
minutes. The electropherogram displayed two distinct displacement zones. The first representing the primary displacement of the fastest probe, 4-hydroxy-3,5-dinitrobenzoic acid. The second zone represented the primary displacement of p-nitrophenol. The first zone was evident by chloride exhibiting a fronted profile followed by the tailing of acetate, signifying the end of the optimal window for 4-hydroxy-3,5-dinitrobenzoic acid. The second zone was most noticeable with benzoate exhibiting a large fronting profile followed by octanesulphonate showing a tailing profile which signified the end of p-nitrophenol’s window.

Interestingly, the electropherogram produced, was quite similar to that recorded for the single probe BGE containing 4-hydroxy-3,5-dinitrobenzoic acid (figure 4-6) with respect to analytes 1-8. Noticeably, benzoate in this system, migrated before pentane and octanesulphonate. The p-nitrophenol window was studied to see what effect the probe had on the overall detection limits. The LOD produced for pentanesulphonate, octanesulphonate and benzoate (table 5-3) were 0.008, 0.010 and 0.010 mM, respectively. These LOD were better than for 4-hydroxy-3,5-dinitrobenzoic acid on its own (0.020, 0.020 and 0.030 mM), however, they were not as good as those found for p-nitrophenol system (0.004, 0.003 and 0.003 mM), see table 4-2. As previously mentioned an analyte displaces the probe that is closest to its own mobility, therefore the increased LOD for the afore mentioned slower analytes in the combined system compared to the 4-hydroxy-3,5-dinitrobenzoic acid system must be directly related to the contribution of p-nitrophenol. The level of noise associated with this system is similar to that previously obtained for the individual systems and therefore is not a contributing factor to the improved LOD. No system optimisation with respect to probe concentration was completed with this multiple co-ion BGE, therefore the less than expected LOD for the slower analytes, when compared to a single probe BGE containing p-nitrophenol, could also be attributed to the fact that only 10 mM p-nitrophenol was used.
Chapter 4

Probe performance

Figure 4-8 – Electropherogram of a separation of anion mix 2 using a co-ionic BGE. Run conditions: 10 mM p-nitrophenol / 10 mM 4-hydroxy-3,5-dinitrobenzoic acid buffered to pH 8.1 with Tris; 428 nm LED; 30 second hydrostatic injection at 10 cm; -25 kV potential; analytes are as figure 4-6.

Table 5-3- LOD for analytes separated in a multiple co-ion BGE (10 mM p-nitrophenol/10 mM 4-hydroxy-3,5-dinitrobenzoic acid); conditions as figure 4-8.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOD (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride</td>
<td>0.030</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.020</td>
</tr>
<tr>
<td>Sulphate</td>
<td>0.010</td>
</tr>
<tr>
<td>Oxalate</td>
<td>0.010</td>
</tr>
<tr>
<td>Formate</td>
<td>0.010</td>
</tr>
<tr>
<td>Succinate</td>
<td>0.002</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.003</td>
</tr>
<tr>
<td>Acetate</td>
<td>0.010</td>
</tr>
<tr>
<td>Pentanesulphonate</td>
<td>0.008</td>
</tr>
<tr>
<td>Octanesulphonate</td>
<td>0.010</td>
</tr>
<tr>
<td>Benzoate</td>
<td>0.010</td>
</tr>
</tbody>
</table>

It was thought that given the suitability of chromate for the faster analytes and p-nitrophenol for the slower analytes that a combination of the two probes might result in better LOD across the whole mobility range. Chromate and p-nitrophenol both absorb strongly at 370 nm (figure 4-7). Therefore, a 370 nm LED was chosen to monitor the system. A 5 mM chromate / 5 mM p-nitrophenol BGE buffered with Tris to pH 8.1 was prepared. The resulting electropherogram can be seen in figure 4-9. Clearly, the separation was not ideal as only six of the eleven analytes were identified. In addition, a large system peak and baseline disturbance was evident around 4 minutes. Peaks 1-4 representing the faster...
analytes, chloride, nitrate, sulphate and oxalate exhibited peak shapes expected for a chromate BGE. Similarly, peaks 9 and 10 representing the slower analytes pentanesulphonate and octanesulphonate exhibited peak shapes expected for a \( p \)-nitrophenol system. The likely displacement of the analytes with the probe closest to its own mobility re-enforced the results obtained for the \( p \)-nitrophenol/4-hydroxy-3,5-dinitrobenzoic acid system. The magnitude and position of the system peak may be explained by the difference in mobility's between chromate and \( p \)-nitrophenol (37x10\(^{-5}\) cm\(^2\)/V.s). However, for this system to be useful, further optimisation is required to minimise the baseline disturbance.

![Electropherogram](image)

Figure 4-9- Electropherogram of a separation of anion mix 2 using a co-ionic BGE. Run conditions: 5 mM \( p \)-nitrophenol / 5 mM chromate buffered to pH 8.1 with Tris; 370 nm LED; 30 second hydrostatic injection at 10 cm; -25 kV potential; analytes are as in figure 4-6

In summary, multiple co-ions provide opportunities to extend the range of analytes that can be analysed. However, mixing of multiple co-ions also presents significant challenges as baseline disturbances and system peaks can interfere with analytes of interest. Optimisation of these systems may minimise these disturbances.
5.1 Determination of oxalate in industrial samples

The two probes, p-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid, were applied to the analysis of real samples. Oxalate in alumina samples was chosen as the analyte to investigate. Although it is a relatively fast anion ($-58.8 \times 10^{-5} \text{cm}^2/\text{Vs}$, [48]), its peak shape was only slightly fronted when the probes under investigation were used (Figure 4-5a and 4-5b). As the Alumina industry is very significant in Western Australia, this application also had local significance.

A local alumina industry provided a number of samples with varying concentrations of oxalate in the presence of other anionic and cationic species. These samples included typical Bayer liquor samples where the oxalate content is required in order to optimise the process. The exact nature of these samples are not provided on the request of the company, therefore, the samples were categorised into three groups based on their oxalate content: those containing relatively high levels of oxalate, those with levels that are typical of Bayer liquor samples and those containing very low levels of oxalate. The analysis of these three groups of samples by ID and CZE using the probes identified as part of this project is discussed below.

5.2 Samples with a high oxalate concentration

The samples with a high oxalate concentration, typically 20 g/L disodium oxalate were investigated first. The sample was diluted (100 fold and 1000 fold) to reduce the interferences from the high alkaline background. In addition, dilution by 100 or 1000 fold maximises field amplified sample stacking (FASS). A 15 mM p-nitrophenol BGE buffered with Tris to a pH of 8.1 was used for the analysis of both samples. A typical 30 second hydrostatic injection at 10 cm was
applied to both samples. In both cases, an oxalate peak was resolved, with peak heights well above detection limit (Figure 5-1). However, the peak recorded for the sample diluted 100 fold displayed more electro-migrational dispersion, indicating that some efficiency was lost. This was re-enforced by the theoretical plates calculated for oxalate. The theoretical plates for the oxalate peak in the sample diluted 100 fold was $8.6 \times 10^3$ and for the sample diluted 1000 fold were 4 times higher, $35.0 \times 10^3$.

![Electropherograms](image)

Figure 5-1- Electropherograms of a high oxalate concentration sample (a) diluted 100 fold and (b) diluted 1000 fold. Run conditions: 15 mM p-nitrophenol buffered with Tris to a pH of 8.1; 30 second hydrostatic injection; -25kV potential.

Ideally for quantitative purposes a signal at least 10 times the detection limit (quantifiable detection limit, QDL) is desirable. As the sample diluted 1000 fold recorded a peak for oxalate which was 20 times LOD, this dilution was used for
quantitation. The method of standard additions was used to determine the oxalate concentration. The calibration curve was linear ($r^2 = 0.99$) and an oxalate concentration of 19 g/L was calculated. This agreed with that recorded for IC (data supplied by industry). However, no attempt was made to validate the method as this was outside the scope of the study.

5.3 Samples with a medium oxalate concentration (Bayer liquor)

A number of Bayer liquor samples with oxalate concentrations typical of those samples that have previously been analysed by CZE and reported in the literature were investigated next. These samples typically contained 2-3 g/L oxalate. While these samples contained less oxalate than the high oxalate concentration samples, their electrical conductance (EC) was approximately three times higher (58 mS vs 17 ms, measurements recorded for samples diluted 10 fold). The higher EC was due to the higher alumina and hydroxide concentrations in the sample. Therefore, maximising the dilution was a priority to reduce the conductivity and allow some FASS to occur. A sample was diluted 1000 fold and injected (30 second hydrostatic injection at 10 cm) into a BGE with 15 mM $p$-nitrophenol as the probe and buffered with Tris to a pH of 8.1. A negative potential of 25 kV was applied throughout the run. Oxalate was fully resolved; however the signal was only 5 times LOD. While this project was not about developing an optimised method for the quantitative analysis of oxalate, it was about evaluating the potential of these probes for such work. Precision is optimised when peaks are at least 10 times LOD, therefore, approaches that would achieve this were investigated [51].
Chapter 5  The analysis of oxalate in relevant alumina industry samples

Figure 5-2- Electropherogram for a Bayer liquor sample, diluted 1000 fold. Conditions: 15 mM p-nitrophenol buffered to pH 8.1 with Tris; 30 second hydrostatic injection at 10 cm; -25 kV potential.

The sample was diluted 100 fold and the resulting electropherogram is presented in Figure 5-3. The oxalate signal was 27 times LOD, however, due to the larger concentrations of oxalate and other constituents, oxalate was no longer resolved.

Figure 5-3- Electropherogram of a Bayer liquor sample, diluted 100 fold. Conditions: 15 mM p-nitrophenol buffered to pH 8.1 with Tris; 30 second hydrostatic injection at 10 cm; -25 kV potential.

A -5 kV voltage was applied during injection (electro-migrational injection) of the Bayer liquor sample diluted 1000 fold. The signal for oxalate was enhanced (24 times LOD), however, the oxalate peak was only just baseline resolved and slight differences in process samples (and the concentration of the closely eluting
analytes) may result in loss of resolution of the oxalate peak, reducing the precision required for quantitative analysis [42].

Figure 5-4: Electropherogram of a Bayer liquor sample, diluted 1000 fold. Conditions: 15 mM p-nitrophenol buffered to pH 8.1 with Tris; 30 second -5kV injection; -25 kV potential

As 4-hydroxy-3,5-dinitrobenzoic acid provided better resolution of the faster anions compared to p-nitrophenol (figure 4-6), the Bayer liquor sample, diluted 1000 fold was separated using a BGE containing 20 mM 4-hydroxy-3,5-dinitrobenzoic acid (buffered to pH 8.1 with Tris). The oxalate peak was fully resolved from the earlier eluting peak and LOD were not compromised (27 times LOD).

Figure 5-6: Electropherogram of a Bayer liquor sample, diluted 1000 fold. Conditions: 20 mM 4-hydroxy-3,5-dinitrobenzoic acid buffered to pH 8.1 with Tris; 60 cm x 50μm (I.D.) fused silica capillary; 30 second -5kV injection; -25 kV potential
Reproducible injection of the analyte using electro-migrational injection requires that the sample matrix has a constant conductivity. However, the Bayer liquor samples conductivity can vary significantly between sampling and batches, therefore an alternative injection method was sought.

The Bayer liquor sample, diluted 100 fold was separated again this time using 4-hydrox-3,5-dinitrobenzoic acid as the probe. In contrast to the p-nitrophenol system, by using hydrostatic injection the oxalate was fully resolved and a peak 50 times LOD was recorded. Therefore, typical Bayer liquor samples can be analysed for oxalate using 4-hydroxy-3,5-dinitrobenzoic acid with more than adequate LOD.

![Figure 5-5: Electropherogram of a Bayer liquor sample, diluted 100 fold. Conditions: 20 mM 4-hydroxy-3,5-dinitrobenzoic acid buffered to pH 8.1 with Tris; 30 second hydrostatic injection at 10 cm; -25 kV potential](image)

**5.4 Samples with a low oxalate concentration**

A low concentration oxalate sample was then analysed. The low oxalate concentration samples provided by the alumina industry contained between 0.02-0.03 g/L oxalate. These samples are a significant challenge for analysis by CZE as the neat sample (0.4 μM) is well below detection limit 10 μM (p-nitrophenol) and 17 μM (4-hydroxy-3,5-dinitrobenzoic acid). Unlike the high oxalate concentration samples and the Bayer liquor samples, the neat low oxalate concentration sample had a relatively low conductivity (16.26 mS). Therefore,
diluting the sample was unlikely to improve stacking efficiency, rather reduce the ability to detect oxalate at all.

Therefore, the undiluted sample was injected directly onto the capillary and separated using a p-nitrophenol BGE system. A trace peak was detected for oxalate which was confirmed by spiking the sample. It was clear, given the low concentration of oxalate and the low conductivity of the sample itself, other pre-concentration methodologies must be explored if CZE is to be used for the quantitative determination of oxalate. One method that has shown significant increases in detection limits in a variety of applications is isotachophoresis. It is an attractive option as it provides better conditions for stacking than FASS and if time permitted would have been an avenue to explore.

Figure 5-7. Electropherogram of (a) neat low oxalate concentration sample and (b) low oxalate concentration sample spiked with oxalate. Conditions: 15 mM p-nitrophenol buffered to pH 8.1 with Tris; 60 cm x 50 μm fused silica capillary; 30 second hydrostatic injection at 10 cm; -25 kV potential applied.
In summary, two types of relevant industrial alumina samples, a high oxalate concentration sample and a medium oxalate concentration (Bayer liquor) sample have been analysed using the two probes studied. A methodology was demonstrated for both of the samples that was suitable for quantitative work. The low oxalate concentration sample requires further pre-concentration to firstly bring the oxalate peak into detectable range and then into quantifiable range. The work shown in this chapter has displayed the potential for both p-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid to be used for challenging applications. Therefore, future work should involve the validation of this study and the use of these probes in additional applications.
Chapter 6

Conclusions

The work carried out throughout this study allowed the following conclusions to be made:

- Two probes, \( p \)-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid have been successfully identified for the analysis of inorganic and organic anions.

- \( p \)-nitrophenol displayed a suitability for slow anions and 4-hydroxy-3,5-dinitrobenzoic acid for moderately mobile anions.

- A multiple co-ionic electrolyte containing \( p \)-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid was successfully used to analyse an anion mix, showing an improvement in detection limits over a wider range of analytes. However, the use of a chromate / \( p \)-nitrophenol electrolyte proved to be inadequate for the same anion mix.

- \( p \)-nitrophenol was successfully applied to the analysis of oxalate in a high oxalate concentration sample and 4-hydroxy-3,5-dinitrobenzoic acid was successfully applied to the analysis of oxalate in a Bayer liquor sample.

- LED's were used as light sources and provided a strong emission line in the region of interest. In addition, they showed that by matching the maximum absorbance of the probe with the emission maximum of the source, results in significant improvements in detection limits.

- 2,4-dinitrobenzoic acid and 4-hydroxy-3,5-dinitrobenzoic acid were identified as possible alternate probes for moderate to slow analytes to be monitored by a UV light source at 254 nm.

Some findings are worthy of further work:

- Validation of both \( p \)-nitrophenol and 4-hydroxy-3,5-dinitrobenzoic acid is required with respect to the alumina samples studied, to ensure precision of the methodology as this is required for industrial application.

- Further evaluation of 2,4-dinitrobenzoic acid and 4-hydroxy-3,5-dinitrobenzoic acid as a suitable alternate probes for work using a 254 nm UV source is required.
Chapter 7

References


