STUDIES ON SEPARATION SELECTIVITY IN CAPILLARY ZONE ELECTROPHORESIS OF INORGANIC ANIONS

by

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DEDICATION

To the memory of my mother

who passed away whilst I was absent attempting this PhD;

with love, honour and respect.

Lawolawo Napaii-tae Ainako Harakuwe *nee* Hero

(? —— 8th February, 1993)

Also dedicated to my maternal grandmother

for her love, patience and unfailing confidence and optimism.

Izo-izo Gepae Hero

Finally, dedicated too to the memory of

my paternal grandfather who passed away also in my absence.

Titiyo Harau-wone Gihuti.

(? —— 3rd September, 1991).

DECLARATION

I hereby declare that this submission is my own work and to the best of my knowledge and belief, contains no material which has been accepted for the award of a higher degree in any tertiary institution and that, the thesis contains no previously published or written material by another person, except where due reference is made in the text of the thesis.

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PREFACE

- In all cases, adaptation in this thesis of published material has been with the permission of the publisher and principal author. Due acknowledgements have been made in the captions according to the formats required by the publishers.

- The literature review is compiled so that it is not merely a compilation of facts and figures. Where deemed appropriate, critical comments and opinions have been expressed.

- All electropherograms shown in this thesis are devoid of any form of computerised manipulation and are shown as acquired.

- Some applications have been mentioned only but not included in the thesis due to space constraints.

ABSTRACT

Parameters influencing the separation selectivity of low molecular-mass anions using free-solution reversed electroosmotic flow capillary zone electrophoresis (FRECZE) have been studied. First, detailed preliminary investigations were performed to address two major limitations of FRECZE, namely, imprecision in migration times and variability of phosphate response. A capillary conditioning regime suitable for the generation of stable migration times and optimal phosphate response was developed. Also, it was established that dried electroosmotic flow (EOF) modifiers generated more stable migration times and gave improved resolution.

Second, a wide range of selectivity-influencing parameters was studied. These parameters included electrolyte pH, EOF modifier chain length, nature of the EOF modifier counter-anion, EOF modifier concentration, binary EOF modifier mixtures, type of indirect detection probe, probe concentration, 1-butanol as an electrolyte additive, and instrumental variables (e.g. detection wavelength and separation voltage). The trends of migration order observed with the above parameters are discussed and possible mechanisms outlined. Major migration order changes were caused by pH and EOF modifier effects. Electrolyte pH variation changed the migration order of weak acid anions at pH values close to their pK_a points by altering their charge to mass ratios. Migration order changes due to either increased or reduced ion-pairing effects were pronounced for lipophilic anions and could be induced particularly with EOF modifier changes, electrolyte concentration and 1-butanol as additive. Migration order changes due to increased effective charge of anions were influenced particularly by 1-butanol as additive as well as electrolyte ionic strength.

Third, information from the above studies was applied to the separation of inorganic anions in samples having varying levels of matrix complexity, e.g. tap water, Bayer liquor, acid-digested concrete, toothpaste, urine, a formulation for prevention of gall-stone formation, corned beef, seawater and Antarctic saline lake water. Analytical performance characteristics are discussed for the separation of anions in Bayer liquor, concrete and toothpaste.

The highlights of this study were that the useful pH range of chromate-based electrolyte could be extended by 20% by incorporation of 1-butanol in the electrolyte; the resolution between fluoride and phosphate could be improved by over 400% making possible the separation of 1 $\mu g.mL^{-1}$ fluoride in the presence of over 800 $\mu g.mL^{-1}$ phosphate, and the use of binary EOF modifier mixtures was introduced and applied to the analysis of Bayer liquor. Two electrolyte compositions capable of simultaneously separating chloride, sulfate, oxalate, malonate, fluoride, formate, phosphate, succinate, tartrate, carbonate and acetate in under 4 minutes were identified. Calibrations were linear in the range 1 - 10 $\mu g.mL^{-1}$, detection limits as low as 0.09 $\mu g.mL^{-1}$ were obtained and near quantitative recoveries (except for phosphate) were recorded.

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PUBLICATIONS* FROM THIS THESIS

1. Harakuwe, A.H., Haddad, P.R. and Buchberger, W., 'Optimisation of separation selectivity in capillary zone electrophoresis of inorganic anions using binary cationic surfactant mixtures', *Journal of Chromatography A*, **685** (1994) 161 - 165.

   (Work from Chapters 5 and 7.)

2. Haddad, P.R., Harakuwe, A.H. and Buchberger, W., 'Separation of inorganic and organic ionic components of Bayer liquor using capillary zone electrophoresis. I. Optimisation of resolution using electrolytes containing surfactant mixtures', *Journal of Chromatography A*, **706** (1995) 571 - 578.

   (Work from Chapter 5 and 7.)

3. Harakuwe, A.H., Haddad, P.R. and Thomas, R., 'Capillary Zone Electrophoresis Method for the Determination of Sulfate in Concrete', *Analytical Proceedings*, **32**, no. 12 (1995) 493 - 494.

   (Work from Chapters 6 and 8.)

4. Harakuwe, A.H. and Haddad, P.R., 'Manipulation of Separation Selectivity for Inorganic Anions in Capillary Zone Electrophoresis using Control of Electrolyte pH', *Journal of Chromatography A*, accepted for publication.

   (Work from Chapters 6 and 8.)

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* Only the papers that have been published, accepted for publication, submitted for publication or are actually being prepared are listed. Other papers (from this study) are under consideration for publication and may be included later if they are submitted before the hard-bound thesis is submitted to the University of Tasmania.

5. Haddad, P.R., Harakuwe, A.H. and Jackson, P.E., 'Quantitative Determination of Oxalate in Bayer Liquor by Capillary Electrophoresis: A Validative Study', *Journal of Chromatography A*, accepted for publication.

(Work from Chapter 7.)

6. Harakuwe, A.H. and Haddad, P.R., 'Optimisation of the Response of Phosphate Separated by Capillary Zone Electrophoresis', *Analytical Proceedings*, submitted.

(Work from Chapter 4.)

7. Haddad, P.R. and Harakuwe, A.H., 'Review: Parameters Influencing the Separation Selectivity of Anionic Solutes using Capillary Zone Electrophoresis with Reversed Electroosmotic Flow', paper in preparation.

(Work from Chapters 2 - 8.)

8. Harakuwe, A.H., Haddad, P.R. and Thomas, R., 'The Simultaneous Separation of Chloride and Sulfate in Concrete with a High Acid and Nitrate Background', *Journal of Chromatography A*, paper in preparation.

(Work from Chapter 8.)

9. Harakuwe, A.H. and Haddad, P.R., 'Stabilisation of Absolute Migration Time of Anions Separated by Capillary Zone Electrophoresis', *Journal of Chromatography A*, paper in preparation.

(Work from Chapter 4.)

10. Harakuwe, A.H., Haddad, P.R. and Andersson, P., 'The influence of 1-butanol as an electrolyte additive on the separation selectivity and migration time precision of anionic solutes using capillary zone electrophoresis', *J. Chromatogr. A.*, submitted.

(Work from Chapter 6.)

11. Harakuwe, A.H. and Haddad, P.R., 'Separation of ionic components of Bayer Liquor using Capillary Zone Electrophoresis. II. Optimisation of the response of fluoride using on- and off-capillary complexation', *Journal of Chromatography A*, paper in preparation.

(Work from Chapters 5 and 7.)

12. Harakuwe, A.H. and Haddad, P.R., 'Effect of mesityl oxide and formamide 'neutral' electroosmotic flow markers on the separation of inorganic anions using capillary zone electrophoresis', paper in preparation.

(Work from Chapter 4.)

13. Harakuwe, A.H., Haddad, P.R. and Davis, N., 'The effect of drying on cationic surfactants and their use in improving the separation of anionic solutes using capillary zone electrophoresis', paper in preparation.

(Work from Chapter 4.)

14. Harakuwe, A.H. and Haddad, P.R., 'Performance characteristics of the separation of fluoride in toothpaste by capillary zone electrophoresis using a chromate-based electrolyte at pH 7.5', paper in preparation.

(Work from Chapters 4, 5 and 6.)

PRESENTATIONS FROM THIS THESIS

POSTERS

1. Haddad, P.R. and Harakuwe, A.H., 'Factors Affecting Analytical Performance in the Determination of Inorganic Anions by Capillary Zone Electrophoresis', *Programme and Abstracts, 12th RACI* Symposium on Analytical Chemistry*, 26th September - 1st October (1993). Perth, WA, Australia: Poster 1/33, p. 80.

   (Work from Chapters 4 - 8.)

2. Haddad, P.R. and Harakuwe, A.H., 'Factors Affecting Inorganic Anion Selectivity in Capillary Ion Electrophoresis', *Programme and Abstracts, 1st RACI Analytical Chemistry Group Research and Development Topics Meeting*, 6th and 7th December (1993). Deakin University, Geelong, Victoria: Poster 19.

   (Work from Chapters 4 - 8.)

3. Harakuwe, A.H., Haddad, P.R. and Kampermann, H., 'Control of Separation Selectivity of Anionic Solutes in Capillary Zone Electrophoresis and Application to Phosphocitrate and Bayer Liquor Analysis', *Abstracts, Chromatography '94*, 19th - 21st July (1994), AJC, Randwick, Sydney, Australia: Poster P05, p. 34.

   (Work from Chapters 4 - 8.)

4. Harakuwe, A.H., Haddad, P.R. and Kampermann, H., 'Parameters Influencing Selectivity of Separation of Anionic Solutes in Capillary Zone Electrophoresis and Application to Phosphocitrate and Bayer Liquor Analysis', *Abstracts,*

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* Royal Australian Chemical Institute.

International Ion Chromatography Symposium '94, 19th - 22nd September (1994). Torino Incontra Congress Centre, Turin, Italy: Poster 74.

(Work from Chapters 4 - 8.)

5. Harakuwe, A.H. and Haddad, P.R., 'Parameters Affecting Anion Selectivity in Free Solution Reversed Electroosmotic Flow Capillary Zone Electrophoresis and Selected Applications', *Programme and Abstracts, 2nd RACI Analytical Chemistry Group Research & Development Topics Meeting*, 5th - 6th December (1994). University of Canberra, Belconnen, ACT, Australia.

   (Work from Chapters 4 - 8.)

6. Harakuwe, A.H. and Haddad, P.R., 'Determination of fluoride and other anions in Bayer liquor using free solution reversed electroosmotic flow CZE', *Programme and Abstracts, 13th RACI Symposium on Analytical Chemistry*, 9th - 14th July (1995). Darwin, NT, Australia: Poster 44, p. 112.

   (Work from Chapters 5 and 7.)

7. Haddad, P.R., Harakuwe, A.H. and Jackson, P.E., 'Quantitative Determination of Oxalate in Bayer Liquor by Capillary Electrophoresis', *International Ion Chromatography Symposium '95*, 1st - 5th October (1995). The Grand Kempinski, Dallas, Texas, USA: Poster 67.

   (Work from Chapter 7.)

8. Harakuwe, A.H. and Haddad, P.R., 'Some Parameters Influencing the Separation Selectivity of Anions using CZE and Selected Applications', *Program and Abstracts, 3rd RACI Analytical Chemistry Group Research and Development*

Topics Meeting, University of Newcastle, Callaghan, NSW, 4th - 6th December (1995): Poster 9.

(Work from Chapters 4 - 8.)

9. Harakuwe, A.H. and Haddad, P.R., 'The direct determination of iodide and iodate in seawater using capillary zone electrophoresis with on-capillary chloride-matrix elimination', *Chromatography '96*, 9th - 11th July, 1996, Rosehill, Sydney, Australia: to be presented.

(Work from Chapters 5 and 7.)

ORAL

10. Harakuwe, A.H., Haddad, P.R. and Jackson, P.E., 'Factors affecting selectivity of anions in reversed electroosmotic flow capillary zone electrophoresis', *Programme and Abstracts, 13th RACI Symposium on Analytical Chemistry*, 9th - 14th July (1995). Darwin, NT, Australia: p. 86.

(Work from Chapters 4 - 8.)

11. Harakuwe, A.H. and Haddad, P.R., 'Selectivity Studies in Capillary Zone Electrophoresis and Extension to the Determination of Anionic Solutes in Difficult Samples', *Program and Abstracts, 3rd RACI Analytical Chemistry Group Research and Development Topics Meeting*, University of Newcastle, Callaghan, NSW, 4th - 6th December (1995).

(Work from Chapters 4 - 8.)

TECHNICAL CONTRIBUTION

12. Harakuwe, A.H., 'Analysis of Anions in Bayer Liquor', *PowerChrom Chromatography System for Macintosh and PowerPC*, company brochure prepared by ADInstruments, Castle Hill, Sydney, NSW, Australia (1995), p. 5.

TABLE OF CONTENTS

LIST OF SYMBOLS

λ_{anion}	conductivity of the anion
λ_{bge}	conductivity of the background electrolyte
γ_i	correction factor for relaxation and retardation
Δh	difference in height of the capillary ends during hydrostatic sampling
λ_0^*	limiting equivalent conductance
ν	migration rate of ion
μ_o^*	mobility of EOF
ε_{net}	overall extinction coefficient
σ^2	variance of peak area
η	viscosity of electrolyte or solution
ρ	density of solution
$(t_R)_x$	retention time of faster moving solute
$(t_R)_y$	retention time of slower moving solute
A	cross-sectional area of the capillary
α	selectivity factor
α_i	degree of dissociation
C	concentration of solution injected into the capillary
$C_{det.\ lim.}$	achievable limit of detection
C_{probe}	concentration of probe or visualising agent
D	anion diffusion coefficient or dielectric constant
δ	thickness of the double-layer
d_o	compact layer of set thickness
E	electric field
e	charge on electron
ε	dielectric constant

ε_{eo}	permittivity of free space
F	F-test
F	Faraday constant
F	force
f_c	frictional resistance of BGE
g	acceleration due to gravity
K	Boltzmann constant
K'	constant
K_{wall}	equilibrium constant between electrolyte and silanol groups on the capillary wall
L	length of capillary
l	length of sample plug introduced into the capillary
ℓ	optical path length
l_+	mobility of cation
l_	mobility of anion
m	mobility of solute
M^+	monovalent BGE ion
$m_{abs.}$	absolute ionic mobility
m_{anion}	mobility of anion
$m_{eff.}$	effective ionic mobility
m_{eof}	mobility of EOF
m_i	mobility of ion
N	number of theoretical plates
v_{HD}	mean hydrodynamic flow during hydrostatic sampling
Q	amount of sample introduced into the capillary through sampling
Q	charge density
Q	see q

q	effective charge on ion
Q_o	total number of ionised silanol groups on the capillary surface
r	radius of capillary
R_S	resolution
T	absolute temperature
t	migration time of solute
t	student t-test
t_{anion}	migration time of anion
t_i	sampling time
$t_{inj.}$	injection time
t_{marker}	migration time of (neutral) marker
t_n	migration time of neutral marker
t_o	retention time of unretained solute
U	voltage
u	mobility of solute
U_{NET}	migration rate of solute (anion)
V	voltage or mobility.
$V_{applied}$	applied voltage
$V_{inj.}$	voltage applied for sampling by electrostacking
W	width of peak
ω	Joule heat or intrinsic mobility
X	see E
Z	see q
z	valence of ion
ζ	zeta or phase boundary potential
μ	mobility of solute
μ_{app}	apparent mobility

μ_e	electrophoretic mobility
μ_{ea}	electrophoretic mobility of anionic solute
μ_{EEO}	mobility of EOF
μ_{el}	electrophoretic mobility
$\mu_{el\ obs}$	net mobility
μ_{eo}	mobility of EOF
μ_{EP}	electrophoretic mobility
μ_{obs}	observed mobility
μ_{osm}	mobility of EOF
μ_p	mobility of particles

LIST OF ABBREVIATIONS

A	absorbance
ACMS	anti-convective media-supported
ACN	acetonitrile
AMT	absolute migration time
AR	analytical grade reagent
BGE	background or running electrolyte
bge	see BGE
CAPS	3-cyclohexylamino-1-propanesulfonic acid
CC	capillary conditioning
CE	capillary electrophoresis
CEC	capillary electrochromatography
CGE	capillary gel electrophoresis
cGE	see CGE
CIA	capillary ion analysis
CIE	capillary ion electrophoresis
CIEF	capillary isoelectric focussing
cIEF	see CIEF
CITP	capillary isotachophoresis
cmc	critical micelle concentration
CTA	cetyltrimethylammonium
CTAB	cetyltrimethylammonium bromide
CTAC	cetyltrimethylammonium chloride
CTAHS	cetyltrimethylammonium hydrogensulfate
CZE	capillary zone electrophoresis
DC	direct current

DR	dynamic reserve
DTAB	dodecyltrimethylammonium bromide
E	electric field
EC	electrochromatography
EEO	see EOF
EI-MS	electron-impact mass spectroscopy
EOF	electroosmotic (or electroendoosmotic) flow
eof	see EOF
EOFM	EOF modifier
FRECZE	free solution reversed electroosmotic flow capillary zone electrophoresis
FZE	free zone electrophoresis
GC	gas chromatography
GC-MS	gas chromatography-mass spectroscopy
HI-EKC	hydrophobic interaction electrokinetic capillary chromatography
HPCE	high performance CE
HPLC	high performance liquid chromatography
HVCZE	high voltage CZE
IC	ion chromatography
ICE	see CIE
IR	infra red
ISE	ion selective electrode
ITP	isotachophoresis
KHP	potassium hydrogen phthalate
LC	liquid chromatography
LI-CV	laser induced capillary vibration
LOD	limit of detection
LR	laboratory grade reagent

LSIMS	liquid secondary ion mass spectroscopy
m-NBA	*m*-nitrobenzylalcohol
m/z	mass/charge
MBCZE	moving boundary capillary zone electrophoresis
MBE	moving boundary electrophoresis
MECC	micellar electrokinetic capillary chromatography
MEKC	see MECC
MeOH	methanol
MOx	mesityl oxide (neutral marker)
MS	mass spectroscopy
MT	migration time
NDC	2,6-naphthalenedicarboxylate
PAGE	polyacrylamide gel electrophoresis
RMT	relative migration time
RSD	relative standard deviation
TR	transfer ratio
TRIS	tris(hydroxymethyl)aminomethane
TTAB	tetradecyltrimethylammonium bromide
ZE	zone electrophoresis

CHAPTER 1

INTRODUCTION AND AIMS

1.1 INTRODUCTION

Capillary zone electrophoresis (CZE) is a rapidly developing separation technique of relatively recent introduction [1-7]. Presently very topical, it is experiencing an exponential growth in publication output. This has meant the inevitable introduction and coinage of new terms and acronyms that can in turn be a source of confusion and misunderstanding, especially to the novice. Recently, Knox [8] has attempted to standardise terminology for separations using capillaries. Inconsistent and at times conflicting and contradictory symbols can cause confusion. Two important and obvious examples are the symbols "V" and "ω" which have been used to denote totally different things by different authors, e.g. "V" can denote either electrophoretic mobility [9] or separation voltage [e.g. 10] whilst "ω" can denote either the work done by Joule heat output from the electrophoretic separation [11] or intrinsic mobility [12]. For clarity, the symbols used in this thesis have intentionally been kept simple and used consistently throughout. Where clarity is believed to be assisted, the author has incorporated and/or altered scripts to equations. Other symbols are mentioned only for completeness.

CZE falls under the broad category of capillary electrophoresis (CE) (i.e. akin to chromatography and its various analogues). Confusion of CZE with the other formats and modes of CE is a real possibility. Therefore to minimise this possibility and to

provide an overall feel and perspective of the method used in this study and its place in the scheme of electrophoretic techniques, an overview is given in Fig. 1.1.

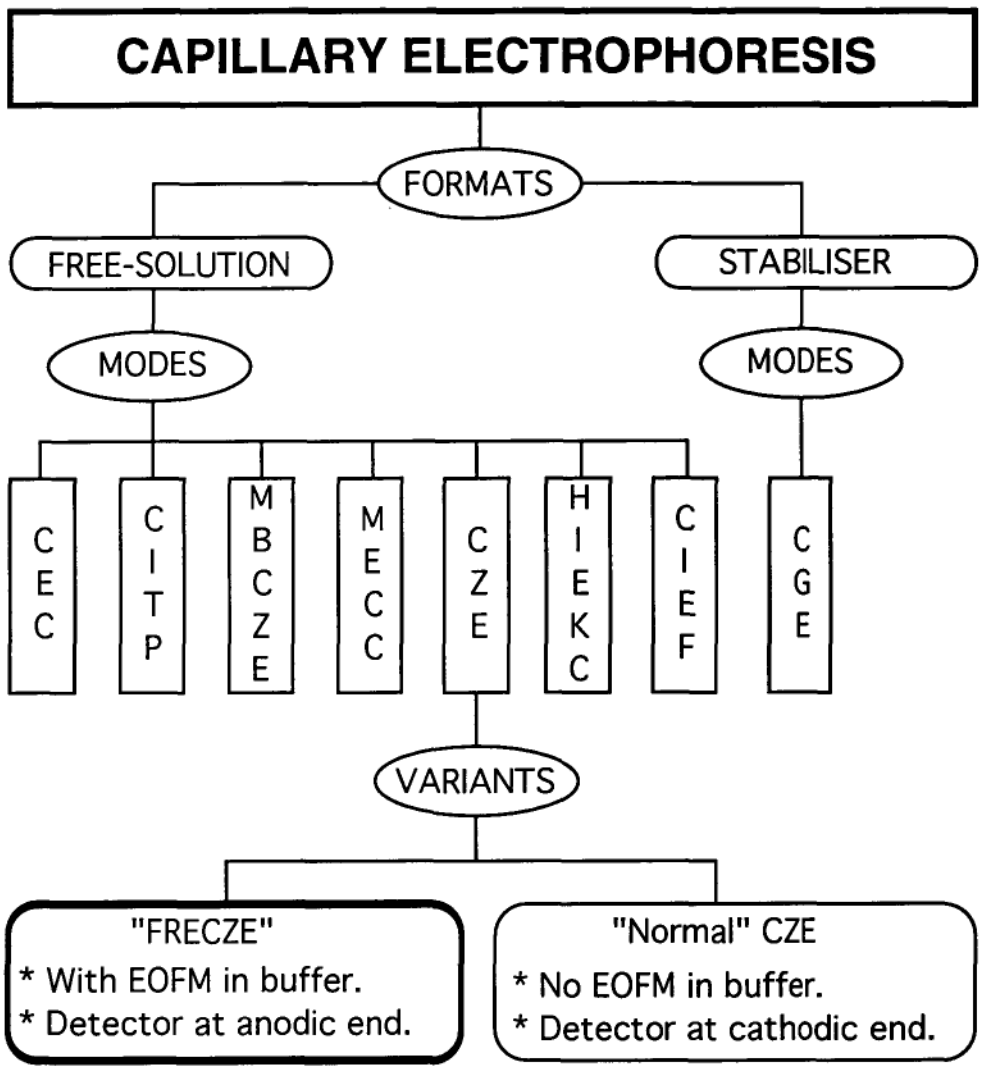

Fig. 1.1: Schematic of 'FRECZE' in relation to other CE formats and modes. Key: CEC = capillary electrochromatography, CITP = capillary isotachophoresis, MBCZE = moving boundary CZE, MECC = micellar electrokinetic capillary chromatography, CZE = capillary zone electrophoresis, HI-EKC = hydrophobic interaction electrokinetic capillary chromatography, CIEF = capillary isoelectric focussing, CGE = capillary gel electrophoresis and EOFM = electroosmotic flow modifier. 'FRECZE' is the technique used in this study. It is explained in Section 1.5.

This thesis comprises nine chapters containing material related to the study of factors affecting the separation selectivity of low molecular weight inorganic anions using CZE. The value and usefulness of such a study can be judged by its successful application to 'real' samples. To this end, the thesis also contains material related to the logical extension of the study to the analysis of such samples under optimised conditions.

1.2 THESIS OVERVIEW

Chapter 1:

In this chapter, the total thesis is surveyed (Section 1.2) and the "why" of inorganic anion determination discussed, i.e. the relevance and importance of inorganic anion analysis (Section 1.3). This is followed by a discussion of the "how" of inorganic anion determination, i.e. some analytical techniques for inorganic anion analysis. CZE being the mode used in this study, it is briefly contrasted with other (standard) methods, especially liquid chromatography (LC) (Section 1.4). The CZE variant used in this study is clarified with the aid of a new acronym, 'FRECZE', introduced to circumvent the confusion and literature review complications that can easily arise due to the abundance of similar and/or inconsistent terms and acronyms (Section 1.5). Next, the aims of the study are outlined (Section 1.6); and as this study specifically involved the manipulation of selectivity in CZE, selectivity is defined (Section 1.7).

Chapter 2:

This chapter contains a review of literature relevant to this study and is presented in three main parts dealing with (i) aspects common to traditional electrophoresis and CE, (ii) traditional electrophoresis and (iii) CE. Note that methods of anion analysis have been omitted as they have been dealt with in Chapter 1.

Chapter 3:

This chapter contains the overall experimental details of the study. The chemicals, reagents, instrumentation, conditions and procedures common to all the investigations are detailed. Where necessary, deviations from these experimental details have been indicated in the other chapters.

Chapter 4:

This chapter contains the results of preliminary investigations dealing with (i) effect of modifier dryness on anion resolution, (ii) stabilisation of absolute migration time, (iii) the use and effect of neutral compounds as electroosmotic flow (EOF) markers and (iv) effect of capillary conditioning regimes on anion (phosphate) response.

Chapter 5 onwards:

The rest of the chapters are concerned with investigations executed relating to selectivity manipulation (Chapters 5 and 6) and application to 'real' samples (Chapters 7 and 8). These chapters are structured to comprise (i) an introduction, (ii) the chapter aim, (iii) experimental details where any deviations from those stated in Chapter 3 are noted, (iv) results and discussion, (v) conclusion and (vi) references sourced. Conclusions and recommendations made from this study are outlined in Chapter 9.

Appendix:

This final section contains appended material.

Having overviewed the thesis, the importance of inorganic anion determination will be discussed, followed by how such determinations can be accomplished.

1.3 Is Inorganic Anion Analysis Important?

The obvious answer to the above question is yes. Inorganic anion analysis is important as it serves vital purposes in a wide area of applications. This can be demonstrated by the following ten random examples:- (i) *Forensic* work, e.g. testing for chloride and chlorate in explosive residues [13]. (ii) *Water* analysis, e.g. characterisation of boiler, potable and waste water [14] and monitoring of oxyhalide [15], primary (such as fluoride and nitrite) and secondary[1] (such as chloride and sulfate) pollutant anions with primary pollutants having the potential to adversely affect health [16]. (iii) *Pulp and paper* industry, e.g. effluent monitoring [14], (iv) *Industrial* analysis and monitoring of toxic waste, e.g. metal cyanides in metal plating solutions [17], analysis of gold and silver cyanide complex anions present in the extraction of precious metals from their ores [18] and analysis of chloride and sulfate in concrete. (v) *Clinical* and *biochemical* analysis, e.g. determination of body fluid electrolyte ions such as chloride, sulfate and phosphate for diagnostic purposes [19]. (vi) *Environmental* toxicology and hazard monitoring, e.g. analysis and speciation of toxic anions such as cyanide [20] and cultural eutrophication chemicals such as phosphate detergents [21]. (vii) *Agriculture*, e.g. analysis of nutrient anions in fertilizers. (viii) *Food* analysis, e.g. analysis of preservatives such as metabisulfite in wines and nitrite in processed meats for quality control and nutritional assay [22]; and quality process control, e.g. fermentation optimisation and control [23]. (ix) *Legal compliance* monitoring, e.g. preservative levels in food [22]. (x) *Electronics* industry, e.g. determination of phosphate and sulfate traces in HF acid used in etching semiconductors [24].

The above broad spread of important fields underscores the relevance and importance of sensitive and accurate methods for inorganic anion analysis. Implications of this type

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1 Termed secondary due to their organoleptic value.

of analysis extend to economic costs, maximisation of production, legal consequences of non-compliance, health hazards, failure of civil structures, etc that can result because of inadequate determination of inorganic anions. On the other hand, the range of applications means the anions are present in widely differing and complex matrices that pose their own particular associated analytical problems. The analyst needs to be mindful of this and use the most appropriate analytical method.

With the importance of inorganic anion analysis impressed, the next step is to discuss how such anions can be determined. Some analytical methods that can be employed are discussed below (Section 1.4).

1.4 Methods for Inorganic Anion Determination

It would be ideal for every analytical technique to provide accurate and precise qualitative and quantitative data about an analyte in as short a time as possible. However, when important parameters like time, equipment robustness, purchase and operating costs, sample matrix effects, precision, accuracy, sensitivity and detection limits, portability, expertise needed, equipment availability, number of analyses required, amount of sample that can be expended, concentration range of analytes, convenience [25], etc are considered, it is more than likely that a method will be deficient in at least one aspect. Obviously, no single method is perfect and compromises may need to be made. In the complex matrices of 'real' samples, it may be necessary to determine anions individually. Furthermore, different methods can be a mandatory requirement, e.g. in the analysis of anions of nutritional significance, chloride, iodide and nitrate are analysed using titrimetry, microdistillation and ion-specific electrodes, respectively [22]. In other cases, it may mean using standard

methods where litigation may result or the customer desires, e.g. APHA[2] methods are used by most laboratories for water analysis.

For various reasons, proponents of a given technique can overstate the positive aspects and down-play (or conveniently omit) the limitations. To present a balanced view, the negative as well as the positive aspects of the technique used in this study are discussed in greater detail in the Literature Review (Chapter 2). It is the author's belief that the limitations of a method need to be appreciated for appropriate and maximum utilisation of that technique.

A number of analytical techniques can be used to analyse inorganic anions. The main methods are discussed below. It must firstly be noted that all these methods require relatively long analysis times and - apart from CZE and LC - do not offer the ability to simultaneously determine multiple anions.

1. ***Classical wet chemical (titrimetric or volumetric) methods***, e.g. Mohr method for chloride and bromide, Volhard method for thiocyanate, chromate, carbonate and phosphate, Kjeldahl method for nitrate and nitrite and the Winkler method for carbonate-hydroxide mixtures [25]. Sulfate can be titrated with barium perchlorate but suffers severe interference from phosphate [26]. Expensive reagents (e.g. silver nitrate) can be required and precision is subject to operator bias and judgement (e.g. reading of end-point color change). Furthermore, classical methods are generally tedious, time consuming and require expert technicians to achieve reliable analytical results. On the other hand, specificity and selectivity are well known and interferences are well documented.

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2 American Public Health Association.

2. ***Gravimetry***, e.g. sulfate determination after precipitation with barium chloride. Although used as standard reference methods, these procedures suffer from occlusion (or co-precipitation of cations, anions and water), adsorption onto precipitate surface and solubility[3] of precipitate. Furthermore, results depend on reagent concentration and rate of reagent addition [26]. Gravimetric methods are usually highly selective for specific anions, but this quality makes impossible the simultaneous separation of multiple anions. Nitrate, another common anion, can be precipitated using nitron.[4] Although very sensitive, other anions (e.g. bromide, iodide, chromate and thiocyanate) do interfere by also precipitating with the nitron [27].

3. ***Electrochemical methods***. These include polarography, voltammetry, amperometry, conductivity, coulometry, potentiometry and ion selective electrodes (ISE). Bromate, nitrite and iodate can be detected polarographically [28]. Cyanide and fluoride ions can be detected using ion selective electrodes. Common anions in water such as sulfate, carbonate, chloride, nitrate and bicarbonate can be detected using conductivity [29]. Sulfate can be analysed using potentiometry/ISE, amperometry and polarography [26], whilst chloride can be detected using potentiometry and amperometry. Chloride, bromide and iodide can be determined using coulometry [25]. Nitrate and nitrite have historically being differentiated using DC polarography. However other anions such as sulfate and phosphate can interfere by precipitating electrolyte cations [30]. Differential pulse polarography can be used to differentiate nitrate and nitrite, but only in a sequential mode. Polarographic determination of anions is pH dependent and needs careful

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3 Small but not negligible.

4 It is also known as diphenyl-endo-anilo-dihydrotriazole and has the formula $C_{20}H_{16}N$.

buffering to give reproducible results [28]. Electrochemical methods are limited to those species able to undergo redox reactions or conduct electricity.

4. ***Spectroscopic methods***, e.g. (i) UV spectroscopy to detect nitrate at 313 nm, carbonate at 217 nm and nitrite at 280 nm and 360 nm [28]. Different wavelengths afford anion differentiation but measurement is limited to those ions that can undergo $n \longrightarrow p^*$ electronic transitions, i.e. anions of good chromophoric quality; (ii) fluorescence spectroscopy to detect fluoride down to the 0.001 ppm level [28]. The sensitivity is excellent but it suffers from interfering species that are likely concomitants (e.g. phosphate, copper and iron) of aqueous samples. Simultaneous determination with other anions, such as phosphate, in samples is therefore not possible; (iii) Indirect atomic absorption spectroscopy to determine sulfate after precipitation with barium ions and the excess cations measured [31]; (iv) Standard colorimetric (photometric absorbance) methods for anion determination also have their limitations, e.g. the AOAC (xylenol - oxidation) [32] and sulphanillic (reduction) [33] methods for nitrate and nitrite differentiation are tedious and relatively costly due to time and reagent expenditure. The many steps required increase the chance of errors due to spillage, contamination, etc.

5. ***LC methods***, e.g. ion chromatography (IC) and high performance liquid chromatography (HPLC). Introduced by Small, Stevens and Bauman [34], IC is an established method for inorganic ion determination. IC is also the recommended method of some environmental regulatory agencies [20] and currently is the most prevalent method for (low molecular weight) inorganic anion analysis [35-37]. Stationary phases and mobile phase systems are more developed relative to CE and allow the user to optimise separation selectivity for a given requirement [38]. Furthermore, the amount of sample injected in LC is not dependent on solution

viscosity, unlike CE. On the other hand, LC has some significant limitations including [37]:- (i) Requirement for gradient elution when there is a wide range of solute retention times under isocratic conditions. Gradient elution introduces a higher cost factor and system complexity [14, 37]. (ii) Limited separation selectivity requires the system to be complex [37]. (iii) The peak capacity [2-4] and efficiency are low compared to CE. (iv) Hydrodynamic flow profiles in the detector cell are complicated by the small eddies created by the pump(s) [37]. (v) Inability to separate samples of high ionic strength and extreme pHs. When injected directly, samples of extreme acidity (pH<1) or alkalinity (pH>11) reduce column life and degrade chromatographic performance [36]. Baseline noise can be severe due to the disturbance of the acid-base equilibria in the system [39]. Concentrated samples of extreme pH values require clean-up or pre-treatment, e.g. dialysis [40, 41]. (vi) Interferences are relatively common. (vii) Additionally, some samples may be difficult to analyse by IC/HPLC, e.g. bitter acids in beer hop extracts can complex with metal ions in the system (e.g. injector and frits). This requires that the stationary phase be given special treatment [42].

6. ***Capillary electrophoresis***. As the name suggests, CE involves electrophoresis in separation chambers of capillary dimensions (usually ≤100 μm I.D.). CE is a broad term (similar to chromatography) and encompasses distinct variants or modes such as those outlined in Fig. 1.1 and detailed later in Chapter 2. The main mode used in this study was CZE with the electroosmotic flow (EOF) reversed by the addition of cationic surfactants to the electrolyte. The main advantages of CZE are its high number of theoretical plates (N) and speed. These give it the ability to separate multiple inorganic anions rapidly with a high level of efficiency and separating power as epitomised by the much publicised separation of over 30 anions in a time window of less than 2 minutes [43].

As the premier method for inorganic anion determination, it is not surprising that IC (including HPLC) has been used as the yardstick to extol the virtues of CE [14, 37]. No separation technique is perfect and both IC and CE have their advantages and limitations. The separation mechanisms of IC and CE are orthogonal [e.g. 44] and it is increasingly apparent from the literature that CE complements IC [14, 44-46], but with the added bonus of efficiencies some two orders of magnitude higher [47].

1.5 WHAT IS 'FRECZE'?

This study utilised CZE in the free solution mode. With the addition of organic modifiers or surfactants in the running electrolyte to reverse the EOF, the technique has been termed 'FRECZE' for the purposes of this thesis. Like gas-liquid chromatography, IC and HPLC; 'FRECZE' is a differential migration separation method in which the analytes are separated by being caused to move at different rates [48, 49]. The term, FRECZE, is suggested to assist with clarification and is not intended to replace other acronyms such as CIA®, ICE [14, 50] and co-EOF CZE [51]. Other closely related acronyms are HVCZE [52-54] when elevated potentials are employed, HPCE [1, 10, 55, 56], and FZE/EEO meaning free zone electrophoresis combined with electroendoosmosis [44]. FRECZE is capable of determining around 130 inorganic anions, organic acids, inorganic cations and negatively charged soaps [14, 37, 50, 57-59].

The acronym 'FRECZE' clarifies concisely the method of main use in this research, i.e.;

F. Free-solution: Separation is done in the free-solution or aqueous mode.

R. Reversed: The direction of EOF is reversed (using a cationic surfactant) compared to 'normal' zone electrophoresis. This also signifies that the detection is done at the anodic end of the CE electrolytic circuit.

E. Electroosmotic flow: This is the EOF that has been reversed.

C. Capillary: A capillary is used as the separation chamber or "column".

Z. Zone: The 'zone' electrophoresis mode is used (i.e. catholyte and anolyte are identical). It also signifies that a necessarily small sample band is separated.

E. Electrophoresis: Separation occurs under the influence of an applied electric field.

1.6 Objectives of This Study

According to Buchberger and Haddad [60], there is little information on the parameters affecting selectivity changes of inorganic anions in CZE. The primary aim of this study therefore was to study the parameters affecting inorganic anion selectivity in FRECZE. Equipped with the migration order trends from the above studies, a further aim was application of these trends to method development and analysis of 'real' samples of varying complexity.

1.7 Selectivity and Resolution

As this study involved the direct manipulation of anion separation selectivity, it is instructive to define selectivity. Selectivity is the ability of an analytical technique to differentiate between analytes. In chromatography, selectivity can also be taken as the

elution order of the analytes. It is derived from basic chromatographic parameters and is usually represented by the selectivity factor, α [25]. See Eqn. 1.1.

$$\alpha = \frac{(t_R)_y - t_o}{(t_R)_x - t_o} \qquad \text{Eqn. 1.1}$$

where $(t_R)_y$ = retention time of anion y, $(t_R)_x$ = retention time of anion x and t_o = retention time of unretained species.

Unlike IC, CZE has no t_o as there is no peak due to "unretained" species. Therefore another peak (e.g. a reference anion, a system peak or the electroosmotic flow) can be used as the reference point to calculate relative migration times and ultimately α. The EOF may be unsuitable as a reference peak since it is highly variable and it can take too long to migrate to the detector, especially without a suitable cationic surfactant in the running electrolyte.

For CE, selectivity can be taken as the relative difference in electrophoretic mobilities of adjacent peaks or as relative migration times. This is represented mathematically as follows [10, 61];

$$\text{Selectivity} = \frac{2\,(m_{\text{faster anion}} - m_{\text{slower anion}})}{(m_{\text{faster anion}} + m_{\text{slower anion}})} \qquad \text{Eqn. 1.2a}$$

$$\text{Selectivity} = \frac{\text{AMT}_{\text{slower anion}}}{\text{AMT}_{\text{faster anion}}} \qquad \text{Eqn. 1.2b}$$

where m = electrophoretic mobility and AMT = absolute migration time.

The basic aim of selectivity studies is to obtain the best resolution for a desired analyte or analytes. Thus, selectivity is inherently closely related to resolution (Eqn. 1.3).

$$R_s = \frac{2\,[(t_R)_y - (t_R)_x]}{W_x + W_y} \qquad \text{Eqn. 1.3}$$

where W_x and W_y are the peak widths of anions x and y.

Eqn. 1.1 may not necessarily hold true for FRECZE as experience has shown that full resolution can be obtained when $\alpha < 1$ (~0.9). This is supported by the literature, e.g. protonated and deuterated isomers can be separated using CE with virtual baseline resolution at α = 1.009 [47]. For 'FRECZE', a relationship for resolution incorporating the selectivity expression (Eqn. 1.2a) can be indicated as follows [10, 62];

$$R_s = \left(\frac{2\,(m_{\text{faster anion}} - m_{\text{slower anion}})}{(m_{\text{faster anion}} + m_{\text{slower anion}})}\right) \cdot \left(\frac{\sqrt{N}}{4}\right) \qquad \text{Eqn. 1.4}$$

where R_s = resolution and N = number of theoretical plates.

1.8 REFERENCES

1 Mikkers, F.E.P., Everaerts, F.M. and Verheggen, Th. P.E.M., *J. Chromatogr.*, **169** (1979) 11.

2 Jorgenson, J.W. and Lukacs, K.D., *J. Chromatogr.*, **218** (1981) 209.

3 Jorgenson, J.W. and Lukacs, K.D., *Clin. Chem.*, **27** (1981) 1551.

4 Jorgenson, J.W. and Lukacs, K.D., *Anal. Chem.*, **53** (1981) 1298.

5 Jorgenson, J.W. and Lukacs, K.D., *Journal of HRC & CC*, **4** (1981) 230.

6 Jorgenson, J.W. and Lukacs, K.D., *Science*, **222** (1983) 266.

7 Jorgenson, J.W., *Science*, **226** (1984) 254.

8 Knox, J.H., *J. Chromatogr. A.*, **680** (1994) 3.

9 Wieland, T. and Dose, K., 'Electrochromatography (Zone Electrophoresis, Pherography)' in Berl, W.G., editor, Physical Methods in Chemical Analysis (Academic Press, New York, 1956), pp. 29 - 65.

10 Jones, H.K. and Ballou, N.E., *Anal. Chem.*, **62** (1990) 2484.

11 Smith, I., 'General Principles of Zone Electrophoresis' in Smith, I., editor, Chromatographic and Electrophoretic Techniques (William Heinemann Medical Books, London, 1976), pp. 1 - 15.

12 Adamson, A.W., Physical Chemistry of Surfaces, 2nd edition (Interscience Publishers, New York, 1967), p. 93.

13 Hargadon, K.A. and McCord, B.R., *J. Chromatogr.*, **602** (1992) 241.

14 Jones, W.R. and Jandik, P., *American Laboratory* (June, 1990) 51.

15 Hautman, D.P. and Bolyard, M., *J. Chromatogr.*, **602** (1992) 65.

16 Romano, J.P. and Krol, J., *J. Chromatogr.*, **602** (1992) 205.

17 Aguilar, M., Huang, X. and Zare, R.N., *J. Chromatogr.*, **480** (1989) 427.

18 Aguilar, M., Farran, A. and Martinez, M., *J. Chromatogr.*, **635** (1993) 127.

19 Tietz, N.W., 'Electrolytes' in Tietz, N.W., editor, Fundamentals of Clinical Chemistry (W.B. Saunders Co., Philadelphia, 1970), pp. 612 - 650.

20 Kalambaheti, C. and Haddad, P.R., *Chemistry in Australia*, **58** (1991) 550.

21 Stoker, H.S. and Seager, S.L., Environmental Chemistry: Air and Water Pollution, 2nd edition (Scott, Foresman & Co., Illinois, 1976), pp. 125 - 127.

22 Southgate, D.A.T., *Food and Nutrition Bulletin*, **5** (1983) 30.

23 Joergensen, L., Weimann, A. and Botte, H.F., *J. Chromatogr.*, **602** (1992) 179.

24 Vanderford, G., *J. Chromatogr.*, **602** (1992) 75.

25 Skoog, D.A. and West, D.M., Fundamentals of Analytical Chemistry, 4th edition (Saunders College Publishing, Philadelphia, 1982).

26 Williams, W.J., Handbook of Anion Determination (Butterworths, London, 1979), pp. 529 - 563.

27 Vogel, A.I., Qualitative Chemical Analysis, 3rd edition (Longmans, Green & Co. Ltd, London, 1945), p. 276.

28 Skoog, D.A., Principles of Instrumental Analysis, 3rd edition (Saunders College Publishing, Philadelphia, 1985), pp. 189, 243, 694.

29 APHA/AWWA/WPCF, Standard Methods for the Examination of Water and Waste Water, 16th edition (APHA, Washington, 1985), p. 34.

30 Holak, W. and Specchio, J.J., *Anal. Chem.*, **64** (1992) 1313.

31 Hassan, S.S.M., Organic Analysis using Atomic Absorption Spectrometry (Ellis Horwood, Sussex, UK, 1984), p. 94.

32 Horwitz, W., editor, Official Methods of Analysis of the Association of Analytical Chemists, 12th edition (AOAC, Washington, 1975).

33 Crank, G., personal communication, 1991, UNSW Food and Drugs Course.

34 Small, H., Stevens, T.S. and Bauman, W.C., *Anal. Chem.*, **47** (1975) 1801.

35 Gjerde, D.T. and Fritz, J.S., Ion Chromatography, 2nd edition (Hüthig Verlag, Heidelberg, Germany, 1987).

36 Haddad, P.R. and Jackson, P.E., Ion Chromatography: Principles and Applications, (Journal of Chromatography Library - volume 46, Elsevier, Amsterdam, 1990).

37 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **546** (1991) 431.

38 Li, S.F.Y., Capillary Electrophoresis: Principles, Practice and Applications, volume 52 (Journal of Chromatography Library, Elsevier, Amsterdam, 1992).

39 Laksana, S., personal communication, 1993.

40 Laksana, S. and Haddad, P.R., *J. Chromatogr.*, **602** (1992) 57.

41 Laksana, S. and Haddad, P.R., *Chemistry in Australia*, **60** (1993) 276.

42 Vindevogel, J., Sandra, P. and Verhagen, L.C., *J. High Resolut. Chromatogr.*, **13** (1990) 295.

43 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **546** (1991) 445.

44 Dülffer, T., Herb, H. and Kobold, U., *Chromatographia*, **30** (1990) 675.

45 Zhu, M., Rodriguez, R., Wehr, T. and Siebert, C., *J. Chromatogr.*, **608** (1992) 225.

46 Schöneich, C., Kwok, S.K., Wilson, G.S., Rabel, S.R., Stobaugh, J.F., Williams, T.D. and Vander Velde, D.G., *Anal. Chem.*, **65** (1993) 67R.

47 Goodall, D.M., Lloyd, D.K. and Williams, S.J., *LC•GC*, **8** (1992) 788.

48 Heftmann, E. 'Survey of Chromatographic and Electrophoretic Techniques' in Heftmann, E., editor, Chromatography: Fundamentals and Applications of Chromatographic and Electrophoretic Techniques. Part A: Fundamentals and Techniques (Elsevier Scientific Publishers, Amsterdam, 1983), pp. A1 - A2.

49 Janini, G.M., Chan, K.C., Barnes, J.A., Muschik, G.M. and Issaq, Benz, H.J., *Chromatographia*, **35** (1993) 497.

50 Romano, J., Jandik, P., Jones, W. and Jackson, P.E., *J. Chromatogr.*, **546** (1991) 411.

51 Jandik, P. and Bonn, G., Capillary Electrophoresis of Small Ions and Molecules (VCH Publishers, New York, 1993).

52 Altria, K.D. and Simpson, C.F., *Chromatographia*, **24** (1987) 527.

53 Fujiwara, S. and Honda, S., *Anal. Chem.*, **59** (1987) 487.

54 Tsuda, T., Nomura, K. and Nakagawa, G., *J. Chromatogr.*, **264** (1983) 385.

55 Cohen, A.S., Terabe, S., Smith, J.A. and Karger, B.L., *Anal. Chem.*, **59** (1987) 1021.

56 Otsuka, K. and Terabe, S., *J. Chromatogr.*, **480** (1989) 91.

57 Kenney, B.F., *J. Chromatogr.*, **546** (1991) 423.

58 Wildman, B.J., Jackson, P.E., Jones, W.R. and Alden, P.G., *J. Chromatogr.*, **546** (1991) 459.

59 Weston A., Brown P.R., Jandik P., Jones, R. and Heckenberg A. L., *J. Chromatogr.*, **593** (1992) 289.

60 Buchberger, W. and Haddad, P.R., *J. Chromatogr.*, **608** (1992) 59.

61 Kuhn, R. and Hoffstetter-Kuhn, S., Capillary Electrophoresis: Principles and Practice (Springer-Verlag, Berlin, 1993), p. 36.

62 Ewing, A.G., Wallingford, R.A. and Olefirowicz, T.M., *Anal. Chem.*, **61** (1989) 292A.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION AND OVERVIEW

The literature review (Section 2.2) has been structured to consider a number of factors. Firstly, electrophoresis is defined (Section 2.2.1). Electrophoretic formats (Section 2.2.2) and modes (Section 2.2.3) are highlighted to distinguish (FRE)CZE from the other classes of electrophoresis (see Fig. 1.1 in Chapter 1 for an overall outline). The classes of electrophoretic mobility are then outlined (Section 2.2.4) and a brief background to traditional electrophoresis is given along with some of its main limitations (Section 2.2.5). CZE is one way of overcoming the major problems of diffusion and long analysis times in traditional electrophoresis (Section 2.2.6). Section 2.2.6 covers background and terminology, applications, sample matrices amenable to CZE and pre-treatment, instrumentation, sampling methods, detection, separation mechanics and basic theory, parameters affecting anion selectivity (Section 2.2.6.7), factors affecting analytical performance, advantages and limitations of CZE, and a listing of relevant reviews and books. Alternative methods of anion determination were reviewed in Chapter 1 and have therefore been omitted here. Only traditional electrophoresis has been included in this chapter as it is the direct precursor of FRECZE.

Terminology associated with both traditional and modern electrophoresis (CZE) has been included because (i) it is important that this terminology be understood and appreciated for its similarities, differences, contradictions, etc, to enable the reader

to be conversant with this field of study; and (ii) terms[1] and symbols denoting important electrophoretic parameters have changed over time and there does not seem to be a convention, even in the recent literature. An important example is the symbol for electrophoretic mobility. Some symbols used include V [2], l_+ for cations and l_- for anions [3], μ_p for particles [4], μ_{osm} [5], μ_o^* [6], μ_{eo} and μ_{EEO} for EOF [1], u [7], μ [8], m [9], m_i [10], μ_{EP} [1], μ_{ea} [8], μ_{el} [11], μ_{app} for apparent mobility [12], $\mu_{el\ obs}$ for net mobility [13], μ_{obs} for observed mobility [13] and μ_e [6, 14].

Nomenclature has not been universally adopted whilst designations for techniques refer to materials and methodology rather than underlying separation mechanisms [15]. Also, novices are often confused by taking such terminology literally. Thus the different terms (and symbols) need to be appreciated to avoid needless confusion. Symbols (related to theory) have been intentionally kept simple and used with consistency throughout the thesis. Where clarity is felt to be aided, the author has added and/or modified scripts to equations. Other symbols are noted for completeness only. A glossary of symbols and abbreviations, acronyms has been appended on pages xxiii - xxix.

2.2 LITERATURE REVIEW

2.2.1 WHAT IS ELECTROPHORESIS?

Coined in 1909 by L. Michaelis [16], *electrophoresis* is usually defined as the movement of charged species through a fluid medium [17] under the influence of an electric field [18-20] applied between an anode and cathode [21] in an

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[1] One example is the electrolyte which is also known as background electrolyte, buffer, working electrolyte, supporting electrolyte or running electrolyte. Another example is electroosmosis which is also known as electroendoosmosis [1].

electrolytic circuit. Although analytes traditionally were *charged* colloids (e.g. proteins), analytes amenable to today's CE include inorganic ions, organic ions, neutral chemicals as well as charged colloidal particles. The traditional definition can therefore be considered obsolete and inaccurate. Also, the traditional definition includes techniques in which separation does not depend solely on electrophoretic mobility (e.g. isoelectric focussing [22]) and techniques that include a combination with chromatography (e.g. immunoelectrophoresis [23]).

2.2.2 FORMATS OF ELECTROPHORESIS

Electrophoresis can be executed in two basic formats, *viz.*; (i) 'free-solution' (Fig. 2.1) or (ii) stabiliser supported (Fig. 2.2) formats [17]. The former is carried out in aqueous ("free") [24] solution whilst the latter is performed with the aid of stabilisers like paper, cellulose acetate [25], starch, agarose and polyacrylamide gels [17, 23]. Polyacrylamide is usually the medium of choice as cross-linking can be easily manipulated and controlled [25] to suit given solute sizes. Such stabilisers are known as sizing or sieving media [26]. Traditionally, electrophoresis was executed predominantly in the stabiliser-supported format [10]. A quick browse through most pre-CE literature indicates that when the term electrophoresis was used, it meant or automatically inferred stabiliser or anti-convective media-supported (ACMS) electrophoresis.

Fig. 2.1 illustrates 'normal' free-solution CZE in which the surface of the capillary is negatively charged (dashed lines) due to the ionisation of surface silanol groups on the fused-silica capillary wall [27]. The electroosmotic flow (EOF) 'plug' migrating to the cathode (negative electrode) has, except for a few nanometres at the capillary surface, a flat profile [28]. (EOF generation and its effect on anion migration rate are discussed in Section 2.2.6.6.) Cationic species migrate to the cathode whilst anionic species do the opposite. Fig. 2.2 depicts specifically an

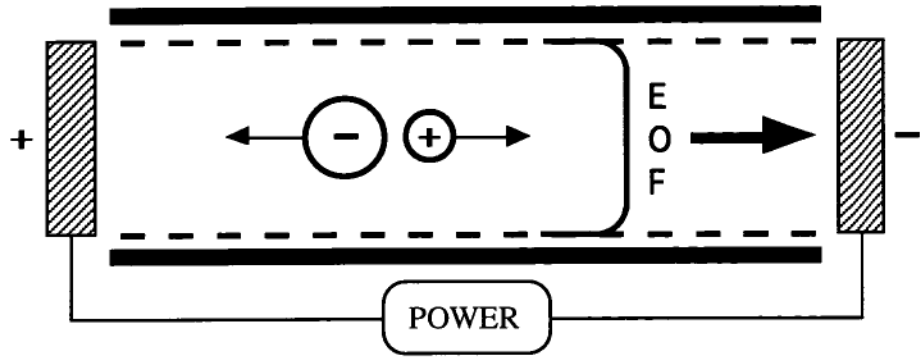

Fig. 2.1: Schematic of free-solution electrophoresis format. Key: Circle with + sign = cation, circle with - sign = anion, - signs = charge on capillary surface and shaded rectangle with appropriate signs = electrodes. The arrows indicate migration direction. [Knox, J.H., *Chromatographia*, **26** (1988) 329. Adapted with permission of the author and publisher. Copyright © (1988) Friedrich Vieweg & Sohn, Pergamon Press.]

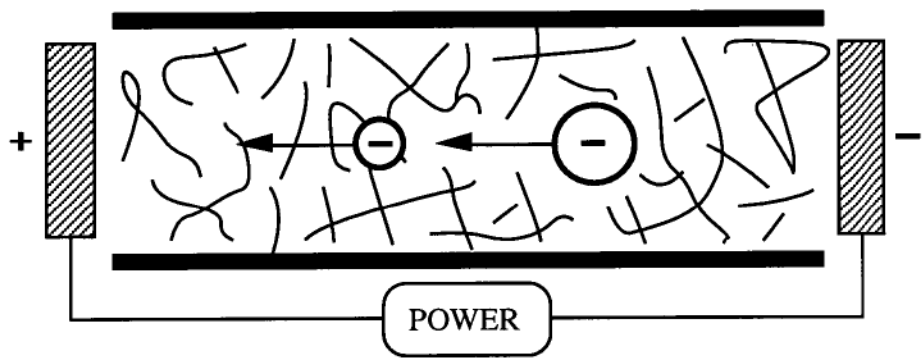

Fig. 2.2: Schematic of stabiliser (or anti-convective media) supported electrophoresis format. Key: See Fig. 2.1. [Knox, J.H., *Chromatographia*, **26** (1988) 329. Adapted with permission of the author and publisher. Copyright © (1988) Friedrich Vieweg & Sohn, Pergamon Press.]

ACMS electrophoresis example, namely capillary gel electrophoresis (CGE). The smaller anion is less restricted and therefore migrates faster to the anode. Note that the EOF has been suppressed.

2.2.3 MODES OF ELECTROPHORESIS

2.2.3.1 Isoelectric Focussing (IEF)

The mechanism of separation for IEF has been illustrated by Dülffer, Herb, Herrmann and Kobold [29] and Widmer [17]. IEF involves separation of

amphoteric substances under a pH gradient [25]. Although an electric field is applied, separation does not depend entirely on electrophoretic mobility [22] but also on the analyte's isoelectric point (p*I*). Solutes migrate between an acidic anolyte and a basic catholyte until the p*I* is reached (i.e. the position at which the compound is neutral). When done in capillaries, it is known as CIEF [30] or cIEF [31]; and sharp peaks with good resolution are obtained. Care however must be taken to prevent analyte precipitation [32] which can cause blockage.

2.2.3.2 Isotachophoresis (ITP)

Also known as isotacho-electro-phoresis, ITP uses two different electrolytes, i.e. it is a discontinuous system with 'leading' and 'terminating' electrolytes. The leading electrolyte has the ion with the highest mobility whilst the terminating electrolyte has the ion with the lowest mobility [10, 25]. The sample is positioned between the two electrolytes and separation occurs with the application of a potential gradient [25] with constant current [10]. Terminology concerning ITP has changed over time, e.g. it has been known variously as 'ion migration method' (1923), 'displacement electrophoresis' (1942) [10], 'steady-state stacking' (1964) and 'cons electrophoresis' (1966) [22]. Its use has been illustrated by Jorgenson [25] and Widmer [17]. When performed in capillaries it is known by the acronym CITP [32, 33]. With non-UV detection, peak step traces are recorded rather than the typical Gaussian-type output.

2.2.3.3 Gel Electrophoresis

Of the ACMS electrophoresis formats, gel electrophoresis is known too as gel sieving or molecular sieving [25]. A minute sample band is introduced and separation based on electrophoretic mobility proceeds under an applied electric field. Pore sizes in the gel network allow for smaller ions to migrate faster and larger solutes to be retarded. Selectivity is based on size. Note Fig. 2.2.

When performed in capillaries, it is represented by the acronym 'CGE' [33, 34]. CGE is also known as HPCGE [17] or gel HPCE [35] with HP representing High Performance, and PAGE[2] when polyacrylamide gel is used as stabiliser. In CGE, EOF is reduced and diffusion is minimal. The absorption of solutes onto the capillary surface is prevented [32]. Neutral compounds will not be separated however as they will not migrate. CGE can suffer from the loss of gel matrix during the separation and formation of air bubbles upon gel shrinkage [32]. Also, polyacrylamide gel coating in capillaries is unstable at alkaline pH[3] [29] and batch to batch variation in gel characteristics results in variation of analyte mobility which may necessitate the use of internal standards [34].

2.2.3.4 Multi-Dimensional Electrophoresis

This involves electrophoretic separation in one dimension followed by isoelectric focussing in another [23, 25] or vice-versa. An example is immunoelectrophoresis. This is cognate to 'fingerprinting' and is suitable for the separation of complex mixtures.

2.2.3.5 Moving Boundary Electrophoresis (MBE)

MBE is analogous to frontal chromatography. Its separation mechanics have been illustrated by Jorgenson [25]. Solutes are separated under the influence of an electric field after a long band of sample is introduced and surrounded by a buffer solution. Complete separation is never fully realised [25].

2.2.3.6 Zone Electrophoresis (ZE)

Here a narrow sample band is applied and surrounded by electrolyte. Separation occurs under the influence of an electric field. Separation is dependent on the

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2 This stands for PolyAcrylamide Gel Electrophoresis.

3 A unique hydrophilically- and hydrolytically-stable monomer coating that accounts for this problem has recently been described by Righetti and co-workers, e.g. see *Electrophoresis*, **15** (1994) 177.

specific (net) mobility of analyte. An elution chromatography analogue, ZE is very similar to MBE - differing only by the smaller amount of sample applied. Sometimes, ZE and MBE are grouped together [14] due to their similarity as elution chromatography analogues. A feature of ZE is that the electrolytes in the anode (anolyte) and cathode (catholyte) compartments are identical, unlike IEF and ITP. When carried out in separation chambers of capillary dimensions, it is known as CZE [30]. It has been schematically illustrated by Jorgenson [25], Widmer [17] and Jandik and Bonn [36].

2.2.3.7 Electrochromatography (EC)

This format is also known as electrokinetic capillary chromatography or EKC [37]. It uses packed capillaries and selectivity is governed by distribution between two phases as in chromatography. EOF is involved in anion separation and is a function of pH, field strength, ionic strength, organic modifier and ionic modifier [32]. CEC has high efficiency and speed and is not limited by pressure restrictions. However, capillary bleeding can be a problem at extreme pH.

2.2.3.8 Micellar Electrokinetic Capillary Chromatography (MECC)

Introduced by Terabe and co-workers [38, 39], MECC is the most popular of the electrokinetic chromatographic techniques [40]. CZE and MECC are similar in all respects except in the amounts of surfactants used to alter the electroosmotic flow (EOF). CZE uses concentrations under the critical micelle concentration (cmc) whilst MECC uses concentrations ≥cmc. This seemingly minor difference is significant as it affords different separation mechanisms to be at play. Separation in MECC is based on a combination of electrophoretic mobility and chromatographic partitioning between the slower moving micellar "pseudo stationary phase" and the aqueous front (EOF). MECC is seen as a complexation and inclusion method [34]

and can separate both ionic and 'neutral' analytes. MECC's separation mechanics has been illustrated by Dülffer *et al.* [29].

2.2.3.9 Hydrophobic Interaction Electro-kinetic Capillary Chromatography (HI-EKC)

Although formally introduced in 1994 by Ahuja and Foley [37] as HI-EKC, similar work was done previously by Walbroehl and Jorgenson [41]. HI-EKC is suitable for the separation of very hydrophobic neutral solutes. Separation is based on hydrophobic interaction with free monomers (e.g. of CTAB). It does not involve micelles as in MECC.

2.2.4 Electrophoretic Mobility

Electrophoretic mobility is fundamental to this study. However, it is clear that confusion does arise easily, especially when one is attempting to correlate mobility with conductivity and realises that actual migration orders do not match orders indicated by standard ionic conductances. Three classes of mobility can be distinguished. They are [22]:-

(a) *IONIC MOBILITY*

This is the mean migration rate at which an anion travels under the influence of an applied electric field. It is represented by m_i [10].

(b) *ABSOLUTE IONIC MOBILITY*

Absolute ionic mobility ($m_{abs.}$) is the mobility at infinite dilution and under unit field strength. It is proportional to limiting equivalent ionic conductance or aqueous charge-to-radius ratios [36] tabulated in many handbooks.

$$m_{abs.} = \frac{v}{V} = \frac{\lambda_0^*}{F} \qquad \text{Eqn. 2.1}$$

where v = migration rate of ion, λ_0^* = limiting equivalent ionic conductance at infinite dilution, V = applied voltage and F = Faraday constant.

(c) *EFFECTIVE IONIC MOBILITY*

Effective ionic mobility ($m_{eff.}$) is the ionic mobility after being corrected for ion-ion interaction, partial dissociation where appropriate, contribution of EOF, etc. It is the (net) mobility measured experimentally. Eqn. 2.2 (Tiselius equation) is applicable for weak electrolytes [10].

$$m_{eff.} = \sum_{i}^{n} \alpha_i \, \gamma_i \, m_i \qquad \text{Eqn. 2.2}$$

where α_i = degree of dissociation of ion (i.e. ionisation of weak acids and bases). Chang and Yeung [42] have defined how α can be calculated for mono- and di-valent ions; m_i = ionic mobility of individual ion present and γ_i = correction factor for relaxation and retardation effects.

Electrophoretic 'retardation' is a reduction in migration rate caused by the solvent molecules which have been energised by the ions which in turn have been energised by the applied potential. Electrophoretic 'relaxation' is related to the ions being energised, becoming non-uniformly distributed and then reforming. The time taken for reformation is the relaxation time [22].

2.2.5 BACKGROUND (TRADITIONAL ELECTROPHORESIS)

Electrophoresis is an established and mature technique dating back almost two centuries to 1807 [16] (or 1809 according to Warner [43]) when the EOF phenomenon was first illustrated by the Russian physicist F. Reuss [17].

Traditional electrophoresis is also known by other names such as electrochromatography, pherography and ionophoresis or ionography. Ionography is used when the analytes are of low molecular mass [2]. When charged colloidal particles are separated, the term cataphoresis [22] is often used. These various terms can be useful in indicating analyte type though their assistance to overall clarity is doubtful.

Historically, early (1800s) electrophoresis was done in the free-solution format [44, 45], usually employing U-tubes [19], columns or rods [44]. However, due mainly to diffusion [25, 44] and the absence of fast and sensitive detection systems [22], free-solution electrophoresis took a back-seat to ACMS electrophoresis - over time the latter becoming synonymous with the terms *'electrophoresis'*, *'conventional electrophoresis'*, *'classical electrophoresis'* and *'traditional electrophoresis'*. For the purposes of this thesis, traditional electrophoresis will mean separation done with the aid of stabilisers [1, 10] or in relatively large columns in the free-solution format.

Separation and detection in traditional electrophoresis have been reviewed by Jorgenson [25]. The separation mechanics of traditional ACMS electrophoresis have been briefly discussed in Section 2.2.2. For the purposes of this thesis, that discussion is deemed adequate for comparison and warrants no further comment.

Although labour intensive, conventional ACMS electrophoresis is powerful [5]. Its resolving power for charged analytes is superior to separation techniques like HPLC [25] and it is of no surprise that electrophoresis is a standard method for the separation and analysis of complex biochemical species [27, 44]. This is evidenced by >50% of publications in biochemistry involving the use of electrophoresis [21]. Although traditional ACMS electrophoresis is used primarily for the separation of

biochemical analytes (e.g. proteins[4] [27], immune bodies [16] and snake venom [46]), it can also be used for the separation of anions [23].

Traditional ACMS electrophoresis and large-column free-solution electrophoresis were hampered by several significant limitations with respect to the following;

(a) *Detection:* Detection was difficult, tedious [10] and time consuming [47] unless solutes were chromophoric. Detection usually entailed specialised staining [47], blotching or densitometry [29] which made for imprecise [29, 44, 47] quantification and made automation of the method difficult [44]. Specialised staining required for low levels [47] added to the labour intensity of the method [25]. Staining can be analyte-specific which on the other hand limits simultaneous detection of other analytes.

(b) *Diffusion:* Large-column and ACMS electrophoresis dissipated heat poorly [48]. This meant Joule heat [25] generated during the separation could cause electrolyte temperature gradients which consequently altered density and viscosity. (In extreme cases, bubbling or evaporation of the electrolyte could occur.) This in turn induced convection which resulted in migration time imprecision, increased solute dispersion [10, 49] and poor resolution [27]. Dispersion was compounded by the inability to achieve ultrathin starting zones [50]. Manual sample injection was another source of dispersion, i.e. when injected, the centre of the sample band moved faster than the edges and eventually attained parabolic (in cylindrical tubes) or complex (in coiled tubes) concentration profiles [51]. Furthermore, eddy migration due to more tortuous routes in gel structures introduced a new source of dispersion [5].

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4 Awarded the 1948 Nobel Prize for work involving electrophoresis, Tiselius was the first to use electrophoresis as an analytical tool [27]. Ion separation with electrophoresis however was known well before that time [17].

(c) *Separation efficiency:* Relatively inefficient separations (lower N) compared to modern CE were observed. Resolution was poor due to the convective disturbances and dispersion caused by the Joule heat [27]. This was compounded by the unavailability of efficient means for heat dissipation.

(d) *Analysis time:* Being a gradual and tedious method, analysis times were long [10, 25, 47]. This in turn resulted in low sample throughput and low productivity. Difficulty of automation [25] and specialised staining [47] and densitometry were contributing factors. The obvious option to reduce separation time would be to increase the applied voltage. This however would be at the risk of increased dispersion (and poor resolution) due to Joule heat and possible denaturation of heat labile solutes.

(e) *Precision and accuracy:* Precision of migration time was poor [47], e.g. 5% [52] and was due to gel to gel variation [1] and Joule heat-induced dispersion [10]. Reproducibility and quantification accuracy were poor [10, 25, 27].

(f) *Automation:* Automation was almost impossible [10, 25], making the method labour intensive [25, 47]. Cumbersome equipment needed to be placed in a horizontally level position [25, 52]. The presence of gel complicated adaptation to on-line sample application, detection [5, 8], quantification and automation [5].

In summary, the main impediments to ACMS and large-column free-solution electrophoresis were inefficient Joule heat dissipation, diffusion, relatively long analysis times and low detection sensitivity. The following are some ways to overcome the above limitations:-

(a) For free-solution electrophoresis in large columns, use of stabilisers like agar, gels and agarose [53] can minimise diffusion and convection [54, 55]. Stabilisers also act as anchoring points for buffer ions, resulting in a stable pH [25]. The problems of long assay time and detection (staining) still remain if the separation is not done in capillaries and using on-line detection. Accurate quantification is difficult whilst interactions between the solutes and stabiliser is a source of error [54, 55]. Furthermore, electrophoresis using stabilisers is largely a manual, labour-intensive method and thus has poor reproducibility [5, 25]. Although usually done on slabs, it can also be performed using capillaries and is known as capillary gel electrophoresis (CGE). In contrast to open-bed gel electrophoresis, CGE offers increased resolution, high sensitivity, high operating voltage and high speed when coupled with on-line detection [27]. CGE however can suffer from loss of gel matrix from the capillary during electrophoresis and shrinkage of the stabiliser during polymerisation which can result in the formation of air pockets [32].

(b) The employment of additional centrifugal, gravitational and electromagnetic force fields to overcome dispersion [54] in large-column free-solution electrophoresis. One way of doing this is to rotate the column along its horizontal axis [56, 57]. More recent methods include application of an external electric field [58] or a radial potential gradient [59].

(c) Perform the separation in space where effects due to gravity and thermal convection are minimal or absent. NASA demonstrated on the Apollo-Soyuz mission that a 'flat' flow profile could be obtained [60] in space which meant minimal dispersion and high separation efficiency could be achieved. The obvious limitation with this approach is its impracticability.

(d) Using capillaries as the separation chamber [10] as suggested by Hjertén [56]. Without stabilisers, this is known as "free-solution" CE and is distinct from CGE. The high surface area:volume ratios [8, 27, 61] and large resistances [62] of capillaries allows for application of high voltages [8] and efficient Joule heat dissipation [8, 27, 34]. This translates directly to short analysis times [25], especially when coupled with on-line detection methods. Furthermore, dispersion effects are no longer a significant limitation with CE [49] due to the "wall effect" [8].

2.2.6 FREE SOLUTION CAPILLARY ZONE ELECTROPHORESIS

2.2.6.1 Background and Terminology

The following sources outline in chronological order historically significant developments in electrophoresis:- Li [45] has covered the period 1886 - 1989 whilst Jandik and Bonn [36] have included the interval 1791 - 1984. Vesterberg [21] has outlined historical developments relative to IEF from 1791 (when Faraday presented his laws on electrolysis) to 1989. Everaerts, Beckers and Verheggen [22] have given an historical account of electrophoresis (relative to isotachophoresis) for the period 1856 - 1972. Jandik, Jones, Weston and Brown [63] have briefly discussed the origins of CE related to ions. The above references cover both traditional and modern electrophoresis (CE). Although many reviews have been published to date (see Table 2A.1 in Appendix 2A), the first fundamental review of CE by Kuhr [48] appears most relevant for this section. It discusses comprehensively the background to CE development (along with instrumentation, principles, detection, formats/modes and applications).

About a decade ago, electrophoresis was considered to be largely a qualitative technique [64]. In 1986 [25], literature in analytical chemistry relating to electrophoresis was considered rare and there was relatively little information on

electrophoresis in standard analytical chemistry textbooks. The scarcity of analytical electrophoresis information has now well and truly passed as a result of the advent and rapid expansion of capillary electrophoresis.

The idea of doing electrophoresis in capillaries has been around since at least the mid-sixties when Hjertén [56] carried out separations in *columns* with small internal diameters (I.D.). For this pioneering work, he was given the first Capillary Electrophoresis Award [65]. Work on electrophoresis in small I.D. *capillaries* was reported initially by Virtanen in 1974 [48]. However, C(Z)E's development was initially stagnated by the absence of efficient insulating materials [22] or means of efficient Joule heat dissipation and sensitive detection methods [5, 8, 22]. Narrow-bore ZE was taken up by Mikkers, Everaerts and Verheggen [54] who were the first to report ZE in capillaries. Further advancement was made with the reduction in I.D. by Jorgenson and Lukacs [66]. Jorgenson [8] also introduced complete instrumental format with on-line detection. All the above developmental work was done in "free-solution"[5] form without the addition of EOF modifiers and was used to analyse ionogenic chemicals. Jorgenson and Lukacs are credited overwhelmingly as the 'pioneers' of modern CE [4, 5, 10, 17, 47] as widespread application has occurred after their disclosures [10, 34] in the early eighties [5, 67-69]. For his outstanding contribution to CE, Jorgenson was awarded the second Capillary Electrophoresis Award [65].

Like LC and traditional ACMS electrophoresis, CE is a broad term encompassing a number of distinct electrophoresis variants and modes. One such mode is CZE and unless one is versed in the subject, misunderstanding can easily occur as a consequence of confusing, interchangeable (e.g. CZE and HPCE [27]), indiscriminate, misleading or incorrect use of terminology. Occasionally,

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5 The 'plug' flow due to the "wall effect" [8] meant that stabilisers could now be dispensed with.

distinctions between CE variants are not made clear. Presented as HPCE variants [70, 71], CZE and MECC are the two most likely to be confused. This is not surprising given that CZE and MECC are identical in all respects (including instrumentation) except that the amount of surfactant used is ≥cmc in the latter method. An overview of CE operational formats and modes has been given in Fig. 1.1 in Chapter 1 and elaborated on in Sections 2.2.2 and 2.2.3. Outlined by Ewing *et al.* [14] and categorised in a review by Schöneich *et al.* [30], the main modes are CZE [14, 17, 25, 30, 61, 72-75], CITP [17, 32, 61], MECC [17, 25, 30, 61], HI-EKC [37], MBE [25], CIEF/cIEF [30-32, 37, 61] and CGE [14, 17, 30, 43, 61]. CZE, CGE and MECC are presently the most topical methods for automated analysis [34], with CZE being most widely used [33].

Although relatively new, CZE's features of high speed and high resolving power make it an attractive method for the separation of anionic solutes. Its separating power is such that it is able to resolve Cl^{35} and Cl^{37} isotopes [76]. The creation of wide interest [17] in CZE and rapid growth [1] in its usage is not surprising. For a technique considered to be in its infancy as recently as 1992 [47], the growth has been phenomenal as attested to by such facts as (i) more than 50% of cited literature being post-1988 [14], (ii) rapid commercialisation with instruments being made by at least 10 manufacturers [33, 34, 77] today in contrast to no commercial instruments as recently as 1987 [43], (iii) being proposed for consideration as an ASTM-EPA standard method [78] and (iv) a database with 400 entries[6] was presented in 1991, and as expected [79], has grown exponentially [80].

The technique used in this study was dubbed 'FRECZE' for clarity. (See Chapter 1 for justification and explanation.) It is a variant of CZE wherein the EOF has been reversed with the aid of a suitable cationic surfactant. Since the CZE mode was used for this study, only terminology related to it will be noted. Various relevant

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6 Databases on CE in Professor Haddad's group held over 1000 entries in 1994.

synonymous terms are (i) continuous-pH ZE [50], (ii) HVCZE[7] [1, 81] when high voltages are used, (iii) High Performance CE [47, 54, 82, 83], (iv) FZE/EEO [29] where the EOF has been altered, (v) ICE[8] or CIE is used when separating inorganic anions [75, 84]. More recently, it has been termed CIA®,[9] [12, 85, 86] when ions are analysed and (vi) free-solution CE [61].

2.2.6.2 Applications, Sample Matrices and Sample Pre-treatment

CZE is a powerful tool for the separation of inorganic anions, small ions and biomacromolecules [48]. Its application area is wide, ranging from simple samples (e.g. tap water) to complex samples (e.g. Bayer liquor). Jones and Jandik [12] have tabulated anions that have been characterised using FRECZE. Over 130 solutes including inorganic anions, organic acids and negatively charged surfactants can be separated [63, 75, 84, 87-89]. As it is not possible to give an exhaustive listing of applications, some random examples from each of the main application areas of CZE are shown in Table 2.1. Almost all the examples are from the study period (1992 - 1995) and although most solutes are anions, others have been included to illustrate the versatility of CZE.

Basic considerations for industrial applications of CE have been discussed by Dülffer *et al.* [29]. Jorgenson [8] has discussed applications demonstrating the use of high voltages. Karger, Cohen and Guttman [90] and Goodall, Lloyd and Williams [34] have reviewed comprehensively applications, particularly in the biochemical sciences. In the first fundamental review of CE, Kuhr [48] has indicated some applications. For the purposes of this study, the comprehensive 76-page review by Wallingford and Ewing [123] is deemed most informative.

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7 This stands for High Voltage Capillary Zone Electrophoresis.

8 This stands for Inorganic Capillary Electrophoresis.

9 This stands for Capillary Ion Analysis.

Table 2.1

SOME APPLICATIONS OF CZE, SAMPLE MATRICES AMENABLE TO CZE AND SAMPLE PRE-TREATMENT

Key: ***a*** = All filtrations were done to pass at least 0.2 μm. ***b*** = Filtered or filtration. ***c*** = Extraction. ***d*** = Dilution. ***e*** = Procedure not fully described. ***f*** = Dissolved.

Solute(s)	Sample Matrix and Note(s)	Sample Pre-treatment[a]	Reference(s)
Water, waste-water treatment and monitoring			
Carbonate, Cl^-, SO_4^{2-}, etc.	*Mineral spring water.*	Not treated. Direct injection.	[91]
Aromatic amines.	*Ground water.*	Adjust pH, fil.[b] and extr.[c] using solid phase.	[92]
Nitrite, chloride, sulfate, etc.	*Tap water.*	Fil. [93] or simple dil.[d] [94, 95].	[91]
Cyanides and hydroxides.	*Industrial waste water.*	Dilution.	[96]
Chloride, sulfate, nitrate, etc.	*Power plant water.*	Not treated. Direct injection.	[97]
Environmental and Toxicology			
Bromide, acetate, lactate, formate, etc.	*Atmospheric aerosols.*	Collect on filters, fractionation, wet with isopropanol and extr. with water under ultrasound.	[98]

Table 2.1 - *continued*

Palytoxin.	*Marine coral; the most toxic non-protein marine toxin known yet.*	Extraction from coral.[e]	[61]
Acetate, glycolate, etc.	*Air (filter). Acidic.*	Butyric acid extr. and 5000-fold dil.	[75]
Chloride, nitrate, sulfate, etc.	*Rain water.*	Frozen and handled in inert-gas box.	[99]
Fluoride, carbonate, etc.	*Ground water.*	Passage through Milli-Trap H^+ cartridge.	[100]
Biochemical			
Chloride, acetate, lactate, etc.	*Saliva.*	10% dil. in deionised water.	[75]
Oxalate, arsenate, Cl^-, etc.	*Urine.*	Dilution as appropriate.	[89]
Globins.	*Human blood.*	Precipitate proteins with acidic acetone, centrifuge, wash twice with acetone and dil. in electrolyte.	[101]
Catecholamines	*Single cell.*	Purified and stored at 4 °C. Washed 5 times with balanced salt solution and dil. (2.5% v/v).	[102].
Formate, sulfate, chloride, etc.	*Dental plaque.*	Diss.[f] and filtration.	[75]
Sulfonamides.	*Commercial drug formulation.*	Diss. in 4% v/v methanol.	[61]

Table 2.1 - *continued*

Food, Beverages and Process Monitoring			
Caffeine, aspartame and benzoic acid preservatives.	*Soft drink and Diet Pepsi.*	Dilution and degassing.	[103, 104]
Artificial sweeteners.	*Soft drink.*	Dissolution, filtration and dilution.	[103]
Citrate, oxalate, tartrate, etc.	*Red wine.*	Dilution in running electrolyte.	[27]
Hop 'Bitter' acids.	*Beer. High ionic strength. Responsible for beer's taste.*	Dissolution in methanol.	[105]
Proteins.	*Egg white.*	1:50 diss. in phosphate buffered saline solution.	[106]
Phosphate, carbonate, Cl^-, etc.	*"Light" beer. High ionic strength.*	10% dilution and degassed.	[84]
Malic, acetic and lactic acids.	*Fruit juices of orange, tomato, apple and grape. High ionic strength.*	Dilution, internal standard addition and filtration.	[87, 95]
Agriculture and Fisheries			
Halofuginone.	*Feedstuffs.*	Add concentrated HAc, sonicate, dilute and filter.	[107]
Nucleotides.	*Fish extract. Acidic and high ionic strength.*	Blend, dil. 1:1 with 10% trichloroacetic acid, centrifuge, neutralise and dil. with phosphate.	[108]

Table 2.1 - *continued*

Tobacco mosaic virus.	*Viral culture. High ionic strength and phosphate content. Neutral.*	Fil. through G-25 Sephadex gel, equilibrate in electrolyte and dil.	[49]
Nitrate and nitrite.	*Vegetables.*	Cut, warm, homogenise, cool and filter.	[109]
Sulfonyl urea, etc.	*Herbicides in soil.*	Extr. with dichloromethane, dehydrate with sodium sulfate and distil off dichloromethane.	[110]
Industrial and Mining			
Gold and silver cyanides.	*Ore leachate with excess free cyanide. High pH and ionic strength.*	Shake, centrifuge and filter.	[111]
Hydroxide, formate, Cl^-, etc.	*Paper and pulp process liquor.*	1:1000 dilution.	[112]
Sulfate, propanesulfonate and butanesulfonate.	*Petroleum refinery extract.*	Iso-propyl alcohol extr. and 100-fold dil. in deionised water.	[75]
Metal cyanides	*Electroplating solution and waste water. High pH and ionic strength.*	Adjustment of pH to 9.5 and 1:6 dil. in water or running electrolyte.	[96]
Chloride, sulfate, oxalate, etc.	*Bayer liquor. High ionic strength and pH.*	Dilution and filtration.	[e.g. 113-115]

Table 2.1 - *continued*

Sulfate.	*Granular detergents.*	Riffled, ground, dissolved and filtered.	[116]
Chloride and sulfate.	*Acid digested concrete. Low pH (~2) and high ionic strength.*	Digest with conc. nitric acid, fil. (Whatman 451), dil., ultrasonication and fil.	[117]
Chloride and sulfate.	*Green dye. Sulfonated.*	1:50 dilution.	[97]
Fluoride, Cl^- and sulfate.	*Caustic solution.*	Pass through Novo-Clean IC-H cartridge.	[118]
Forensic and Trace Analysis			
Chloride and chlorate.	*Pipe bomb explosive residues.*	Washing, marker addition and filtering.	[119]
Phosphate and sulfate traces.	*HF used in etching semiconductors.*	Simple dilution. Plastic ware required.	[120]
C_6 - C_{10} sulfonates.	*Shampoo base.*	200-fold dilution in deionised water.	[75]
Chloride, sulfate and nitrate.	*Silicon wafer surfaces.*	Clean bench conditions used.	[121]
Methylphosphonic acids. (Banned material.)	*Nerve agent degradation products. Dangerous; used in warfare.*	Passed through resin bed.	[100]
Trace anions.	*Deionised water.*	Not treated. Injection by electromigration.	[122]

All samples analysed by free-solution CE must be in the solution form. This can be a limitation where the sample is in either a solid or a gas. Generally, sample treatment in FRECZE is minimal relative to other techniques.

2.2.6.3 Instrumentation

For FRECZE, the eight main instrumental components [10, 22, 29, 124] and configuration are as shown in Fig. 2.3. The same instrumentation can be adapted for IEF and ITP [125].

(1) The cathode compartment with catholyte (electrolyte).

(2) The separation chamber consisting of a capillary. The capillary can be made of Pyrex borosilicate glass [5, 68], fused silica, glass, teflon [5], poly(fluoroethylpropylene) [126], polyethylene, poly(vinyl chloride), polypropylene and polyfluorocarbon [127]. The capillary can be bare or treated (bonded) [128, 129]. Capillaries used in FRECZE are more often than not, made of fused silica [10] as it has the best transparency and compatibility [17] with UV detection.

(3) The anode compartment with anolyte (electrolyte).

(4) The sample injection system which can operated in the hydrostatic, electromigration, manual or other modes. Not shown in Fig. 2.3.

(5) A high voltage power source of interchangeable polarity. The applied field must be in the *direct current* mode to avoid 'rippling' effects due to *alternating current* [130].

(6) An on-line detector.

(7) Computer to record and manipulate data and to control the instrument. Some authors [e.g. 5, 22, 61, 84] have illustrated systems without a computer. The computer should be, in this author's view, considered as essential and not as a peripheral component. With the very small time frames [51], resolutions and high data acquisition rates (e.g. 20 data points per second [75]) normally encountered in CE, the computer becomes a necessity.

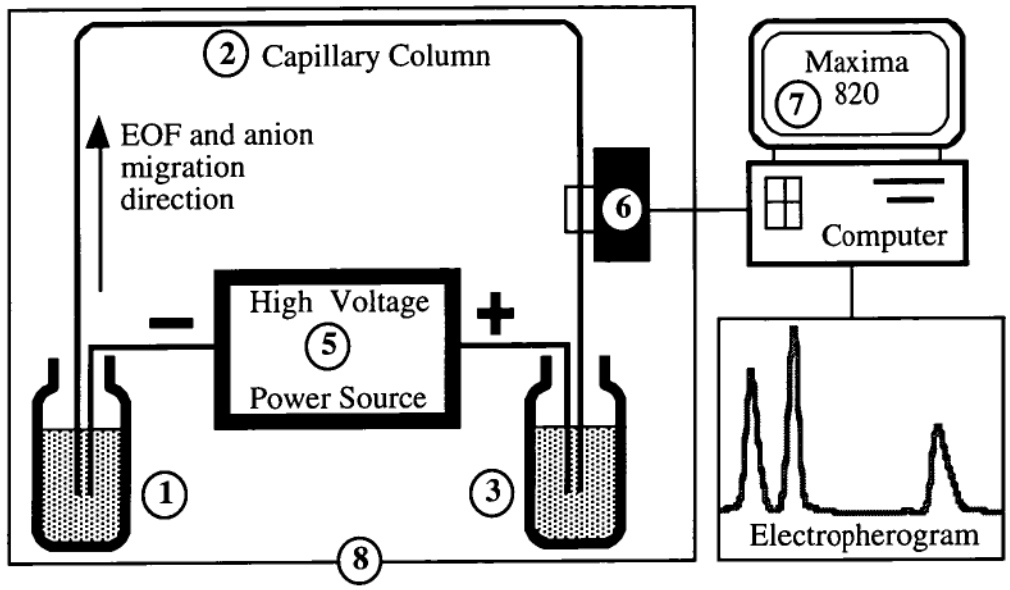

Fig. 2.3: Schematic of main instrumental components in FRECZE. Note that the above illustration shows the configuration in which the EOF is reversed, necessitating the positioning of the detector at the anodic end and the sample injection at the opposite end. (In 'normal' electrophoresis, the order would be reversed.) Key: 1 = cathodic vial, 2 = fused silica capillary, 3 = anodic vial, 4 = sampling system (not shown), 5 = power supply, 6 = detector, 7 = data station, 8 = safety housing. [Jones, W.R. and Jandik, P., "Electrophoresis", *American Laboratory* (June, 1990) 56. Adapted with the permission of the authors[10] and publisher. Copyright © (1990), International Scientific Communications Inc.]

(8) Due to the danger of electric shock, the system is earthed and normally enclosed, usually in a plexiglass [8] housing or Faraday cage [124].

(9) Optional extras [124] such as (i) liquid [131] or forced air thermostating and (ii) pressurising the inlet and/or outlet ends of capillaries for flushing and sampling.

Commercial systems are available from: (i) Waters Millipore, (ii) Dionex, (iii) Beckman, (iv) Applied Biosystems, (v) Bio-Rad, (vi) Spectra-Physics [34, 77], (vii) Thermo Separation Products [132], (viii) ATI Unicam, (ix) Isco, (x) Hewlett

10 Permission given by Jim Krol of Waters Millipore on behalf of W.R. Jones and P. Jandik.

Packard, (xi) Kontron Instruments [33, 77], GTI/Spectrovision and Zeta Technology [77].

2.2.6.4 Sample Introduction Methods

A number of techniques are used for sample introduction in CZE. They include:- (i) *Manual injection* [5, 54]. This method is prone to undesirable mixing of the sample with the buffer. Consequently precision is poor [54], e.g. 13.4% RSD [133]. Peaks are broad [5], probably due to parabolic flow of the injection. (ii) *Microinjection* which is suited to sampling from single cells [123]. However, it is labour intensive, delicate and may require sophisticated micromanipulation [134]. (iii) *On-column fracture* [135]. Solutes are introduced with the aid of the "electroosmotic syringe" and the method gives good precision, e.g. 2% RSD. (iv) *Electric-splitting* using rotary-type devices. Although it has good precision (3% RSD), this method is difficult to adapt to capillaries of <100 μm I.D. [123, 133]. (v) *Electromigration* [e.g. 10, 11, 27, 29, 89, 133, 136-139]; (vi) *Hydrodynamic* or *hydrostatic flow* injection [e.g. 10, 27, 29, 89, 103, 136]; and (vii) *spontaneous fluid displacement* [140]. This is done by forming a droplet at the capillary tip upon removing from the sample. The droplet is pushed into the capillary by pressure differences across the droplet surface. Volumes down to 3.5 nL can be injected with a precision of 5.8 ± 0.7% RSD [140]. The amount injected is varied by changing the capillary's outer diameter. Noise and background vibrations are likely to interfere by causing droplet break-up. Furthermore, the capillary end must be square.

The choice of sample injection method will depend on relative concentrations of solutes, available sample size, precision required, sample matrix, sensitivity required, electrophoresis mode and solutes of interest. For enhanced resolution, the applied sample size must be minute relative to the capillary volume [27].

The main modes of sample introduction are hydrodynamic injection and electromigration [14, 139]. They are therefore covered in more detail in Sections 2.2.6.4.1 and 2.2.6.4.2. These two methods are suitable for quantitative analysis as they give good linearity for peak area response [133]. For the most precise work, hydrostatic injection is desirable whilst for the most sensitive work, electromigration is desirable. For both injection modes, the sample plug must be under ~1% of the total capillary length for efficiency to be maintained [80].

2.2.6.4.1 Hydrodynamic Injection

Although the instrumental design needed for hydrodynamic or hydrostatic flow injection is complex relative to that required for electromigration [10], it is the most common method of sample introduction in CE [84]. It can be achieved by using pressure drop differences [10] created using positive pressure, negative pressure (suction or vacuum) or height difference between the capillary ends (gravity feed). Picolitre to nanolitre sample volumes are possible [27]. When automated, this method gives excellent precision. Values of 2.11% RSD [123], 2.9% RSD [136] and 3% RSD [133] have been quoted. The Quanta 4000 system[11] has been evaluated by Rahn [141] to have a precision of <2% RSD. Peak area response linearity is good [133]. Furthermore, the solute composition of the sample has no discriminatory effect on the relative amounts of solutes injected [123].

The equations relating sampling time, height, solution viscosity, capillary radius and length, etc to the amount of sample injected using hydrodynamic flow sampling have been discussed by Rose and Jorgenson [136], Wallingford and Ewing [123] and Dose and Guiochon [142]. The mean hydrodynamic flow (v_{HD}) in the capillary during hydrodynamic sampling is given by the Poiseuille equation [123, 136, 142].

$$v_{HD} = \frac{\rho g r^2 \Delta h}{8 \eta L} \qquad \text{Eqn. 2.3}$$

[11] This system is identical to the one used in this study.

where ρ = solution density, r = capillary radius, Δh = difference in height of the capillary ends, η = electrolyte viscosity and L = capillary length.

The sample zone length (l) introduced into the capillary using a sampling time of t_i is defined by Eqn. 2.4 [123]. Thermal and electrokinetic effects neglected, diffusion dictates that the most suitable sample band length is a maximum 3 mm [48, 143].

$$l = v_{HD}\, t_i \qquad \text{Eqn. 2.4}$$

As can be seen from the above equations, hydrodynamic injection sensitivity is affected by sample viscosity [103]. This means temperature control is vital [29]. It also means that internal standards may be more reliable than external standards which bear little resemblance to the sample matrix.

To obtain the amount of sample (Q) introduced into the capillary, Eqn. 2.4 is multiplied by the capillary cross-sectional area (πr^2) and the sample concentration (C) [123].

$$Q = \left(\frac{\rho g r^2 \Delta h}{8 \eta L} \right) . t_i . C . \pi . r^2 = \frac{\rho g r^4 \Delta h\, t_i\, C\, \pi}{8 \eta L} \qquad \text{Eqn. 2.5}$$

Diffusion is a source of error to be noted with all sample introduction methods. Generally for hydrostatic injection, diffusion is minimised when capillary ends are positioned evenly, and capillaries of increased length and reduced radii are used. Increasing the viscosity of solutions is not advisable as the chemistry of the system can be altered [142]. Also, the amount of sample introduced into the capillary will be altered thereby affecting sensitivity [103]. Reduced sample volume results in a

reduced amount of loaded sample. A possible cause has been suggested as the surface tension playing a more significant role [136].

2.2.6.4.2 Electromigration Injection

The instrumental configuration for electromigratory injection is simpler than for hydrodynamic injection. It is thus easier to control [27, 136]. Furthermore, hydrodynamic initiated diffusion at the sampling stage has little, if any, effect on sample amount injected. To inject samples by electromigration, voltage is applied for a short time to 'pump' the solutes into the capillary [88]. For optimal injection ('stacking') performance, solutes must be injected from a lower concentration environment into a higher concentration electrolyte [63]. A 10-fold dilution in background electrolyte has been found to be ideal by Beckers and Ackermans [138] who have also studied the effect stacking has on linearity, resolution and pH. In the absence of adequate dilution, reversed stacking can occur, resulting in peak broadening [71]. Also termed 'transient CITP/CZE' [144], stacking simultaneously involves CZE and ITP processes and has the advantage of removing interfering matrix components and preconcentrating solutes [10, 145]. Sample presentation is >100 times more sensitive than hydrodynamic flow injection [88]. It has been noted through the course of this study that a smoother baseline is obtained as compared to hydrodynamic sampling. This is due to the capillary being kept clean by the introduction of solutes of the required charge [29]. It must be noted that in ITP stacking, concentration boundaries between electrophoretic zones are indicated by strong UV shifts due to the different absorbances of the electrolytes [138]. This may interfere with anion detection.

Biases in electrokinetic sampling were noted early in the development of CE by Tsuda and co-workers [126, 146]. Later, definitive data presented by Huang, Gordon and Zare [139] showed that there are two biases, *viz.* (i) different mobilities of sample ions alter peak areas and (ii) electrical resistance of the

medium, which governs the mobilities of anions and EOF and ultimately the amounts injected. The lower the electrical resistance, the greater the amount of sample injected due to the corresponding high field strength. Although simple, electromigration injection has the disadvantage of being discriminating and may not introduce the same amount of anions due to differences in their mobilities [27, 133]. Enrichment is biased towards smaller, highly charged anions [29, 89]. Furthermore, quantification is difficult due to the dependency of injection quantity on sample composition [10]. Electromigration is unsuitable for studies requiring EOF monitoring using neutral markers. A drawback of electrokinetic sampling is contamination of the sample by electrochemical reaction products [136]. Localised depletion of solutes is another drawback that has the potential to diminish reproducibility between replicate injections of the same sample.

The amount (Q) of sample introduced using electromigration can be calculated as follows [5, 147];

$$Q = \left(\frac{(m_{anion} + m_{eof})\, C\, A\, t_{inj.}}{1} \right) \cdot \left(\frac{V_{inj.}}{L} \right) \cdot \left(\frac{\lambda_{bge}}{\lambda_{anion}} \right) \qquad \text{Eqn. 2.6}$$

where C = molar sample concentration and $t_{inj.}$ = injection duration, A = circular cross-sectional area, $V_{inj.}$ = injection voltage and λ = conductivity of the background electrolyte and anion. The other symbols have their usual meanings. Eqn. 2.6 has also been shown without the C term [136].

2.2.6.5 Detection

It is all well and good to say that CZE is an extremely powerful separation technique but if the separated analytes are not detected sensitively, the power of the separation is not fulfilled. Due to the minute sample zones separated and detection limitations imposed by the small diameter of the capillary, it is widely recognised

that detection is a limiting factor of CZE when circular capillaries and on-line optical detection are used [10, 11, 79, 80, 148, 149].

2.2.6.5.1 Detection Methods

Detection methods have been reviewed by Jorgenson [25], Gordon *et al.* [27] and Widmer [17]. Yeung [150] has reviewed indirect detection methods in particular. Detection methods can be divided into six main groups. They are; (i) optical methods (e.g. absorbance, fluorescence, RI [151], scattering [25], Raman spectroscopy [90] and luminescence [152]); (ii) electrochemical methods (e.g. amperometry and potentiometry), (iii) thermal methods (e.g. thermooptical absorbance [17, 153]); (iv) electrical methods (e.g. conductivity [25]); (v) radiometric detection [154]; and (vi) others (e.g. mass spectroscopy and photothermal techniques like LI-CV[12] [155]).

Some detection methods used in CE and their detection limits are listed in Table 2.2, in order of increasing sensitivity for ease of comparison. Most of the data in Table 2.2 are from Goodall *et al.* [34].

The choice of method may be defined by the nature of the solute (e.g. amperometry for electroactive species and direct spectroscopy for strong chromophoric species). It may also depend on the sample concentration and sensitivity required (e.g. RI can detect in the low femtomole region [163]). Selectivity enhancement may also be a vital consideration (e.g. electrochemical methods offer excellent selectivity as only a handful of species in a mixture are likely to be electroactive [10, 27]). Commercial availability is another factor (e.g. UV and fluorescence detection are well established and commercially available [29, 131]).

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12 This stands for Laser Induced Capillary Vibration. It is a technique that does not require derivatisation [155]. It was the first publication to determine amino acids at the ultrasensitive level without derivatisation. Also, it has good linearity with concentration.

Table 2.2[§]

DETECTION METHODS AND SENSITIVITY

Detection Method	Detection Limit*	Reference(s)
Potential Gradient	10^{-4}	[54, 156]
Direct UV[a]	10^{-6}	[27, 80, 96]
Conductivity	10^{-6}	[95, 156, 157]
RI[b]	10^{-6}	[151]
Indirect UV detection	10^{-7}	[158, 159]
Thermooptical absorbance[c]	5×10^{-8}	[153]
Electrochemistry[d]	2.1×10^{-8}	[149]
Lamp-based Fluorescence	10^{-8}	[80]
Luminescence[e]	3×10^{-9}	[152]
Amperometry	10^{-9}	[160]
Mass spectroscopy	10^{-9}	[161]
Radiochemical[a, g]	10^{-11}	[80]
Laser-based Fluorescence	10^{-12}	[162]

*in moles.L^{-1}, [a]maximum, [b]for saccharose, [c]for methionine, [d]gluthathionate, [e]for nitrite in the indirect time-resolved mode, [g]gamma-ray detection is linear over two orders of magnitude [154].

[§ Adapted with permission from *LC•GC*, 8, 10 (1992). Copyright (1992) by Advanstar Communications. Printed in U.S.A.]

For this study, on-capillary indirect UV absorbance detection was employed. Other on-line methods are optical (fluorescence, RI and scattering), electrical (electric field, conductivity), thermal methods [25] and radiometry [27]. Being performed on-line ensures analysis is fast [8, 27] and band broadening [155] is minimised. On-line detection also removes diffusion resulting from post-column detection [8] and avoids decrease in resolution due to the dead volume of the detector [27].

Indirect UV absorbance detection is used widely [e.g. 63, 89, 94, 164] for solutes (including most inorganic anions) exhibiting poor UV absorbing properties [12, 75, 164, 165]. Optimised indirect UV detection for anions has been discussed by Jandik and Jones [88] and Ma and Zhang [166]. Other indirect methods used in CE include amperometry [167], fluorescence [e.g. 150, 158, 168, 169] and luminescence [152]. The main advantage of indirect detection is its universality [86, 150]. It has wider application and is suitable for both strongly and weakly chromophoric solutes. Additionally, it is non-destructive, has the possibility of sensitivity transfer, has ease of quantification, instrumentation is simplified and tedious sample manipulation is avoided [150].

2.2.6.5.2 On-Line Indirect UV Detection

On-line optical UV and fluorescence detection are most frequently used [10], probably because of their compatibility with fused-silica [17]. For indirect UV detection, the theory related to quantification using (corrected) peak area has been discussed by Ackermanns *et al.* [74] :-

$$A_{net} = A_{bge} - A_{anion} = [anion] \,.\, \varepsilon_{net} \,.\, \ell \qquad \text{Eqn. 2.7}$$

where A_{net} = observed absorbance, A_{bge} = absorbance due to the background electrolyte, A_{anion} = absorbance due to the anion, [anion] = Molar concentration of anion, ε_{net} = overall extinction coefficient and ℓ = path length (cm).

For indirect UV detection, the achievable detection limit ($C_{det.\ lim.}$) is [150, 152, 166, 170, 171];

$$C_{det.\ lim.} = \frac{C_{probe}}{DR \,.\, TR} \qquad \text{Eqn. 2.8}$$

where C_{probe} = probe or visualising agent concentration [152], DR = dynamic reserve and TR = transfer ratio, i.e. the number of equivalents of solutes displaced

by 1 equivalent of probe. It should be noted that the parameters in Eqn. 2.8 are not necessarily independent [171, 172].

C_{probe} must be as low as possible whilst still being able to generate a sufficient background signal. The *TR* depends on the mobilities of the analyte and probe or background ion. It has been indicated as unity for anions [171], but can deviate significantly [152, 173] from this value. The *DR* should be as high as possible. It is the ability to measure minute changes on top of a large background. In other words, it is the ratio of the background signal to background noise [150]. Sensitivity of indirect UV detection depends on the *DR* and this may create problems when complex samples are analysed [173].

Quantitative aspects and limitations of indirect UV detection in CZE have been discussed by Nielen [173]. Some problems include (i) low useful dynamic range of solutes with mobilities different from the probe, (ii) a TR of unity holds true only for cases where mobility of the solute and probe are equal and (iii) stability is poor. The second limitation can be addressed by using binary mixtures of probes [174].

2.2.6.5.3 Methods for Improvement of Sensitivity

In real samples, anions can be present at levels below the limit of detection. This creates the need for detection methods with better sensitivity or considering ways of improving the detectability with the available instrumentation.

For the indirect UV method employed in the study, the parameters comprising the Beer-Lambert law can be manipulated to give increased sensitivity. Some routes to improving sensitivity have been discussed by Albin *et al.* [80] and include:-

(a) Using detection methods with higher sensitivity [80], e.g. laser-induced fluorescence is 100- to 1000-fold more sensitive [8, 61]. See Table 2.2 for other methods with lower detection limits.

(b) Concentrating the sample. This can be done off-line (e.g. extraction and volume reduction) or on-line (e.g. electromigration 'stacking' [80, 88, 86] (see Section 2.2.6.4.2)).

(c) Increasing the optical path length through geometry variation, e.g. (i) rectangular paths are more sensitive by more than an order of magnitude compared to circular (or square) paths due to increased length [148]. Also circular capillaries cause optical distortions due to their curvature [148, 164] so that absorption path lengths are different and an average response is measured [164] which means that absorbance may not be a linear function of concentration [56]; (ii) expanded path capillaries (bubble cells) [86]; (iii) Z-shaped path [175]; (iv) normal axial illumination [176, 177]; (v) axial illumination with fluorescence [178] using ball lenses [177] to focus radiation and (vi) multi-reflection cell [179].

(d) Other routes to sensitivity enhancement are to increase N (e.g. by matching mobilities of probe and solute [164], using as high an electric field as possible and reducing temperature, and use of a probe with a high molar absorptivity [88].

2.2.6.6 Separation Mechanics and Theory

Like gas chromatography, IC and HPLC, CZE is a differential migration [180] separation method in which analytes are separated by being caused to move at different rates [15]. As CZE in the free-solution format was used in this study, its separation theory and mechanics will be outlined. Other electrophoretic formats and modes are disregarded as they are of no relevance to this study.

2.2.6.6.1 Mechanics and Basic Equation

The separation mechanics of CZE using both bare and coated capillaries have been discussed by Dülffer *et al.* [29]. The mechanics and theory discussed here hold true only for the ZE separation of small ions in the 'free-solution' format (as done in

this study). Corrections need to be made when stabilisers are used or when larger solutes are separated [2]. Corrections in this study were therefore not applicable and will not be reviewed.

The separation mechanics of ZE [25] are illustrated in Fig. 2.4 for the separation of a sample band comprising two anions, a neutral compound and a common cation. In step (1), the sample is applied as a thin band or 'plug' and is surrounded by a homogeneous electrolyte. In step (2), a high electric field[13] is applied [182] and the components begin to migrate based mainly on their electrophoretic mobilities [1]. Anions and cations migrate to the electrode of opposite charge [27] at rates shown in Eqn. 2.9. This general equation is applicable to all forms of electrophoresis [84].

$$v_{ion} = m_{ion} E = m_{ion} \left(\frac{V_{applied}}{L} \right) \qquad \text{Eqn. 2.9}$$

where v_{ion} = ion migration rate[14] or velocity, m_{ion} = electrophoretic mobility[15] ($V^{-1}.cm^2.\ min.^{-1}$) of solute, $V_{applied}$ = applied voltage[16], L = total length of capillary and E = electric field strength[17].

In step (3), the zones are partially resolved and the electric field is maintained. In step (4), the zones are fully resolved (in the ideal experiment) and subsequently detected for identification and quantification. Although not clear from Fig. 2.4, it must be kept in mind that cationic and anionic species in the buffer migrate to the cathode and anode respectively. Assuming that the surface of the capillary is negatively charged under most conditions (pH ≥2), any neutral solutes present will

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13 One driving force in FRECZE is the EOF which is due to the zeta or phase boundary potential [181]. The other driving force is the applied potential.

14 Which may also represented by U_{EP} [1].

15 Which may also be represented by m and μ_{EP} [1] and u [183].

16 Which may also be represented by U [10] and V.

17 Which may also be represented by X [183].

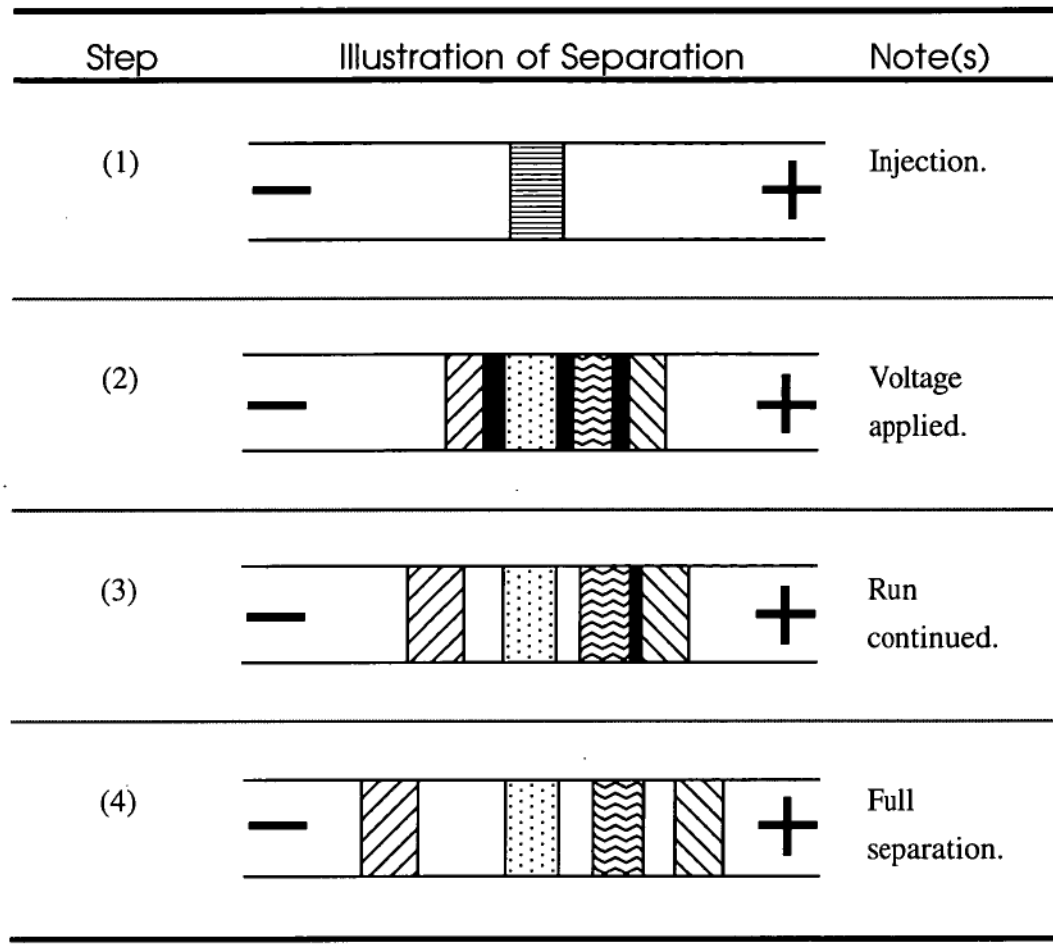

Fig. 2.4: Schematic of component separation in free-solution capillary zone electrophoresis without electroosmotic flow. Key: = mixture with one common cation, a neutral chemical and two different anions, = neutral solute, = overlap between adjacent zones or bands, = cation, = slower migrating anion, = faster migrating anion, = anodic end, = cathodic end. See text for description of separation mechanics. [Jorgenson, J.W., *Anal. Chem.*, **58** (1986) 743A. Adapted with permission of the author and publisher. Copyright © (1986) American Chemical Society.]

be transported with the EOF to the cathode. (For separations using electrolytes containing cationic surfactants, the EOF migration is to the anode.)

The migration velocity of an ion can also be represented in other forms as shown in Eqn. 2.10 [183].

$$v_{ion} = z \,.\, e \,.\, \omega \,.\, X \qquad \text{Eqn. 2.10a}$$

$$v_{ion} = z \,.\, e \,.\, \left(\frac{1}{6\,\pi\, r\, \eta}\right) .\, X \qquad \text{Eqn. 2.10b}$$

$$v_{ion} = \frac{z \,.\, e \,.\, \omega \,.\, X}{300} = \frac{z \,.\, m_{ion} \,.\, X}{1} \qquad \text{Eqn. 2.10c}$$

where z = valence of ion, e = charge on electron, ω = intrinsic mobility, X = electric field strength[18] and m_{ion} = mobility of the ion. Eqn. 2.10b is known as Stoke's law and is applicable to spherical ions.

The contribution of EOF is fundamental to FRECZE as it affects the actual (net) migration rate of the anion. This means that the migration rate of an anion will not necessarily be as indicated in Eqn. 2.9 and Eqn. 2.10.

2.2.6.6.2 Addition of Cationic Surfactants

Reijenga *et al.* [184] were the first to report EOF reversal with a cationic surfactant. The addition of cationic surfactants (or "EOF modifiers") to the electrolyte alters properties such as surface tension, viscosity, dielectric constant, etc of the solution. Surface tension is usually reduced sharply. Furthermore, the EOF modifier accumulates on the capillary surface and changes the surface charge and thickness of the charged layer. This results directly in altering the magnitude and direction of EOF. EOF manipulation using surfactants has been studied by Altria and Simpson [1]. EOF decreases linearly with log concentration of C_1 - C_{16} cationic surfactants. Fig. 2.5 illustrates the effect of CTAB concentration on EOF [42].

The surfactants can be non-ionic, cationic (e.g. TTAB) or anionic (e.g. sodium dodecyl sulfate) and are also known as soaps. These soaps are compounds (e.g.

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18 Which may also be represented by E [84].

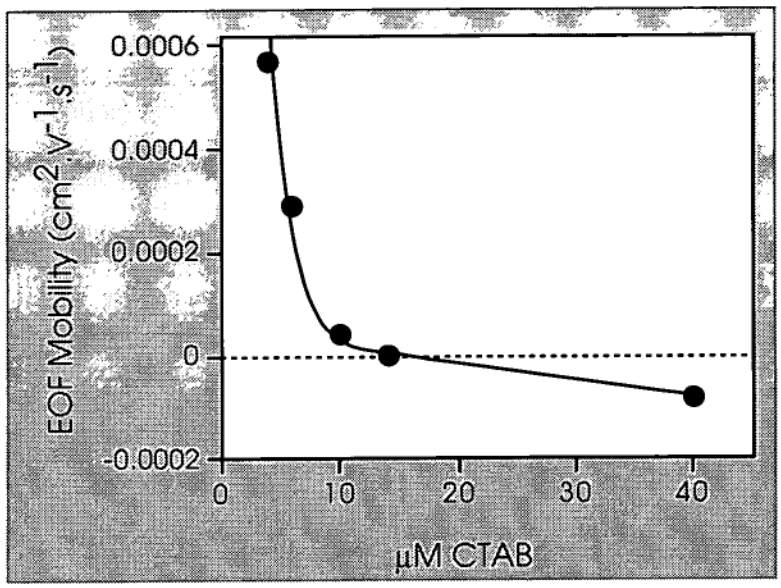

Fig. 2.5: Effect of CTAB concentration on EOF mobility. [H-T Chang and E. S. Yeung, *J. Chromatogr.*, **608** (1992) 69. Adapted with the permission of the authors and publisher.

alkyl sulfonates) that do not form precipitates with Ca and Mg ions - or else samples such as hard water cannot be analysed or the capillary will become blocked with precipitates. When positive absorption of EOF modifier onto the capillary wall occurs, three systems can be formed (Fig. 2.6). The systems are (a) unsaturated monolayers on the capillary surface, (b) saturated monolayers (or hemimicelles) on the capillary surface and (d) micelles in the electrolyte [185]. The formation of these systems can be monitored via measurements of surface tension, conductivity, etc.

In FRECZE, only the first two systems come into play. The formation of the third (micellar) system initially had its detractors [e.g. 186] but is now widely accepted, especially after the pivotal work of Terabe and co-workers [38].

2.2.6.6.3 EOF Generation, Monitoring and Profile

EOF (which is also known as electroendoosmosis [1, 187]) plays a significant role in the separation of anions by FRECZE. It has a major influence on anion resolution and migration time and it has a direct bearing on band width [188] and

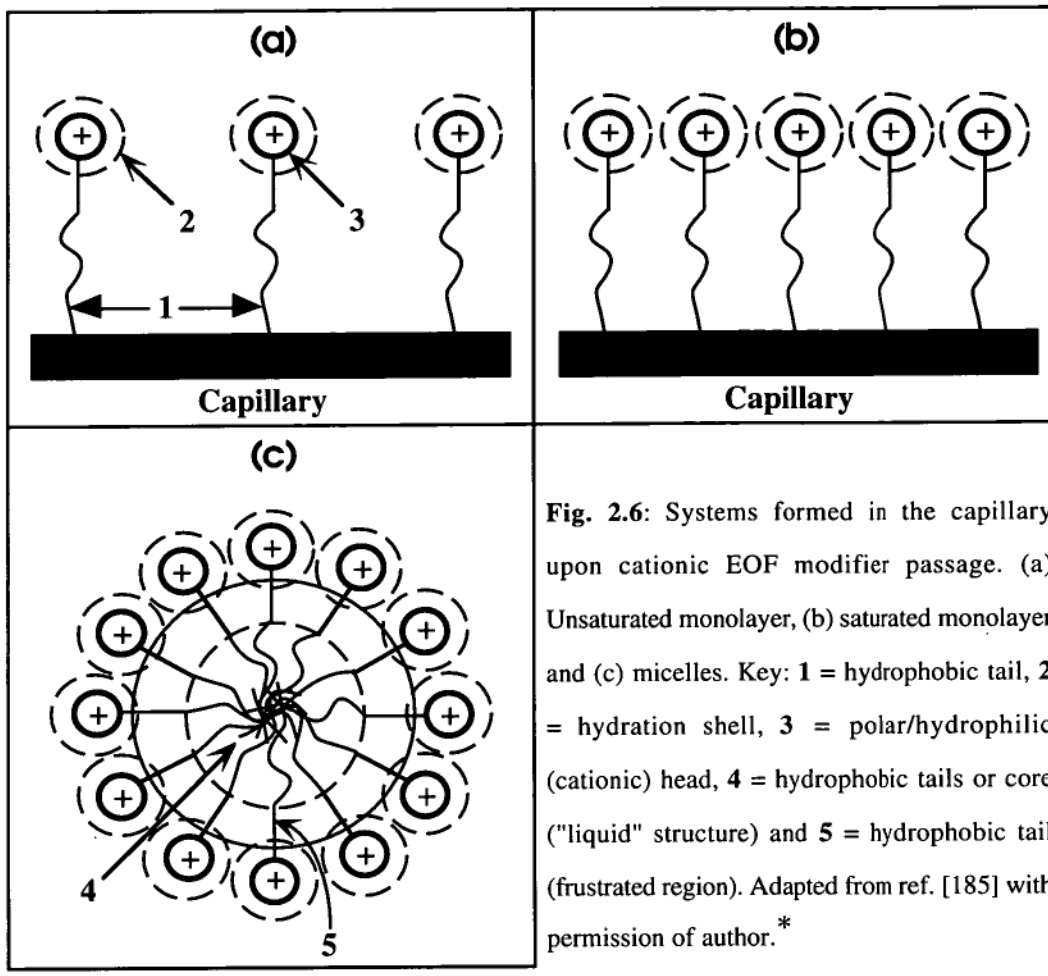

Fig. 2.6: Systems formed in the capillary upon cationic EOF modifier passage. (a) Unsaturated monolayer, (b) saturated monolayer and (c) micelles. Key: **1** = hydrophobic tail, **2** = hydration shell, **3** = polar/hydrophilic (cationic) head, **4** = hydrophobic tails or core ("liquid" structure) and **5** = hydrophobic tail (frustrated region). Adapted from ref. [185] with permission of author.*

therefore efficiency (N). Precise measurements of EOF are vital for accurate determination of electrophoretic mobility of solutes [1].

EOF generation has been described by a number of authors including Adamson [183], Altria and Simpson [1] and Schwer and Kenndler [10]. The theory and mathematics of EOF in cylindrical capillaries has been discussed by Rice and Whitehead [189] who noted that the assumption of the double layer being much smaller than the capillary I.D. may not be true in some cases.

* Publisher indicated that the rights had reverted to the author.

Chang and Yeung [42] and Salomon *et al.* [190] have outlined the factors that govern EOF. The main variable is the zeta potential (ζ) on the capillary wall. EOF magnitude and direction is a function of the electrolyte composition, the nature of the capillary and the nature of solvent [1, 191], which also govern the sign and magnitude of the ζ potential [189]. EOF is suppressed by the addition of methylhydroxyethylcellulose [74] or methanol [165]. Addition of polyethylene glycol eliminates EOF [28]. EOF flow can be positive, zero or negative as a result of pH variation (e.g. zero EOF can be achieved by using s-benzyl thiouronium chloride at pH 4.5 for both cations and anions [1] or an external electric field applied to control the ζ potential at the capillary-background electrolyte (BGE) interface [58]). EOF can also be controlled by application of a radial potential gradient [59]. EOF increases linearly [1] with increasing pH [10] due to the ionisation of surface silanol groups. EOF is reduced at high electrolyte concentration and a model for this has been developed by Salomon *et al.* [190].

EOF is generated when an interface between two different charges is subjected to applied potential [1]. The Stern-Gouy-Chapman model of the electrical double layer at a charged interface [192] is the model usually employed to explain EOF (see Fig. 2.7). The EOF is fixed in its direction by the charge at the surface of the capillary. For bare fused silica capillaries at pH $\geq$2, the surface charge is negative due to the ionisation of the surface silanol groups [28]. Therefore the direction of EOF is to the negative electrode (cathode) in 'normal' CZE. In FRECZE, the charge at the capillary surface is reversed by the positive absorption of cationic EOF modifier and thus the flow is to the anode. For electroneutrality, negative counter-ions are present in the double-layer adjacent to capillary surface. EOF is induced by these hydrated counter-ions [28, 193] migrating to the appropriate electrode. Reijenga *et al.* [184] were the first to reverse EOF using cationic surfactants.

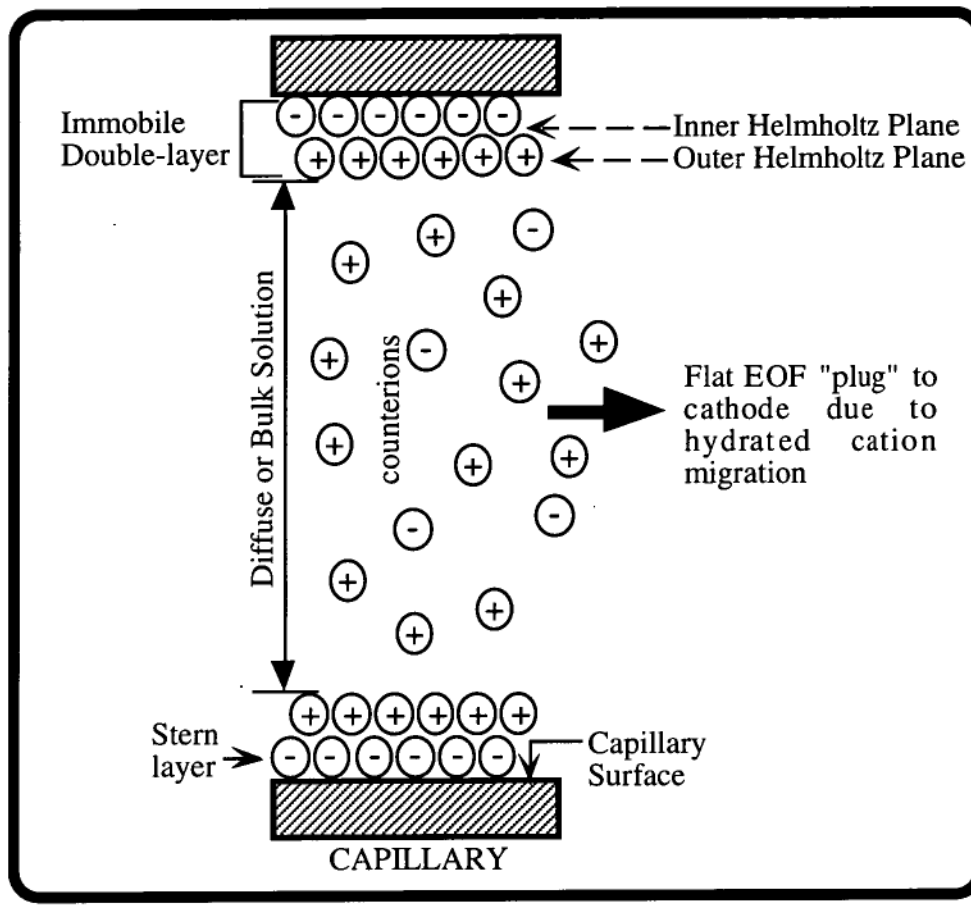

Fig. 2.7: Schematic of CZE without the addition of EOF modifier. The diffuse or bulk solution layer is also known as the Gouy layer. [Adamson, A.W., Physical Chemistry of Surfaces, 2nd edition (Interscience/John Wiley & Sons, New York, 1967), p. 215. Adapted with permission of the author and publisher. Copyright © (1967) John Wiley & Sons.]

EOF generation is dependent on the ζ potential across the immobile double layer. (See Eqn. 2.11 which is also known as the Helmholtz equation in its various representations.) The ζ potential is a function of the concentration and valency of the electrolyte and capillary material [1]. For silica capillaries in aqueous solutions, the ζ potential is 900 $V.cm^{-1}$ [191] and is proportional to the double-layer thickness (δ) , the charge and charge density on the capillary, excess charge (e) in the electrolyte (Eqn. 2.11c) [190, 194], the type of electrolyte and whether organic solvents are present. EOF was noted to increase with 50% ACN in phosphate buffer. However 50% ACN in borate buffer reduced EOF. The roles of phosphate and borate in creating this effect are not understood at present [195]. The addition

of methanol reduces EOF (and improves resolution) but the mechanism is presently unclear [190]. It is noteworthy that the immobile compact (Helmholtz) layer structure is open to speculation [190]. The ζ potential is also known to be proportional to the inverse of the square root of the electrolyte concentration. Electrolyte addition compresses the double layer [191], resulting ultimately in reduced EOF and migration rate for anions. High electrolyte concentration reduces EOF and improves resolution [190].

$$\zeta = \frac{4 \pi \eta m_{eof}}{\varepsilon} \qquad \text{Eqn. 2.11a}$$

$$\zeta = \frac{4 \pi \eta v_{anion}}{\varepsilon E} = \frac{4 \pi \eta v_{anion}}{\varepsilon} \cdot \left(\frac{L}{V_{applied}} \right) \qquad \text{Eqn. 2.11b}$$

$$\zeta = \frac{4 \pi \delta e}{\varepsilon} \qquad \text{Eqn. 2.11c}$$

where ε = dielectric constant[19] of solution, η = viscosity, m_{eof} = coefficient (or mobility) of EOF[20], δ = thickness of double layer, L = capillary length and e = excess charge in electrolyte [194].

The EOF mobility is, by re-arrangement [1];

$$m_{eof} = \frac{\zeta \varepsilon}{4 \pi \eta} \qquad \text{Eqn. 2.12a}$$

$$m_{eof} = \frac{\varepsilon \varepsilon_{eo} \zeta}{\eta} \qquad \text{Eqn. 2.12b}$$

$$m_{eof} = \frac{Q \delta}{\eta} \qquad \text{Eqn. 2.12c}$$

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19 The dielectric constant is also represented by the symbol D [1, 183] and Σ [191].

20 Which may also be represented by μ_{eo} and μ_{EEO} [1].

where ε_{eo} = permittivity of free space, δ = Debye-Hückel thickness[21] and Q = charge density. Eqn. 2.12b is from Salomon *et al.* [190] and van de Goor *et al.* [196]; and Eqn. 2.12c is from Salomon *et al.* [190]. Note that Eqn. 2.11a and Eqn. 2.12b are approximate and a more reliable alternative has been proposed by Salomon *et al.* [190]. This is shown in Eqn. 2.12d.

$$m_{eof} = \frac{Q_o}{\eta\,(1 + K_{wall}\,[M^+])} \cdot \left(d_o + \frac{1}{K' \sqrt{[M^+]}} \right) \qquad \text{Eqn. 2.12d}$$

where Q_o = total number of ionised silanol groups on the capillary surface, K_{wall} = equilibrium constant between (monovalent) buffer ion and hydrated silica groups on the capillary wall, M^+ = monovalent buffer ion, d_o = compact layer of set thickness and K' = a constant which is equal to 3.2 x 10^9 m^{-1} $(mol\ l^{-1})^{-1/2}$ for dilute aqueous systems at 25 °C [190].

From Eqn. 2.9, the EOF migration rate (v_{eof})[22] can be shown as follows;

$$v_{eof} = m_{eof} \cdot E = \frac{\zeta\,\varepsilon\,E}{4\,\pi\,\eta} \qquad \text{Eqn. 2.13}$$

A variety of methods are used to monitor the EOF. These include:- (i) Measurement of change in level of solution in the receiving vial [1]. (ii) Taking mass measurements, which is a tedious off-line method that has been described by Altria and Simpson [1] with a precision of 1.3% RSD. It has now been modified for rapid on-line measurement [187, 196]. (iii) Measuring the time required for the electrolyte level in one vial to reach a specific height [191]. (iv) Measurement of streaming potential [184], which has been developed into an automated method for real-time measurement [196]. (v) Monitoring colloidal particles of the same material

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21 Which may also be represented by x.

22 Which may also be represented by v_{eo} and U_{EEO} [1, 10].

as the capillary [197]. (vi) Measuring a (fluorescent) marker downstream from the detector. This method is very fast (~ 1 s) and can measure changes as small as 1% in the flow rate [198]. (vii) Microscopic imaging onto a camera upon EOF flow across a capillary junction [134]. (viii) On-line photometric detection of 'neutral' markers [11, 187, 191, 199]. This is the easiest method and markers employed include benzyl alcohol [28, 195], adenosine [96], mesityl oxide [49, 136, 180, 199-203], iodine [204], acetophenone [205], formamide [206], 5-(hydroxymethyl)-2-furaldehyde [207], acetone [4, 190], MeOH [208], umbelliferon, riboflavin and 4-nitroaniline [10], pyridine [126], phenol [11], toluene [191], benzene and guanosine [202]. α-Napthol has been used as a marker between pH 3.0 and 6.5 [42], i.e. in the region where it does not ionise. The marker must not ionise in the background electrolyte [1].

When done off-line, the precision is 6% RSD [1]. However, the separation can be affected by adsorption of the marker onto the capillary surface, conductivity effects and the time required before detection may be long [1].

Except for a few nanometres at the capillary surface [28], the generated EOF has a 'plug' [1] or 'flat' [28] flow profile due to the "wall effect" [54, 66] (see Fig. 2.8). This is true when the electrical double-layer is small and the capillary radius is ≥7 times larger than the double-layer [17, 191]. EOF flow is such that dispersion due to kinetic effects is minimal, which results in high N (efficiency) [1]. Ideally, EOF should cause no dispersion in CZE [45].

A 'flat' flow profile is ideal for the prevention or minimisation of anion zone (band) broadening. Unlike HPLC/IC, the flow profile in CE is not dependent on the performance of the pump used to drive the mobile phase and solutes. However, the 'flat' flow profile in CE can be distorted by a number of variables such as hydrodynamic deviations that can be a consequence of uneven positioning of the

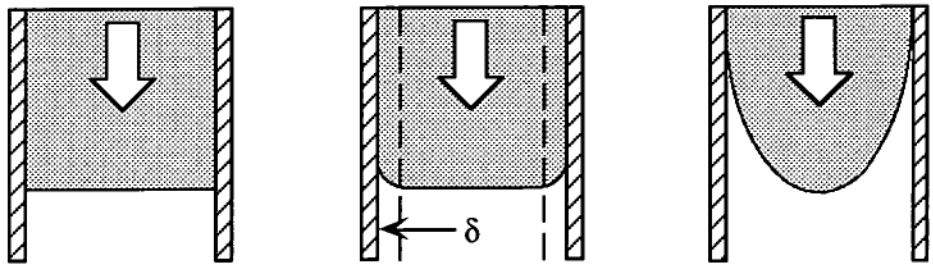

Fig. 2.8: Schematic of flow profiles in (**a**) 'ideal' plug, (**b**) FRECZE and (**c**) conventional free-solution zone electrophoresis or IC (which is also known as laminar flow [10]). The arrows indicate flow direction which in all cases is to the detector and δ denotes the electrical double layer thickness. [Adapted from *Chromatographia*, **30**, 9/10 (1990) 549 with the permission of the author and publisher. Copyright © (1990), Friedrich Vieweg and Sohn, Pergamon Press.]

capillary ends [14], viscosity changes caused by radial temperature differences in the capillary [188] and modulation of the applied voltage which can cause the flow profile to alternate between parabolic and 'flat' profiles [130].

2.2.6.6.4 Anion Migration Rate

The four main forces simultaneously governing anion migration have been outlined by Widmer [17]. These forces include those of attraction, friction, retardation and relaxation. The latter two forces are considered as having negligible effect on anion migration [22].

The contribution from the EOF to the migration rate of an anion (v_{anion})[23] is clear from Eqn. 2.14.

$$v_{anion} = \frac{(m_{eof} + m_{anion}) \cdot V_{applied}}{L} \qquad \text{Eqn. 2.14}$$

where m_{anion} = mobility of the anion.[24]

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23 Which may also be represented by the symbol U_{NET} [1].

24 Which may also be shown by μ_{ea} [8] and other symbols as shown in Section 2.1.

The anion migration rate can also be related to the properties of the electrolyte or buffer in which it is being migrated. The properties of the anion (e.g. size and charge) also influence migration rate. They are considered below.

2.2.6.6.4.1 Electrolyte Contribution

Relating the anion migration rate to the viscosity and frictional properties of the electrolyte and the effective anion charge (q_{anion})[25], Eqn. 2.14 becomes:

$$v_{anion} = \frac{q_{anion} \cdot E}{f_c} \qquad \text{Eqn. 2.15}$$

where f_c = frictional resistance of the electrolyte. f_c is also known as the translational friction coefficient [49].

Assuming negligible contributions from electrophoretic retardation and relaxation [22], this means that migration times are shorter using electrolytes with lower viscosity and frictional resistance. It should be noted that for FRECZE, ion-pairing effects [209] can influence net migration times of anions.

2.2.6.6.4.2 Anion Nature

The two main forces involved in anion migration are due to the applied electric field (Eqn. 2.16) and the frictional resistance or drag of the electrolyte. The electric field drives the anion towards the detector whilst friction (Eqn. 2.17) resists this migration. At equilibrium, they are equal to each other [27].

$$F_1 = q_{anion} E \qquad \text{Eqn. 2.16}$$

where F_1 = force due to applied field.

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25 Which may also be represented by Z [45].

$$F_2 = f_c \, v_{anion} = 6 \, \pi \, r \, \eta \, v_{anion} \qquad \text{Eqn. 2.17}$$

Eqn. 2.17 is known as Stoke's law and is limited only to rigid spherical anions separated in free-solution [17, 49]. For non-spherical anions and separations using stabilisers, correction factors have to be applied to account for shape (e.g. the mobility of a rod-shaped analyte has been reported by Grossman and Soane [49]), size of solute, orientation and impedance by the stabiliser. The friction factor (f_c) nonetheless would still be proportional to the electrolyte viscosity.

To take into account the contribution of the anion charge on its migration rate, Eqn. 2.16 and Eqn. 2.17 are equated and re-arranged as shown in Eqn. 2.18. This holds true after the anion has reached a finite migration rate and steady-state (equilibrium).

$$q_{anion} \, E = 6 \, \pi \, r \, \eta \, v_{anion} \qquad \text{Eqn. 2.18a}$$

$$v_{anion} = \frac{q_{anion} \, E}{6 \, \pi \, r \, \eta} = \frac{q_{anion}}{6 \, \pi \, r \, \eta} \cdot \frac{V_{applied}}{L} \qquad \text{Eqn. 2.18b}$$

This means that a high migration rate is achieved when the anion is highly charged, of a small effective size, high voltages are used and the capillary is short. See Eqn. 2.19 [18].

$$m_{anion} = \frac{q_{anion}}{6 \, \pi \, r \, \eta} \qquad \text{Eqn. 2.19a}$$

$$m_{anion} = \frac{\zeta \, \varepsilon}{6 \, \pi \, \eta} \qquad \text{Eqn. 2.19b}$$

$$m_{anion} = \frac{L \, . \, \ell \, . \, (t_{marker} - t_{anion})}{V_{applied} \cdot (t_{marker} \cdot t_{anion})} \qquad \text{Eqn. 2.19c}$$

where r = hydrodynamic radius of anion [45], L = total capillary length, ℓ = distance to detector window, t_{marker} = migration time of marker[26], t_{anion} = anion migration time.[27] Eqn. 2.19b is known as the Hückel rule [18].

2.2.6.7 Parameters Affecting Separation Selectivity of Inorganic Anions

Selectivity in FRECZE can be defined as the relative difference in effective electrophoretic mobilities between adjacent anions [4, 10]. According to Buchberger and Haddad [210], there is little information on the parameters affecting selectivity of inorganic anions in CZE. The parameters that have been published to date can be classed into three main groups involving; (i) chemical, (ii) instrumental and (iii) miscellaneous effects. The basic theory covered previously (Section 2.2.6.6) gives an indication of the variables that can be used to manipulate the selectivity of anions.

In 'normal' HVCZE, the general migration order is cations, neutral compounds, slightly polar chemicals and anions [1, 84]. For FRECZE, the migration order is reversed. For anionic solutes in particular, the general migration order is hydroxide, inorganic anions, carbonate and organic acids [84]. Generally, selectivity can be predicted according to equivalent ionic conductances [12]. Computer simulation of migration order and migration time using equivalent conductances compares favourably with actual results [211], at least in simple systems devoid of effects from surfactants, complexation, etc. It must be noted though that observed migration orders do not strictly follow equivalent conductance values [137] which suggests that effects other than pure electrophoretic mobility also influence anion separation.

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26 Which may also be represented by t_n [4].

27 Which may also be represented by t_i [4].

2.2.6.7.1 Chemical Parameters

Chemical parameters governing selectivity are essentially those having an impact on the size and charge - and ultimately the charge:mass ratio - of anions. These parameters can be subdivided into (i) the anion and its nature, and (ii) the chemical environment or the background electrolyte.

2.2.6.7.1.1 Nature of Anion

Anion electrophoretic mobility [190] plays a major role in selectivity. Electrophoretic mobility in turn depends on the nature of the anion and its chemical environment. On the anion's nature, its pK_a (or pK_b) [1], hydrophobicity (or hydrophilicity), Stoke's radius, effective charge, effective size and shape are important factors [12, 27, 94, 111, 137, 212]. Molecular orientation and aspect ratio[28] also govern electrophoretic mobility and selectivity, especially for non-spherical polyions [49]. 'Spherical' analytes are unaffected by orientation effects [49].

The form in which an anion exists and ultimately its electrophoretic mobility and selectivity depend overwhelmingly on electrolyte pH [1, 211]. Changes in migration performance are pronounced when electrolyte pH matches the pK_a of an analyte [213]. Weak acids and bases are best separated using a pH at or near their dissociation constants (pK_a or pK_b) [42]. This effect is pronounced for anions such as carbonate [137]. If an ion is not in the free form, the effective mobility will be lower thereby impacting directly on selectivity.

Anionic solutes with close electrophoretic mobilities can not be resolved fully in CZE [214]. This may mean taking advantage of their hydrophobic (or hydrophilic) characteristics, e.g. iodide and thiosulfate migration order can be altered

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[28] Represented by the symbol ø, it is mathematically defined as $L.(2R)^{-1}$ where L = solute length and R = solute radius [49].

significantly by varying the concentration of surfactant. Separation under an MECC environment is another selectivity option, particularly for samples containing anions and neutral analytes. In such a case, symmetry (shape) of solute influences selectivity, e.g. the more symmetrical solutes partake in more interaction with the slow moving micelle and thus migrate later [214] than less symmetrical solutes.

2.2.6.7.1.2 Chemical Environment

Background electrolyte composition [61, 94, 111, 201, 212] has a significant effect on the separation selectivity of anionic solutes.

Hydrogen Ion Concentration

The pH of either the sample or background electrolyte impacts significantly on anion selectivity. There is ample material on the latter in the literature. Material on the former appears limited. The probable reason could be the assumption that the electrolyte concentration being ≥1000 times greater than the sample [14] would have an overriding influence on the behaviour (charge) of the anion. Work done by the author* has shown however that anions in treated (buffered) samples behave differently. This observation may be of direct relevance to naturally buffered samples, e.g. seawater.

For the most part, electrolyte pH determines the degree of dissociation and the electroactive form in which anions exist. The variation of pH is a common way of manipulating selectivity [10] and has been studied by many authors and groups [e.g. 10, 42, 61, 87, 137, 190, 201, 202, 211, 213, 215]. Selectivity changes are obvious at or near the pK_a or pK_b values [1, 42, 137, 213, 215] where charge transitions occur and the effective charge and charge:mass ratios change. As expected, selectivity changes using pH variation resemble a titration curve [137].

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* Unpublished work on the treatment of Bayer liquor using tetraborate (1994).

Akin to cIEF, dynamic pH changes can be used to manipulate selectivity [42, 72, 104, 213, 216-219] using H^+, OH^-, ligands, counter-ions or co-ions. The pH change can be effected using pulsing effects [218], step changes [219, 220] or gradient variations [72, 216, 217, 221] and can be done manually or automatically with computer control [104]. All of the above work involving non-step pH variation were performed on solutes (e.g. proteins) other than inorganic anions, although this approach has been applied for the separation of weak organic acid anions [42, 104, 213]. There is nothing however to suggest it not being applicable to inorganic anions.

A practical aspect to be noted is that, although at high pH the OH^- ion performs a cleaning and surface re-generation role by removing adsorbed cations from the capillary surface [190], care should be exercised as pH extremes can be detrimental to bare capillaries [215] and the baseline can be deteriorated. Also practicalities of the operating conditions can limit the operational pH range, e.g. precipitation occurs at pH <10 for electrolytes with vanadate and TTAB [88].

Electrolyte Cation and Anion

Firstly, it can be noted that for indirect detection employed in this study for anions, the co-ion is known as the carrier anion, probe or visualising agent [152] and is the anion present in the highest concentration [165] in the electrolyte. In FRECZE, the counter-ions are cations. Atamna *et al.* [202] have studied the role of buffer cation and its influence on selectivity (at least for dansylated amino acids) and have noted that migration time increased with cation size. Significant selectivity changes were not demonstrated. However, EOF migration rates were markedly affected, e.g. 37% difference in migration time of neutral markers using lithium acetate and caesium acetate [201] under the same conditions. Buffer anion effects have been studied by Salomon *et al.* [190] and Atamna *et al.* [201].

Various carrier anions have been studied, including chromate [121, 137, 210, 222-225]; vanadate, KHP, trimesate [88]; benzoate and benzoic acid [88, 164]; pyromellitic or 1,2,4,5-benzenetetracarboxylic acid [36, 165, 226]; sorbic acid [164]; (tetra)borate [201, 227]; phosphate in both the mono- and di-hydrogen forms [201, 202]; mono-, di- and tri-naphthalene sulfonates[29] [228]; *p*-hydroxybenzoate [63]; *p*-anisate, *o*-phthalate, mellitate [36]; salicyclate [36, 121]; iodide [128]; molybdate [121]; chloride [210, 223]; acetate, citrate, nitrite, carbonate [201]; nitrate [201, 210]; sulfate [210, 229]; and 2,6-naphthalenedicarboxylate (NDC) [98]. NDC and chromate showed different migration orders for anions [98]. The co-ion can be chosen to suit given anionic solutes, e.g. *p*-hydroxybenzoate for slowly migrating anions, phthalate for anions with intermediate mobility and chromate for very mobile anions [63].

Selectivity changes due to variation in electrolyte concentration seem to depend on the type of solute ion and electrolyte ion. Only minor changes were noted for inorganic anion solutes using chromate as carrier anion [137, 210]. The migration order for sulfate and chloride was reversed with increasing concentration of electrolyte [128]. Similarly, thiosulfate (and other divalent anions) were retarded more than monovalent solutes with increasing probe concentration [121] and the cause for this has been suggested as shifts in the solvation equilibrium [121, 230]. For anions demonstrating marked selectivity changes with variation of the concentration of chromate, Jimidar and Massart [224] have indicated that migration order changes are due to ion-exchange partitioning effects.

Electrolyte Media, Solvation and Organic Solvents

There is ample material on selectivity in aqueous media where the main governing parameter is acid-base equilibria [211]. CZE of anions in non-aqueous media is

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29 The di- form performs best compared to the other two forms. However, selectivity changes were minor [228].

largely unexplored. Non-aqueous media can allow for manipulation over a wider pH range than aqueous systems and may also be useful in ionising species not easily ionisable in water, i.e. hydrophobic samples [5].

Solvation is a common way of manipulating selectivity [10] through changes in the hydration volume [231], which in turn alters the charge:mass ratio of the solute. Solvation changes can be effected by the addition of organic solvents to the BGE. Some solvents used in CE include methanol [165, 180, 204, 206, 210, 223, 232, 233], acetonitrile [180, 190, 206, 210, 223, 232], tetrahydrofuran [180, 210, 232], ethylene glycol, acetone [210], ethanol [180, 206, 223, 232], 2-propanol [160, 180, 206, 232] for catechols and serotonin [160], 1-butanol [234], 1-propanol [206], dimethylformamide [204] and dioxane [232].

The concentrations of organic solvents used vary widely and the choice will depend on the effect of the solvent on detection, baseline noise, etc. Walbroehl and Jorgenson [235] used a non-aqueous electrolyte (0.05M tetraethylammonium perchlorate and 0.01 M HCl in acetonitrile). On the other hand, only small amounts of 1-butanol are recommended for anion separation [236]. The concentration of the solvent is usually varied in a step-wise manner, but can be programmed to yield gradient migration (as has been demonstrated for the MECC mode [237]).

The addition of organic solvents causes major changes in the physicochemical, chemical and electrochemical properties of the BGE [206]. Apart from causing changes in the migration time through reduction of the compact (double) layer, possibly due to improved solvation of the charges in this region [190] and facilitating selectivity changes through solvation effects, the addition of organic solvents can also influence the selectivity of solutes by partially suppressing the interaction between the solutes and the surfactant [206].

Electroosmotic Flow Modifiers

To separate anions rapidly in CZE, cationic surfactants are included in the BGE to alter the EOF to migrate in the same direction as anions. This approach has been extended to manipulate selectivity in CZE. The effect of EOF modifier on anions has been studied extensively [e.g. 1, 10, 42, 74, 81, 137, 164, 210, 238, 239]. The type of quaternary amines used include TTAB[30] [74, 116], tetradecyltrimethylammonium hydroxide [99], DTAB [59], CTAB [1, 42, 118, 214], hexamethonium bromide or hydroxide [121, 165, 226, 240, 241] (i.e. a di-quaternary amine), diethylenetriamine [165], tertiary amines (e.g. triethylamine [207]) and benzyltrimethylammonium bromide [154]. The effect of surfactant size on anion separation selectivity has been studied by Buchberger and Haddad [210] who noted only minor changes. Cationic polymers or polyelectrolytes like hexadimethrine[31] are a unique group of additives that have been used to alter anion selectivity [206, 222, 223], particularly for sulfate. Ion-exchange has been indicated as the likely mechanism [222].

The concentration of cationic surfactant in the electrolyte can influence the mechanism effecting selectivity changes. At concentrations above the cmc, chromatographic partitioning effects with the slow moving micelle also play a part in addition to mobility. Although this is especially useful for neutral solutes, separation of anions under an MECC environment has been demonstrated [82, 242]. At concentrations under the cmc, ion-pairing is the predominant mechanism [12, 137, 209, 239]. Ion-pairing, or ion-association as it is sometimes known, is an equilibrium effect and the theory relating to it has been derived by Kaneta *et al.* [209], at least for monovalent anions. Ion-pairing effects are pronounced for lipophilic anions like iodide, thiocyanate, thiosulfate and perchlorate [137, 239].

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30 Also known as CIA-Pak OFM Anion-BT, a registered trademark of Waters Millipore.

31 Also known as polymethobromide [206].

Ion-pairs separate also according to differences in retention rather than pure electrophoretic mobility [243].

Cationic polymers show different selectivity, e.g. the migration order of sulfate is altered markedly with rising concentration [222, 223] and the likely cause for this has been suggested as ion-exchange [222]. In contrast, cationic surfactants show no significant change in the migration order of sulfate with increasing concentration.

Although most selectivity studies have involved step changes in concentration of surfactant, a concentration gradient (using CTAB) has been used to manipulate separation selectivity of weak acid anions [42] and anions [244].

2.2.6.7.2 Instrumental Parameters

Sample introduction by electromigration offers a selectivity option where only charged solutes are selectively injected. Stacking can alter migration order of solutes [138]. The detection mode (see Section 2.2.6.5) also offers another option, e.g. the selective detection of UV absorbing anions in the presence of other anions has been demonstrated for the chromate-TTAB system by Jones [239]. Temperature variation as a means of manipulating selectivity has been reported [188, 201, 213]. Bonded or treated capillaries [e.g. 10, 28, 129, 201, 210] are being used increasingly in CE. Although any effect on selectivity will depend on the nature of the bonded phase, treated capillaries can offer a wider accessible pH range [129]. Bare, C_1 and C_{18} bonded capillaries have been compared for performance by Chen and Cassidy [28]. Although this may not be relevant for most inorganic anions, it is interesting to note that applied electric field strength affects the orientation of solutes within the applied field, with the mobility of rod-shaped solutes increasing with increased field [49]. Additionally, the electric field can

effect selectivity changes via Joule heat-induced pH changes [127, 245] and ion-pair or ion-exchange equilibria.

2.2.6.7.3 Miscellaneous Parameters

Other ways of altering the separation selectivity of anionic solutes include having different electrolytes in the anodic and cathodic vials [138] and complexation and ion-ligand interactions [10]. Complexation has been used in the manipulation of cationic solutes [e.g. 13, 246] and uncharged molecules, e.g. sugars and catechols complexed with borate [151, 160]. Its corresponding application to inorganic anions appears to be rare although it has been done successfully in the ITP mode [156] to alter the selectivity of chloride, nitrate and sulfate with Cd^{2+} as complexing ion. With cadmium complexation, nitrate migrated before chloride and nitrite, which is in contrast to chloride migrating first using chromate-based systems containing no metal ions. Copper ion has been used as a complexing agent for polycarboxylic acids [247]. Resolution between nitrate and sulfate was improved using Ba^{2+} to form ion-pairs [99].

2.2.6.8 Factors Affecting Analytical Performance

For the successful application of selectivity studies to the analyses of real samples, an appreciation of some of the more important parameters affecting analytical performance is essential.

2.2.6.8.1 Separation Voltage, Joule Heat and Diffusion

For rapid separation of anions, the application of high voltages is desirable. However, the application of ever increasing voltage is limited by the generation of Joule heat [10, 44]. Furthermore, disproportionately high rises in temperature can result from small changes in the applied voltage. Joule heat causes parabolic temperature gradients which cause diffusion by altering BGE viscosity and kinetic processes [10, 44, 190, 248-250]. The decrease in the viscosity of the BGE adds

further to the high speed of migration at high voltage [190]. Mobility is constant at ≤15 kV. At >15 kV, mobility increases due to reduction in electrolyte viscosity [1], especially when heat removal is inefficient. (See Eqn. 2.12.)

Anion zone dispersion is assumed to be virtually non-existent in CZE, being limited only by diffusion, Joule heat and initial sample width [164, 200]. In practice, dispersion in CZE can result from solute-wall interactions or sorptions [5, 13], Joule heat (2%/°C [5]), molecular diffusion, injection effects [13], electromigration dispersion [54, 251] and sample concentration [5]. The effect of analyte concentration on peak shape has been illustrated by Jorgenson and Lukacs [5]. Assuming only longitudinal diffusion to be significant in FRECZE, the variance (σ^2) of the anion peak area can be represented as shown in Eqn. 2.20. The standard deviation of the Gaussian peak [10] can be obtained by taking square roots of the variance.

$$\sigma^2 = 2\,D\,t_{anion} = \frac{2\,D\,L^2}{(m_{eof} + m_{anion})\,.\,V_{applied}} \qquad \text{Eqn. 2.20}$$

where D = anion diffusion coefficient. The above equation assumes the capillary surface being non-absorptive towards solutes [8].

Peak broadening or tailing can be caused by differences in the mobility of solute ions and electrolyte ions. The conditions for symmetrical peaks have been indicated by Chen and Cassidy [28].

To reduce diffusion (and increase separation efficiency), high voltages and reduced capillary lengths would be desirable. Band broadening outlined above has a direct bearing on the number of theoretical plates and resolution, which are discussed next.

2.2.6.8.2 Separation Efficiency

Separation efficiency in FRECZE may be in the millions of theoretical plates (N) [63]. This is usually calculated on the assumption that the peak is Gaussian, which can be a risky assumption since some peaks can be triangular in shape [211]. N can be influenced by the capillary-washing sequence [108] and N can even be reduced by solutes adsorbed onto the capillary surface (e.g. proteins - see Section 2.2.6.8.6) [34]) or when the anion concentration is ≥1% of the electrolyte [1, 95].

Separation efficiency in FRECZE can be represented by the number of theoretical plates (N) or the height equivalent to a theoretical plate [10]. For the calculation of N [8, 10, 34, 44], the following equations can be applied;

$$N = (m_{eof} + m_{anion}) \cdot \left(\frac{V_{applied}}{2D} \right) \qquad \text{Eqn. 2.21a}$$

$$N = \frac{L^2}{\sigma^2} = \frac{m_{eff.\ anion} \cdot V_{applied}}{2D} = \frac{V_{applied} \cdot Q}{2KT} \qquad \text{Eqn. 2.21b}$$

where $m_{eff.\ anion}$ = effective mobility of the anion, D = anion diffusion coefficient, Q = anion charge, K = Boltzmann constant and T = absolute temperature. Eqn. 2.21a holds true only when axial diffusion is the main source of band broadening [11, 44].

Obviously, high voltage [44] and a low anion diffusion coefficient would permit high N and therefore greater efficiency. The capillary length makes no significant difference to efficiency [8].

2.2.6.8.3 Resolution

Resolution of anions is a function of N and differences in their electrophoretic mobilities. See Eqn. 2.22 [8, 14].

$$R_s = \frac{\sqrt{N}}{4} \cdot \left(\frac{(v_{\text{anion 1}} - v_{\text{anion 2}})}{\left(\frac{(v_{\text{anion 1}} + v_{\text{anion 2}})}{2} \right)} \right) \qquad \text{Eqn. 2.22a}$$

$$R_s = \left(\frac{m_{\text{anion 1}} + m_{\text{anion 2}}}{5.65685} \right) \cdot \left(\frac{\sqrt{\left[\left(\frac{m_{\text{eof}} + m_{\text{anion}}}{1} \right) \cdot \left(\frac{V_{\text{applied}}}{2\,D} \right) \right]}}{(m_{\text{eof}} + m_{\text{anion}})} \right) \qquad \text{Eqn. 2.22b}$$

The subscripts of 1 and 2 indicated in the mobilities refer to anion 1 and anion 2. Eqn. 2.22b is adapted from Ewing *et al.* [14].

Resolution between anions can also be defined in terms of differences in distance travelled [64] or time differences [10]. It can also be represented as follows [1, 5, 42];

$$R_s = 0.177\ (m_{\text{anion 1}} - m_{\text{anion 2}}) \cdot \left(\frac{V_{\text{applied}}}{D\ .\ (m_{\text{av.}} + m_{\text{eof}})} \right)^{\frac{1}{2}} \qquad \text{Eqn. 2.22c}$$

where $m_{\text{av.}}$ = average mobility[32] of the two adjacent anions. The other symbols have their usual meanings.

Resolution is dependent on temperature, separation voltage, EOF rate, concentration of electrolyte, mobilities of adjacent solutes, etc. Resolution is best at low temperatures [131, 213], low EOF rate and high concentration of electrolyte [190]. Resolution is also better at high voltages according to some reports [44, 201] and this may be due to the mobility reaching a plateau at the high field strength [4]. Others have indicated that resolution can be compromised at high speed (voltage) [138]. Baseline resolution can be achieved as long as the difference in mobilities of adjacent solutes is ≥0.5% [6].

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32 Which may also be represented by $\bar{\mu}$ [5].

Having covered the relevant basic theory, the following criticism may be in order. Some authors have used equations in which the effective length (i.e. the distance from injection to the detector) and the total capillary length are not distinguished. This is especially relevant for systems with on-line photometric detection. Non-differentiation between the two lengths can result in serious errors, e.g. (i) migration rate of EOF (see Eqn. 2.14), (ii) migration time and mobility of solute anions (see Eqn. 2.19c) and (iii) peak response variance (see Eqn. 2.20). Another pertinent point that has been noted is that resolution values can have errors due to Gaussian shape assumption when fronted peaks occur [4].

2.2.6.8.4 Migration Time Precision

Short migration time is a much highlighted feature of CZE and is achieved with the application of high separation voltages [e.g. 44]. On the other hand, the precision of absolute migration time is generally poor, ranging between ~1 - >4% RSD [1, 166, 226, 229, 252] for simple solutions and from 2 - 14% RSD [87] for real samples. Possible contributing factors for the poor precision of migration time include dispersion due to Joule heat generated upon application of the high voltage, sorption of solutes or impurities onto the capillary surface [12, 196] and low stability of the dynamically coated double-layer.

As anion response is a function of the migration rate of solutes [241], it is important that migration time is stable. However, a detailed study of the causes for and solution(s) to the poor precision of absolute migration time has not been sighted by the author. A study addressing this important aspect was executed and is detailed in Chapter 4.

2.2.6.8.5 Quantitative Accuracy and Precision

CZE gives accurate quantification [5, 108, 109, 116] for most anions. Quantification is usually based on peak areas [88, 111, 253], although peak heights

too can be used but with greater care as linearity is restricted to a limited (low) concentration range. At high concentrations of solutes, the relationship between peak height and concentration is not linear and the fit approximates a parabolic function [254].

On the other hand, the precision with peak heights can be better than with peak areas [125]. The precision for both peak area and peak height vary depending on the anion and the conditions used. For peak areas, figures ranging from 2% - 11% RSD have been reported [84, 166, 229] whilst for peak heights, figures ranging from 0.7% - 3% RSD [131, 229, 252] have been reported.

The quantification accuracy for silanophilic solutes (e.g. fluoride, phosphate and proteins) can be poor. Phosphate is a strong silanophile [45, 255, 256] and its response in CE is very variable [115, 257], e.g. variations of over 50% in peak area and over 30% in peak height have been reported [257]. The poor response of phosphate and how it can be improved is detailed in Chapter 4.

2.2.6.8.6 Sources of Error

The following are some sources of error that can affect the separation of anions using fused silica capillaries:-

- When analysing anions in samples containing proteins (e.g. nitrite in corned beef or anions in bodily fluids), the adsorption of proteins onto the surface of the capillary [5, 10, 25, 34, 71] is a major source of error. The adsorption of proteins alters the double-layer which in turn causes variations in the observed migration time of anions. The mechanism of adsorption, consequences and remedies have been reviewed by Schöneich *et al.* [30]. Protein adsorption is suggested as being due to titration of acid/base sites on the capillary surface [183] and ionic, hydrophobic and H-bonding effects [127]. Some of the adverse

effects of protein adsorption include poor precision of the migration time of solutes [25, 30, 34], decrease in the peak area [10, 34], band broadening, tailing and reduced efficiency [25, 34, 47, 71], non-linearity between response and concentration [47] and poor quantitative precision [30, 34]. The adsorption of proteins onto the surface of the capillary can be prevented by using electrolytes with low pH or pH greater than the *p*I of the protein [34] or using additives [34] like hydroxypropylmethylcellulose [47] and poly(vinyl alcohol) [127].

- Poor precision of migration time. This can be caused by foaming, presence of traces of detergents or soaps in glassware, low concentration of surfactant, Joule heat generation and desorption of the surfactant. Foaming is to be avoided as the modifier concentration is higher in the foam phase relative to the bulk solution [258], so that soaps and detergents should be avoided when washing glassware since large variations in EOF can result from the sorption of minute amounts of impurities onto the capillary surface. Washing with ethanolic KOH is recommended [28].
- Extreme pH being detrimental to untreated capillaries [96, 215].
- Peak distortion can occur as a result of the sampling sequence used [131] and can be removed by going through a sequence of injection, washing the outside of capillary with buffer, running in fresh buffer and rinsing with fresh buffer before re-starting the sequence [131].
- The production of Joule heat leads to changes in the density, viscosity, etc of the electrolyte. This directly affects anion mobility, e.g. mobility increases by 2% for every ^{o}C rise in temperature [10]. This error source can be eliminated by effective thermostating.

2.2.6.9 Advantages and Limitations of CZE

Through the course of this study, it has become obvious that in declaring the advantages of CZE some qualification and clarification may be in order. Specifically, these are:-

(a) *Matrix independence*

CZE is not truly matrix independent, as illustrated in the analysis of complex samples, e.g. Bayer liquor. The sample matrix (e.g. viscosity and ionic strength) affects the amounts injected and thereby the accuracy of quantification. Baseline noise due to degradation of the capillary surface is also a function of sample matrix and pH.

(b) *Minute sample requirement*

It should be clarified that this refers to the 'plug' that is actually separated. The sample injection mode should also be indicated. Although low, there are minimum sample quantities (which are not the nL and µL ranges often cited) that are required for analysis. For the Quanta™ 4000 used in this study, the sample volume required is about 4 mL. Manual injection can introduce minute sample quantities from limited samples (e.g. single cells) but dispersion is inherently worse. With electromigration, there must be a certain minimum volume to cover the end of the capillary and the electrode.

(c) *Efficiency*

Calculated values for resolution and efficiency can be in error due to the assumption of peaks being Gaussian when fronted peaks are present [4]. Furthermore, the detection method should be indicated when indicating N, e.g. the 1 million plates often cited was achieved using fluorescence detection of non-inorganic anionic solutes.

(d) *Migration time precision*

Precision calculated using relative migration time (RMT) data may be misleading and does not convey the fact that absolute migration time is very variable. Precision calculated from RMTs should be indicated clearly.

(e) *Quantification*

There is some confusion regarding the use of peak areas and heights. Generally, peak areas are considered to give accurate results [111, 253], i.e. good linearity with concentration. Recently however, the use of peak heights for quantification has been demonstrated [152, 173]. Appropriate care needs to be exercised (see Section 2.2.6.8.5) when this method is used.

Table 2.3 shows some published advantages of CE. Although some advantages are quoted more often than others, the list is impressive. However, CZE is not without its limitations and some of these evident when on-line UV detection is used are given in Table 2.4. Several of the points listed were not published as limitations and some reading between the lines was required to pick out esoteric or alluded limitations. Although some of the limitations may seem minor, the consequences of overlooking them cannot be underestimated. Furthermore, an appreciation of the limitations along with the obvious advantages of CZE would give a better balance.

2.2.6.10 Reviews and Books

With the recent rapid developments in the use of CE, it is not surprising that it is a popular topic of review with approximately 100 reviews being published since 1990 [259]. The reviews pertinent to CE are listed in chronological order in Table 2A.1 (Appendix 2A). Many are general and are not confined to FRECZE. However, FRECZE is a variant of CE and therefore the reviews outlined all have some relevance. Some reviews deal with specific topic(s) whilst others address more than one aspect.

Already at least thirteen books on CE have been written. Of these, those by Li [45], Grossman and Colburn [260], Jandik and Bonn [36], Guzman [261], Foret, Krivánková and Boček [262] and Kuhn and Hoffstetter-Kuhn [227] were present

Table 2.3

SOME ADVANTAGES OF CAPILLARY ZONE ELECTROPHORESIS

1. High number of theoretical plates [4, 10, 12, 44, 27, 61, 80, 84, 88, 155, 213]. Values in the millions [1] have been indicated in the literature for given cases [263]. For inorganic anions detected using UV photometry, values in the 100,000s [44, 84, 210] are more realistic. Nevertheless, efficiency is better than IC, e.g. N = 160, 000 for sulfate using C(Z)E in contrast to 5,000 for IC using a good quality column [84].

2. Simultaneous separation of multiple anions [78] with direct quantification [30].

3. High speed [4, 5, 8, 17, 27, 29, 34, 47, 61, 71, 84, 96, 108, 111, 141, 155, 190, 213, 264-266]. This is made possible by the capillary's high heat dissipation efficiency [5, 8, 27, 28, 80, 131] due to its high area:volume ratio [8, 61]. This permits high voltages to be applied, resulting in reduced migration time. Compatibility with on-line detection [10, 80] and easy and full automation [8, 10, 17, 27, 30, 47, 61, 108, 264] are other factors that allow for CZE to be fast.

4. Small sample volume required for separation [10, 17, 27, 30, 34, 47, 61, 80, 155, 166, 170, 265]. Injection volumes are up to 4 orders of magnitude smaller than IC [84, 88] and volumes are in the nL [166] region. Even pL can be sufficient as long as the detector is sufficiently sensitive [27]. This makes CZE ideal for application to situations where sample quantity is limited (e.g. cytoplasma and single nerve cells [17] and tear drops [267]) and sample conservation is required [265].

Table 2.3 - *continued.*

5. High mass sensitivity [80] and detection limits comparable with IC [84].

6. Requirement for minimal sample pre-treatment [71, 87, 111]. Some samples can be injected directly (e.g. ore leachate [111]) or after simple dilution (e.g. Bayer liquor [113, 241]). This leads to high sample throughput and a reduction in overall analysis time.

7. Low operating cost [87] due to its efficiency and high sample throughput and short change-over time between analyses [61]. Furthermore, use of solvents, electrolytes, reagents, etc are minimal and capillaries are re-useable [8] or cheap to replace, e.g. a capillary costs ~$US5-00 in contrast to >$US300-00 for columns [267].

8. Anion dispersion is minimal [1, 27]. This is due to the 'wall effect' [8] and the absence of connections (with the capillary acting as the UV cell) [84]. Plug flow minimises dispersion [1]. The dispersive effects of Joule heat are not a limiting factor [49] due to the efficient heat dissipation by capillaries. Small I.D. capillaries reduce dispersion due to mass transfer and Joule heat. It is assumed that only longitudinal diffusion contributes to anion diffusion [66, 68, 69].

9. Generally good analytical performance characteristics for most anions. The method is quantitative [34, 47], accurate [5, 27, 47, 108, 109, 116], has good area precision [29, 84], good linearity of calibration [84] over 2 - 3 orders of magnitude [29].

10. Simple [5, 268], convenient [17] and easily automated. Additionally, the low dead volume of the capillary (e.g. 2.65 µL compared to +600 µL for IC [84]), allows for rapid flushing and cleaning [265], and rapid change over between separations.

Table 2.4

SOME LIMITATIONS OF CAPILLARY ZONE ELECTROPHORESIS

The following limitations of CZE apply to the separation (particularly) of anions and detection using UV-Vis absorbance.

No.	Limitation	Possible Solution(s) and Note(s)
1.	Reproducibility of EOF [10, 12] and migration time is poor. Interaction between many sample components and the capillary wall is unpredictable [108]. Certain sample types (e.g. brewed coffee) interact with capillary thereby altering migration time [12].	See Chapter 4. Conditioning the capillary between runs, which on the other hand will also increase analysis time.
2.	Low concentration sensitivity [10, 80, 155, 200, 269].	Use more sensitive detection methods (see Section 2.2.6.5.3) or increase sample loading.
3.	Quantification accuracy is linear only with respect to peak areas and not to peak heights [111, 251].	This may not be valid according to Nielen [173] who has used both heights [152, 173] and areas [173].

Table 2.4 - *continued.*

4.	Neutral solutes cannot be resolved from each other as they co-migrate with the EOF front [27, 28, 41]. When a neutral analyte is being determined, a positive error will result from the presence of other neutral solutes [103].	However, neutral species can be put to good use as EOF markers. This is especially useful in the use of relative migration times for solute identification.
5.	Limited linear dynamic range [10, 119] and capillary easily overloaded [86, 119, 163], especially with high concentration of anions [10].	Increase probe concentration. Use small amounts of sample, dilute or reduce injection time.
6.	Resolution is poor for adjacent anions present at high concentrations, e.g. 100 $\mu g.mL^{-1}$ each of chloride and sulfate [116].	Dilute or alter selectivity, e.g. use polyelectrolytes to move sulfate away from chloride [222, 223].
7.	Bromide system peak 'dip' [116] due to the surfactant anion can interfere with the separation of adjacent anions, e.g. chloride.	Use bromide as a diluent [116] or convert the surfactant to the hydroxide form.
8.	Difficult to find correct buffers (and conditions) for complex samples [104]. Trial-and-error optimisation may be time consuming [108].	Use temperature gradient [188], pH-gradient [104] or couple with IC/HPLC.

Table 2.4 - *continued.*

9.	Limited sample concentration without unacceptable peak deformation [211].	Dilute sample.
10.	Difficult to separate solutes with very similar electrophoretic mobilities [42, 105, 214].	Use pH gradient, EOFM concentration gradient or MECC format.
11.	Cannot analyse certain samples, e.g. (i) trace anions in HF acid [120] (ii) oxalate in acidified urine [228] using chromate electrolyte, (iii) solutes that breakdown in the electrolyte (e.g. alkaline hydrolysis of free cyanide (to formate) [270] and phosphocitrate and (iv) capillophiles, i.e. solutes having a propensity to interact with or adsorb onto the capillary surface (e.g. phosphate).	Use IC [120] or use a suitable probe, e.g. trimellitate. See Chapter 4 for the optimised detection of phosphate.
12.	The maximum concentration of surfactants and pH range of the electrolyte to be used for selectivity variation can be restricted by the limited solubility and formation of precipitates [63, 88].	Use organic solvents or reduce the concentration of the electrolyte.

in the author's laboratory during the execution of the research. The book by Jandik and Bonn [36] was particularly useful for this study.

2.3 REFERENCES

1 Altria, K.D. and Simpson, C.F., *Chromatographia*, **24** (1987) 527.

2 Wieland, T. and Dose, K., 'Electrochromatography (Zone Electrophoresis, Pherography)' in Berl, W.G., editor, Physical Methods in Chemical Analysis (Academic Press, New York, 1956), pp. 29 - 65.

3 Moore, W.J., Physical Chemistry (Longmans Green and Co., London, 1958), pp. 442 - 446.

4 Jones, H.K. and Ballou, N.E., *Anal. Chem.*, **62** (1990) 2484.

5 Jorgenson, J.W. and Lukacs, K.D., *Science*, **222** (1983) 266.

6 Cohen, A.S., Najarian, D., Smith, J.A. and Karger, B.L., *J. Chromatogr.*, **458** (1988) 323.

7 Weast, R.C., CRC Handbook of Chemistry and Physics, 58th edition (The Chemical Rubber Company Press, Cleveland, Ohio, 1977-78), p. F130.

8 Jorgenson, J.W., *Trends Anal. Chem.*, **3** (1984) 51.

9 Andrews, A.T., Electrophoresis: Theory, Techniques and Biochemical and Clinical Applications, 2nd edition (Clarendon Press, Oxford, 1986).

10 Schwer, Ch. and Kenndler, E., *Chromatographia*, **30** (1990) 546.

11 Lukacs, K.D. and Jorgenson, J.W., *J. High Resolut. Chromatogr.*, **8** (1985) 407.

12 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **608** (1992) 385.

13 Swaile, D.F. and Sepaniak, M.J., *Anal. Chem.*, **63** (1991) 179.

14 Ewing, A.G., Wallingford, R.O and Olefirowicz, T.M., *Anal. Chem.*, **61** (1989) 292A.

15 Heftmann, E. 'Survey of Chromatographic and Electrophoretic Techniques' in Heftmann, E., editor, Chromatography: Fundamentals and Applications of Chromatographic and Electrophoretic Techniques. Part A: Fundamentals and Techniques (Elsevier Scientific Publishers, Amsterdam, 1983).

16 Reiner, M., 'History of Electrophoresis' in, Lewis, L.A. and Opplt, J.J., editors, CRC Handbook of Electrophoresis. Volume I. Lipoproteins: Basic Principles and Concepts (CRC, Florida, 1980), pp. 3 - 8.

17 Widmer, H.M., *Chimia*, **43** (1989) 134.

18 Wieme, R.J., 'Theory of Electrophoresis', in Heftmann, E., editor, Chromatography: A Laboratory Handbook of Chromatographic and Electrophoretic Methods, 3rd edition (Van Nostrand Reinhold & Co., New York, 1975), pp. 228 - 281.

19 Skoog, D.A., Principles of Instrumental Analysis, 3rd edition (Saunders College Publishing, Philadelphia, 1985), pp. 189, 243, 694.

20 Warner, M., *Anal. Chem.*, **60** (1988) 1159A.

21 Vesterberg, O., *J. Chromatogr.*, **480** (1989) 3.

22 Everaerts, F.M., Beckers, J.L. and Verheggen, Th. P.E.M., Isotachophoresis: Theory, Instrumentation and Applications, Journal of Chromatography Library, Volume 6 (Elsevier Scientific, Amsterdam, 1976).

23 Michl, H., 'Techniques of Electrophoresis', in Heftmann, E., editor, Chromatography: A Laboratory Handbook of Chromatographic and Electrophoretic Methods, 3rd edition (Van Nostrand Reinhold & Co., New York, 1975), pp. 282 - 311.

24 Grossman, P.D., Colburn, J.C., Lauer, H.H., Nielsen, R.G., Riggin, R.M., Sittampalam, G.S. and Rickard, E.C., *Anal. Chem.*, **61** (1989) 1186.

25 Jorgenson, J.W., *Anal. Chem.*, **58** (1986) 743A.

26 Pentoney, Jr., S.L., Zare, R.N. and Quint, J.F., *Anal. Chem.*, **61** (1989) 1642.

27 Gordon, M.J., Huang, X., Pentoney, Jr., S.L. and Zare, R.N., *Science*, **242** (1988) 224.

28 Chen, M. and Cassidy, R.M., *J. Chromatogr.*, **602** (1992) 227.

29 Dülffer, T., Herb, H. and Kobold, U., *Chromatographia*, **30** (1990) 675.

30 Schöneich, C., Kwok, S.K., Wilson, G.S., Rabel, S.R., Stobaugh, J.F., Williams, T.D. and Vander Velde, D.G., *Anal. Chem.*, **65** (1993) 67R.

31 Caslavska, J., Molteni, S., Chmelík, J., Šlais, K., Matulík, F. and Thormann, W., *J. Chromatogr. A.*, **680** (1994) 549.

32 Australian Government Analytical Laboratories (AGAL) seminar on Capillary Gel Electrophoresis (Sydney, November, 1992). Paper courtesy of Professor Paul R. Haddad.

33 Warner, M., *Anal. Chem.*, **66** (1994) 1137A.

34 Goodall, D.M., Lloyd, D.K. and Williams, S.J., *LC•GC*, **8** (1992) 788.

35 Guttman, A., Cohen, A.S., Heiger, D.N. and Karger, B.L., *Anal. Chem.*, **62** (1990) 137.

36 Jandik, P. and Bonn, G., Capillary Electrophoresis of Small Ions and Molecules (VCH Publishers, New York, 1993), pp. 2, 18.

37 Ahuja, E.S. and Foley, J.P., *J. Chromatogr. A.*, **680** (1994) 73.

38 Terabe, S., Otsuka, K., Ichikawa, K., Tsuchiya, A. and Ando, T., *Anal. Chem.*, **56** (1984) 111.

39 Terabe, S., Otsuka, K. and Ando, T., *Anal. Chem.*, **57** (1985) 834.

40 Terabe, S., Ishihama, Y., Nishi, Y., Fukuyama, T. and Otsuka, K., *J. Chromatogr.*, **545** (1991) 359.

41 Walbroehl, Y. and Jorgenson, J.W., *Anal. Chem.*, **58** (1986) 479.

42 Chang, H-T. and Yeung, E.S., *J. Chromatogr.*, **608** (1992) 65.

43 Warner, M., *Anal. Chem.*, **61** (1989) 795A.

44 Karger, B.L., *Journal of Research of the National Bureau of Standards*, **93** (1988) 406.

45 Li, S.F.Y., Capillary Electrophoresis. Principles, Practice and Applications (Elsevier, Amsterdam, 1992), pp. 2, 3, 203, 204.

46 Heftmann, E., 'History of Chromatography', in Heftmann, E., editor, Chromatography: A Laboratory handbook of Chromatographic and Electrophoretic Methods, 3rd edition (Van Nostrand Reinhold Co., New York, 1975), pp. 1 - 13.

47 Lindner, H., Helliger, W., Dirschlmayer, A., Talasz, H., Wurm, M., Sarg, B., Jaquemar, M. and Puschendorf, B., *J. Chromatogr.*, **608** (1992) 211.

48 Kuhr, W.G., *Anal. Chem.*, **62** (1990) 403R.

49 Grossman, P.D. and Soane, D.S., *Anal. Chem.*, **62** (1990) 1592.

50 Catsimpoolas, N., 'Basic principles of different types of electrophoresis' in, Lewis, L.A. and Opplt, J.J., editors, CRC Handbook of Electrophoresis.

Volume I. Lipoproteins: Basic Principles and Concepts (CRC, Florida, 1980), pp. 11 - 23.

51 Guiochon, G. and Colin, H., 'Narrow-Bore and Micro-Bore Columns in Liquid Chromatography', in Kucera, P., editor, Microcolumn High-Performance Liquid Chromatography, Volume 28 (Elsevier, Amsterdam, 1984), pp. 1 - 38.

52 Srivastava, S.K., Gupta, V.K., Tiwari, B.B. and Ali, I., *J. Chromatogr.*, **635** (1993) 171.

53 Nerenberg, S.T., Electrophoretic Screening Procedures (Lea and Febiger, 1973), pp. 8, 11.

54 Mikkers, F.E.P., Everaerts, F.M. and Verheggen, Th. P.E.M., *J. Chromatogr.*, **169** (1979) 11.

55 Waters (Millipore) Operators Manual #250119, Millipore Corporation, Waters Chromatography Division, Milford, MA, USA (199), p. E-1.

56 Hjertén, S., *Chromatogr. Rev.*, **9** (1967) 122.

57 Hjertén, S., *Electrophoresis*, **11** (1990) 665.

58 Lee, C.S., Blanchard, W.C., Wu, C-T., *Anal. Chem.*, **62** (1990) 1550.

59 Tsai, P., Patel, B. and Lee, C.S., *Anal. Chem.*, **65** (1993) 1439.

60 Vanderhoff, J.W., Micale, F.J. and Krumrine, P.H., "Electrophoresis '78" proceedings (MIT, Cambridge, MI), April 19 - 21, 1978, edited by Catsimpoolas, N., pp. 405 - 425.

61 Burge, G.L., *Chemistry in Australia*, **59** (1992) 253.

62 Heiger, D.N., Carson, S.M., Cohen, A.S. and Karger, B.L., *Anal. Chem.*, **64** (1992) 192.

63 Jandik, P., Jones, W.R., Weston, A. and Brown, P.R., *LC•GC.*, **9** (1991) 634.

64 Fifield, F.W. and Kealy, D., Principles and Practice of Analytical Chemistry, 2nd edition (International Textbook Company, Glasgow, 1983).

65 Anonymous, *Anal. Chem.*, **66** (1994) 901A.

66 Jorgenson, J.W. and Lukacs, K.D., *Anal. Chem.*, **53** (1981) 1298.

67 Jorgenson, J.W. and Lukacs, K.D., *Clin. Chem.*, **27** (1981) 1551.

68 Jorgenson, J.W. and Lukacs, K.D., *JHRC & CC*, **4** (1981) 230.

69 Jorgenson, J.W. and Lukacs, K.D., *J. Chromatogr.*, **218** (1981) 209.

70 Desbène, P.L., Rony, C., Desmazières, B. and Jacquier, J.C., *J. Chromatogr.*, **608** (1992) 375.

71 Vinther, A., Petersen, J. and Søeberg, H., *J. Chromatogr.*, **608** (1992) 205.

72 Bočcek, P., Demhl, M., Pospíchal, J. and Sudor, J., *J. Chromatogr.*, **470** (1989) 309.

73 Giddings, J.C., *J. Chromatogr.*, **480** (1989) 21.

74 Ackermans, M.T., Everaerts, F.M. and Beckers, J.L., *J. Chromatogr.*, **549** (1991) 345.

75 Romano, J., Jandik, P., Jones, W. and Jackson, P.E., *J. Chromatogr.*, **546** (1991) 411.

76 Lucy, C.A. and McDonald, T.L., *Anal. Chem.*, **67** (1995) 1074.

77 Oda, R.P., Spelsberg, T.C. and Landers, J.P., *LC•GC*, **12** (1994) 50.

78 Romano, J.P. and Krol, J., *J. Chromatogr.*, **602** (1992) 205.

79 Dunn, M.J., *Trends Anal. Chem.*, **10** (1991) 6.

80 Albin, M.A., Grossman, P.D. and Moring, S.E., *Anal. Chem.*, **65** (1993) 489A.

81 Tsuda, T., *JHRC & CC,* **10** (1987) 622.

82 Cohen, A.S., Terabe, S., Smith, J.A. and Karger, B.L., *Anal. Chem.*, **59** (1987) 1021.

83 Otsuka, K. and Terabe, S., *J. Chromatogr.*, **480** (1989) 91.

84 Jones, W.R. and Jandik, P., *American Laboratory* (1990) 51.

85 Jandik, P. and Jones, W.R., Waters International IC Symposium Presentation (1992).

86 Weston, A., Brown, P.R., Jandik, P., Heckenberg, A.L. and Jones, W.R., *J. Chromatogr.*, **608** (1992) 395.

87 Kenney, B.F., *J. Chromatogr.*, **546** (1991) 423.

88 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **546** (1991) 431.

89 Wildman, B.J., Jackson, P.E., Jones, W.R. and Alden, P.G., *J. Chromatogr.*, **546** (1991) 459.

90 Karger, B.L., Cohen, A.S. and Guttman, A., *J. Chromatogr.*, **492** (1989) 585.

91 Li, K. and Li, S.F.Y., *J. Liq. Chromatogr.*, **17** (1994) 3889.

92 Cavallaro, A., Piangerelli, V., Nerini, F., Cavalli, S. and Reschiotto, C., *J. Chromatogr. A.*, **709** (1995) 361.

93 Nielen, M.W.F., *J. Chromatogr.*, **608** (1992) 85.

94 Weston, A., Brown, P.R., Jandik, P., Heckenberg, A.L. and Jones, W.R., *J. Chromatogr.*, **602** (1992) 249.

95 Avdalovic, N., Pohl, C.A., Rocklin, R.D. and Stillian, J.R., *Anal. Chem.*, **65** (1993) 1470.

96 Aguilar, M., Huang, X. and Zare, R. N., *J. Chromatogr.*, **480** (1989) 427.

97 Oehrle, S.A., *J. Chromatogr. A.*, **671** (1994) 383.

98 Dabek-Zlotorzynska, E. and Dlouhy, J.F., *J. Chromatogr. A.*, **671** (1994) 389.

99 Röder, A. and Bächmann, K., *J. Chromatogr. A.*, **689** (1995) 305.

100 Oehrle, S.A. and Bossle, P.C., *J. Chromatogr. A.*, **692** (1995) 247.

101 Zhu, M., Rodriguez, R., Wehr, T. and Siebert, C., *J. Chromatogr.*, **608** (1992) 225.

102 Chang, H-T. and Yeung, E.S., *Anal. Chem.*, **67** (1995) 1079.

103 Jimidar, M., Hamoir, T.P., Foriers, A. and Massart, D.L., *J. Chromatogr.*, **635** (1993) 179.

104 Purghart, V. and Games, D.E., *J. Chromatogr.*, **605** (1992) 139.

105 Vindevogel, J., Sandra, P. and Verhagen, L.C., *J. High Resolut. Chromatogr.*, **13** (1990) 295.

106 Chen, F-T. A and Tusak, A., *J. Chromatogr. A.*, **685** (1994) 331.

107 Krivánková, L., Foret, F. and Boček, P., *J. Chromatogr.*, **545** (1991) 307.

108 Mitsyuk, B.M., *Russ. J. Inorg. Chem.*, **17** (1972) 471.

109 Jimidar, M., Hartmann, C., Cousement, N. and Massart, D.L., *J. Chromatogr. A.*, **706** (1995) 479.

110 Dinelli, G., Vicari, A. and Brandolini, V., *J. Chromatogr. A.*, **700** (1995) 201.

111 Aguilar, M., Farran, A. and Martinez, M., *J. Chromatogr.*, **635** (1993), 127.

112 Salomon, D.R. and Romano, J., *J. Chromatogr.*, **602** (1992) 219.

113 Harakuwe, A.H., Haddad, P.R. and Buchberger, W., *J. Chromatogr. A.*, **685** (1994) 161.

114 Jackson, P.E., *J. Chromatogr. A.*, **693** (1995) 155.

115 Haddad, P.R., Harakuwe, A.H. and Buchberger, W., *J. Chromatogr. A.*, **706** (1995) 571.

116 Jordan, J.M., Moese, R.L., Johnson-Watts, R. and Burton, D.E., *J. Chromatogr. A.*, **671** (1994) 445.

117 Harakuwe, A.H., Haddad, P.R. and Thomas, R., 'The simultaneous determination of chloride and sulfate in concrete digested with nitric acid using CZE', paper in preparation.

118 Saari-Nordhaus, R. and Anderson, Jr., J.M., *J. Chromatogr. A.*, **706** (1995) 563.

119 Hargadon, K.A. and McCord, B.R., *J. Chromatogr.*, **602** (1992) 241.

120 Vanderford, G., *J. Chromatogr.*, **602** (1992) 75.

121 Boden, J., Bächman, K., Kotz, L., Fabry, L. and Pahlke, S., *J. Chromatogr. A.*, **696** (1995) 321.

122 Bondoux, G. and Jones, T., *LC•GC*, **13** (1995) 144.

123 Wallingford, R.A. and Ewing, A.G., *Advances in Chromatography*, **29** (1989) 1.

124 Knox, J., *personal communication*, 1994.

125 Hjertén, S. and Zhu, M-D., *J. Chromatogr.*, **346** (1985) 265.

126 Tsuda, T., Nomura, K. and Nakagawa, G., *J. Chromatogr.*, **264** (1983) 385.

127 Monnig, C.A. and Kennedy, R.T., *Anal. Chem.*, **66** (1994) 280R.

128 Tindall, G.W. and Perry, R.L., *J. Chromatogr. A.*, **696** (1995) 349.

129 Huang, M., *The Reporter*, **14** (1995) 10. (Supelco International)

130 Demana, T., Guhathakurta, U. and Morris, M.D., *Anal. Chem.*, **64** (1992) 390.

131 Lux, J.A., Yin, H.-F. and Schomburg, G., *Chromatographia*, **30** (1990) 7.

132 Wong, T.M., Carey, C.M. and Lin, S.H.C., *J. Chromatogr. A.*, **680** (1994) 413.

133 Schwartz, H.E., Melera, M. and Brownlee, R.G., *J. Chromatogr.*, **480** (1989) 129.

134 Kuhr, W.G., Licklider, L. and Amankwa, L., *Anal. Chem.*, **65** (1993) 277.

135 Linhares, M.C. and Kissinger, P.T., *Anal. Chem.*, **63** (1991) 2076.

136 Rose, D.J. and Jorgenson, J.W., *Anal. Chem.*, **60** (1988) 642.

137 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **546** (1991) 445.

138 Beckers, J.L. and Ackermans, M.T., *J. Chromatogr.*, **629** (1993) 371.

139 Huang, X., Gordon, M.J. and Zare, R.N., *Anal. Chem.*, **60** (1988) 375.

140 Fishman, H.A., Scheller, R.H. and Zare, R.N., *J. Chromatogr. A.*, **680** (1994) 99.

141 Rahn, P.C., *International Laboratory*, **20** (1990) 44.

142 Dose, E.V. and Guiochon, G., *Anal. Chem.*, **64** (1992) 123.

143 Huang, X., Coleman, W.F. and Zare, R.N., *J. Chromatogr.*, **480** (1989) 95.

144 Thompson, T.J., Foret, F., Vouros, P. and Karger, B.L., *Anal. Chem.*, **65** (1993) 900.

145 Hjertén, S., *J. Chromatogr.*, **347** (1985) 191.

146 Tsuda, T., Mizuno, T. and Akiyama, J., *Anal. Chem.*, **59** (1987) 799.

147 Rose, Jr., D.J. and Jorgenson, J.W., *J. Chromatogr.*, **447** (1988) 117.

148 Tsuda, T., Sweedler, J.V. and Zare, R.N., *Anal. Chem.*, **62** (1990) 2149.

149 O'Shea, T.J. and Lunte, S.M., *Anal. Chem.*, **65** (1993) 247.

150 Yeung, E.S., *Acc. Chem. Res.*, **22** (1989) 125.

151 Bruno, A.E., Krattiger, B., Maystre, F. and Widmer, H.M., *Anal. Chem.*, **63** (1991) 2689.

152 Nielen, M.W.F., *J. Chromatogr.*, **608** (1992) 85.

153 Yu, M. and Dovichi, N.J., *Anal. Chem.*, **61** (1989) 37.

154 Miyagishi, S., Kurimoto, H., Ishihara, Y and Asakawa, T., *Bull. Chem. Soc. Jpn.*, **67** (1994) 2398.

155 Wu, J., Odake, T., Kitamori, T. and Sawada, T., *Anal. Chem.*, **63** (1991) 2216.

156 Gebauer, P., Deml, M., Boček, P. and Janak, J., *J. Chromatogr.*, **267** (1983) 455.

157 Huang, X., Pang, T-K.J., Gordon, M.J. and Zare, R.N., *Anal. Chem.*, **59** (1987) 2747.

158 Kuhr, W.G. and Yeung, E.S., *Anal. Chem.*, **60** (1988) 1832.

159 Sepaniak, M.J., Swaile, D.F. and Powell, A.C., *J. Chromatogr.*, **480** (1989) 185.

160 Wallingford, R.A. and Ewing, A.G., *Anal. Chem.*, **61** (1989) 98.

161 Moseley, A.M., Deterding, L.J., de Wit, J.S.M., Tomer, K.B., Kennedy, R.T., Bragg, N. and Jorgenson, J.W., *Anal. Chem.*, **61** (1989) 1577.

162 Wu, S. and Dovichi, N.J., *J. Chromatogr.*, **480** (1989) 141.

163 Zhao, J.Y., Waldron, K.C., Miller, J., Zhang, J.Z., Harke, H. and Dovichi, N.J., *J. Chromatogr.*, **608** (1992) 239.

164 Foret, F., Fanali, S., Ossicini, L. and Boček, P., *J. Chromatogr.*, **470** (1989) 299.

165 Harrold, M.P., Wojtusik, M.J., Riviello, J. and Henson, P., *J. Chromatogr.*, **640** (1993) 463.

166 Ma, Y. and Zhang, R., *J. Chromatogr.*, **625** (1992) 341.

167 Olefirowicz, T.M. and Ewing, A.G., *J. Chromatogr.*, **499** (1990) 713.

168 Ma, Y.F. and Yeung, E.S., *Mikrochim. Acta.*, **III** (1988) 327.

169 Gross, L. and Yeung, E.S., *J. Chromatogr.*, **480** (1989) 169.

170 Wang, T. and Hartwick, R.A., *J. Chromatogr.*, **607** (1992) 119.

171 Yeung, E.S. and Kuhr, W.G., *Anal. Chem.*, **63** (1991) 275A.

172 Wilson, S.A. and Yeung, E.S., *Anal. Chim. Acta.*, **157** (1984) 53.

173 Nielen, M.W.F., *J. Chromatogr.*, **588** (1991) 321.

174 Wang, T. and Hartwick, R.A., *J. Chromatogr.*, **589** (1992) 307.

175 Chervet, J.P., van Soest, R.E.J. and Ursem, M., *J. Chromatogr.*, **543** (1991) 439.

176 Grant, I.H. and Steuer, W., *J. Microcolumn Sep.*, **2** (1990) 74.

177 Xi, X. and Yeung, E.S., *Appl. Spectrosc.*, **45** (1991) 119.

178 Taylor, J.A. and Yeung, E.S., *Anal. Chem.*, **64** (1992) 1741.

179 Wang, T., Aiken, J.H., Huie, C.W. and Hartwick, R.A., *Anal. Chem.*, **63** (1991) 1372.

180 Janini, G.M., Chan, K.C., Barnes, J.A., Muschik, G.M. and Issaq, Benz, H.J., *Chromatographia*, **35** (1993) 497.

181 McEldoon, J.P. and Datta, R., *Anal. Chem.*, **64** (1992) 227.

182 Smith, I., 'General Principles of Zone Electrophoresis' in Smith, I., editor, Chromatographic and Electrophoretic Techniques (William Heinemann Medical Books, London, 1976), pp. 1 - 15.

183 Adamson, A.W., Physical Chemistry of Surfaces, 2nd edition (Interscience Publishers, New York, 1967), 93.

184 Reijenga, J.C., Aben, G.V.A., Verheggen, Th. P.E.M. and Everaerts, F.M., *J. Chromatogr.*, **260** (1983) 241.

185 Sparnaay, M.J., The Electrical Double Layer (Pergamon Press, Oxford, 1972).

186 Mukerjee, P., *Journal of Physical Chemistry*, **66** (1962) 1375.

187 Wanders, B.J., van de Goor, A.A.A.M., Everaerts, F.M., *J. Chromatogr.*, **470** (1989) 89.

188 Grushka, E., McCormick, R.M. and Kirkland, J.J., *Anal. Chem.*, **61** (1989) 241.

189 Rice, C.L. and Whitehead, R., *J. Phys. Chem.*, **69** (1965) 4017.

190 Salomon, K., Burgi, D.S. and Helmer, J.C., *J. Chromatogr.*, **559** (1991) 69.

191 Stevens, T.S. and Cortes, H.J., *Anal. Chem.*, **55** (1983) 1365.

192 Cantwell, F.F. and Puon, S., *Anal. Chem.*, **51** (1979) 623.

193 Weiss, C.S., Hazlett, J.S., Datta, M.H. and Danzer, M.H., *J. Chromatogr.*, **608** (1992) 325.

194 Tsuda, T., Nomura, K. and Nagakawa, G., *J. Chromatogr.*, **248** (1982) 241.

195 Chadwick, R.R. and Hsieh, J.C., *Anal. Chem.*, **63** (1991) 2380.

196 van de Goor, A.A.A.M., Wanders, B.J. and Everaerts, F.M., *J. Chromatogr.*, **470** (1989) 95.

197 Herren, B.J., Shafer, S.G., van Alstine, J., Harris, J.M. and Snyder, R.S., *J. Colloid Interface Sci.*, **115** (1987) 46.

198 Lee, T.T., Dadoo, R. and Zare, R.N., *Anal. Chem.*, **66** (1994) 2694.

199 Lauer, H.H. and McManigill, D., *Anal. Chem.*, **58** (1986) 166.

200 Pálmarsdóttir, S. and Edholm, L.-E., *J. Chromatogr. A.*, **693** (1995) 131.

201 Atamna, I.Z., Metral, C.J., Muschik, G.M. and Issaq, H.J., *J. Liq. Chromatogr.*, **13** (1990) 3201.

202 Atamna, I.Z., Metral, C.J., Muschik, G.M. and Issaq, H.J., *J. Liq. Chromatogr.*, **13** (1990) 2517.

203 Atamna, I.Z., Issaq, H.J., Muschik, G.M. and Janini, G.M., *J. Chromatogr.*, **588** (1991) 315.

204 Salimi-Moosavi, H. and Cassidy, R.M., *Anal. Chem.*, **67** (1995) 1067.

205 Terabe, S., *Trends Anal. Chem.*, **8** (1989) 129.

206 Masselter, S.M. and Zemann, A.J., *Anal. Chem.*, **67** (1995) 1047.

207 Corradini, D., Cannarsa, G., Fabbri, E. and Corradini, C., *J. Chromatogr. A.*, **709** (1995) 127.

208 Ong, C.P., Ng, C.L., Lee, H.K. and Li, S.F.Y., *J. Chromatogr.*, **588** (1991) 335.

209 Kaneta, T., Tanaka, S., Taga, M. and Yoshida, H., *Anal. Chem.*, **64** (1992) 798.

210 Buchberger, W. and Haddad, P.R., *J. Chromatogr.*, **608** (1992) 59.

211 Poppe, H., *Anal. Chem.*, **64** (1992) 1908.

212 Zhu, M., Hansen, D.L., Burd, S. and Gannon, F., *J. Chromatogr.*, **480** (1989) 311.

213 Whang, C-W. and Yeung, E.S., *Anal. Chem.*, **64** (1992) 502.

214 Lukkari, P., Jumppanen, J., Holma, T., Siren, H., Jinno, K., Elo, H. and Riekkola, M.-L., *J. Chromatogr.*, **608** (1992) 317.

215 Ng, C.L., Lee, H.K. and Li, S.F.Y., *J. Chromatogr.*, **598** (1992) 133.

216 Pospíchal, J., Deml, M., Gebauer, P. and Boček, P., *J. Chromatogr.*, **470** (1989) 43.

217 Sustácek, V., Foret, F. and Boček, P., *J. Chromatogr.*, **480** (1989) 271.

218 Boček, P., Deml, M. and Pospíchal, J., *J. Chromatogr.*, **500** (1990) 673.

219 Foret, F., Fanali, S. and Boček, P., *J. Chromatogr.*, **516** (1990) 219.

220 Sudor, J., Pospíchal, J., Deml, M. and Boček, P., *J. Chromatogr.*, **545** (1991) 331.

221 Transfiguracion, J.C., Dolman, C., Eidelman, D.H. and Lloyd, D.K., *Anal. Chem.*, **67** (1995) 2937.

222 Stathakis, C. and Cassidy, R.M., *Anal. Chem.*, **66** (1990) 667.

223 Stathakis, C. and Cassidy, R.M., *J. Chromatogr. A.*, **699** (1995) 355.

224 Jimidar, M. and Massart, D.L., *Anal. Chim. Acta.*, **294** (1994) 165.

225 Salomon, D.R. and Romano, J., *J. Chromatogr.*, **602** (1992) 219.

226 Dabek-Zlotorzynska, E., Dlouchy, J.F., Houle, N., Piechowski, M. and Ritchie, S., *J. Chromatogr. A.*, **706** (1995) 469.

227 Kuhn, R. and Hoffstetter-Kuhn, S., Capillary Electrophoresis: Principles and Practice (Springer-Verlag, Berlin, 1993), p. 75.

228 Shamsi, S.A. and Danielson, N.D., *Anal. Chem.*, **66** (1994) 3757.

229 Song, L., Ou, Q., Yu, W. and Xu, G., *J. Chromatogr. A.*, **696** (1995) 307.

230 Bächmann, K., Boden J. and Haumann I., *J. Chromatogr.*, **626** (1992) 259.

231 Evans, K.P. and Beaumont, G.L., *J. Chromatogr.*, **636** (1993) 153.

232 Fujiwara, S. and Honda, S., *Anal. Chem.*, **59** (1987) 487.

233 Colburn, J., Black, B., Chen, S-M., Demorest, D., Wiktorowicz, J. and Wilson, K., *Research News*, Applied Biosystems, winter issue 1 (1990).

234 Mitsyuk, B.M., *Russ. J. Inorg. Chem.*, **17** (1972) 471.

235 Walbroehl, Y. and Jorgenson, J.W., *J. Chromatogr.*, **315** (1984) 135.

236 Benz, N.J. and Fritz, J.S., *J. Chromatogr.*, **671** (1994) 437.

237 Balchunas, A.T. and Sepaniak, M.J., *Anal. Chem.*, **60** (1988) 617.

238 Huang, X., Luckey, J.A., Gordon, M.J. and Zare, R.N., *Anal. Chem.*, **61** (1989) 766.

239 Jones, W.R., *J. Chromatogr.*, **640** (1993) 387.

240 Wojtusik, M.J. and Harrold, M.P., *J. Chromatogr. A.*, **671** (1994) 411.

241 Cousins, S.M., Haddad, P.R. and Buchberger, W., *J. Chromatogr. A.*, **671** (1994) 397.

242 Wallingford, R.A. and Ewing, A.G., *J. Chromatogr.*, **441** (1988) 299.

243 Pfeffer, W.D. and Yeung, E.S., *J. Chromatogr.*, **557** (1991), 125.

244 Chang, H-T. and Yeung, E.S., *J. Chromatogr.*, **608** (1992) 65.

245 Bondoux, G., Jandik, P. and Jones, W.R., *J. Chromatogr.*, **602** (1992), 79.

246 Saitoh, T., Hoshino, H. and Yotsuyanagi, T., *J. Chromatogr.*, **469** (1989) 175.

247 Wiley, J.P., *J. Chromatogr. A.*, **692** (1995) 267.

248 Knox, J.H., *Chromatographia*, **26** (1988) 329.

249 Bello, M.S. and Righetti, P.G., *J. Chromatogr.*, **606** (1992) 95.

250 Bello, M.S. and Righetti, P.G., *J. Chromatogr.*, **606** (1992) 103.

251 Mikkers, F.E.P, Everaerts, F.M. and Verheggen, Th. P.E.M., *J. Chromatogr.*, **169** (1979) 1.

252 Martí, V., Aguilar, M. and Yeung, E.S., *J. Chromatogr. A.*, **709** (1995) 367.

253 McGhie, T.K., *J. Chromatogr.*, **634** (1993) 107.

254 Wätzig, H., *J. Chromatogr. A.*, **700** (1995) 1.

255 McCormick, R.M., *Anal. Chem.*, **60** (1988) 2322.

256 Tran, A.D., Park, S., Lisi, P.J., Huynh, O., Ryall, R.P. and Lane, P.A., *J. Chromatogr.*, **542** (1991) 459.

257 Rhemrev-Boom, M.M., *J. Chromatogr. A.*, **680** (1994) 675.

258 APHA/AWWA/WPCF, Standard Methods for the Examination of Water and Waste Water, 16th edition (APHA, Washington DC, 1985), p. 578.

259 Kuhr, W.G. and Monnig, C.A., *Anal. Chem.*, **64** (1992) 389R.

260 Grossman, P.D. and Colburn, J.C., editors, Capillary Electrophoresis (Academic Press, San Diego, 1992).

261 Guzman, N.A., editor, Capillary Electrophoresis Technology (Marcel Dekker, New York, 1993).

262 Foret, F., Kriványková, L. and Boček, P., Capillary Zone Electrophoresis, in Radola, B.J., editor (VCH, Weinheim, FRG, 1993).

263 Lauer, H.H. and McManigill, D., *Trends Anal. Chem.*, **5** (1986) 11.

264 Kraak, J.C., Busch, S. and Poppe, H., *J. Chromatogr.*, **608** (1992) 257.

265 Li, W., Moussa, A. and Giese, R.W., *J. Chromatogr.*, **608** (1992) 171.

266 Soini, H., Riekkola, M-L. and Novotny, M.V., *J. Chromatogr.*, **608** (1992) 265.

267 Chadwick, R.R., Hsieh, J.C., Resham, K.S. and Nelson, R.B., *J. Chromatogr. A.*, **671** (1994) 403.

268 Jackson, P.E. and Haddad, P.R., *J. Chromatogr. A.*, **640** (1993) 481.

269 Soucheleau, J. and Denoroy, L., *J. Chromatogr.*, **608** (1992) 181.

270 Marsh, J.D.F. and Martin, M.J., *J. Appl. Chem*, **7** (1957) 205.

271 Snopek, J., Jelínek, I. and Smolková-Keulemansová, E., *J. Chromatogr.*, **452** (1988) 571.

272 Niessen, W.M.A., Tjaden, U.R. and van der Greef, J., *J. Chromatogr.*, **636** (1993) 3.

273 Xu, Y., *Anal. Chem.*, **65** (1993) 425R.

274 Heiger, D.N. and Majors, R.E., *LC•GC*, **13** (1995) 12.

CHAPTER 3

EXPERIMENTAL

3.1 INTRODUCTION

Wide ranging experimental conditions and procedures were used in this study and are detailed in each chapter. Some conditions and procedures were however more general and applied to all investigations undertaken. Except where indicated otherwise in the appropriate chapters, figures, tables, etc; the following generalities applied:- (i) All results were obtained using the instrumental conditions shown in Table 3.1. (ii) All reagents were of AR grade. (iii) All reagents were dried overnight at 100 ^{o}C and cooled in a desiccator before use. (iv) Milli-Q® water[1] (Millipore, Bedford, MA, USA) was used in all instances; and (v) All solutions (standards, samples and electrolytes) were filtered to pass 0.45 μm (Millex®-HA, Millipore, Bedford, MA, USA) and degassed by ultrasound before use.

3.2 INSTRUMENTATION

The following instruments were used:- (a) Waters Quanta™ 4000 Capillary Electrophoresis system fitted with a high voltage power source (Spellman, Plainview, N.Y., USA) of interchangeable polarity, interchangeable UV lamps and filters, and an Accusep® bare fused silica capillary coated with polyimide. The polyimide gives the capillary strength and flexibility by preventing the strained siloxy bonds from being hydrated [1]. (b) Blue Chip 80286 computer (IBM compatible) for instrument control,

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1 From here on, reference to 'water' will mean Milli-Q® water.

data acquisition, processing and management with the Maxima 820 (software) data station (Dynamic Solutions, Millipore, Ventura, CA, USA). (c) Ultrasonic bath (Unisonics, Sydney). (d) Model 8520 digital pH meter (Hanna Instruments, Singapore).

Quanta™ 4000 operating conditions

The operating conditions in Table 3.1 were standard for all analyses except where alterations are clearly indicated in the later chapters.

Table 3.1

Quanta™ 4000 CE OPERATING CONDITIONS

Capillary type:	Waters Accusep® polyimide-coated fused silica.[a]
Capillary dimensions:	75 µm I.D. x 52 cm effective[b] x 60 cm total length.
Power supply:	Interchangeable polarity (Spellman, Plainview, NY).
Separation voltage:	- 20 000 volts
Injection mode:	Hydrostatic (held at 10 cm for 30 seconds).
Detection mode:	Indirect UV at 254 nm (Hg lamp).
Detector Range:	0.001 AUFS
Temperature:	Ambient. Active cooling by in-built fan.
Time constant:	0.1 second.
Data Acquisition rate:	20 points per second.[c]
Purge Time:	5 minutes.

a Replacement capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA).

b This is the distance from the injection point to the detector.

c This rate was necessary due to the very narrow peak widths achieved in CZE [2, 3].

3.3 REAGENTS AND CHEMICALS

Anion stock solutions

Stock solutions were made accurately by weighing to 4 decimal places the appropriate amounts of the salts shown in Table 3.2, which were dissolved and diluted to volume and filtered to pass 0.45 μm and stored in clean sample bottles. Standard mixtures were made as required by appropriate dilution of the stocks. No single standard mixture was used throughout the study and different compositions were employed in order to suit given applications.

EOF modifiers (surfactants)

To reverse the EOF direction, the following AR quaternary ammonium salts (Aldrich Milwaukee, WI, USA) were used: dodecyltrimethylammonium bromide (DTAB), tetradecyltrimethylammonium bromide (TTAB), cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTAC) and cetyltrimethylammonium hydrogensulfate (CTAHS). All surfactants were dried at 100 ^{o}C for ≥ 1 hour except for CTAC which came as a 20% w/v solution. Where conversion from the bromide to the hydroxide form was required, Ag_2O (AR, Aldrich, Milwaukee, WI, USA) or high capacity ion-exchange resin in the hydroxide form were used.

Chromate probe stock solution

Chromate was the major UV-absorbing probe employed in this study. A 100 mM stock solution prepared by dissolving 8.0995g sodium chromate (LR, Ajax, NSW, Australia), followed by ultrasonification and dilution to 500 mL. Sulfuric acid [4-6] was not used to dissolve the chromate as it was noted that there was a propensity for rapid crystal formation in electrolytes made from sulfuric-acid treated chromate stock.

Table 3.2

SOLUTE ANIONS USED IN STUDY

Anion	*Salt used*	*Grade*[§]	*Source*
bromate	$KBrO_3$	AR	Ajax Chemicals, Sydney.
bromide	NaBr	AR	Sigma, St. Louis, MO, USA.
carbonate	Na_2CO_3	AR	Ajax Chemicals, Auburn, NSW.
chloride	NaCl	AR	BDH, Victoria.
cyanide	KCN	AR	BDH, London.
fluoride	NaF	AR	Rhône-Poulenc, Manchester, U.K.
iodate	KIO_3	AR	Fluka AG, Switzerland.
iodide	NaI	AR	Merck, Darmstadt, Germany.
nitrate	$NaNO_3$	AR	Ajax Chemicals, Auburn, NSW.
nitrite	$NaNO_2$	AR	Ajax Chemicals, Sydney.
phosphate[a]	$Na_3PO_4.12H_2O$	AR	Ajax Chemicals, Auburn, NSW.
sulfate	Na_2SO_4	AR	Rhône-Poulenc, Manchester, U.K.
thiocyanate	NaSCN	LR	Hopkin & Williams, Essex, England.
thiosulfate	$Na_2S_2O_3.5H_2O$	AR	BDH, Victoria, Australia.

[§]AR and LR stand for Analytical Grade and Laboratory Grade, respectively. [a]Undried as decomposition occurs under 100 ^oC [7].

Additionally, the presence of sulfate in the BGE would have been detrimental to the analysis of samples (e.g. Bayer liquor) for sulfate.

3.4 PROCEDURES

Capillary conditioning and maintenance

There is no single commonly used 'standard' procedure in the literature for the conditioning and maintenance of bare fused-silica capillaries, with the frequency and sequence for conditioning being varied. All published procedures involve washing the capillary with up to 1 M hydroxide of sodium [8-10], potassium, lithium or rubidium [11].

With the aim of stabilising absolute migration times, the regime outlined below was used to condition and maintain the bare fused-silica capillaries used in this study. The manufacturer's suggestions for capillary conditioning were not used since absolute migration times were found to be unstable (i.e. increased velocity with repeats).

Prior to separation with each background electrolyte (BGE), the capillary was conditioned as follows: flushed for 5 min. each with water, absolute ethanol and water; 10 min. with 0.5N KOH (AR, May and Baker, Sydney); 5 min. with water; and 10 min. with BGE. Water blanks were then analysed in triplicate under the running conditions for 1 min. each. This routine was necessary to avoid poor phosphate response [12]. The capillary was flushed with water before each shut-down and the vials with electrolyte replaced with those containing filtered water to avoid electrolyte crystallisation and consequent capillary blockages.

Running or background electrolyte preparation

All working electrolytes were prepared freshly [2] as required. Appropriate amounts of 100 mM chromate and 50 mM EOF modifier stock solutions were diluted to 200.0 mL. The pH of the electrolyte was adjusted with a dilute mineral acid (depending on solutes

of interest) or 0.001M KOH (AR, May & Baker, Sydney, Australia), filtered and degassed to give the final working electrolyte. Where buffering was required, Tris(hydroxymethyl)aminomethane (TRIS) (AR, Aldrich, Milwaukee, WIS, USA) and 3-cyclohexylamino-1-propanesulfonic acid (CAPS) (AR, Aldrich, Milwaukee, WIS, USA) were also used as pH adjustment reagents.

3.6 REFERENCES

1 Gordon, M.J., Huang, X., Pentoney, Jr., S.L. and Zare, R.N., *Science*, **242** (1988) 224.

2 Romano, J., Jandik, P., Jones, W.R. and Jackson, P.E., *J. Chromatogr.*, **546** (1991) 411.

3 Guiochon, G. and Colin, H., 'Narrow-Bore and Micro-Bore Columns in Liquid Chromatography', in Kucera, P., editor, Microcolumn High-Performance Liquid Chromatography, Volume 28 (Elsevier, Amsterdam, 1984), pp. 1 - 38.

4 Waters Ion Analysis Method # N-601, Waters Millipore, Bedford, MA, USA (1991).

5 Jordan, J.M., Moese, R.L., Johnson-Watts, R. and Burton, D.E., *J. Chromatogr. A.*, **671** (1994) 445.

6 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **546** (1991) 445.

7 Aylward, G.H. and Findlay, T.J.V., SI Chemical Data, 2nd edition (John Wiley & Sons, Milton, Qld, 1974), p. 62.

8 Nguyen, A.-L., Luong, J.H.T. and Masson, C., *Anal. Chem.*, **62** (1990) 2490.

9 Chadwick, R.R. and Hsieh, J.C., *Anal. Chem.*, **63** (1991) 2380.

10 Purghart, V. and Games, D.E., *J. Chromatogr.*, **605** (1992) 139.

11 Salomon, K., Burgi, D.S. and Helmer, J.C., *J. Chromatogr.*, **559** (1991) 69.

12 Harakuwe, A.H., Haddad, P.R. and Buchberger, W., *J. Chromatogr. A.*, **685** (1994) 161.

CHAPTER 4

PRELIMINARY STUDIES

4.1 INTRODUCTION

Obvious advantages like short separation time and high efficiency make FRECZE an attractive method for the separation of inorganic anions. On the other hand, FRECZE is not devoid of limitations as discussed earlier (Chapter 2, Section 2.2.6.9). Two major limitations are imprecision of migration time and large variability in the response of phosphate. These main problems (and other related issues) gave impetus to the preliminary investigations in this chapter. The nature of each of these problems is summarised below.

Effect of Neutral Marker on Migration Time

To calculate mobilities (and to construct reliable selectivity plots), the measurement of EOF is essential. For rapid monitoring of EOF, UV-active neutral markers are usually incorporated into the background electrolyte (BGE) or sample and monitored photometrically on-capillary. As the separation of anions depends, among other factors, on BGE conductivity and viscosity, it was necessary to establish the effect(s), if any, that neutral markers have on separation. Although neutral markers play a vital role in the calculation of mobilities, the effect on important analytical variables like absolute migration time (AMT), AMT precision and resolution (R_S) due to their presence is yet to be investigated in detail. For this study, mesityl oxide [1-6] (as the most commonly used marker) and formamide (supplied by Waters) were chosen as model neutral markers.

Effect of Surfactant Dryness

For accuracy, all reagents where possible should be dried. Drying of reagents, salts, etc is a fundamental operation in analytical chemistry. It is known that EOF modifier concentration governs EOF rate [7-10] and thus anion migration velocity. From work done on the determination of chloride and sulfate in concrete, it was noted that increasing the EOF modifier concentration from 2.5 mM to 2.6 mM was sufficient to alter the migration order of anions with respect to the system peak. It was therefore deemed important to make up EOF modifier concentrations correctly and accurately. It seems from nearly all the publications to date on separation of anions using FRECZE that more often than not, undried cationic surfactants have been used. It was considered worthwhile to investigate the effect of surfactant dryness on R_S, stability of migration times and separation efficiency.

Variability of Phosphate Response

The response of anions must be optimised for reliable quantification. The response however for phosphate is very variable [11], especially at low levels, and this has a major impact on detection limits. Recently, it was reported that the variability of the response of phosphate was as high as 32% RSD for heights and 56% RSD for areas [12]. The current literature does not address adequately this important aspect, i.e. the high variability of the response of phosphate and how that variability can be minimised or eliminated.

Phosphate is known to bind strongly [13-16] onto fused silica. The binding of phosphate onto the capillary surface affects the migration rate of sample anions (including phosphate itself) due to the alteration of the electrical double-layer, the zeta potential and consequently the EOF. Furthermore, the amount of phosphate reaching

the detector can be lowered significantly for samples containing low levels of phosphate. Consequently a false (lower) value for the phosphate content of a sample will be reported.

The variability of phosphate response appears to result from differing capillary treatment regimes and BGE compositions. Also, the times required for these conditioning regimes vary considerably, from a few minutes [17] to up to an hour [18] and even overnight [19]. Depending on the sample matrix being separated, conditioning between each run may be necessary [20]. Even after conditioning for extended periods, the AMT stability achieved has often not been detailed. The impetus of this study was therefore to devise a capillary conditioning regime able to provide optimal response for phosphate and AMT stability without unduly extending the total analysis time.

Imprecision of Absolute Migration Time

The AMT must be as precise as possible, i.e. the EOF generated must be stable. Stable migration times yield reliable selectivity plots and are of primary importance in optimising separations and calculating anion mobilities [21]. Unfortunately, AMT imprecision can be considerable in FRECZE, e.g. for real samples, precision can range from 2 - 14% RSD [22]. AMTs have been found to decrease with replicate measurement [23] and this could not be explained in terms of minor changes in pH, temperature and BGE composition [23]. Possible causes are effects that dynamically alter the thickness of the double-layer (and hence the zeta potential) and the conductivity of the BGE.

The aim of this preliminary investigation was to address the limitations noted above and to develop a capillary conditioning routine that would generate stable AMTs.

4.2 EXPERIMENTAL

Instrumentation and Conditions

Chapter 3 shows details of instrumentation and general separation conditions. For mass spectral analysis of dried and undried TTAB using electron impact (EI-MS) and liquid secondary ion mass spectrometry (LSIMS), a Kratos ISQ mass spectrometer was used. The conditions for EI-MS fragmentation were; 70 eV electron energy, 8 kV accelerating voltage, source temperature of 200 °C and scanning at 2 s per decade between m/z = 35 - 800. For the LSIMS measurement, the same instrument was used under the following conditions; the sample was dissolved in *m*-nitrobenzylalcohol (*m*-NBA) and desorbed and ionised with a Cs^+ ion primary beam. The accelerating voltage was 5.3 kV and accurate mass scans were performed using 7000 resolution (5 s per decade) and internally referenced to *m*-NBA. For mass spectral analysis using GC-MS, a Hewlett-Packard 5890 GC fitted with a HP5970 mass selective detector was used. The conditions were: the carrier gas was He (15 psi), injections were made in the splitless mode and the GC oven was programmed from 60 °C to 290 °C at 10 °C/min. The separation was performed on a HP1 column measuring 25 m x 0.32 mm O.D. x 0.52 µm film thickness. Full mass spectra were recorded from m/z = 35 - 550 at 1 scan/sec. For infra-red scans of dried and undried TTAB, a Bruker IFS 66 FTIR spectrophotometer was used. Samples were prepared in KBr disks and scanned at standard 4 cm^{-1} resolution using a rate of 32 scans per sample.

Reagents and Chemicals

The chemicals and reagents required to carry out this preliminary investigation are shown in Table 4.1.

Table 4.1

CHEMICALS AND REAGENTS NEEDED FOR PRELIMINARY STUDIES.

COMPOUND ($\mu g.mL^{-1}$)	GRADE	MANUFACTURER
Neutral Marker Study		
Mesityl oxide	AR	Aldrich, Dorset, England.
Formamide	AR	Waters, Milford, MA, USA.
Potassium cyanide (10)	AR	BDH, Poole, England.
Sodium chloride (10)	AR	BDH, Victoria, Australia.
Sodium sulfate (10)	AR	Rhône-Poulenc, Manchester, UK.
Migration Time Stability		
1-butanol	LR	Ajax Chemicals, Sydney, Australia.
Phosphate Response		
sodium chloride	AR	Rhône-Poulenc, Victoria, Australia.
sodium sulfate	AR	Rhône-Poulenc, Manchester, UK.
$Na_3PO_4.12H_2O$	AR	Ajax, Sydney, Australia.
sodium hydroxide	AR	BDH, Victoria, Australia.
Kieselgel 60 (230 mesh) silica	AR	Merck, Darmstadt, Germany.
Surfactant Dryness		
DTAB	AR	Aldrich, Milwaukee, WI, USA.
CTAB	AR	Aldrich, Milwaukee, WI, USA.
Common Reagents		
TTAB	AR	Aldrich, Milwaukee, WI, USA.
KOH	LR	Ajax, Sydney, Australia.
TRIS	AR	Aldrich, Milwaukee, WI, USA.
sodium chromate	LR	Ajax, Sydney, Australia.

Neutral Markers

Chloride and sulfate were used as model anions to test effects on AMT, AMT precision and resolution. For the study of the effect on detection sensitivity, cyanide ion was also used along with the above model anions.

Surfactant Dryness

Undried and dried (100 ^{o}C overnight) TTAB were compared. Two BGEs were made up to contain 2.6 mM surfactant, 5 mM chromate and pH 8.44. The capillary was conditioned by manually flushing with 0.3 mL absolute ethanol, 0.3 mL water, 0.2 mL 0.5 M KOH and 0.2 mL BGE. Instrumental operating conditions were identical to those shown in Table 3.1 (Chapter 3) except that the flush time was set at 6 min.

Phosphate Response

Reagents and chemicals

Unless otherwise specified, all chemicals used were of AR grade and sourced from Ajax Chemicals (Sydney, Australia). All salts (except trisodium orthophosphate) were dried at 100 ^{o}C overnight and desiccated before use. Making appropriate dilutions, a series of standards as shown in Table 4.2 were prepared. A litre of 5 $\mu g.mL^{-1}$ phosphate was made for the phosphate-to-silica adsorption simulation. NaOH (0.5M) and silica were also required for this simulation. Other chemicals required were sodium chromate for BGE preparation and KOH for pH adjustment and capillary conditioning.

Capillary conditioning regimes

The following **common sequence** was used to condition the capillary by vacuum flushing for 5 minutes each with water, ethanol and water; 8 minutes with 0.5N KOH; 5 minutes with water; and finally for 10 minutes with the BGE. Variations were then included to obtain the six capillary conditioning regimes shown in Table 4.3.

Table 4.2

STANDARD MIXTURES USED FOR PHOSPHATE RESPONSE STUDY.

Standard #	$\mu g.mL^{-1}$ Chloride	$\mu g.mL^{-1}$ Sulfate	$\mu g.mL^{-1}$ Phosphate
1	60	5	20
2	40	10	40
3	20	1	60
4	10	20	10

Table 4.3

CAPILLARY CONDITIONING REGIMES

Regime No.	PROCEDURE, NOTES, ETC
1	Standard #4 run continuously after common sequence but without the 10 min. purge with the BGE.
2	After the common sequence, the capillary was flushed manually with 1 mL of standard mixture containing 10 $\mu g.mL^{-1}$ phosphate prior to running solutions.
3	Same as regime no. 2 except 1 mL of 1000 $\mu g.mL^{-1}$ phosphate was used rather than the standard with 10 $\mu g.mL^{-1}$ phosphate.
4	After the common sequence, 1000 $\mu g.mL^{-1}$ phosphate was run 6 times (4 min. purge, 3 min. run) prior to separating standard mixture 4.
5	Same as regime no. 4 except saturated phosphate was substituted for 1000 $\mu g.mL^{-1}$ phosphate.
6	Same as regime no. 4 except water was used instead of 1000 $\mu g.mL^{-1}$ phosphate.

Data acquisition and peak integration

All measurements were made in replicates of ≥2 using fresh BGE per separation at 20 points per second. Peak positions were confirmed by spiking with standards. The peak integration parameters shown in Table 4.4 were used to process all data.

Table 4.4

MAXIMA 820 PEAK INTEGRATION PARAMETERS

Detection Parameters	
Baseline Points:	18
Filter Window (in Points):	25
Integration Sensitivity (Coarse):	303.0 $\mu V.s^{-1}$
Integration Sensitivity (Fine):	1259 $\mu V.s^{-2}$
Skim Ratio:	8.000
Rejection Criteria	
Minimum Area:	477.6 $\mu V.s$
Minimum Height:	755.8 μV
Minimum Width:	2.100 s

Phosphate-to-silica adsorption simulation

This involved passing an aliquot of standard phosphate through hydrated amorphous silica and comparing the filtrate with a untreated aliquot of the same standard. An initial trial involved passing 5 $\mu g.mL^{-1}$ phosphate through 140g of thoroughly washed and hydrated (>48 hrs) silica in a #4 sintered glass funnel (column vol. = 170 mL). The first 190 mL of filtrate was discarded to ensure that any reduction in the response of phosphate was not due to dilution. Another 10 mL was used for rinsing the collection tube and finally, ~10 mL of filtrate was collected for analysis. The final simulation involved passing 5 $\mu g.mL^{-1}$ phosphate through 400g (column vol. = 390 mL) of

washed hydrated (>24 hrs) silica which had been conditioned with 390 mL of 0.5 M NaOH and rinsed with at least 1170 mL of water to neutral pH. The first 395 mL of filtrate was discarded and the collection tube rinsed twice with 5 mL filtrate each. After this, ~10 mL of filtrate was collected for analysis.

4.3 RESULTS AND DISCUSSION

4.3.1 'NEUTRAL' MARKERS

Ideally, neutral EOF markers should be separated concurrently with analyte anions to ensure that the solutes and marker are subjected to the same forces and effects during the separation. The model EOF markers (MOx and formamide) were tested at different concentrations and pH. Ten replicate measurements were made under all conditions tested. The effect of the presence of markers on AMT, AMT precision and R_S are summarised in Table 4.5 (MOx) and Table 4.6 (formamide). Further details are included in Appendices 4.3.1.1 - 4.3.1.5.

All statistical comparisons were made at the 95% confidence level. For the criteria involving significance of effect on AMT and R_S between the test anions, mean values were compared [24]. The comparison involved use of the student t-test. Student t-test values at 18 degrees of freedom ($n_1 + n_2 - 2$) were obtained from Johnson [25]. For comparison of AMT precision, the F-test [24, 25] was applied. Upon comparison of the experimental and computed criterion (t or F) values, a pass (✔) or fail (✖) was assigned depending on whether or not the null hypothesis was rejected. A pass meant that the difference between the response variables obtained with and without the presence of the neutral marker were not statistically significant.

Table 4.5

EFFECT OF MESITYL OXIDE ON SOME SEPARATION CRITERIA

Key: $\bar{x}$ = mean of AMT (min.), Δ AMT = Difference in AMT (min.), SD = standard deviation using n-1 degrees of freedom, R_S = resolution, n = number of readings, ✔ = pass and ✖ = fail.

% v/v	pH	Anion	Migration Time (min.)						Critical Values		
			No Marker (n = 10)		With Marker (n = 10)		Pooled Data (n = 20)				
			$\bar{x}$	SD^{n-1}	$\bar{x}$	SD^{n-1}	$\bar{x}$	SD^{n-1}	AMT	Precision	R_S
0.001	8.5	chloride	2.9605	0.01503	2.9752	0.01050	2.9679	0.01469	✖	✔	-
"	8.5	sulfate	3.1416	0.01467	3.1564	0.01170	3.1490	0.01497	✖	✔	-
"	8.5	Δ AMT	0.1811	0.00110	0.1812	0.00155	0.1812	0.00131	-	-	✔
"	11.9	chloride	2.8857	0.03198	2.8995	0.02344	2.8926	0.02820	✔	✔	-
"	11.9	sulfate	3.0971	0.03486	3.1119	0.02597	3.1045	0.03087	✔	✔	-
"	11.9	Δ AMT	0.2114	0.00299	0.2124	0.00267	0.2119	0.00281	-	-	✔
"	12.2	chloride	2.8393	0.01317	2.8467	0.01394	2.8430	0.01373	✔	✔	-
"	12.2	sulfate	3.0640	0.01533	3.0703	0.01563	3.0670	0.01545	✔	✔	-
"	12.2	Δ AMT	0.2244	0.00227	0.2236	0.00196	0.2240	0.00210	-	-	✔

Table 4.6

EFFECT OF FORMAMIDE ON SOME SEPARATION CRITERIA

See Table 4.5 for key.

% v/v	pH	Anion	Migration Time (min.)						Critical Values		
			No Marker (n = 10)		With Marker (n = 10)		Pooled Data (n = 20)				
			$\bar{x}$	SD^{n-1}	$\bar{x}$	SD^{n-1}	$\bar{x}$	SD^{n-1}	AMT	Precision	R_S
0.0020	8.5	chloride	3.0420	0.00115	3.0288	0.00432	3.0354	0.00744	✖	✖	-
"	"	sulfate	3.2495	0.00184	3.2336	0.00510	3.2416	0.00897	✖	✖	-
"	"	Δ AMT	0.2075	0.00143	0.2048	0.00123	0.2062	0.00190	-	-	✖
0.0004	11.9	chloride	3.0481	0.00591	3.0500	0.00350	3.0491	0.00482	✔	✔	-
"	"	sulfate	3.2597	0.00560	3.2624	0.00341	3.2611	0.00470	✔	✔	-
"	"	Δ AMT	0.2116	0.00084	0.2124	0.00070	0.2120	0.00086	-	-	✖

Effect of Mesityl Oxide

At ≥ 0.001% v/v and pH 8.5, the presence of MOx significantly altered the AMT of chloride and sulfate (test anions). Without totally disregarding possible changes in the dielectric constant, conductivity, viscosity, etc of the BGE, the most likely cause for this is alteration of the double-layer (that defines EOF and net anion migration rate). This can be due to either MOx adsorbing onto the capillary surface or MOx solubilising the TTAB adsorbed onto the capillary surface. Either way, the double-layer thickness, charge density, zeta potential and EOF are reduced, resulting in the increases in AMT noted. At elevated pH (≥11.9) however, there was no significant effect on AMT of the test anions resulting from the presence of MOx (0.001% v/v). AMT precision and R_S on the other hand, were not compromised at either pH value.

Effect of Formamide

Formamide at pH 8.5 and a concentration of 0.002% v/v caused a statistically significant difference with regard to AMT, AMT precision and R_S of the test anions (Table 4.6). The detrimental effects on AMT and AMT precision could be avoided by increasing the pH (11.9) and reducing the concentration of formamide to 0.0004% v/v. In contrast to the effect of MOx at elevated pH, it was noted that R_S was still reduced even when the formamide concentration was decreased. For safety, the routine use of formamide as an EOF marker is not recommended due to it being a teratogen. Also, formamide was unsuitable for detection with the chromate-TTAB system at 254 nm. A change to 214 nm was necessary to detect it.

Variability of EOF Position and Interference by the Injection Peak

For reliable calculation of mobilities, it is imperative that the EOF position be determined accurately. In the separation of aqueous samples using BGE with chromate-quaternary ammonium bromide surfactants, a large peak is usually observed well after

the anionic components have migrated. This large peak (hereafter called the 'plug' peak) varied in size with injection time (Fig. 4.1) and was due to the water component or 'plug' of the sample. This peak was not observed when the BGE or samples dissolved in the BGE were injected hydrostatically, or when electromigration was used.

The 'plug' peak was initially accepted as being due to the water front carried by the EOF and could therefore be used in the calculation of anion mobility. However, as reported by Jones and Jandik [20], mobilities calculated using the 'plug' peak as the EOF are likely to be inaccurate due to the broadness of the peak and the irregular shape of the 'plug' peak which varies with the matrix of the sample [20]. Also, the use of the 'plug' peak in calculating mobilities may have limited applicability and validity because the migration positions indicated by the 'plug' peak and neutral marker were noted to vary considerably depending on surfactant concentration and type, e.g. at 0.5 - 1 mM TTAB, the 'plug' peak migrated with the marker peak (Fig. 4.2 (a)). Above 1 mM TTAB, the MOx[§] migrated well before the 'plug' peak (Fig. 4.2 (b)). It was also noted that at the same concentration (2.6 mM), the MOx migration time and relative position varied with the type of surfactant used (Fig. 4.3).

The MOx marker migrated after (DTAB - trace 1 in Fig. 4.3), before (TTAB - trace 2 in Fig. 4.3) or with (CTAB - trace 3 in Fig. 4.3) the 'plug' peak. The differences in migration position using the different surfactants can be ascribed to the different extents and rates at which they saturate the capillary surface, i.e. EOF reversal and effectiveness in shielding the MOx marker from the capillary surface. The MOx AMT for TTAB (2.6 mM) and CTAB (2.6 mM) were about the same (*ca.* 15 min.). With CTAB though, the large 'plug' peak is likely to interfere through swamping.

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§ Formamide could not be detected at the concentrations and wavelength (254 nm) used.

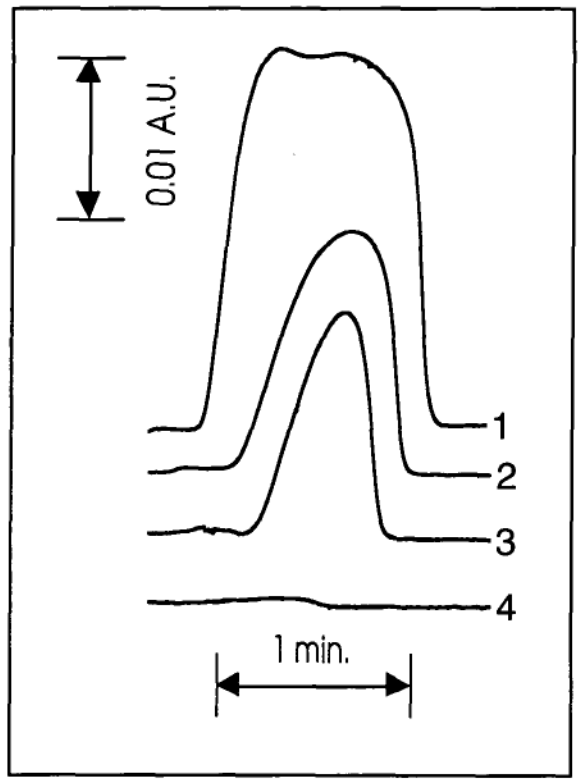

Fig. 4.1: Effect of hydrostatic injection time on 'plug' peak size. Conditions: 5 mM chromate, 2.6 mM TTAB (dried overnight) and pH 9.5. Sample injection height was 10 cm. Sampling time: 1 = 60 s, 2 = 30 s, 3 = 15 s and 4 = 30 s BGE. For 1 - 3, MilliQ® water blank was injected.

The above results show the possible interference on the apparent position of the EOF (monitored with neutral markers) by the 'plug' peak. This interference is a function of surfactant type and concentration. To avoid unduly altering separation characteristics and performance, only a small amount of marker should be used. However, detector signal for the neutral marker may be swamped by the 'plug' peak unless the BGE is used as solvent for the neutral marker.

It was noted that at 0.5 mM TTAB and 2.6 mM DTAB, the MOx marker migrated after the 'plug' peak. The interaction of solutes with the capillary surface has been noted previously [e.g. 7, 26] and the seemingly anomalous migration order of MOx

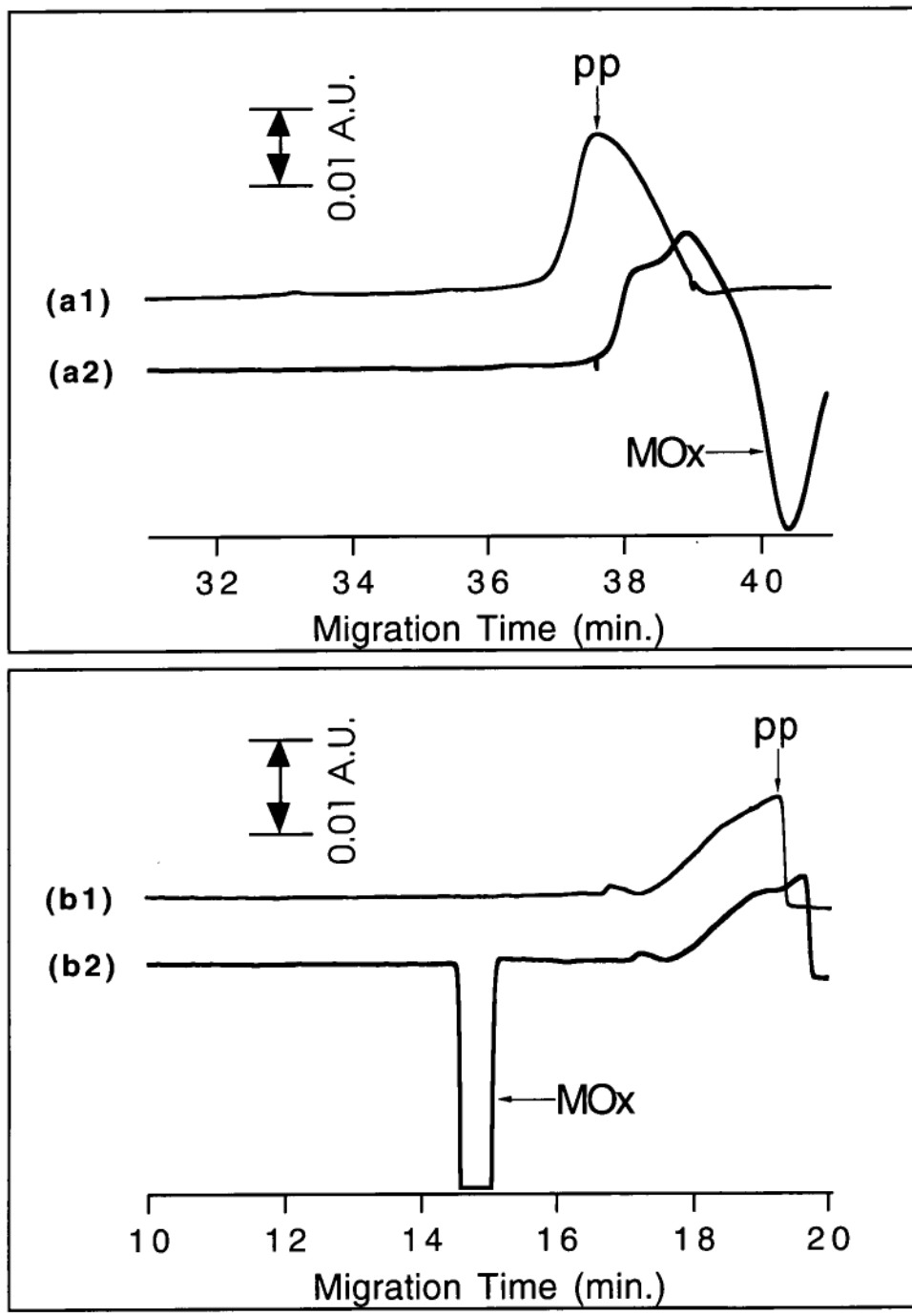

Fig. 4.2: Effect of TTAB concentration on 'plug' peak and MOx marker migration position. Conditions: (a) 0.5 mM TTAB, 5 mM chromate and pH 8.5. Injection was in the hydrostatic mode (sample vial raised to 10 cm and held for 30 s) and indirect UV detection at 254 nm (Hg lamp) was used for detection. Separation voltage was -20 kV. A standard mixture of anions was separated. (b) Conditions as in (a) except TTAB concentration was 2.6 mM. MilliQ® water was separated. Key: a1 = without marker, a2 = spiked with MOx, b1 = without marker, b2 = spiked with MOx and pp = 'plug' peak.

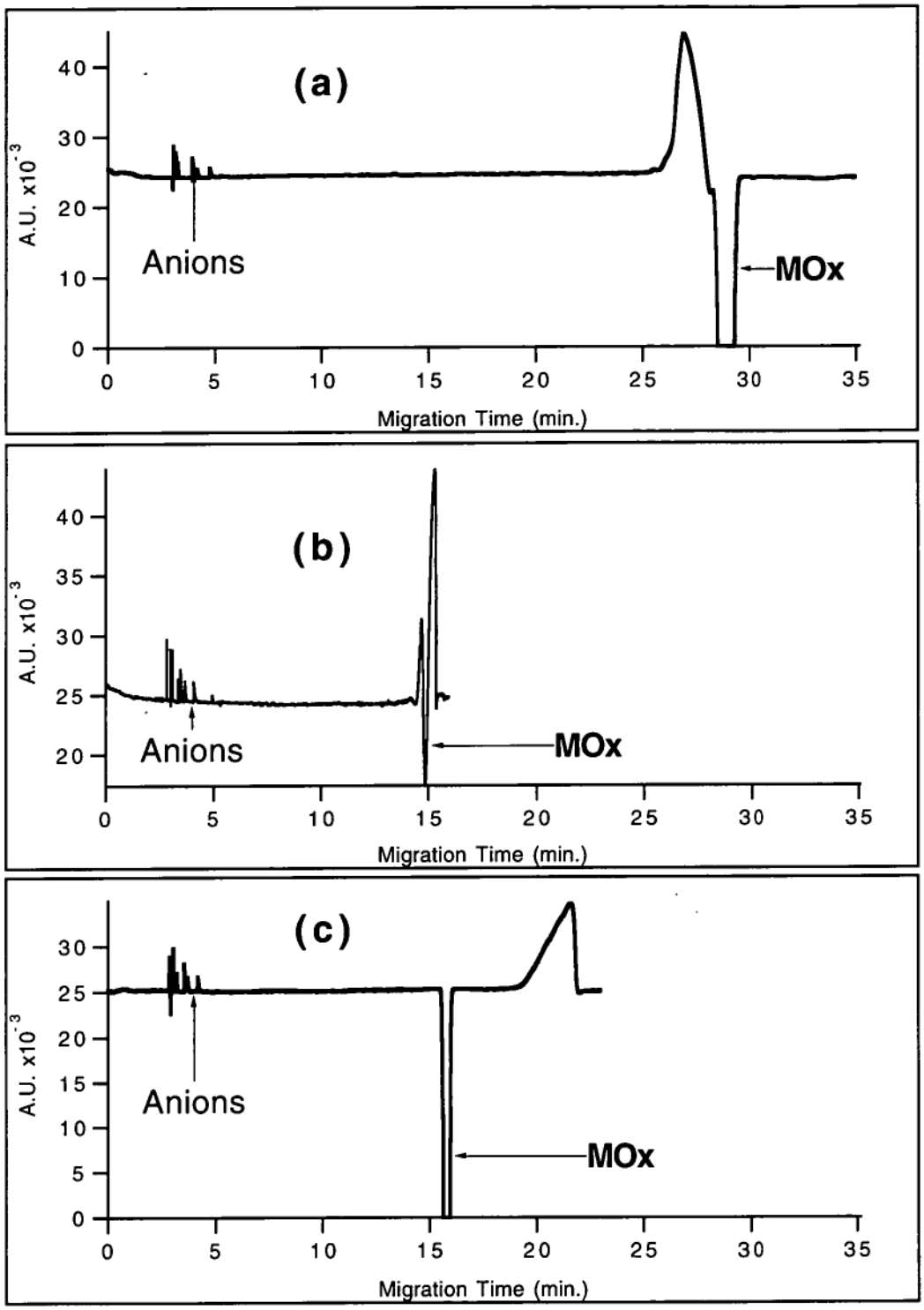

Fig. 4.3: Effect of surfactant type on neutral EOF marker migration position. Conditions: (a) 2.6 mM DTAB, (b) 2.6 mM CTAB and (c) 2.6 mM TTAB. Other conditions as in Fig. 4.2 (a). The solution separated was a standard mixture of anions.

may be occuring through formation of donor complexes in a similar manner to phosphate, through acid-base interaction between the silanol OH groups and the acidic proton on the α-carbon in MOx, or through formation of cationic solvophobic ion-pairs in a similar manner to MOx and tetrahexylammonium ion [27]). At 0.5 mM TTAB and 2.6 mM DTAB, the sites on the capillary surface are not fully occupied by the surfactant which allows for the MOx to interact with the capillary surface, explaining why MOx was migrating after the 'plug' peak. At 1 mM TTAB, MOx was noted to migrate with the 'plug' peak. With further increase in TTAB concentration to 2.6 mM, the available sites at the capillary surface are occupied relatively quickly and the MOx was then not able to interact with the capillary surface.

Effect on Anion Peak Area

Both MOx and formamide were noted to affect anion response depending on the concentration of surfactant used and the type of anion involved. For strong acid anions like chloride and sulfate, the presence of both markers did not influence peak area response regardless of the concentration of surfactant used. For cyanide, there was no reduction in peak area response when 2.6 mM TTAB was used. On the other hand, peak area response was lowered (~67%) and baseline noise increased by the presence of both MOx (≥0.001% v/v) and formamide (≥0.002% v/v) when a BGE with 0.5 mM TTAB was used (Fig. 4.4). One possible cause is sorption of cyanide onto the capillary surface, especially since the low TTAB concentration may not be effective in dynamically saturating the available sites on the capillary surface. Another cause, particularly with MOx, is the possibility of 1,4-nucleophilic addition of the CN^- to the marker which has C=C and C=O bonds in conjugation. A further possibility is the formation of cyanohydrins[1] by addition of CN^- at the C=O carbon.

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1 Dr. C. Moorhoff, *personal communication* (August, 1995).

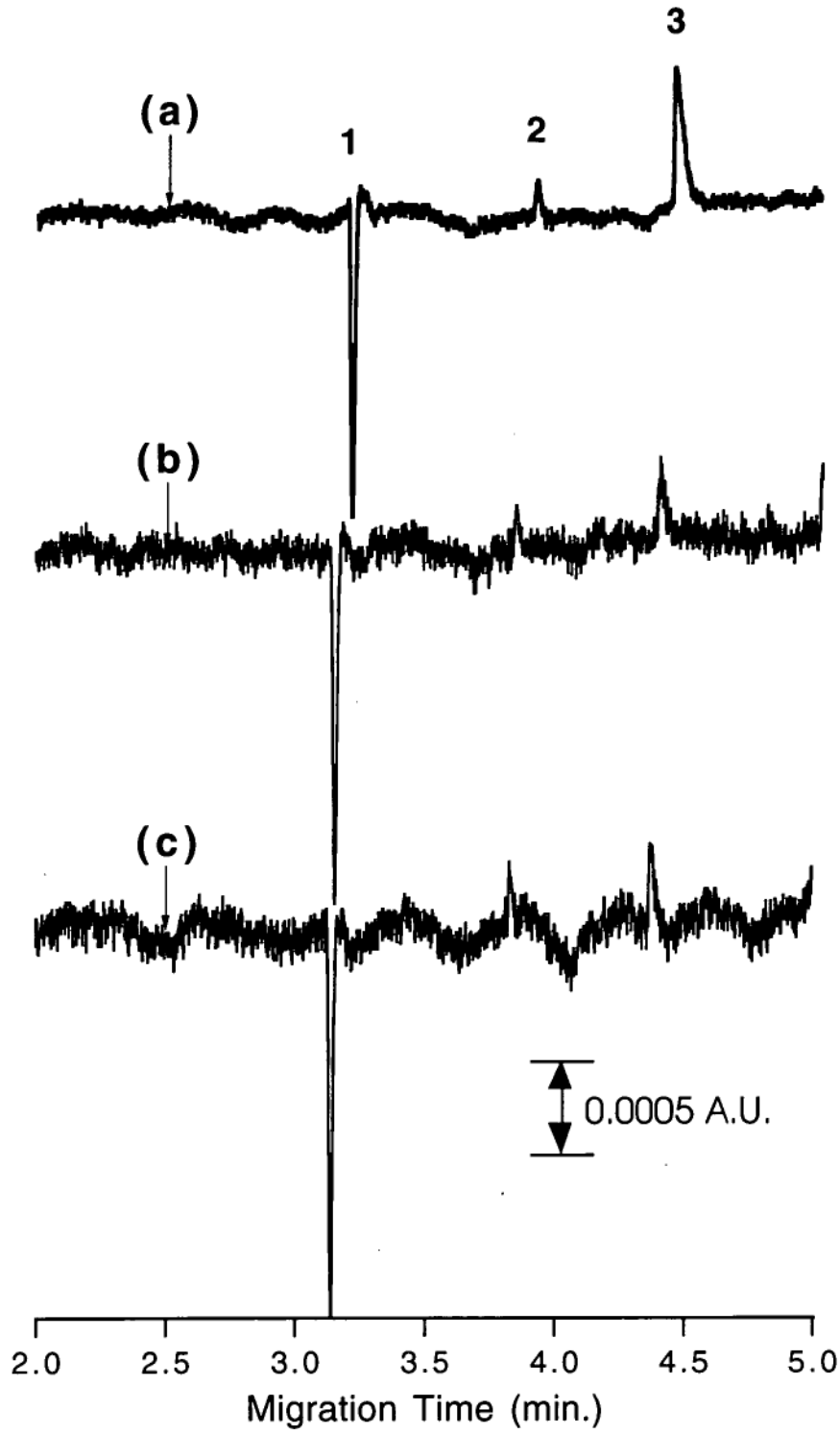

Fig. 4.4: Effect of the presence of neutral EOF markers on the response of CN^- (10 $\mu g.mL^{-1}$). Conditions: (a) without marker, (b) with 0.002% v/v formamide and (c) with 0.001% v/v MOx. Other conditions were as in Fig. 4.2 (a). Key: 1 = system peak, 2 = formate and 3 = cyanide.

Discussion

In all cases, it was noted that the AMTs in the presence of ≥0.001% v/v MOx and 0.002% v/v formamide were longer than AMTs in their absence. This was most probably due to a reduction in EOF. Detrimental effects due to the presence of neutral markers seem to be dependent upon pH, marker type and marker concentration (e.g. at the same pH of 8.5, 0.001% v/v MOx and 0.002% v/v formamide caused a statistically significant change in the AMTs of the test anions).

The use of the 'plug' peak as the EOF to calculate the mobilities of anions using the chromate-based system is valid when the TTAB concentration is 0.5 - 1 mM. The use of the 'plug' peak as the EOF above 1 mM TTAB is unreliable and is not an accurate representation of the EOF.

On the basis of the results of this investigation it was decided that relative migration times (to either the 'plug' peak or a reference anion) would be employed to construct the selectivity plots. The use of RMTs to show selectivity (mobility) in CE is valid [28]. The trends in migration order for the solute anions would be the same regardless of the peak used as the reference.

4.3.2 SURFACTANT DRYNESS

The influence on AMT stability of using dried surfactants is discussed in Section 4.3.4. The emphasis here will be on AMT and R_S. For this study, TTAB was used as the model surfactant. EOF direction and velocity, which ultimately governs anion migration time, is influenced predominantly by surfactant concentration [7, 8]. Since very small changes in concentration of TTAB are known to alter the migration order of

anions (chloride) with respect to the bromide peak [29], it was deemed important that EOF modifier concentrations be made up accurately.

Effect on Migration Time and Resolution

Drying is vital to making solutions of accurate concentrations, yet most (if not all) publications on CE to date do not indicate whether the EOF modifier used was dried or not. The model EOF modifier for this investigation was TTAB. Its melting point is 245 - 250 ^{o}C [30] so the initial expectation was for little decomposition or change to occur to the TTAB upon drying. However when dried overnight at 100 ^{o}C, the colour of the TTAB was altered and a mass loss of 0.26% was recorded.

A standard mixture of anions was then separated under identical conditions but using undried and dried TTAB as EOF modifier. Fig. 4.5 shows that with the dried TTAB, phosphate detectability was good, even from the first separation in comparison to the undried TTAB. Also, the R_s was generally better and the separation time shorter using dried TTAB in comparison to using undried TTAB. The mass loss upon drying was not sufficient to cause a significant change in molar concentration of TTAB and hence EOF. This suggested the involvement of other factor(s).

Infra-red and Mass Spectroscopic Analysis

The drying time was then varied (0 to 8.5 days) and the colour change was noted to be proportional to drying time. The colour changed from white to brownish-grey. Fig. 4.6 shows the change in colour upon drying for the three EOF modifiers. All three EOF modifiers were white powders before drying. The colour suggested decomposition or polymerisation. Infra-red (IR) and mass spectroscopic (MS) determinations were performed on both dried and undried surfactants to see if any chemical change was occurring as a result of drying (heating) at 100 ^{o}C. It is known that a quaternary

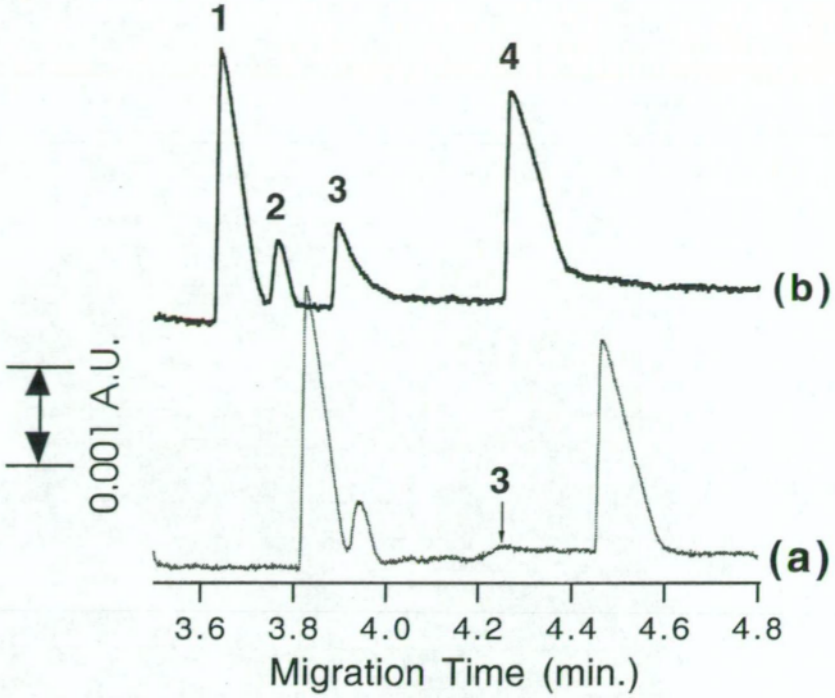

Fig. 4.5: Effect of surfactant dryness on absolute migration time and resolution. Conditions: (a) Run #1 using undried 2.5 mM TTAB, 5 mM chromate and pH ~8. (b) Run #1 using conditions as in (a) except the TTAB was dried at 100 ^{o}C overnight. Key: 1 = fluoride, 2 = bromate, 3 = phosphate and 4 = carbonate.

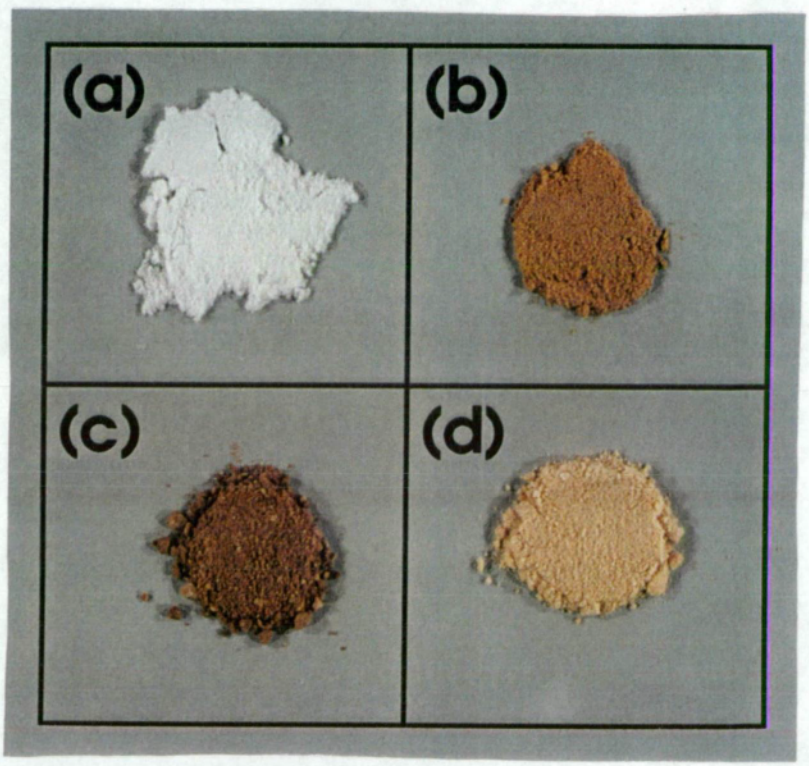

Fig. 4.6: Effect of drying at 100 ^{o}C on the colour of quaternary ammonium bromide salts. Key: (a) = undried TTAB, (b) = TTAB dried for 8.5 days, (c) = DTAB dried for 8.5 days and (d) = CTAB dried for 8.5 days.

ammonium surfactant (e.g. as the hydroxide or iodide form) can undergo Hofmann elimination [31] upon heating to yield an amine, alkene (least substituted) and HX (where X = surfactant anion).

IR scans were performed on TTAB, CTAB and DTAB dried at 100 ^{o}C for varying lengths of time. This was intended to determine if smaller (primary, secondary or tertiary) amines were being formed through degradation by heating. These would have shown absorption at ~3100 cm^{-1} for the primary and secondary amines. IR spectra (Appendix 4.3.2.1 (a)) of the three surfactants before and after drying showed a peak at ~1725 cm^{-1} (Appendix 4.3.2.4.1 (b)) not present in the undried surfactants but present in surfactants dried for ≥ 48 hours. This peak increased in intensity with length of drying time. The likely identities for peaks at this position are C=C, C=O, C=N and N=O [32]. Assuming the surfactants were pure and taking into account the drying conditions, it is probable that the peak was due to C=C or C=O (oxidation product). Evidence of olefinic C-H at >3000 cm^{-1} was inconclusive. If an alkene was formed as postulated above, it may have been lost[2] through volatilisation or through participation in a secondary saturation reaction with the HBr. There was no detectable evidence of primary or secondary amines due to the absence of peaks at ≥3100 cm^{-1}. Essentially, the IR spectra did not support the presence of products expected from a Hofmann type elimination upon heating of the surfactant.

It has been reported that alkylamines can stabilise or even reverse the EOF in bare fused silica capillaries [33]. Alkylamines interact strongly with the silanol functionality [34, 35] in reversed-phase ion-pair chromatography and their effectiveness in masking silanophilic activity in CE has been reported [36-38]. The premise therefore was that

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2 This was supported by the peaks at m/z = 276/278 of the GC-MS spectrum and the absence of peaks at m/z = 196 (alkene) and m/z = 81 (HBr).

the drying process was resulting in the *in-situ* formation of alkylamines (tertiary amines) which were contributing to the superior resolution and migration time precision for separations using dried TTAB in comparison to undried TTAB.

Mass spectrometric analysis of the dried and undried TTAB was then performed using EI-MS, LSIMS and GC-MS to ascertain if any changes in structure were occurring as a result of drying. Previous MS analysis of tetraalkylammonium salts using EI-flash desorption at >1000 °C had demonstrated that these salts could be fragmented to form, amongst others, R_4N^+ and R_4N groups [33]. As the drying of the surfactants used in this work was done at 100 °C, the investigation was geared towards determining if similar changes were occurring during the drying process at the lower temperature, and to determine if there was a basis to link the act of drying the TTAB to the observed superior separation achieved using dried TTAB.

The EI-MS fragmentation patterns for dried and undried TTAB were similar (Appendix 4.3.2.2) but that did not necessarily mean that no changes had occured upon drying at 100 °C because both the dried and undried TTAB were subjected to a source temperature of 200 °C for the MS analysis. The strategic peaks recorded were at:

(a) m/z = 58 $[N(CH_3)_3]$ or more correctly $[C_3H_8N]$.

(b) m/z = 94/96 [MeBr]

(c) m/z = 241 $[CH_3(CH_2)_{13}N(CH_3)_2]$ - tertiary amine.

(d) m/z = 256 $[CH_3(CH_2)_{13}N(CH_3)_3^+]$ - parent ion.

As the EI-MS was performed at a temperature below the melting point of TTAB, the fact that a mass spectrum was recorded at all confirmed the thermal instability [39] of the TTAB and that there was a 'slow decomposition' of the TTAB to form, amongst

other products, the tertiary amine at m/z = 241. It also confirmed that the color changes noted after drying were probably due to bromine-type coloration.

It was important to confirm that the amine at m/z = 241 was indeed a product of heating. The GC-MS output (Appendix 4.3.2.3) showed principal peaks at molecular weight = 241 and 276. The peak at molecular weight = 276 was found to correlate with 1-bromotetradecane from the library of mass spectra of known compounds. The LSIMS spectra (Appendix 4.3.2.4) showed a prominent peak at m/z = 256 (parent ion) but more significantly, no significant peak at m/z = 241/242, i.e. $[M+H]^+$. This proved conclusively that alkylamines (and alkyl bromides) were formed when TTAB was subjected to heating. To determine if the alkylamine (m/z = 241) was in fact formed when the TTAB was dried at 100 °C and was not an artefact from the heat applied for all previous MS analyses (except LSIMS), a confirmatory GC-MS experiment using on-column injection was performed. The spectra from this experiment (Appendix 4.3.2.5) showed conclusively that alkylamine was formed (in detectable quantity) upon drying at 100 °C.

Work is continuing on simulating the stabilisation of AMT using model tertiary amines as BGE additives. For the present purposes of this study, the use of dried EOF modifiers is recommended.

4.3.3 PHOSPHATE RESPONSE

Effect of capillary conditioning regime on phosphate detectability

Fig. 4.7 shows the effect of the six different capillary conditioning regimes on the detectability of phosphate (10 $\mu g.mL^{-1}$). Using capillary conditioning regime no. 1, phosphate was not discernible as a genuine peak and could not be automatically

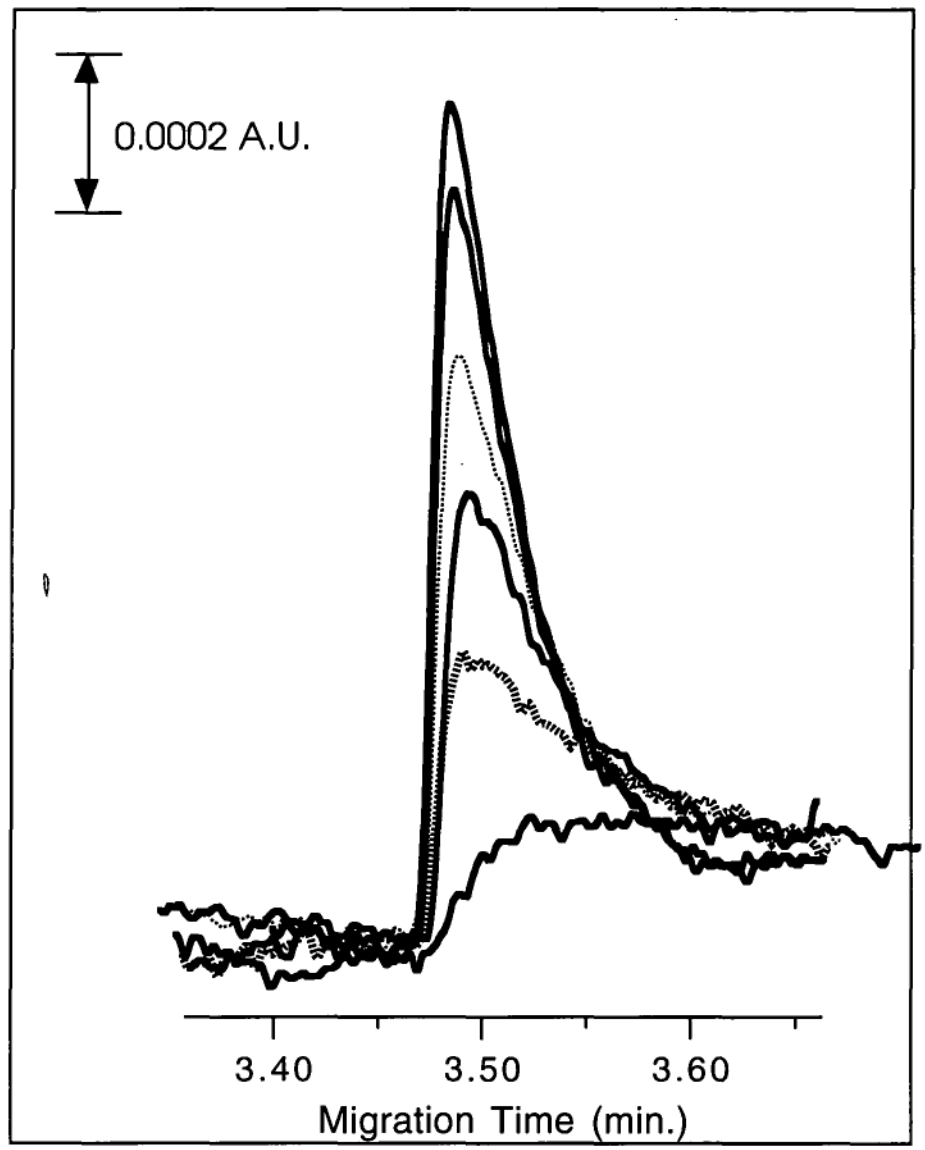

Fig. 4.7: Effect of capillary conditioning regimes on detectability of phosphate (10 $\mu g.mL^{-1}$). Conditions: 2.6 mM dried TTAB, 5 mM chromate and pH 8.8. Other conditions as in Fig. 4.2 (a). The peaks for phosphate going from the bottom to the top correspond to conditioning regimes 1 to 6, respectively

detected using the integration parameters given in Table 4.4. This necessitated manual integration which can be imprecise due to human subjectivity and error. It is obvious that the differences in peak intensity were not due to normal experimental variation which is 1.5% for phosphate [40]. Contrasting the worst and best performance,

phosphate detectability using capillary conditioning regime no. 6 was ~170% and ~550% more sensitive in terms, respectively, of peak area and peak height than capillary conditioning regime no. 1.

It has been noted that increased peak response with repeated injections is profound for phosphate [41]. Comparison of capillary conditioning regimes 1, 2 and 3 suggests that this is likely to be due to adsorption of phosphate onto the bare silica surface, giving rise to low peak areas and heights. Upon occupation of all possible sites, more phosphate ions reach the detector, resulting in increased peak response. The rate at which the phosphate is absorbed seems to depend on phosphate concentration, as shown by comparing capillary conditioning regimes no. 4 and no. 5 (Fig. 4.7). Since the same standard mixture with 10 $\mu g.mL^{-1}$ phosphate was separated using the various capillary conditioning regimes, the peak area should have (ideally) been the same, regardless of dispersion or broadening. However, this was not the case, further supporting the statement that phosphate detectability is affected by possible adsorption onto the capillary surface. It can also be seen that peak areas and heights were consistently higher using capillary conditioning regime no. 6, as compared to the other regimes. It has been known from previous work [42] that system stability can be achieved by pre-running a standard mixture of anions (or even water) under the same experimental conditions, prior to actual separation. Based on this observation, standard no. 4 was separated after conditioning the capillary according to capillary conditioning regimes 4 - 6. Optimal detectability of phosphate and precision of migration time, peak area and peak height precision was achieved with capillary conditioning regime no. 6.

Explanation of observed behaviour

The most probable cause of the variation in phosphate detectability is strong adsorption onto the capillary surface. To confirm phosphate-to-silica adsorption as the cause, a

preliminary trial involving passage of 5 $\mu g.mL^{-1}$ phosphate through 140g of washed silica (giving 0.0002 $\mu g.m^{-2}$ phosphate loading[3]) was performed and the filtrate compared with an untreated portion of the same standard. It was noted that the phosphate solution which had been passed through the silica gave lower peak area (4.5% decrease) and height (7.4% decrease). The reduction in peak area was greater than the normal experimental precision of CZE of 1.5% [40].

This preliminary trial did not involve washing the silica with hydroxide as is done in the normal routine of capillary conditioning. The simulation was then repeated using 440g of silica washed with hydroxide, effectively increasing the phosphate loading to 0.0006 $\mu g.m^{-2}$. This resulted in decreases of 16.5% and 14.3% in peak area and height, respectively. These results are in agreement with the literature [13] where it has been confirmed by radioactive tracing that an increase in phosphate adsorption occurs after alkaline treatment of silica. This is due to the removal of the weakly adsorbing hydrated silica film on the silica surface by the base, permitting increased adsorption of phosphate [13].

From the above discussion, it can be said that the variability of phosphate detectability is significant at the $\leq 10\ \mu g.mL^{-1}$ level. This may not be true at higher levels. The most likely cause is adsorption onto the capillary surface which is magnified by alkaline treatment [13]. It is apparent from this investigation that the prevalent practice of capillary regeneration by flushing with NaOH or KOH prior to inorganic anion separation may be detrimental to the detection of phosphate using CZE with reversed EOF, especially if an inappropriate conditioning regime is used.

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3 The loading is the number of µg of phosphate for each m^2 of silica surface.

4.3.4 STABILISATION OF ABSOLUTE MIGRATION TIME

The generation in CZE of precise absolute migration time (AMT) is vital to the identification of solutes (similar to the use of t_r values in chromatography), the calculation of accurate mobilities and perhaps more importantly for this study, the construction of reliable selectivity plots. Precise AMTs are also useful where sample quantity is limited and accurate identification of solutes has to be achieved using the available sample. Also, precise AMTs are useful in the analysis of unknown solutes and samples in which degradation and formation products are being monitored. Knowing that AMT of anions is a function of the zeta potential at the BGE-capillary interface (and the parameters that influence it), the factors shown below were studied.

Effect of Surfactant Dryness and Concentration

For this study, undried and dried (100 ^{o}C overnight) TTAB was used. Chloride was selected as the model anion to investigate AMT precision (% RSD) under the various conditions studied.

Undried TTAB

Fig. 4.8 shows the AMT for selected anions with repeated separations over two days. The general trend was for AMT to decrease with repeats (chloride: 1.29% RSD for n = 11; 1.42% RSD for n = 6) and with each experiment, the 'starting' and 'stable' migration times were different. These trends (Fig. 4.8) are in agreement with observations reported by other workers [23]. The likely cause of this is dynamic build-up of surfactant at the capillary-BGE interface, which in turn increases the double-layer thickness. The notion of dynamic surfactant accumulation is supported by the fact that the baseline dropped with each series of repeats after it was zeroed at the start of the

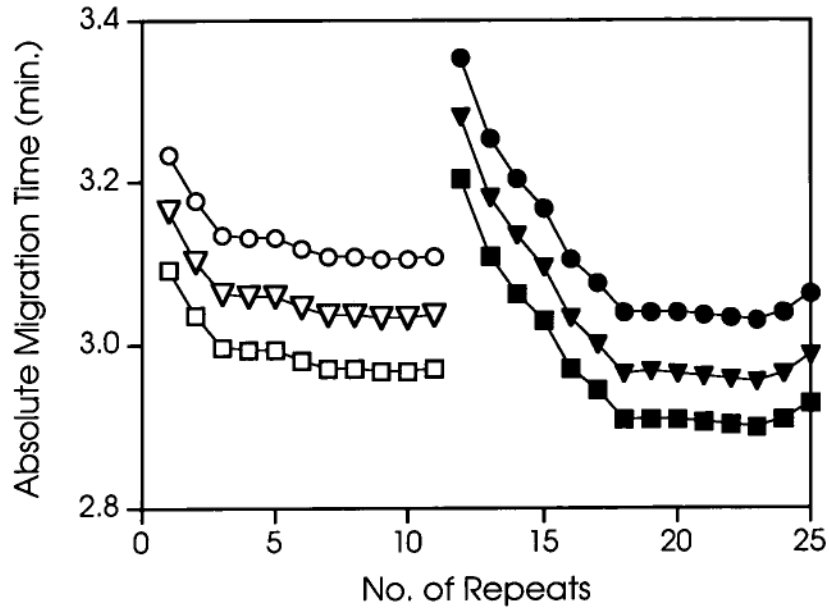

Fig. 4.8: Absolute migration with repeated separations. Conditions as in Fig. 4.2 (a) but with 2.5 mM TTAB and pH 8. Key: □ = chloride on day 1, ∇ = bromide or system peak on day 1, O = nitrite on day 1, ■ = chloride on day 2, ▼ = bromide or system peak on day 2 and ● = nitrite on day 2.

experiment, and by a reduction in AMT as a result of increased EOF (or double-layer thickness).

More than three hours (or six repeats) were needed for AMT to be stabilised and it was noted that starting and stable AMTs varied from day to day. This underscored the dependence of separation performance on capillary history and storage. Further, it shows why there is a real need to find conditions able to generate stable AMTs and serves to highlight why it can be difficult to reproduce published electropherograms.

Dried TTAB

Dried TTAB showed a showed a trend similar to that of the undried TTAB but more importantly, AMT precision was superior (chloride: 0.86% RSD, n = 6) and the

detectability of hydrogenphosphate was improved. (See Section 4.3.3.) The day to day variation in AMT also was similar to that obtained using undried TTAB.

Effect of Surfactant Concentration

For this work 2.6 mM and 0.5 mM TTAB were compared. The former has been discussed above (*Dried TTAB*). The latter (0.5 mM) is the typical concentration used widely in CE separation of anions. The AMT precision for chloride using 0.5 mM TTAB was 3.9% RSD (n = 4). This did not compare well with AMT precision using 2.6 mM TTAB (1.79% RSD, n = 6) and was a reflection of the large variability in EOF rate. After four repeats of the same experiment (i.e. using 0.5 mM TTAB), the difference in AMT of the EOF between the first two separations varied between 6 - 15 min. This obviously is unsuitable for any precise analytical measurement and thus the use of low concentrations are to be avoided where possible.

Effect of Pre-running and Capillary Treatment

Noting from the above that about six repeats (~3 hrs) were required to stabilise AMT, an attempt was made to reduce the time required for AMT to become stable. Separations were performed after the capillary had been subjected to six separations (lasting 5 min. each) before separating the sample mixture (lasting about 20 min. in order to detect the MOx marker). This yielded AMTs which were stable from the first run (chloride: 0.30% RSD, n = 7; 0.33% RSD, n = 6).

Effect of Electrolyte Composition

BGE composition plays a vital role in the separation and migration time of anions [5]. Two means of stabilising AMT through BGE composition were the use of 1-butanol as additive and the use of binary surfactant mixtures. With the former, AMTs were relatively stable (chloride: 1.3% RSD, n = 6) which was in agreement with the

stabilisation of EOF noted by Benz and Fritz [43]. In contrast to Fig. 4.8, it was noted that AMTs were stable virtually from the first run (Fig. 4.9).

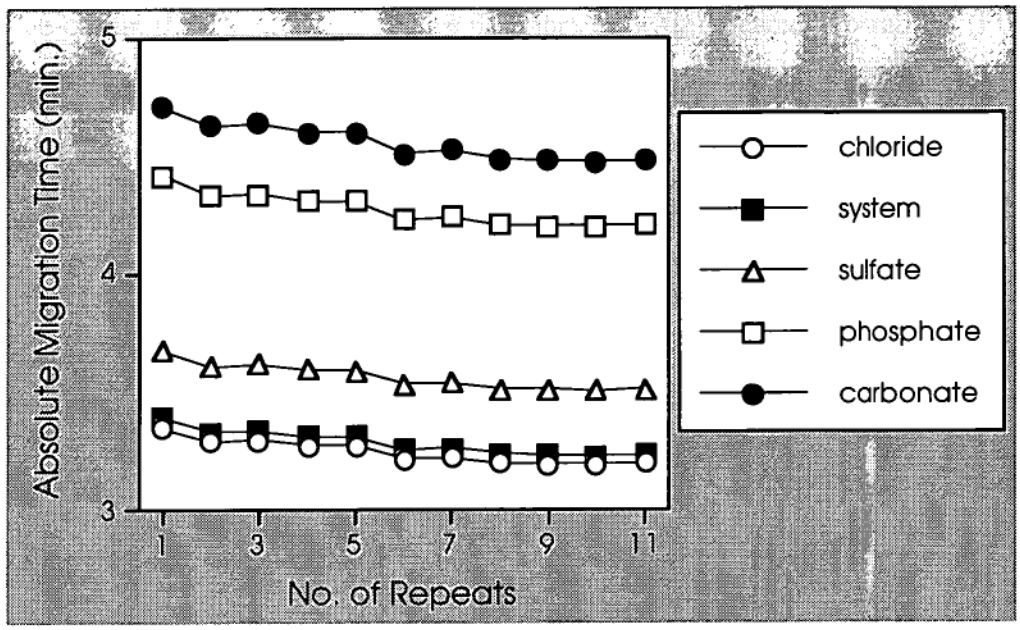

Fig. 4.9: Absolute migration order times for BGE with 5% v/v 1-butanol as additive. Other conditions as in Fig. 4.2 (b). Concentration of anions ($\mu g.mL^{-1}$): chloride = 10, system peak = not applicable, sulfate = 20, hydrogenphosphate = 10 and hydrogencarbonate = ubiquitous.

Chromate-based BGEs containing binary surfactant mixtures [11, 44] also generated excellent AMT precisions (chloride: 0.27% RSD, n = 10 for optimum 1; 1.5% RSD, n = 10 for optimum 2). Apart from being able to generate AMTs with better precision, the use of 1-butanol and binary surfactant mixtures also offered the possibility of manipulating separation selectivity of anions.

Effect of Purge Time

The observation that baseline noise deteriorated with repeated separations (Fig. 4.10) suggested that the BGE in the capillary body which had undergone localised pH changes was not being adequately flushed before the next run, or the formation of fine crystals (as does occur at the anodic end, particularly for BGEs adjusted in pH with

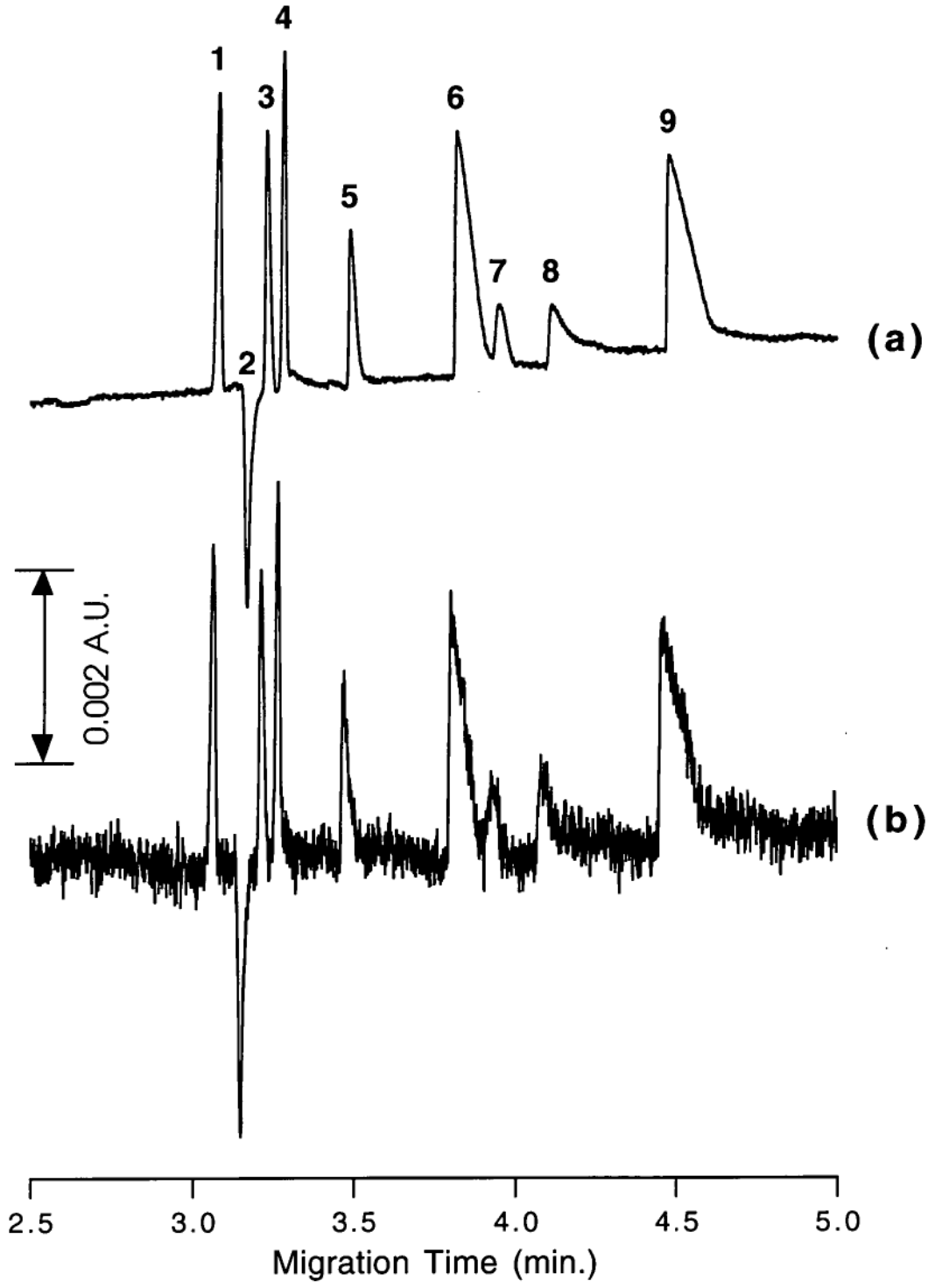

Fig. 4.10: Deterioration of baseline with repeated separation. (a) = run 1 and (b) = run 9. Conditions: The BGE contained 2.6 mM TTAB and 5 mM chromate. Sampling was done hydrostatically (raised to 10 cm and held for 30 s) and indirect UV at 254 nm was used for detection. Separation voltage was -20 kV. Anions: 1 = chloride, 2 = system (bromide), 3 = nitrite, 4 = sulfate, 5 = nitrate, 6 = fluoride, 7 = bromate, 8 = hydrogenphosphate and 9 = hydrogencarbonate.

mineral acids). As it was felt that the flushing period may have been inadequate, the effect of increased purge time on AMT stability was also studied. The idea behind increased purge time is presentation (ideally) of the same surface (double-layer thickness, charge density, etc) for each separation. With a purge time of ≥5 min., AMT stability was reached within the first 3 repeats (chloride: 0.58% RSD, n = 6) in contrast to the first 6 repeats using the default 2 min. purge time setting. It had earlier been concluded that BGEs containing low (0.5 mM) surfactant were unsuitable for generation of stable AMT (see Fig. 4.8). Using the same (0.5 mM) concentration of TTAB but with increased purge time, the AMT precision was improved (Fig. 4.11). Therefore contrasting Fig. 4.8 and Fig. 4.11 (drawn with the same time-scale), it can be seen that increased purge time was conducive to generation of stable AMT.

Summary

A variety of protocols that assist in generating stable AMTs have been discussed above. The common factor with all procedures is to stabilise and maintain the double-layer formed at the BGE-capillary interface. A stable double-layer will yield stable EOF and AMTs for anions.

4.4 OVERALL SUMMARY

From the above investigations, the following conclusions were reached and extended to the execution of the rest of the study:-

- *Neutral Markers:* The use of neutral markers to monitor EOF can be unreliable. Additionally, the AMT of anions, AMT precision and R_S can be compromised. The EOF can be detected reliably under the typical conditions used in this study by the migration time of the 'plug' peak. Therefore for the separation of inorganic anions, the use of neutral markers is not recommended.

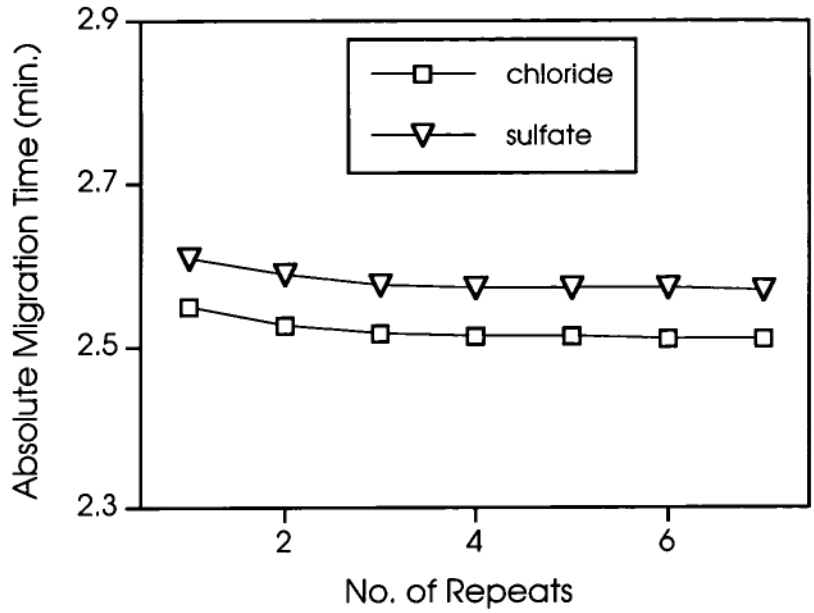

Fig. 4.11: Absolute migration time with repeated separations using 6 min. purge time between runs. Others conditions and time-scale as in Fig. 4.8.

- *Surfactant Dryness:* Where the highest possible detectability, R_S and AMT precision of anions is desired, the use of dried surfactants is recommended.
- *Phosphate Response:* For optimal response for phosphate, the use of dried surfactants and capillary conditioning regime no. 6 are advised.
- *Migration Time Stabilisation:* Capillary conditioning regime no. 6 is recommended for improved AMT precision. Apart from stabilising AMT, it will also be of assistance in the detection of silanophilic anions like phosphate.

4.5 REFERENCES

1 Rose, D.J. and Jorgenson, J.W., *Anal. Chem.*, **60** (1988) 642.

2 Janini, G.M., Chan, K.C., Barnes, J.A., Muschik, G.M. and Issaq, Benz, H.J., *Chromatographia*, **35** (1993) 497.

3 Pálmarsdóttir, S. and Edholm, L.-E., *J. Chromatogr. A.*, **693** (1995) 131.

4 Grossman, P.D. and Soane, D.S., *Anal. Chem.*, **62** (1990) 1592.

5 Atamna, I.Z., Metral, C.J., Muschik, G.M. and Issaq, H.J., *J. Liq. Chromatogr.*, **13** (1990) 3201.

6 Atamna, I.Z., Metral, C.J., Muschik, G.M. and Issaq, H.J., *J. Liq. Chromatogr.*, **13** (1990) 2517.

7 Altria, K.D. and Simpson, C.F., *Chromatographia*, **24** (1987) 527.

8 Chang, H-T. and Yeung, E.S., *J. Chromatogr.*, **608** (1992) 65.

9 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **546** (1991) 445.

10 Buchberger, W. and Haddad, P.R., *J. Chromatogr.*, **608** (1992) 59.

11 Jorgenson, J.W. and Lukacs, K.D., *Science*, **222** (1983) 266.

12 Rhemrev-Boom, M.M., *J. Chromatogr. A.*, **680** (1994) 675.

13 Hensley, J.W., *Journal of the American Chemical Society*, **34** (1951) 188.

14 McCormick, R.M., *Anal. Chem.*, **60** (1988) 2322.

15 Tran, A.D., Park, S., Lisi, P.J., Huynh, O.T., Ryall, R.R. and Lane, P.A., *J. Chromatogr.*, **542** (1991) 459.

16 Li, S.F.Y., Capillary electrophoresis principles, practice and applications, (Elsevier, Amsterdam, 1992).

17 Chadwick, R.R. and Hsieh, J.C., *Anal. Chem.*, **63** (1991) 2380.

18 Salomon, K., Burgi, D.S. and Helmer, J.C., *J. Chromatogr.*, **559** (1991) 69.

19 Nguyen, A. -L., Luong, J.H.T. and Masson, C., *Anal. Chem.*, **62** (1990) 2490.

20 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **608** (1992) 385.

21 Atamna, I.Z., Metral, C.J., Muschik, G.M. and Issaq, H.J., *J. Liq. Chromatogr.*, **13** (1990) 2517.

22 Kenney, B.F., *J. Chromatogr.*, **546** (1991) 423.

23 Lux, J.A., Yin, H.-F. and Schomburg, G., *Chromatographia*, **30** (1990) 7.

24 Skoog, D.A. and West, D.M., Fundamentals of Analytical Chemistry, 4th edition (Holt-Saunders, Philadelphia, 1982).

25 Johnson, R., Elementary Statistics, 4th edition (Duxbury Press, Boston, 1984), p. 524.

26 Gassner, B., Friedl, W. and Kenndler, E., *J. Chromatogr. A.*, **680** (1994) 25.

27 Walbroehl, Y. and Jorgenson, J.W., *Anal. Chem.*, **58** (1986) 479.

28 Kuhn, R. and Hoffstetter-Kuhn, S., Capillary Electrophoresis: Principles and Practice (Springer-Verlag, Berlin, 1993).

29 Harakuwe, A.H., *unpublished results from work on the analysis of chloride and sulfate in concrete.*

30 Aldrich® Catalogue Handbook of Fine Chemicals 1994 - 1995 (Sigma-Aldrich, Castle Hill, NSW, Australia), p. 1016.

31 Fessenden, R.J. and Fessenden, J.S., Organic Chemistry (Willard Grant Press, Boston, MA, 1981), p. 730.

32 Silverstein, R.M., Bassler, G.C. and Morrill, T.C., Spectrometric Identification of Organic Compounds, 4th edition (John Wiley & Sons, New York, 1981).

33 Lee, T.D., Anderson, Jr., W.R., Daves, Jr., G.D., *Anal. Chem.*, **53** (1981) 304.

34 Bij, K.E., Horváth, C., Melander, W.R. and Nahum, A., *J. Chromatogr.*, **203** (1981) 65.

35 Wahlund, K.-G. and Sokolowski, A., *J. Chromatogr.*, **151** (1978) 299.

36 Corradini, D., Rhomberg, A. and Corradini, C., *J. Chromatogr. A.*, **661** (1994) 305.

37 Corradini, D., Cannarsa, G., Fabbri, E. and Corradini, C., *J. Chromatogr. A.*, **709** (1995) 127.

38 Cohen, N. and Grushka, E., *J. Chromatogr. A.*, **678** (1994) 167.

39 Weiss, C.S., Hazlett, J.S., Datta, M.H. and Danzer, M.H., *J. Chromatogr.*, **608** (1992) 325.

40 Jones, W.R. and Jandik, P., *Amer. Lab.*, (1990) 51.

41 Haddad, P.R. and Harakuwe, A.H., poster presented at the 1st RACI Anal. Chem. R&D Meeting, Deakin Uni., Geelong, Australia. 6-7th December, 1993.

42 Harakuwe, A.H. and Haddad, P.R., *unpublished results*, 1993.

43 Benz, N.J. and Fritz, J.S., *J. Chromatogr.*, **671** (1994) 437.

44 Harakuwe, A.H., Haddad, P.R. and Buchberger, W., *J. Chromatogr. A.*, **685** (1994) 161.

CHAPTER 5

SELECTIVITY EFFECTS DERIVING FROM THE SURFACTANT

5.1 INTRODUCTION

Apart from reducing anion migration time through reversal or suppression of EOF, cationic surfactants or EOF modifiers can be used to induce selectivity changes for anions [1]. Quaternary alkylammonium salts are the predominant EOF modifiers used in FRECZE (see Chapter 2 - literature review) and this chapter will involve consideration of the effects due to (i) size or chain length, (ii) counter-anion type and (iii) concentration of modifiers. Previous papers [e.g. see refs. 2-9] have involved the use of single EOF modifiers. The use of binary surfactant mixtures for manipulation of anion separation using FRECZE is a further subject of the work discussed in this chapter.

5.2 EXPERIMENTAL

5.2.1 INSTRUMENTAL, REAGENTS AND CHEMICALS

See Chapter 3 for details of instrumentation and general separation conditions. The EOF modifiers used were dodecyltrimethylammonium bromide (DTAB), tetradecyltrimethylammonium bromide (TTAB), cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium (CTA) chloride and CTA-hydrogensulfate. All EOF modifiers were of AR grade (Aldrich, Milwaukee, WI, USA) and were dried overnight

at 100 °C, with the exception of CTA-chloride which came as a 25% w/v solution. Chromate was used throughout for this work as the UV-absorbing visualising agent.

5.3 RESULTS AND DISCUSSION

5.3.1 EFFECT OF SURFACTANT COUNTER-ANION AND SIZE

CTA in the bromide, chloride and hydrogensulfate forms was used for the investigation of the effect of EOF modifier counter-anion. No significant changes in anion separation selectivity occurred as a function of EOF modifier counter-anion (Fig. 5.1). Nevertheless, appropriate choice of EOF modifier counter-anion is essential to avoid interference since it is this ion that produces the system peak, for example, the use of CTA-chloride would be unwise for the separation of anions in body fluids like urine because of the inability to quantify chloride in such samples.

The effect of the size of the EOF modifier on anion separation selectivity using DTAB (12 C atoms in the alkyl chain), TTAB (14 C atoms in the alkyl chain) and CTAB (16 C atoms in the alkyl chain) are shown in Fig. 5.2. Selectivity changes were pronounced for thiocyanate and iodide. The large changes in absolute migration time (AMT) and migration order for these lipophilic anions can be attributed to formation of ion-pairs [1, 6-11] with the EOF modifier. The lipophilic anions are slowed down due to the lower mobility of these ion-pairs. The trend was for a virtually linear increase in AMT for the lipophilic anions with increasing size of the alkyl group on the EOF modifier. On the other hand, the other (more hydrophilic) anions showed increased migration rate corresponding to increasing size of the alkyl chain in the EOF modifier. This can be attributed to the differences in thickness of the double-layer

formed by the EOF modifier after dynamically coating the capillary surface. The thickness of the double-layer will correspond to the alkyl chain length, i.e. longer chains will form relatively thicker double-layers or hemimicelles. This in turn directly governs the zeta potential and hence the EOF. Shorter AMTs are indicative of larger EOF and by extension, larger double-layer thickness.

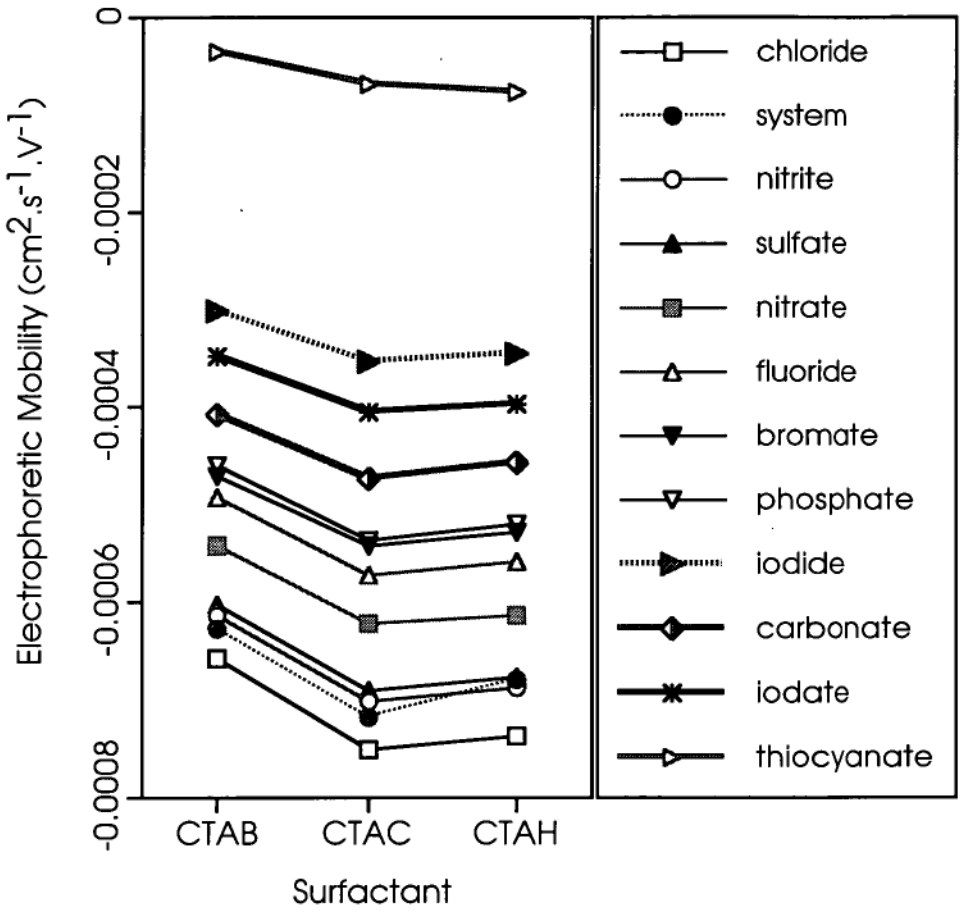

Fig. 5.1: Effect of surfactant counter-anion on anion separation selectivity. Conditions: 2.5 mM surfactant, 10 mM chromate, pH 9.1. Sampling was in the hydrostatic mode (10 cm for 30 s) and indirect UV at 254 nm was used for detection. Separation voltage was -20 kV applied across a 75 µm ID x 52 cm effective length x 60 cm total length fused-silica capillary. Negative mobilities (calculated relative to the 'plug' peak) denote migration to the anode. Key: CTAB = CTA-bromide, CTAC = CTA-chloride and CTAH = CTA-hydrogensulfate. The concentrations in $\mu g.mL^{-1}$ of the anions were chloride = 8, nitrite = 6, sulfate = 8, nitrate = 10, fluoride = 5, bromate = 7, hydrogenphosphate = 12, iodide = 10, (bi)carbonate = 10, iodate = 10 and thiocyanate = 10.

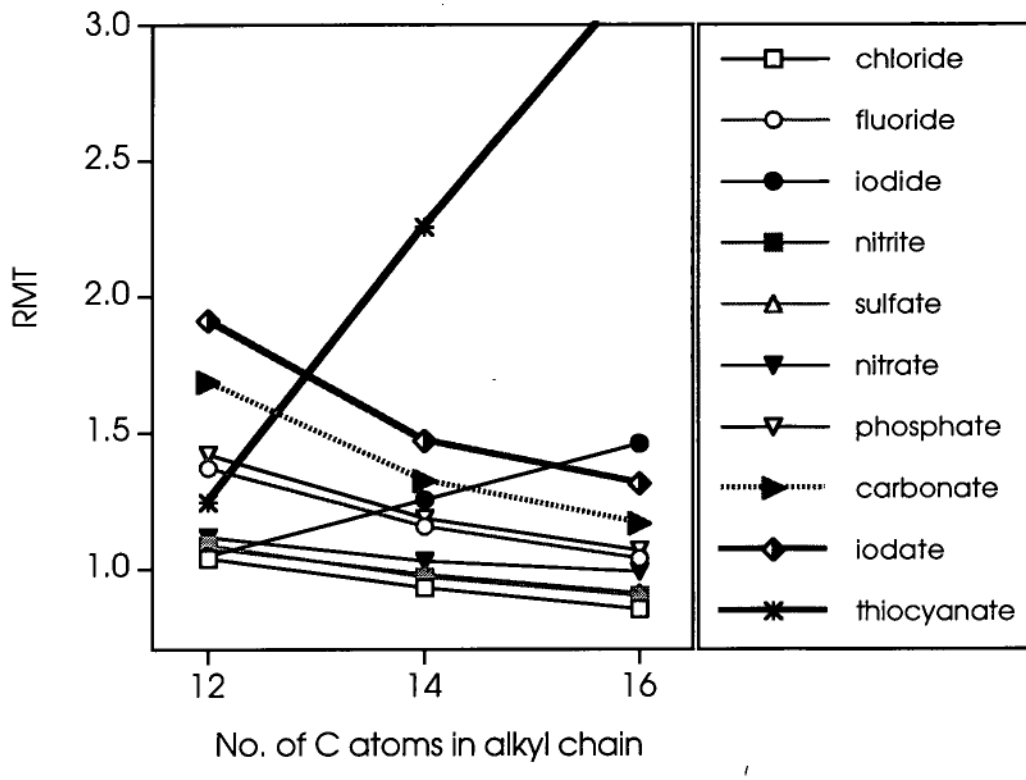

Fig. 5.2: Effect of alkyl chain length of quaternary ammonium bromide surfactants on anion selectivity. Conditions: 2.5 mM surfactant, 5 mM chromate and pH 8.5 ± 0.1. Other conditions were as in Fig. 5.1. Key: RMT = relative migration time (to the system peak), 12 C = DTAB, 14 C = TTAB and 16 C = CTAB. The anion concentrations in $\mu g.mL^{-1}$ were: chloride = 20, bromide = 10, fluoride = 10, iodide = 20, nitrite = 5, sulfate = 8, nitrate = 12, (hydrogen)phosphate = 16, iodate = 20 and thiocyanate = 8.

5.3.2 Effect of Surfactant Concentration

The effect of the concentration of EOF modifier on anion separation selectivity using TTAB as the model surfactant is shown in Fig. 5.3. The selectivity trends as a function of TTAB concentration approximate those evident from Fig. 5.2. Again, the marked selectivity changes for the lipophilic anions were most likely due to their strong affinity towards the hydrophobic portion of the surfactant. The other anions showed decreases

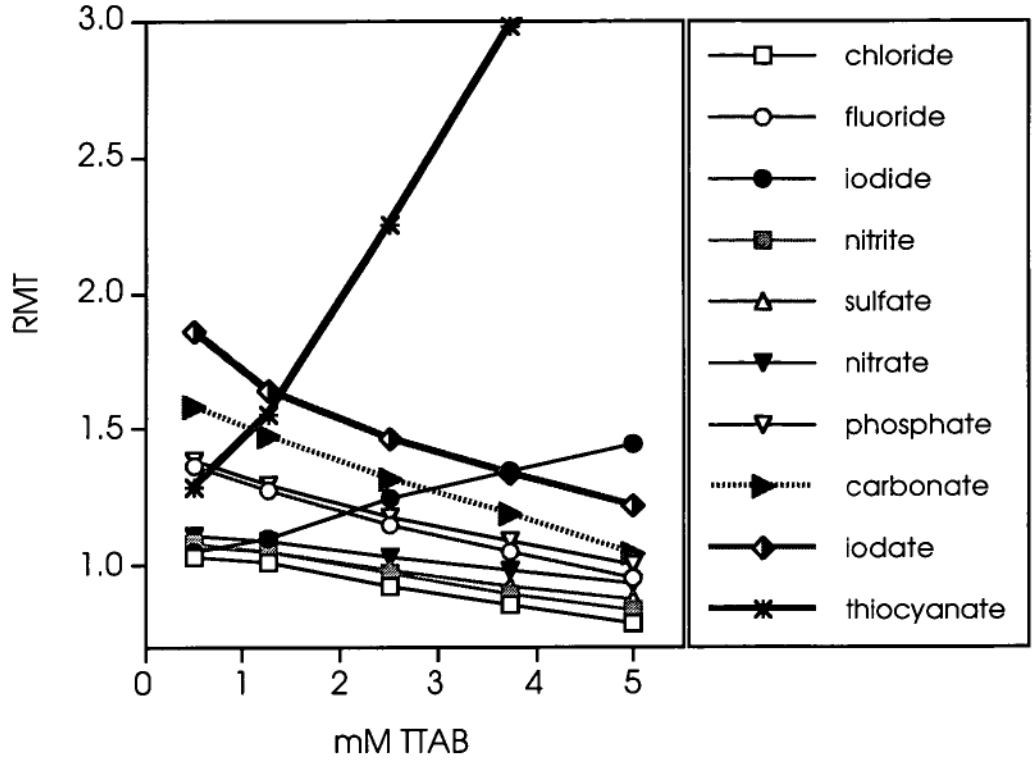

Fig. 5.3: Effect of TTAB concentration on anion selectivity. Apart from the differences in TTAB concentration, the other conditions were as in Fig. 5.2. Key: RMT = relative migration time (to system peak). Anion concentrations were as in Fig. 5.2.

in relative migration time as the concentration of the EOF modifier was increased. Slight changes in migration order were noted for nitrite.

5.3.3 Effect of Surfactant Mixtures

Momentarily disregarding the lipophilic anions, it was seen from Fig. 5.2 that the migration order and AMT trends for the other anions were roughly the same for TTAB and CTAB (e.g. chloride migrated before the system peak) in contrast to DTAB (e.g. chloride migrated before the system peak). It was possible that a "hybrid" separation performance could be achieved using mixtures of DTAB and either TTAB or CTAB.

As an initial study, DTAB and TTAB in binary combinations were studied for their effect(s) on separation selectivity of anions. Chromate was used throughout as the UV-absorbing probe for this investigation. Selectivity changes occurred (notably for chloride, nitrite and fluoride) as a function of total surfactant concentration (Fig. 5.4) and molar ratio of the surfactants (Fig. 5.5). Explanation of these results must take into consideration critical micelle concentrations (cmc) of TTAB and DTAB which are 15 mM and 3.5 mM [12], respectively, in water. It might be concluded that in the range below 6 mM total concentration, ion-pairing effects are mainly responsible for selectivity changes. Above 6 mM total surfactant concentration, the anions may be partaking in an interaction with micelles. Although the study of micelles was outside the scope of this investigation, it is noted that the notion of two different equilibria existing above and below the cmc has been proposed by Kaneta *et al.* [1].

Above the cmc, it may not necessarily be so that the anions are only involved in a distribution between the aqueous electrolyte and the micellar 'pseudo' stationary phase. There may be some ion-pairing between the anions and the micelles (i.e. distinct from pairing between anions and the monomeric EOF modifier). In such a case, the micelles may play a similar role to polymers, which have been used as additives to optimise separation selectivity [13]. Whatever the detailed mechanism of these interactions, the anion separation selectivity changed gradually from the migration order typical of electrophoresis to a migration order resembling in part that occurring in ion-exchange chromatography.

Apart from influencing the separation selectivity of anions, the use of BGEs with binary surfactant mixtures also offered the possibility of improving anion resolution. This is illustrated in Fig. 5.6. A standard mixture separated using BGEs with single

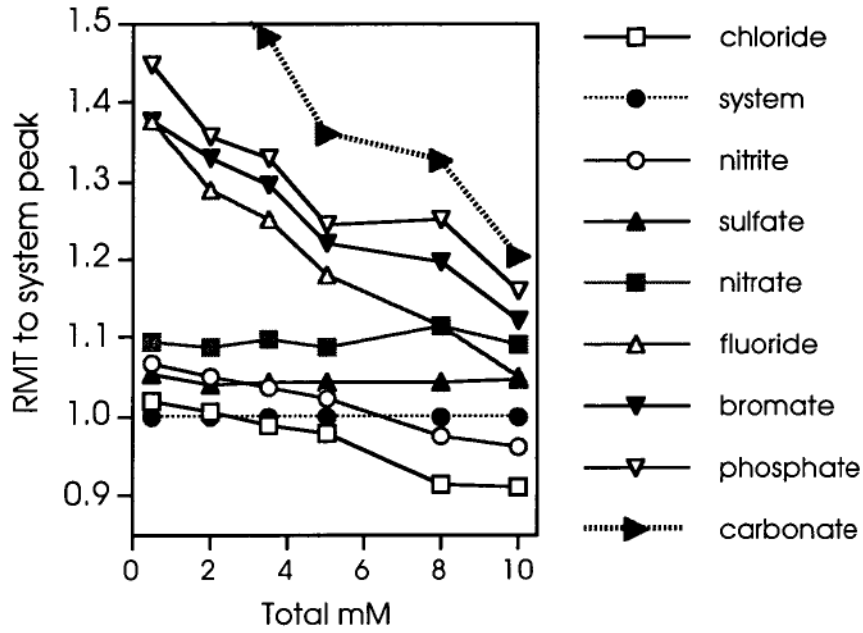

Fig. 5.4: Dependence of anion migration orders on total EOF modifier concentration using 1:1 mixtures of TTAB and DTAB. Conditions: Apart from the varying amounts of TTAB and DTAB, the BGE contained 5 mM chromate at pH 8.8. All anions were 10 $\mu g.mL^{-1}$ in concentration. Other conditions were as in Fig. 5.1.

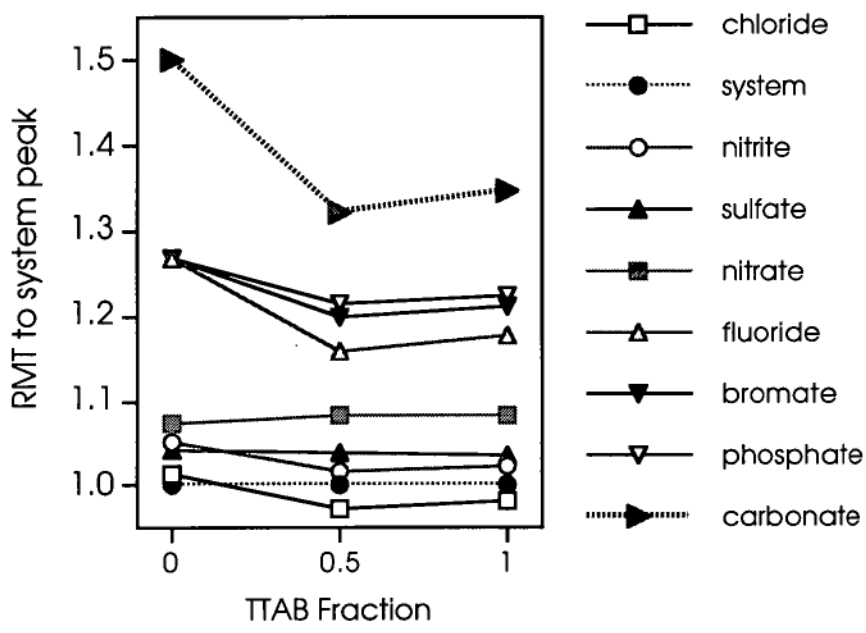

Fig. 5.5: Dependence of anion migration orders on ratios of TTAB and DTAB. Conditions and key as in Fig. 5.4. The total surfactant concentration was 2.6 mM.

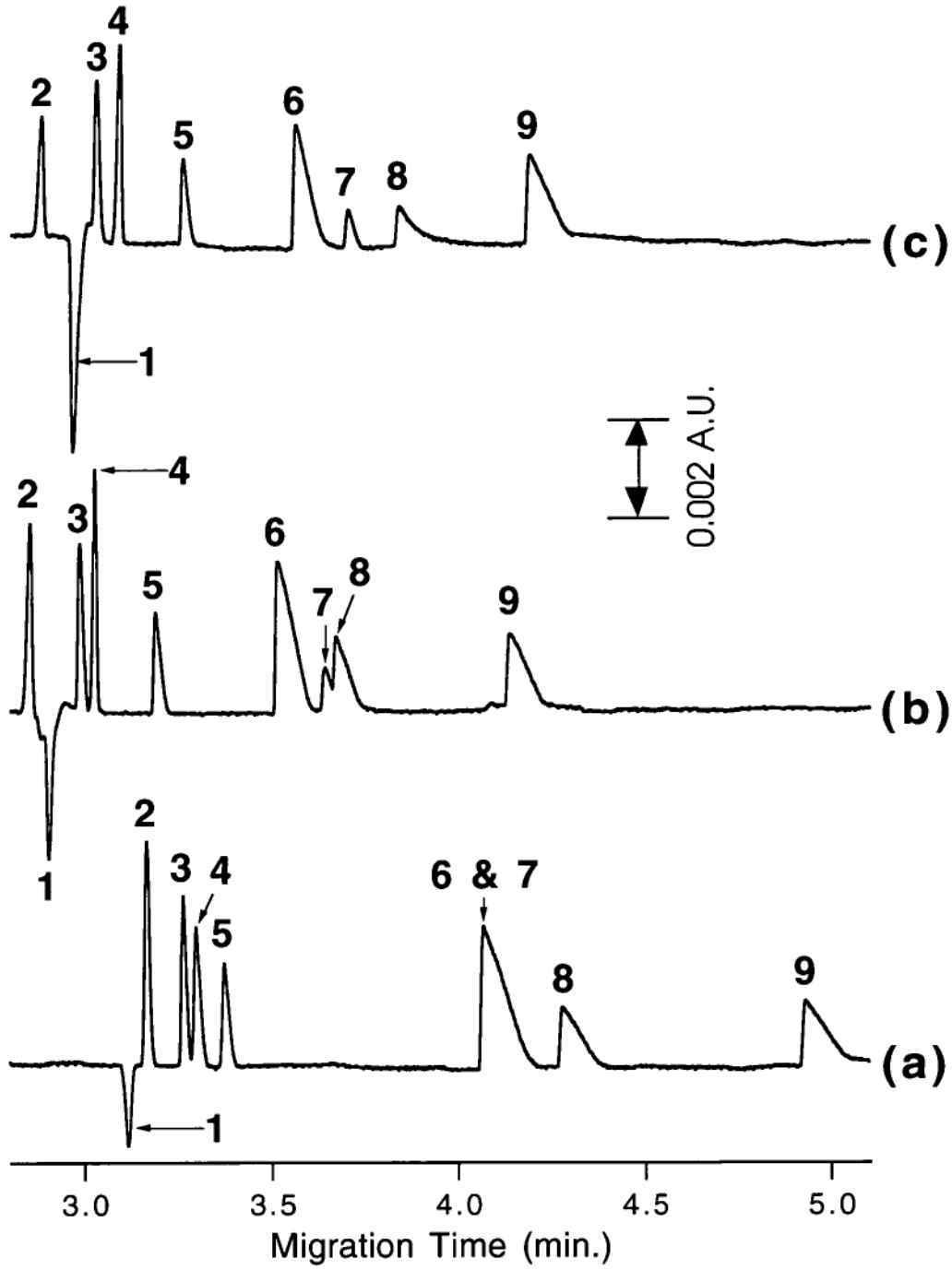

Fig. 5.6: Comparison of separations using DTAB and TTAB singly and as binary mixtures. Conditions: (a) 2.6 mM DTAB, 5 mM chromate and pH 9.1. Other conditions as in Fig. 5.1. (b) 2.6 mM TTAB. Other conditions as in (a). (c) BGE had 2.6 mM TTAB and 2.6 mM DTAB. Other conditions were as in (a). Anions: 1 = system (bromide), 2 = chloride, 3 = nitrite, 4 = sulfate, 5 = nitrate, 6 = fluoride, 7 = bromate, 8 = phosphate, 9 = carbonate.

2.6 mM DTAB (Fig. 5.6 (a)) and 2.6 mM TTAB (Fig. 5.6 (b)) suffered from poor resolution of bromate and nitrite from their respective adjacent anions. However when the same mixture was separated using a BGE with a binary mixture of 2.6 mM each of DTAB and TTAB, the problems noted above were eliminated (Fig. 5.6 (c)).

A common method to improve resolution of closely-migrating or unresolved anions in FRECZE, the obvious and commonly used option is to increase the concentration of the electrolyte. This method however also increases the separation or migration time. In contrast, the use of binary surfactant mixtures in the BGE can improve resolution without unduly increasing migration time. This is demonstrated clearly in Fig. 5.6.

5.4 CONCLUSIONS

The anion of quaternary ammonium EOF modifiers did not appear to alter anion separation selectivity. However, the alkyl chain length of the surfactant significantly altered the separation selectivity, particularly for lipophilic anions via ion-pairing effects. The use of binary surfactant mixtures in the BGE was shown to be a viable method for altering anion separation selectivity and was shown to be dependent on both total concentration and the ratio of the two surfactants used. Using binary surfactant mixtures, resolution of anions was improved without compromising separation time. Future work should consider other surfactants and ratios of surfactants.

5.5 REFERENCES

1 Kaneta, T., Tanaka, S., Taga, M. and Yoshida, H., *Anal. Chem.*, **64** (1992) 798.

2 Altria, K.D. and Simpson, C.F., *Chromatographia*, **24** (1987) 527.

3 Jones, W.R. and Jandik, P., *American Laboratory*, (1990) 51.

4 Romano, J., Jandik, P., Jones, W.R. and Jackson, P.E., *J. Chromatogr.*, **546** (1991) 411.

5 Jandik, P., Jones, W.R., Weston, A. and Brown, P.R., *LC•GC*, **9** (1991) 634.

6 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **546** (1991) 445.

7 Buchberger, W. and Haddad, P.R., *J. Chromatogr.*, **608** (1992) 59.

8 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **608** (1992) 385.

9 Romano, J.P. and Krol, J., *J. Chromatogr.*, **602** (1992) 205.

10 Pfeffer, W.D. and Yeung, E.S., *J. Chromatogr.*, **557** (1991) 125.

11 Jones, W.R., *J. Chromatogr.*, **640** (1993) 387.

12 Fendler, E.J. and Fendler, J.H., in Gold, V., editor, Advances in Physical Organic Chemistry (Academic Press, London, 1970), p. 276.

13 Terabe, S. and Isemura, T., *Anal. Chem.*, **62** (1990) 652.

CHAPTER 6

SELECTIVITY EFFECTS DERIVING FROM OTHER PARAMETERS

6.1 INTRODUCTION

In this chapter, selectivity effects due to factors other than the surfactant will be discussed. These factors are related to the composition of the background electrolyte (BGE) except for the effect of separation voltage (instrumental variable). The factors are:-

6.1.1 PROBE TYPE AND CONCENTRATION

The UV-absorbing probe or visualising agent which is also known as the carrier anion [1] plays a vital role in the detection of inorganic anions, most of which are UV-inactive [2]. Importantly too, probes also act as the conducting medium through which anions traverse during separation.

Probe Type

Buffer anion effects have been studied by Salomon *et al.* [3] and Atamna *et al.* [4]. Various probe anions have been used in CZE, e.g. chromate [5-10], *p*-anisate, salicyclate, *o*-phthalate, mellitate, 1,2,4,5-benzenetetracarboxyllic acid [1, 8], vanadate, KHP, trimesate [11], benzoate and benzoic acid [11, 12], sorbic acid [12], (tetra)borate [4, 13], phosphate in both the mono- and di-hydrogen forms [4, 14],

mono-, di- and tri-naphthalene sulfonates[1] [15], *p*-hydroxybenzoate [16], chloride [6, 9], acetate, citrate, nitrite, carbonate [4], nitrate [4, 9], and sulfate [9]. The carrier ion can be chosen to suit given anionic solutes, e.g. *p*-hydroxybenzoate for slowly migrating anions, phthalate for anions with intermediate mobility and chromate for very mobile anions like chloride [16]. Matching the mobilities of the solutes and the carrier anion also has the added advantage of increasing the detection sensitivity when indirect detection is used.

The choice of probe will depend on sample pH, acid-base dissociation constants of solutes, degree of ionisation required for effective resolution, required sensitivity, etc. For this study, the probes selected were chromate, trimellitate, tetraborate and nitrate. Whilst chromate was chosen as the 'reference' probe, tetraborate and trimellitate were chosen because their use for manipulation of separation selectivity in CZE has not been published previously. Nitrate was chosen for possible application to the analysis of anions in concrete samples digested with concentrated nitric acid (discussed more fully in Chapter 8).

Probe Concentration

As most inorganic anions have poor chromophoric qualities [1, 2, 12, 17, 18], their detection is usually performed in the indirect mode [19-22]. Chromate is a common visualising agent for inorganic anions and it has been used extensively in CZE [e.g. 5, 7-11, 18]. Apart from chromate's function in indirect UV detection and acting as the conducting medium, it also plays a role in the selectivity of the separation.

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1 The di- form performs best compared to the other two forms, however selectivity changes were minor [15].

Electrolytes with various surfactant concentrations and chromate concentration ranges have been studied for their effects on anion selectivity [7-9]. The widest published chromate concentration range is 2.5 - 15 mM [7]. For the present work, the range 2 - 15 mM was used. This is an extension on the above range as well as ranges used by Buchberger and Haddad (1 - 7 mM) [9] and Jones and Jandik (3 - 9 mM) [8]. Although selectivity changes will depend on the nature of the constituent anions of standard mixtures, subtle changes have been noted for iodide, nitrite [9] and sulfate [8, 9]. With the above studies, 0.5 mM surfactant was used in contrast to the ~2.5 mM used for this study.

The present study was undertaken with a view to applying it to the analysis of concrete for sulfate and chloride, seawater and table salt (for iodide and iodate) and Bayer liquor. Apart from changes in selectivity, it was also important to monitor changes in detection sensitivity.

6.1.2 Effect of Electrolyte pH

The extent of dissociation and thus the charge of anions depends predominantly on the pH of the BGE [23, 24]. Since the migration rate of an anion is directly proportional to its net charge, pH variation is an obvious and common [25] parameter for manipulating anion separation selectivity in CZE [4, 8, 26-28].

The pH of either the (i) sample or (ii) running electrolyte impacts significantly on separation selectivity. There is ample material on the latter aspect in the literature whilst material on the former appears limited. The probable reason could be the assumption that the electrolyte concentration being ≥ 1000 times greater than the sample [29] would have an overriding influence on the behaviour (charge) of the anion. Work done by the

author[2] has shown however that anions in treated (buffered) samples behave differently. This observation may be of direct relevance to naturally buffered samples (e.g. seawater), especially for electrolytes such as chromate that have a low buffering capacity.

Selectivity manipulation by variation of the pH of the BGE has been used widely in CZE [e.g. 3, 4, 8, 14, 24, 25-28, 30, 31]. Marked selectivity changes occur at or near the pK_a or pK_b values [8, 23, 27, 28, 30] where the rate of change of ionisation is greatest and charge:mass ratios alter rapidly with pH variation. The changes in pH can be instituted in sequential steps or as a gradient [28, 32-34]. For this study, the former was employed.

Chromate Electrolytes

The extent to which pH can be varied depends on the solute anion(s) of interest, the resolution and analysis time required, the carrier type, and the electrolyte composition (e.g. precipitation occurs at pH <10 for electrolytes with TTAB as EOF modifier and vanadate as probe [11], whereas electrolytes with cetyltrimethylammonium bromide (CTAB) are not affected in the pH range 4.5 to 11.0 [23]).

Use of chromate as both electrolyte and UV-absorbing probe for the separation and indirect detection of inorganic anions is commonplace due to its excellent UV absorbance characteristics [4, 11] and its high mobility [16] which matches that of many inorganic anions. Separation of inorganic anions by FRECZE employing chromate-based BGEs containing TTAB at pH ≥8 has been reported widely [e.g. see refs. 8, 9, 11, 17, 35]. On the other hand, separation of inorganic anions using chromate-TTAB BGEs at pH <8 has not been possible due to the formation of

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2 Unpublished results from work on borate treatment of Bayer liquor.

precipitates between BGE components. While other probes and EOF modifier systems that do not suffer precipitation at pH ≤8 have been used, these are inferior to chromate in terms of detection sensitivity (except for the trimellitic and pyromellitic acids [1, 8, 36]), separation efficiency and speed of separation for inorganic anions.

In FRECZE, it is hard to separate adjacent solutes with similar mobilities [28], such as fluoride and phosphate [31], especially when they are present in significantly disparate amounts (see for instance ref. [37]).

To improve resolution, dilution is usually the first option but is unsuitable when the minor component is present at trace levels. Manipulation of separation selectivity is a better approach and the weakly acidic nature of phosphate suggests that pH variation could be exploited to maximise the migration time difference between phosphate and fluoride. This section will discuss how the pH of a chromate-TTAB electrolyte can be lowered below 8 with subsequent improvement in the resolution between fluoride and phosphate.

6.1.3 Effect of 1-Butanol as Electrolyte Additive

In FRECZE, the separation of anions depends on, among other variables, the effective size of the electroactive solute anion. Variation of anion size through solvation effects can thus be used to manipulate separation selectivity [8, 25]. Variation in effective size through solvation is usually instituted with the aid of organic solvents as BGE additives.

A multitude of protic and aprotic solvents has been studied for their influence on the separation of anionic solutes using capillary electrophoresis. These include methanol [1, 6, 9, 38-42], acetonitrile [3, 6, 9, 38, 39, 41], tetrahydrofuran [9, 38, 39], ethylene glycol, acetone [9], ethanol [6, 38, 39, 41], 2-propanol [38, 39, 41, 43] for catechols and serotonin [43], 1-butanol [44], dioxane [38] and dimethylformamide [40]. Studies related specifically to the effect of organic modifiers or solvents on anionic solutes are those from refs. [9], [41], [44] and [45].

Although 1-butanol has been used previously in CZE to stabilise EOF [44] and to facilitate the improvement of resolution between fluoride and phosphate [46], its utility in manipulating separation selectivity of inorganic anions has not been demonstrated. The objective of this work was to study the influence of 1-butanol on separation selectivity of anions using CZE. The effect on migration time, baseline noise and reproducibility was also considered.

6.1.4 Effect of Separation Voltage

Instrumental variables can also be used to manipulate selectivity in FRECZE. The obvious variables are detection mode, injection mode, separation voltage and capillary type. Detection wavelength offers a means of selective detection of solutes. This facilitates differentiation between anions that are UV-active and those that are not (e.g. thiosulfate and hydrosulfide can be detected directly at 214 nm [10]). The use of separation voltage to manipulate the separation selectivity of anions using FRECZE is rare and this approach was studied further here.

6.2 EXPERIMENTAL

6.2.1 INSTRUMENTAL AND REAGENTS

See Chapter 3 for details of instrumentation and general separation conditions. For all investigations indicated in this chapter, the capillary was conditioned according to regime no. 6 (see Table 4.3 in Chapter 4). Apart from the investigation of the effect of separation voltage on separation selectivity, all investigations in this chapter involved separating the 11-anion standard mixture shown in Table 6.1. The anions were diluted as appropriate from accurately made 100 $\mu g.mL^{-1}$ stock solutions of the individual anions.

Table 6.1

ANION STANDARD MIXTURE

ANION	$\mu g.mL^{-1}$
Phosphate	12
Iodide	10
Carbonate	10
Iodate	10
Nitrate	10
Thiocyanate	10
Chloride	8
Sulfate	8
Bromate	7
Nitrite	6
Fluoride	5

6.2.1.1 Effect of Probe Type and Concentration

Running electrolytes were prepared freshly, filtered to pass 0.45 µm and degassed ultrasonically. Apart from the differences in chromate concentration, all running electrolytes had 2.5 mM TTAB (diluted as required from 50 mM stock solution prepared from TTAB dried at 100 °C for 1 hour) and adjusted to pH 9.0 ± 0.1 with dilute sodium hydroxide.

Under the conditions used for this study, mobilities were calculated according to Eqn. 6.1 [23] and resolution (R_S) was calculated according to Eqn. 6.2 [47, 48].

$$m_{anion} = \left(\frac{-0.0026}{AMT_{anion}}\right) - \left(\frac{-0.0026}{AMT_{EOF}}\right) \qquad \text{Eqn. 6.1}$$

$$R_s = \left(\frac{2\,(m_{faster\ anion} - m_{slower\ anion})}{(m_{faster\ anion} + m_{slower\ anion})}\right) \cdot \left(\frac{\sqrt{N}}{4}\right) \qquad \text{Eqn. 6.2}$$

where m_{anion} = anion electrophoretic mobility ($cm^2.s^{-1}.V^{-1}$), AMT = absolute migration time, EOF = electroosmotic flow front (indicated in this instance by the 'plug' peak) and N = number of theoretical plates.

6.2.1.2 Effect of Electrolyte pH

BGEs were adjusted to the required pH with either dilute sodium hydroxide (AR, BDH, Victoria, Australia), Tris(hydroxymethyl)aminomethane (TRIS) (AR, Aldrich, Milwaukee, WIS), 3-cyclohexylamino-1-propanesulfonic acid (CAPS) (AR, Aldrich, Milwaukee, WIS) or mineral acids.

6.2.1.3 Effect of 1-Butanol as Electrolyte Additive

Preparation of Background Electrolyte

As there is a propensity for chromate-based BGEs containing TTAB to suffer precipitation or cloudiness upon pH adjustment using mineral acids, BGEs were prepared as follows. The required amounts of TTAB and 1-butanol were combined, diluted to ~80% of final volume and mixed thoroughly with a magnetic stirrer until there was no sign of emulsion. The pH was then adjusted to the desired value (≤8) with dilute NaOH, the required amount of chromate was then added and the solution mixed well. At this point, the pH was noted to drop slightly. Final adjustment of pH was then effected and the solution diluted to the final volume. Apart from avoiding precipitation/cloudiness, this procedure also ensured that only hydroxide was used for pH adjustment. This is useful in avoiding interferences (e.g. dips in baseline) that can result from anions introduced through the use of acids for pH adjustment.

BGEs were prepared freshly using the above procedure, filtered to pass 0.45 μm (Millex®-HA, Millipore, Bedford, MA, USA) and degassed ultrasonically. Apart from the differences in the concentration of 1-butanol (LR, Ajax, Sydney), all BGEs contained 5 mM chromate (LR, Ajax, NSW, Australia) and 2.5 mM TTAB. Electrolyte pH was adjusted to the desired value with dilute sodium hydroxide (AR, BDH, Victoria, Australia).

6.2.1.4 Effect of Separation Voltage

The effect of separation voltage on anion separation selectivity was performed with a view to applying it to the analysis of iodide, iodate and thiocyanate in seawater. The BGE was therefore made up to contain 0.56 M NaCl, approximating the chloride

content in seawater. As the solutes of interest are UV-active, detection was performed in the direct mode at 214 nm (Zn lamp).

6.3 RESULTS AND DISCUSSION

6.3.1 EFFECT OF PROBE TYPE AND CONCENTRATION

Conditions and Detection

To compare the migration order of the four selected probes, the pH and concentrations were kept constant. Based on tetraborate, a pH of 9.5 was used for all four probes to ensure that any differences in migration order were not due to differences in pH. The concentration for all four probes was set at 10 mM, based on preliminary work using 5 mM tetraborate. For the tetraborate and nitrate probes, detection was performed at 214 nm using a zinc lamp whilst for the other two probes, detection was at 254 nm (Hg lamp). Although this difference in detection regimes meant varying sensitivities, it did not impede the study of separation selectivity effects.

Effect of Probe Type

Fig. 6.1 shows the effect of probe type on the selectivity of the anions in the mixture. Selectivity changes were plotted using relative migration times rather than mobilities because the EOF for trimellitate was not detected within 30 min. Migration times were normalised to chloride and not the bromide system peak as the latter was undiscernible with tetraborate.

Marked changes in selectivity are evident from Fig. 6.1. A possible cause for the migration behaviour of iodide and thiocyanate (lipophiles) was the effect of ionic strength. Subtle but important changes in migration order were noted with the nitrate

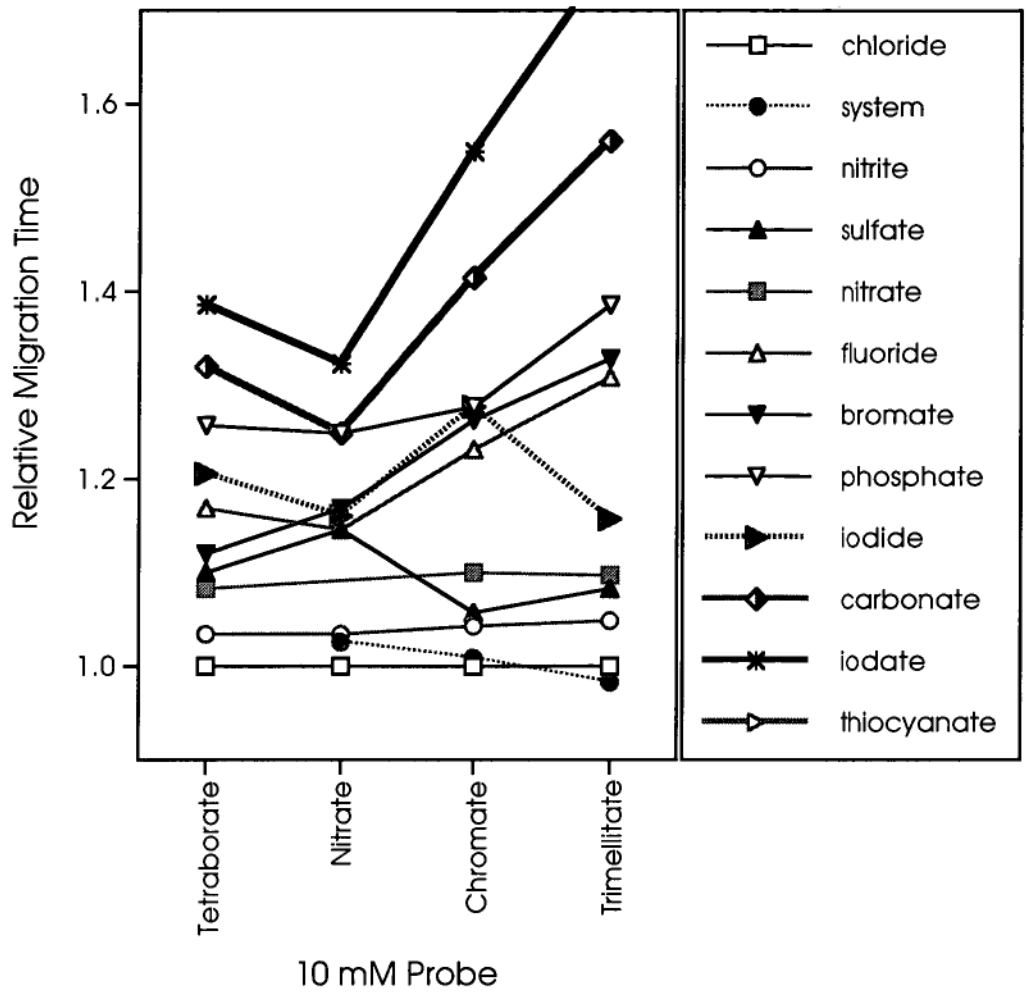

Fig. 6.1: Effect of probe type on separation selectivity of anions. All probes were 10 mM in concentration. Conditions: All electrolytes contained 2.5 mM TTAB at pH 9.0 ± 0.1. Sampling was in the hydrostatic mode (raised to 10 cm for 30 s) and detection was in the indirect mode at 254 nm for chromate and trimellitate and 214 nm for tetraborate and nitrate. Separation voltage was -20 kV. The capillary was of fused-silica (75 µm x 52 cm to detector x 60 cm total length). All times have been normalised to that of chloride. See Table 6.1 for concentration of anions.

BGE, and a change in migration order for bromate was noted when tetraborate was used as probe. Overall, resolution and sensitivity were best with trimellitate. Although it has generally been considered that migration orders are rarely affected by changes in probe type [49], Fig. 6.1 shows otherwise, at least under the conditions used for this study.

Although it was demonstrated that selectivity changes could be altered by using different probes, it is important to point out that the overall resolution of anions using tetraborate and nitrate as probes was not as good as the resolution with either chromate or trimellitate. This obviously means that the analytical utility of tetraborate and nitrate was limited, at least under the conditions used. On the other hand these may be suited to particular applications.

Effect of Probe Concentration

To investigate the effect of probe concentration on separation selectivity, chromate was used as the model probe. For most of the anions in the mixture, migration times increased with increasing chromate concentration (Fig. 6.2). This can be attributed to several factors including; (i) resistance [50] or reduction in migration rate due to 'stacking' [51] that occurs at high chromate concentration [23], (ii) reduction or compression of the double-layer [52] thereby reducing EOF velocity and ultimately increasing anion migration time. The double-layer is a function of probe concentration [53] and (iii) reduction in the surface charge density due to increased ionic strength of electrolyte causing 'negative' adsorption of surfactant on the capillary surface, as occurs similarly at high KCl and NaCl concentrations [54]. Although unlikely due to the efficient heat dissipation capacity of capillaries [55-57], it is nonetheless worth noting that Joule heat generated at elevated chromate concentration may cause the viscosity of the electrolyte to decrease. This would result in a decrease in migration time in accordance with Stoke's law [58] and other relationships [3, 23]. This effect was not apparent under the conditions used. Generally, resolution was better at higher concentration of chromate due to the EOF being reduced [3].

Against the trend discussed above, it can be seen that thiocyanate and iodide migrated faster with increasing chromate concentration so that selectivity changes for these two

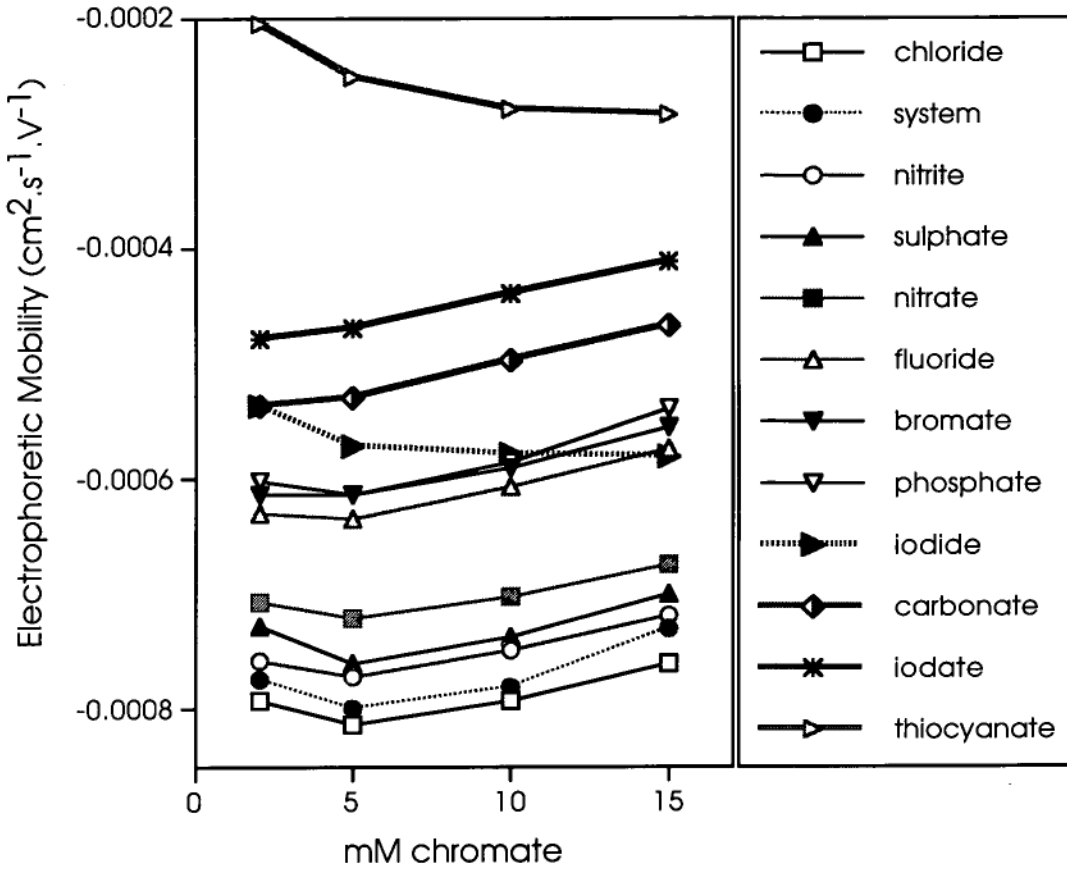

Fig. 6.2: Effect of chromate concentration on separation selectivity of anions. Apart from the differing chromate concentration, the conditions and key were as in Fig. 6.1. Electrophoretic mobilities were calculated relative to the 'plug' peak.

anions were marked. Both anions are lipophilic, so the effect must be related to factors influencing the formation of ion-pairs [8, 18, 45, 59] between these ions and the surfactant. Recently, Jimidar and Massart [7] have indicated that the likely mechanism for the selectivity changes noted for lipophilic anions was due to ion- exchange partitioning. This means that anions are in equilibrium with the surfactant as ion-pairs [7]. The equilibrium between the anions and the surfactant is a competitive process and the introduction of more chromate ions displaces the iodide and thiocyanate from their less charged ion-pair form with TTAB. Their release effectively yields electroactive

species of higher charge. It then follows that the more highly charged solutes will migrate faster, resulting in the selectivity changes noted above.

There was no change in the migration order of sulfate and nitrite at ≥ 7 mM chromate. This is in disagreement with changes noted elsewhere [60]. Also, it is interesting to note that at 5 mM chromate (the typical concentration used in CZE), the migration order for the singly charged halide anions did not follow trends expected from charge:mass ratios (assuming other variables are identical) where the expected order is fluoride, chloride, bromide and iodide. The actual migration order shows fluoride behaving anomalously. This can be explained as being due to the tendency of fluoride to 'form structures' through hydrogen-bonding. In an aqueous BGE, its small size allows it to acquire a relatively higher hydration volume, so that means that the charge:mass ratio of the hydrated fluoride is lower than anticipated. Although highly unlikely due to the capillary conditioning regime used, the formation of donor-acceptor complexes with silicon atoms [61] at the capillary surface can be an additional factor contributing to reduced fluoride migration rate.

Effect of Probe Concentration on Detectability and Resolution

Fig. 6.3 shows the effect of increasing chromate (as the model probe) concentration on the detector response for selected solutes. It shows that there is no quantitative advantage in using high concentrations of chromate since peak area and height responses decrease or level off at elevated chromate concentration. This is in agreement with the literature [7] and is attributable to the limitation defined by the linear dynamic range of the detector.

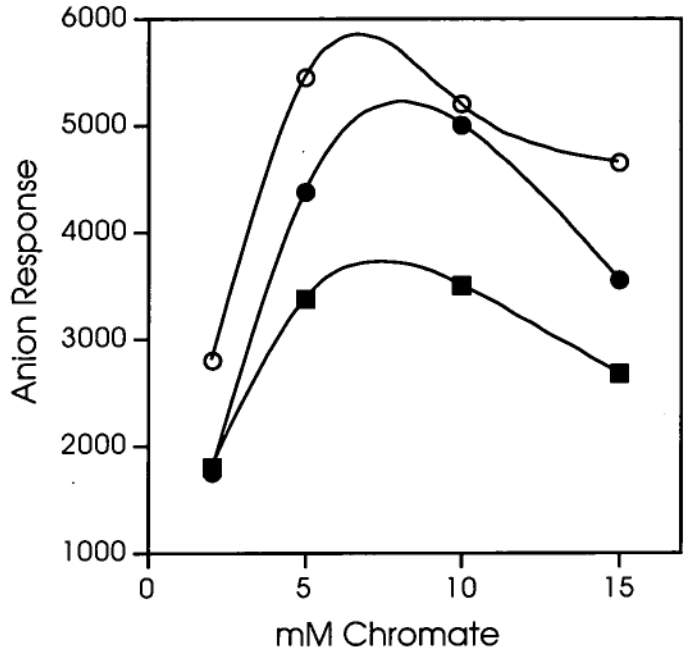

Fig. 6.3: Effect of chromate concentration on sensitivity. Conditions as in Fig. 6.1. Chloride and sulfate were used as the model solutes. Key: O = sulfate peak height (μV), ● = chloride peak height (μV) and ■ = chloride peak area (μV.s).

With real samples, resolution and sensitivity may be over-riding factors in the practical application of CZE. For such cases, it is worth noting that resolution is generally better at higher probe concentrations, but this is usually achieved at the expense of analysis time. Fig. 6.4 shows the effect of chromate concentration on EOF velocity and R_S between selected anion pairs (chloride-sulfate and iodide-carbonate). R_S is a function of N and the differences in anion electrophoretic mobilities and was calculated according to the equation of Ewing *et al.* [48]. For the calculations, N was set at a conservative 100,000 [11] as the intention was to show trends rather than obtaining absolute values. Although it is generally accepted that R_S increases with increasing carrier ion (probe) concentration, Fig. 6.4 shows that R_S also depends on the solutes in question. For the chloride-sulfate pair (i.e. strong acid anions), there was hardly any improvement in R_S. On the other hand, the resolution of the iodide-carbonate pair that co-migrated at 2 mM

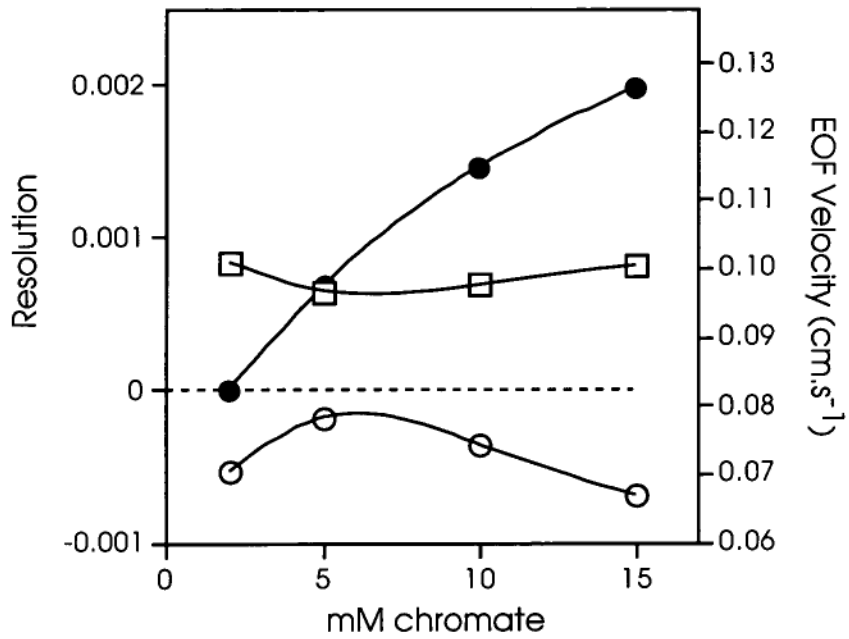

Fig. 6.4: Effect of chromate concentration on resolution of selected anion pairs and EOF migration rate. Conditions as in Fig. 6.1. Key: □ = resolution between chloride and sulfate, ● = resolution between iodide and carbonate and ○ = EOF velocity. The dotted line indicates region of zero resolution.

chromate improved in an approximately linear manner with chromate concentration. This behaviour, at least at ≥ 5 mM chromate, mirrors the reduction in EOF velocity in concert with generally accepted trends [3]. The anomalous EOF velocity at 2 mM chromate is presently unexplained. Finally, baseline noise was noted to be high at extremes of chromate concentration. At the low end, it may have been due to low dynamic reserve [19] whilst at the high end, temperature gradients due to Joule heat was a possible cause.

6.3.2 Effect of Electrolyte pH

Adjustment of pH

Due to possible interference in the separation of anions, it is important to choose carefully the agent (acid, base, buffer) for pH change. In indirect UV detection, the

anion associated with the agent can cause a negative dip[3] in the electropherogram, and hence can interfere with anions migrating nearby.

For this investigation, TRIS, CAPS, hydroxide and mineral acids were used to adjust the pH of the BGE. Fig. 6.5 shows the effect of hydroxide and TRIS as pH-altering agents on the separation of anions. TRIS caused a depression of the carbonate peak (Fig. 6.5 (b)), making it unsuitable for determinations requiring quantification of carbonate. On the other hand, TRIS can be useful where analysis of carbonate is not essential or where interference from carbonate needs to be minimised (e.g. Bayer liquor). CAPS was unsuitable due to generation of extraneous peaks, e.g. at ~5 min. (likely to interfere with thiocyanate) and at ~8 min. CAPS, TRIS and other buffers are essential where BGE needs to be buffered, e.g. in the analysis of polyprotic anions. TRIS is UV-transparent and would have minimal interference on the detection of anions. Additionally, TRIS may be good for probes like chromate that do not have a high buffering capacity. In an experiment involving monitoring of the changes in pH of BGE, a BGE with a starting pH of 9.43 dropped 0.6 pH units to pH 8.83 at the end of the same experiment.

Dilute hydroxide was selected as the agent of choice for adjustment of BGE pH. Although the obvious disadvantage of hydroxide is the introduction or absorption of ubiquitous carbon dioxide, the hydroxide is less likely to interfere with solute anions as it has the highest mobility and migrates well before the common anions. On a practical aspect it is noted that, although at high pH the hydroxide ion performs a cleaning and surface re-generation role by removing adsorbed cations from the capillary surface [3], care should be exercised as pH extremes can be detrimental to bare capillaries [27].

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3 Actually positive but appearing negative due to the reversal of the detector polarity.

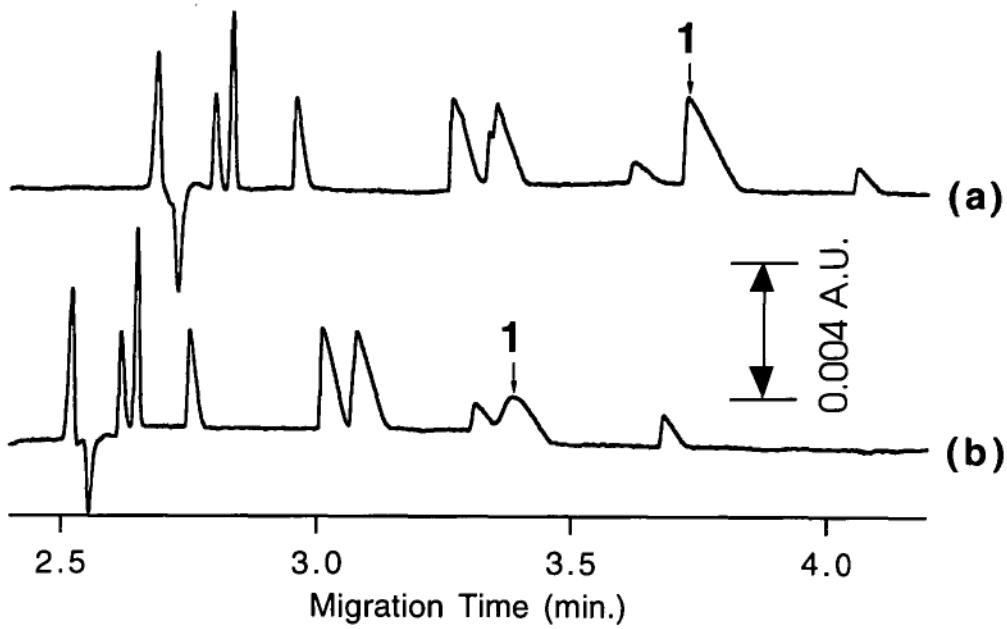


Fig. 6.5: Effect on carbonate response due to the use of hydroxide (a) and TRIS (b) as pH-altering agents. Conditions: 2.5 mM TTAB, 5 mM chromate, pH 9. Other conditions as in Fig. 6.1. Key: 1 = hydrogencarbonate.

Effect of the BGE pH on Separation Selectivity

Fig. 6.6 shows the effect on anion selectivity caused by variation of the pH from 8.09 to 12.67 for a BGE system with chromate and TTAB. Bicarbonate/carbonate showed marked changes for the pH range considered, going from position 10 at pH ~8 to position 6 at pH ~12.5. This is due to the change in charge from -1 (as bicarbonate at pH ~8) to -2 (as carbonate at pH >10.33). Obviously the changes are pronounced at or near the pK_a values where charge transitions occur, leading to a change in the charge:mass ratio of the solute. This behaviour is typical of weak acid or polyprotic anions [8]. In contrast, there was no change in the migration order of fluoride and phosphate at pH >11 in this study, unlike the migration order changes noted for these two anions elsewhere [60]. Changes in selectivity were not observed for the hydrogenphosphate anion as expected at pH 12.67 (third pK_a = 12.36 for phosphoric acid). This could be due to ion-pairing effects arising from the different amounts of

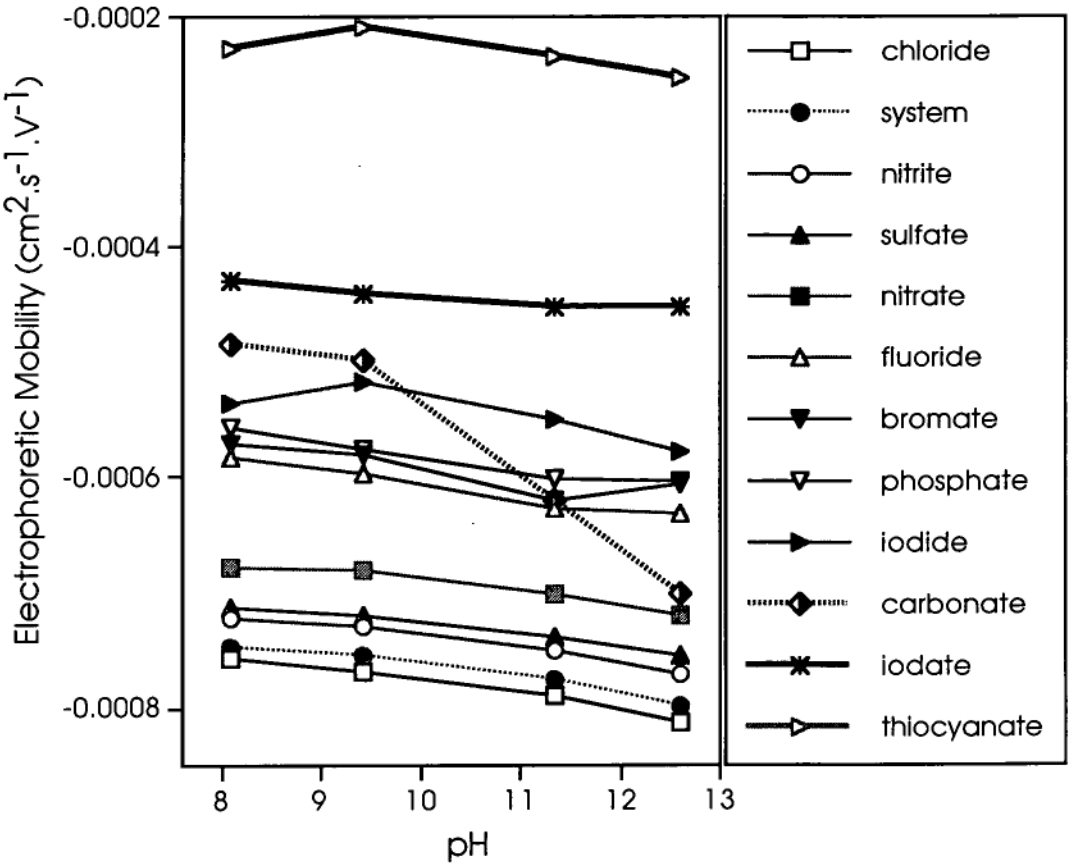


Fig. 6.6: Effect of the BGE pH on separation selectivity of anions. Conditions: All BGEs had 5 mM chromate and 2.5 mM TTAB. Dilute NaOH and HCl were used for pH adjustment. Sampling was in the hydrostatic mode (raised to 10 cm for 30 s) and detection was performed at 254 nm. Separation voltage was -20 kV. The capillary was of fused-silica (75 µm I.D. x 52 cm to detector x 60 cm total length). See Experimental for concentration of anions. Negative mobilities denote anodic migration.

TTAB used or errors in pH measurement caused by the presence of organic surfactant in the BGE [7].

Fig. 6.6 shows a general trend of reduced migration time at high pH due to the higher EOF generated with increasing pH. This is essentially due to a greater zeta potential at the capillary inner surface caused by a greater level of silanol dissociation [49] or an increase in the effective charge of the solute anions.

6.3.3 Effect of 1-Butanol as Electrolyte Additive

The type and concentration of organic solvents used as electrolyte additives in CZE vary widely and the choice of solvent will depend on the effects on detection, baseline noise, baseline drift, aqueous miscibility, etc. With 1-butanol, only small amounts have been recommended for anion separations [44]. For the present study, a maximum 1-butanol concentration of 7.5% v/v was used. Fig. 6.7 shows that increasing amounts of 1-butanol in the BGE caused major changes in the migration order of iodide, thiocyanate and bromate. Minor (but still important) selectivity changes also occurred for sulfate and nitrate. The possible causes for these migration order changes are discussed below.

Lipophilic Anions

The TTAB present in the BGE forms a positively charged double-layer at the capillary surface. The double-layer may be acting as a 'quasi' stationary phase and the migration of lipophilic anions may be retarded through the formation of ion-pairs or ion-exchange effects. Additionally, lipophilic anions may form solution-phase ion-pairs with the 'free' TTAB in the BGE. The strength of the interaction with the adsorbed TTAB depends, amongst other factors, on the TTAB concentration and charge density at the double-layer. When the available sites on the capillary surface are occupied by other species, such as the 1-butanol which forms a monolayer [44], the concentration and charge density of the TTAB 'quasi' stationary phase is reduced. This results in a reduction of the retardation effect and lipophilic anions like iodide and thiocyanate migrate faster - essentially because they are ionised [29] and their effective charges are increased by being freed from the ion-pair entity. This effect can be likened to the influence of increasing chromate concentration on lipophilic anions, as discussed in Chapter 5. For lipophilic anions present as ion-pairs with the 'free' TTAB in the BGE,

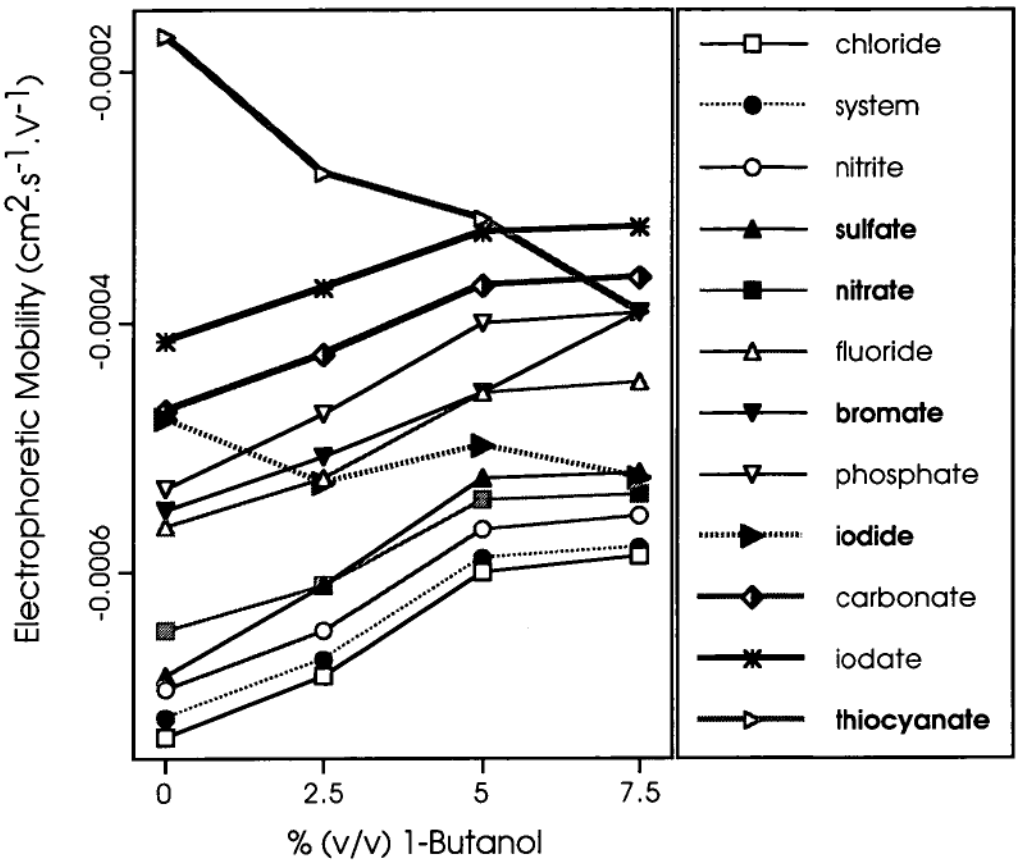


Fig. 6.7: Effect of 1-butanol on separation selectivity of anions. Conditions: Apart from the varying amounts of 1-butanol, all electrolytes contained 5 mM chromate, 2.5 mM TTAB at pH 8.03. Sampling was in the hydrostatic mode (raised to 10 cm for 30 s) and detection was in the indirect mode at 254 nm. For the separation voltage was -20 kV was passed through a polyimide-coated fused-silica capillary measuring 75 µm I.D. x 52 cm to detector x 60 cm total length. See Experimental for concentration of anions. Negative mobilities denote migration to the anode.

a partial suppression of the interaction between the surfactant and the solute anion(s) [41] may yield electroactive species with a higher effective charge - thereby the increased migration rate (e.g. see the trend of migration rate for thiocyanate in Fig. 6.7) in accordance with Stoke's law. Another possible cause for the increased migration rate of lipophilic anions is selective destruction of the water of hydration surrounding the anion [1, 40].

Other Anions

The reversal of the migration order of sulfate and nitrate in the presence of 1-butanol is similar to the results of Buchberger and Haddad [9], who used up to 30% v/v organic solvents. The likely cause for the reversal of the sulfate and nitrate migration order is an increase in the effective charge:mass ratio of nitrate through a selective reduction in the solvation shell [1, 38, 40, 43] surrounding the nitrate, or changes in ion association [40]. Changes in hydration volume as a cause for selectivity change is unlikely as these same changes would have been evident for fluoride and this was not observed.

The behaviour of bromate was interesting and showed a trend opposite to that of thiocyanate (and iodide). Since 1-butanol has been reported to adsorb onto the capillary surface [44] to form a monolayer, it was initially thought that the bromate was being slowed down by participating in some form of interaction (e.g. ion-exchange partitioning [7], distribution or sorption) with the 1-butanol on the capillary surface. In order to validate this notion, a 10 $\mu g.mL^{-1}$ bromate aqueous standard was separated with and without extraction with 1-butanol to see if there would be a reduction in the response of bromate after extraction with 1-butanol. A reduction which would have supported the case for bromate interacting with the 1-butanol was not recorded. On the contrary, the response for bromate was increased after extraction with 1-butanol which was indicative of a concentration effect, but more importantly, it did not give credence to the idea of bromate being slowed down as a result of interaction with the 1-butanol at the capillary surface. Solubility tests were then performed and noted that the bromate was more soluble in water than 1-butanol. All the preceding observations suggested that the altered selectivity of bromate due to its reduced migration speed with increasing concentration of 1-butanol was likely to be due to a reduced effective charge effect. For a hydrophilic solute being subjected to an electrolyte gradually decreasing in its

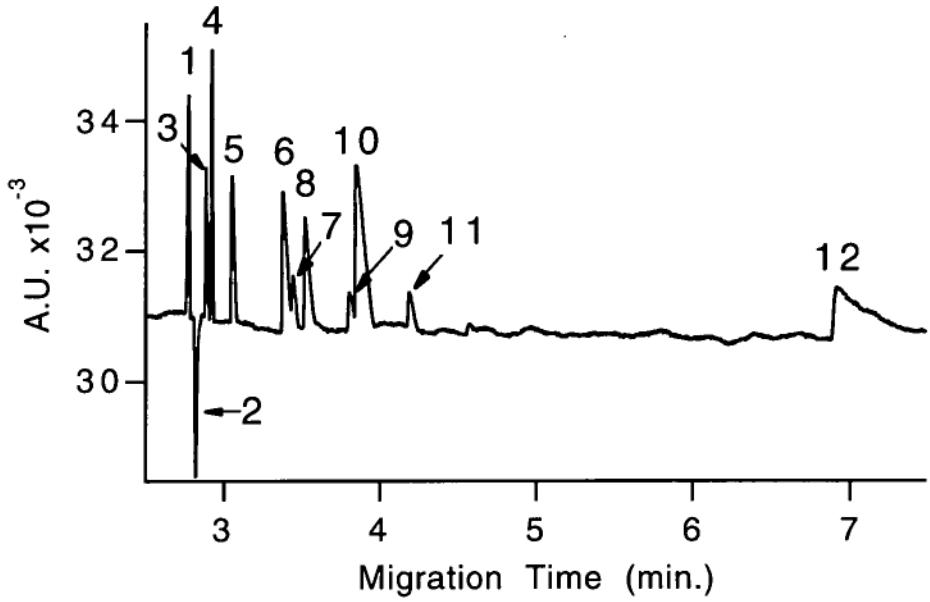
hydrophilicity, the reduced effective charge for bromate is suggested as being brought about by shifts in solubility and solvation equilibria [62, 63].

The EOF velocity decreased with increasing 1-butanol concentration in the BGE. This means either a reduction in the double-layer thickness or charge density of the double-layer and gives credence to the discussion (above) regarding the behaviour of iodide and thiocyanate. The electrophoretic mobilities for most of the anions reached a limit at 5% v/v 1-butanol. Above that, there was no significant improvement in resolution or selectivity changes, except for bromate and the lipophilic anions. At ≥5% v/v 1-butanol, it was likely that the EOF was governed mainly by the electrolyte viscosity. This means that the zeta potential and dielectric constant were diminished [39, 43]. Janini *et al.* [39] have shown that electrolytes with similar viscosities give similar EOF rates regardless of the concentration of organic modifier.

Effect on Migration Time and Resolution

Apart from its influence on anion selectivity, incorporation of 1-butanol in the BGE also resulted in a reduction of total separation time for the anions. Fig. 6.8 shows that the total separation time for the anions in the mixture was reduced from *ca.* 7 min. (without 1-butanol) to *ca.* 5 min. (with 5 and 7% v/v 1-butanol). This reduction in separation time differs from the observations of Benz and Fritz [44] who noted a general increase in migration times. However, closer observation of Fig. 6.8 shows that the migration times for the non-lipophilic anions actually increased and the reduction in analysis time was caused by a large decrease in time for thiocyanate. The migration time trends in this study indicated that separation time was a function not only of EOF velocity but also of ion-pairing effects and solute type.

(a) 0% v/v 1-butanol in electrolyte:



(b) 5% v/v 1-butanol in electrolyte:

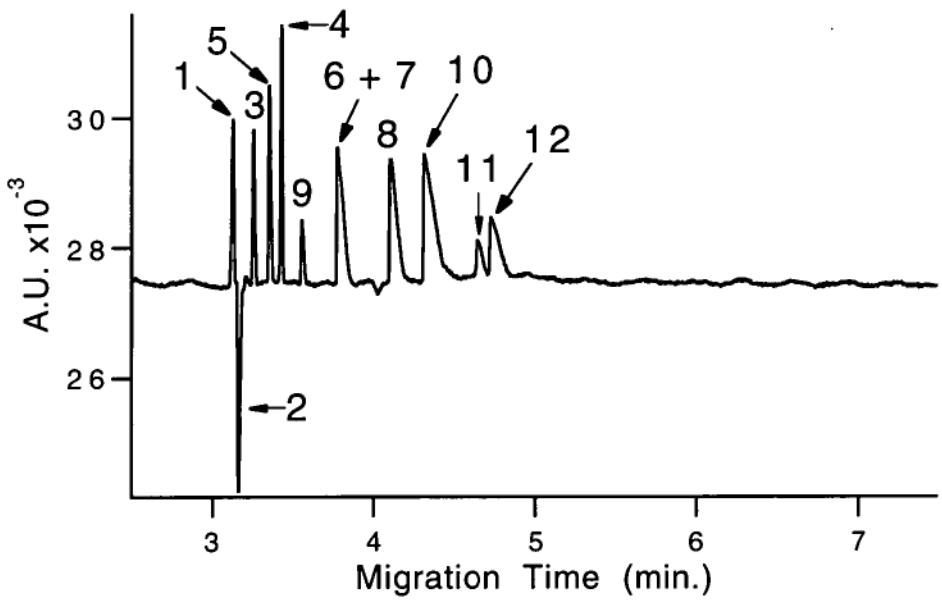


Fig. 6.8: Effect of 1-butanol concentration on separation time and resolution of anions in the standard mixture. Conditions: Both (a) and (b) as in Fig. 6.7 except (b) had 5% v/v 1-butanol added to the electrolyte. Key: 1 = chloride, 2 = system peak, 3 = nitrite, 4 = sulfate, 5 = nitrate, 6 = fluoride, 7 = bromate, 8 = hydrogenphosphate, 9 = iodide, 10 = hydrogencarbonate, 11 = iodate and 12 = thiocyanate.

Precision of Migration Time, Peak Area and Peak Height

Absolute migration time (AMT) imprecision is a major limitation of FRECZE. This appears to be due to unstable EOF which in turn is due to the dynamic variation in the double-layer formed by the accumulation of cationic surfactant(s) at the capillary-BGE interface. It has been indicated by Benz and Fritz [44] that the EOF is stabilised by the addition of small amounts of 1-butanol to the BGE. They have proposed the mechanism as dynamic adsorption of TTAB and 1-butanol onto the capillary surface. No precision data for the separated anions was presented in their work.

Determination of precision of AMTs, peak areas and peak heights was performed using an electrolyte containing 5% v/v 1-butanol since this gave the best overall separation (Fig. 6.8 (b)). A mixture containing chloride, sulfate and hydrogenphosphate was used. Precision of AMT, peak areas and peak heights are shown in Table 6.2 which shows that all values were <1.8% RSD ($n = 11$). Such precision compares well with best values from the literature, e.g. 1.5% RSD at $n = 10$ [23]. The excellent AMT precision was indicative of stable EOF rate and gives quantitative support to the observations of Benz and Fritz [44]. Peak area precision (Table 6.2) compared well with previously published values [64], except for hydrogenphosphate which is known to give variable response in FRECZE. Peak height precision also compared well with published values, e.g. 3% RSD [65]. The worst peak height precision in this study was 1.7% RSD ($n = 11$).

Limits of Detection

Detection limits (calculated at 3 x baseline noise) for BGEs with 0% and 5% 1-butanol (optimal concentration) are listed in Table 6.3. Overall, limits of detection (LOD) for the 11 anions separated with and without 1-butanol were comparable. The notable exceptions were iodide and thiocyanate which showed marked improvements in LOD.

Table 6.2

PRECISION OF ABSOLUTE MIGRATION TIME, PEAK AREA AND PEAK HEIGHT USING AN ELECTROLYTE CONTAINING 5% v/v 1-BUTANOL

Key: § = Absolute migration time (min.), ¶ = Peak area units were μV.s, ß = Peak height units were μV. * = The precision values for carbonate peak area and peak height were not calculated as the carbonate was ubiquitous in nature and was introduced by the use of hydroxide for pH adjustment and from absorption from the air.

ANION	% RSD REPRODUCIBILITY (n = 11)		
	AMT§	Peak Area¶	Peak Heightß
chloride	1.6	2.1	0.9
sulfate	1.6	1.1	1.3
hydrogenphosphate	1.7	4.0	1.7
hydrogencarbonate	1.8	- *	- *

This can be attributed to their increased migration rate, showing increased effective charge. Improvement in the LOD of bicarbonate using the BGE with 1-butanol was noted, although this could have been an artificial change caused by the relatively greater amount of hydroxide used to adjust BGE pH. The LOD for fluoride was reduced using the BGE with 1-butanol, due to co-migration with bromate.

6.3.4 EFFECT OF SEPARATION VOLTAGE

Separation voltage plays an important role in defining the migration rate of anions and in determining the separation 'time-window'. High voltages create higher field strengths thereby reducing migration time (see Eqn. 2.14 in Chapter 2). It is generally

Table 6.3

DETECTION LIMITS USING ELECTROLYTES WITH 0% v/v AND 5% v/v 1-BUTANOL AS ADDITIVE

ANION	LIMITS OF DETECTION ($\mu g.mL^{-1}$)	
	0% v/v 1-Butanol	5% v/v 1-Butanol
bromate	1.5	-
chloride	0.5	0.8
fluoride	0.5	0.6
hydrogencarbonate	1.6	0.4
hydrogenphosphate	1.3	1.4
iodate	3.1	4.0
iodide	4.4	2.4
nitrate	0.8	0.7
nitrite	0.8	0.6
sulfate	0.3	0.5
thiocyanate	3.1	2.7

considered that separation voltage does not influence the separation selectivity of anions. Although this consideration appears to hold under most conditions used in FRECZE for the separation of anions, Fig. 6.9 shows that separation voltage had an impact on the migration order of iodide (1), iodate (2) and thiocyanate (3). Being directly related to work on the determination of iodide, iodate and thiocyanate in seawater, the conditions used in Fig. 6.9 were vastly different from those discussed elsewhere in this thesis.

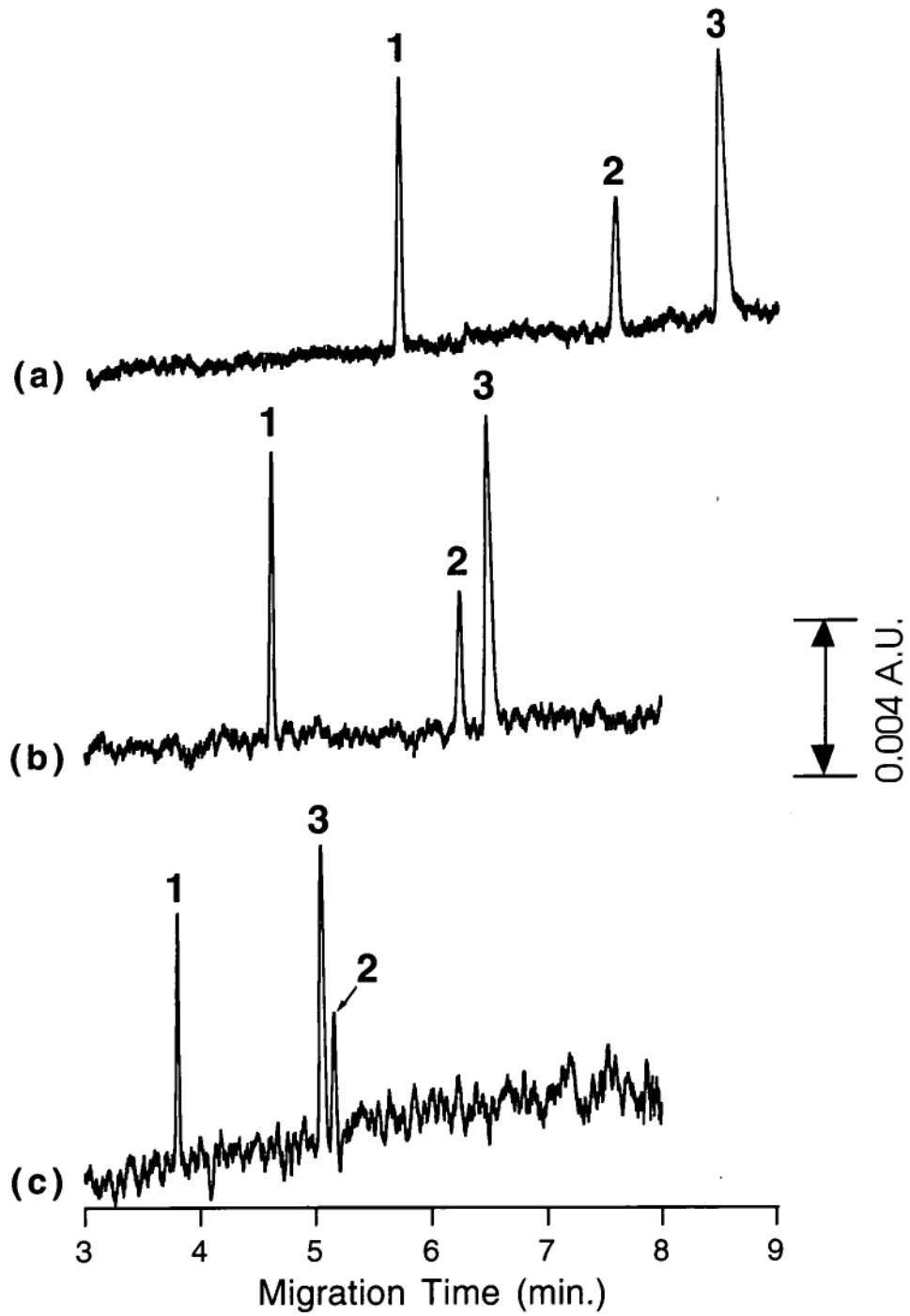


Fig. 6.9: Effect of applied voltage on separation selectivity of anions. Conditions: 560 mM NaCl, 90 mM LiCl, 8 mM TRIS and 10 mM cetyltrimethylammonium chloride. The pH was unadjusted. Sampling was in the hydrostatic mode at 10 cm for 30 s. Detection was performed in the direct mode at 214 nm. Separation voltage: (a) = -8 kV, (b) = -8.5 kV and (c) = -9 kV. Anions (all 10 $\mu g.mL^{-1}$): 1= iodide, 2 = iodate and 3 = thiocyanate.

The change in separation selectivity (as distinct from changes in resolution or migration time) of the solutes in Fig. 6.9 is interesting and almost certainly was an indirect effect resulting from variation of applied voltage. Before attempting to discuss possible causes for the behaviour in Fig. 6.9, it is instructive to firstly note that iodide and thiocyanate are lipophilic whilst iodate is hydrophilic. This means iodide and thiocyanate participate in ion-pair formation. Considering that solutes have to be solvated, the ionic atmosphere surrounding the ion-pairs (e.g. iodide-surfactant) would be relatively more diffuse than ions less prone to ion-pair formation.

As expected, migration times were shorter using higher applied voltage. This can be due to either the increased field strength or a reduction in BGE viscosity [47, 66] caused by temperature gradients at high field strength. The baseline noise was noted to increase with increasing applied voltage. Possible causes for the selectivity change in Fig. 6.9 are;

(i) ***Joule heating*** induced pH gradients [66]. This however seems unlikely as a 40 ^{o}C differential is needed to cause a change of 1 pH unit [30] and the Quanta 4000 instrument used was actively-cooled by fan forced convection. Additionally, the solute anions were singly charged (unlike the weak acids used elsewhere [30]) and any change in pH would have affected the EOF more than the anions.

(ii) ***Orientation effects*** [67] related to the shape (aspect ratio) and polarizability of anions do influence selectivity, especially for rod-shaped solutes. At high field strength, the anion is better aligned with the applied field and migrates faster, experiencing less hindrance or drag resistance. As the model anions were not rod-shaped, this factor seems less important.

(iii) ***Wien effect*** [67]. When the electroactive anion is separated at high field strength, it migrates rapidly through the BGE and the ionic atmosphere enveloping the anion is distorted [47]. The counterions (in this case the surfactant component of the ion-pairs between the surfactant and lipophilic anions) are not allowed to reform [67] and are sheared. This results in the anion not being shielded and hence assuming a higher net effective charge, which in turn increases the mobility. Considering that all three anions are singly charged and that the lipophilic anions are migrated as ion-pairs (see Chapter 5), the changes in effective charge due to the Wien effect would be more pronounced for iodide and thiocyanate relative to iodate. This possibly explains the change in migration order in Fig. 6.9. Although the contribution from a reduction in viscosity is unlikely due to the efficient heat dissipation properties of fused silica capillaries, the Wien effect ties in with the changes expected from Stoke's law (Eqn. 2.11 - Chapter 2). The vital factors being influenced under varying field strengths were effective charge and effective size.

(iv) ***Temperature induced effects*** on ion pairing. The formation of ion pairs between lipophilic anions and the surfactant is a weak association. It is therefore possible that in the presence of Joule heat, the weak association (bond) would be broken thereby allowing for the lipophilic anions to assume a higher mobility.

6.4 CONCLUSIONS

From the investigations executed above, the following conclusions were reached:-

- Changes in migration order of anions can be achieved by changing the probe ion (~10 mM). Although chromate was ideal as a general probe for inorganic anions, trimellitate may be more suited where greater sensitivity is required. Changes in

concentration of the probe caused pronounced selectivity changes as a result of increased effective charge on some anions (e.g. iodide).

- As expected, changes in separation selectivity due to pH variation were pronounced for the weak acid anions. With the aid of 1-butanol as a BGE additive, the useful pH range of the chromate-based BGE was extended by 1 pH unit (i.e. 20% increase in the accessible pH range). This made possible the improvement of resolution between fluoride and phosphate.

- Unlike other organic solvents that appear to affect only the EOF and migration time in CZE separations, 1-butanol was demonstrated to also influence the separation selectivity of anions. In addition to alteration of the double-layer [44], BGE viscosity appeared to play a part in reducing EOF in anion migration times. Changes in effective charge and charge:mass ratios were probably responsible for the migration order changes noted.

- Selectivity changes can be achieved as a result of indirect effects arising from variation of the separation voltage.

6.5 REFERENCES

1 Harrold, M.P., Wojtusik, M.J., Riviello, J. and Henson, P., *J. Chromatogr.*, **640** (1993) 463.

2 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **608** (1992) 386.

3 Salomon, K., Burgi, D.S. and Helmer, J.C., *J. Chromatogr.*, **559** (1991) 69.

4 Atamna, I.Z., Metral, C.J., Muschik, G.M. and Issaq, H.J., *J. Liq. Chromatogr.*, **13** (1990) 3201.

5 Stathakis, C. and Cassidy, R.M., *Anal. Chem.*, **66** (1990) 667 - 676.

6 Stathakis, C. and Cassidy, R.M., *J. Chromatogr. A.*, **699** (1995) 355.

7 Jimidar, M. and Massart, D.L., *Anal. Chim. Acta.*, **294** (1994) 165 - 176.

8 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **546** (1991) 445.

9 Buchberger, W. and Haddad, P.R., *J. Chromatogr.*, **608** (1992) 59.

10 Salomon, D.R. and Romano, J., *J. Chromatogr.*, **602** (1992) 219.

11 Jandik, P. and Jones, W.R., *J. Chromatogr.*, **546** (1991) 431.

12 Foret, F., Fanali, S., Ossicini, L. and Boček, P., *J. Chromatogr.*, **470** (1989) 299.

13 Kuhn, R. and Hoffstetter-Kuhn, S., Capillary Electrophoresis: Principles and Practice (Springer-Verlag, Berlin, 1993).

14 Atamna, I.Z., Metral, C.J., Muschik, G.M. and Issaq, H.J., *J. Liq. Chromatogr.*, **13** (1990) 2517.

15 Shamsi, S.A. and Danielson, N.D., *Anal. Chem.*, **66** (1994) 3757 - 3764.

16 Jandik, P., Jones, W.R., Weston, A. and Brown, P.R., *LC•GC*, **9**, no. 9 (1991) 634.

17 Romano, J., Jandik, P., Jones, W.R. and Jackson, P.E., *J. Chromatogr.*, **546** (1991) 411.

18 Jones, W.R., *J. Chromatogr.*, **640** (1993) 387.

19 Yeung, E.S. and Kuhr, W.G., *Anal. Chem.*, **63** (1991) 275A.

20 Nielen, M.W.F., *J. Chromatogr.*, **588** (1991) 321.

21 Ma, Y. and Zhang, R., *J. Chromatogr.*, **625** (1992) 341.

22 Ackermans, M.T., Everaerts, F.M. and Beckers, J.L., *J. Chromatogr.*, **549** (1991) 345.

23 Altria, K.D. and Simpson, C.F., *Chromatographia*, **24** (1987) 527.

24 Poppe, H., *Anal. Chem.*, **64** (1992) 1908.

25 Schwer, Ch. and Kenndler, E., *Chromatographia*, **30** (1990) 546.

26 Kenney, B.F., *J. Chromatogr.*, **546** (1991) 423.

27 Ng, C.L., Lee, H.K. and Li, S.F.Y., *J. Chromatogr.*, **598** (1992) 133.

28 Chang, H-T. and Yeung, E.S., *J. Chromatogr.*, **608** (1992) 65.

29 Jorgenson, J.W. and Lukacs, K.D., *Science*, **222** (1983) 266.

30 Whang, C-W. and Yeung, E.S., *Anal. Chem.*, **64** (1992) 502.

31 Burge, G.L., *Chemistry in Australia*, **59** (1992) 253.

32 Pospíchal, J., Deml, M., Gebauer, P. and Boček, P., *J. Chromatogr.*, **470** (1989) 43.

33 Boček, P., Deml, M., Pospíchal, J. and Sudor, J., *J. Chromatogr.*, **470** (1989) 309.

34 Sustácek, V., Foret, F. and Boček, P., *J. Chromatogr.*, **480** (1989) 271.

35 Wildman, B.J., Jackson, P.E., Jones, W.R. and Alden, P.G., *J. Chromatogr.*, **546** (1991) 459.

36 Cousins, S.M., Haddad, P.R. and Buchberger, W., *J. Chromatogr. A.*, **671** (1994) 397.

37 Oehrle, S.A. and Bossle, P.C., *J. Chromatogr. A.*, **692** (1995) 247.

38 Fujiwara, S. and Honda, S., *Anal. Chem.*, **59** (1987) 487.

39 Janini, G.M., Chan, K.C., Barnes, J.A., Muschik, G.M. and Issaq, Benz, H.J., *Chromatographia*, **35** (1993) 497.

40 Salimi-Moosavi, H. and Cassidy, R.M., *Anal. Chem.*, **67** (1995) 1067.

41 Masselter, S.M. and Zemann, A.J., *Anal. Chem.*, **67** (1995) 1047.

42 Colburn, J., Black, B., Chen. S-M., Demorest, D., Wiktorowicz, J. and Wilson, K., *Research News (Applied Biosystems)*, winter issue **1** (1990).

43 Wallingford, R.A. and Ewing, A.G., *Anal. Chem.*, **61** (1989) 98.

44 Benz, N.J. and Fritz, J.S., *J. Chromatogr.*, **671** (1994) 437.

45 Kaneta, T., Tanaka, S., Taga, M. and Yoshida, H., *Anal. Chem.*, **64** (1992) 798.

46 Harakuwe, A.H. and Haddad, P.R., 'Manipulation of separation selectivity for inorganic anions in capillary zone electrophoresis using control of electrolyte pH', *J. Chromatogr. A.*, accepted for publication.

47 Jones, H.K. and Ballou, N.E., *Anal. Chem.*, **62** (1990) 2484.

48 Ewing, A.G., Wallingford, R.A. and Olefirowicz, T.M., *Anal. Chem.*, **61** (1989) 292A.

49 Jandik, P. and Bonn, G., Capillary electrophoresis of small ions and molecules (VCH Publishers, New York, 1993), p. 262.

50 Huang, X., Gordon, M.J. and Zare, R.N., *Anal. Chem.*, **60** (1988) 375.

51 Beckers, J.L. and Ackermans, M.T., *J. Chromatogr.*, **629** (1993) 371.

52 Stevens, T.S. and Cortes, H.J., *Anal. Chem.*, **55** (1983) 1365.

53 Widmer, H.M., *Chimia*, **43** (1989) 134.

54 Sparnaay, M.J., The Electrical Double Layer (Pergamon Press, Oxford, 1972).

55 Gordon, M.J., Huang, X., Pentoney (Jr), S.L. and Zare, R.N., *Science*, **242** (1988) 224.

56 Jorgenson, J.W., *Trends Anal. Chem.*, **3** (1984) 51.

57 Goodall, D.M., Lloyd, D.K. and Williams, S.J., *LC•GC*, **8** (1992) 788.

58 Adamson, A.W., Physical Chemistry of Surfaces, 2nd edition (Interscience Publishers, New York, 1967), p. 93.

59 Pfeffer, W.D. and Yeung, E.S., *J. Chromatogr.*, **557** (1991) 126.

60 Gebauer, P., Deml, M., Boček, P. and Janak, J., *J. Chromatogr.*, **267**, no. 2 (1983) 455.

61 Altria, K.D., Simpson, C.F., Bharij, A.K. and Theobald, A.E., *Electrophoresis*, **11** (1990) 732.

62 Boden, J., Bächman, K., Kotz, L., Fabry, L. and Pahlke, S., *J. Chromatogr. A.*, **696** (1995) 321.

63 Bächmann, K., Boden, J. and Haumann, I., *J. Chromatogr.*, **626** (1992) 259.

64 Jones, W.R. and Jandik, P., *American Laboratory*, (June, 1990) 51 - 64.

65 Lux, J.A., Yin, H.-F. and Schomburg, G., *Chromatographia*, **30** (1990) 7.

66 Bondoux, G., Jandik, P. and Jones, W.R., *J. Chromatogr.*, **602** (1992) 79.

67 Grossman, P.D. and Soane, D.S., *Anal. Chem.*, **62** (1990) 1592.

CHAPTER 7

PRACTICAL APPLICATION OF SELECTIVITY EFFECTS DERIVING FROM THE SURFACTANT

7.1 INTRODUCTION

A practical application utilising selectivity effects deriving mainly from the surfactant(s) was investigated, namely the analysis of Bayer liquor for inorganic and organic acid anions. Alumina (Al_2O_3) is made from bauxite ore using the cyclic Bayer process patented in 1888 by Karl Josef Bayer. The alumina is then reduced electrolytically using the Hall-Héroult process [1] to make aluminium metal. Bayer liquors are by-products of these processes and are usually of high pH and ionic strength and contain anions such as sulfate, phosphate, chloride, fluoride, oxalate, malonate, silicate, succinate and formate [2, 3].

The analysis of anions in Bayer liquor is important industrially for two primary reasons, namely (i) toxicology and environmental impact monitoring and (ii) process monitoring (including quality control and optimisation of product yield and purity). On the environmental monitoring side, fluoride is a universal toxin affecting humans, plants and animals. Free and complex cyanides are also present [4] and their potential for harm must be considered. On the process monitoring side, sodium ions associated with anions interfere (i.e. indirectly) with alumina precipitation in the Bayer process,

increase liquor viscosity[1] and reduce oxalate stability, making it difficult for removal of the latter from process liquor [2, 5]. Precipitation of alumina is also inhibited by low amounts of gluconic and tartaric acids [5]. The rapid determination of fluoride is important for the determination of cryolite ratio ($NaF:AlF_3$) [4], with a ratio of 2-3 needing to be maintained for optimal operation [6].

Ion chromatography (IC) is the only technique comparable to FRECZE for simultaneous separation of multiple anions. However, the simultaneous and fully resolved separation of chloride, oxalate, sulfate, malonate, fluoride, formate, carbonate, phosphate, acetate and citrate in Bayer liquor using IC has yet to be published. IC is not routinely used to analyse Bayer liquor most probably due to co-elution of weakly retained species [1, 7]. The high ionic strength and pH of Bayer liquor make it an extremely difficult matrix to separate by IC without prior clean-up, for example by dialysis [8]. When injected directly, untreated samples reduce column life (e.g. as a result of precipitation of alumina on the suppressor [2]) and performance [9], the latter being due to disturbance of the acid-base equilibria in the system.

The high separation speed, power, quantitative accuracy, unique selectivity (orthogonal and complementary to IC), low cost (e.g. unlike IC columns, fused silica capillaries are cheap and can be re-generated using hydroxide), requirement for minimal amount of reagents, etc; make FRECZE an attractive method for the analysis of Bayer liquor. Literature related to the separation of anions in Bayer liquor using CZE is limited, but the separation of sulfate, oxalate, chloride, [2] and fluoride [10] has been reported. However the optimised simultaneous separation of chloride, sulfate, oxalate, malonate, fluoride, formate, carbonate, phosphate, citrate and acetate has not been reported

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1 This may be of some relevance to sampling in the hydrodynamic mode which is affected by solution viscosity.

previously. The main impediments appear to be inadequate resolution of a closely migrating cluster of anions comprising, amongst other anions, fluoride, phosphate and formate, as well as deterioration of the baseline with repeated analyses.

In all previous work on the analysis of Bayer liquor by CZE [2, 10], a single cationic surfactant species has been used to reverse the EOF. In Chapter 5, it was shown that certain selectivity effects in the separation of anionic solutes arose when a binary mixture of surfactants was incorporated in the background electrolyte (BGE). The objective of this investigation was to exploit these selectivity effects with a goal of achieving a fully resolved separation of the inorganic and organic ionic components of Bayer liquor using FRECZE.

7.2 EXPERIMENTAL

See Chapter 3 for details of instrumentation and general separation conditions. To obtain precise migration times, the capillary was conditioned according to conditioning regime no. 6 (see Table 4.3 in Chapter 4). The 'pregnant' Bayer liquor sample from Alcoa (Kwinana, Western Australia) was provided by Dr. Peter E. Jackson (Waters Millipore, Sydney). After a preliminary study of the effect of purge times on baseline stability, the time was set at between 5 - 8 min. For the acquisition of essential data, a purge time of 8 min. was used. For others (e.g. spiking for peak identification), <8 min. purge time was used. Peak positions were confirmed by spiking with known standards.

Standards

Standard stock solutions (1000 $\mu g.mL^{-1}$) of each of the analytes were made from AR grade sodium salts which had been dried at 100 °C overnight (except where indicated). The salts used were *d*-gluconate (Fluka Chemie AG, Switzerland), malonate (undried; LR, BDH, Poole, England), succinate (undried; LR, BDH, Poole, England), citrate (By-Products & Chemicals, Auburn, NSW, Australia), formate, acetate (anhydrous; Strem Chemicals Inc., Newburyport, MA, USA), tartrate (Mallinckrodt, St. Louis, MO, USA), phosphate ($Na_3PO_4.12H_2O$, undried), chloride (Rhône Poulenc, Victoria, Australia), nitrate, sulfate (May and Baker, Manchester, UK), fluoride (Rhône Poulenc, Manchester, UK) and carbonate (anhydrous). An oxalate standard solution was made by dissolving 0.3583g undried $H_2C_2O_4.2H_2O$ (Mallinckrodt, St. Louis, MO, USA) and titrating with NaOH to pH 6.1, followed by dilution to 200 mL. An adipate standard was made similarly by titrating 0.2535g adipic acid (LR) to pH 8.6 and dilution to 200 mL. Working standards between 1 and 10 $\mu g.mL^{-1}$ were made by appropriate dilution of the stock solutions.

Preparation of Background Electrolytes

BGEs were prepared daily using accurately weighed amounts of tetradecyltrimethylammonium bromide (TTAB) and/or dodecyltrimethylammonium bromide (DTAB) dried at 100 °C for 1 hour. After dissolution of the solid material and dilution to ~80% of final volume, the appropriate aliquot of 100 mM chromate was added and the pH adjusted to 9.0 ± 0.1 with HNO_3 or NaOH. Final dilution of the BGE was used to produce 5 mM chromate and the desired concentration(s) of surfactant(s). Obviously the BGE compositions after optimisation were different and are shown under the relevant figures.

7.3 RESULTS AND DISCUSSION

It was shown in Chapter 5 that the separation selectivity of anions using CZE can be manipulated by employing binary TTAB and DTAB mixtures in the BGE. Before extending this to the separation of Bayer liquor, separation using single surfactants was performed to provide a basis for comparison with the use of binary surfactant mixtures. Migration times were determined for the species anticipated to be present in the Bayer liquors (chloride, sulfate, fluoride, phosphate, carbonate, acetate, oxalate, malonate, formate, tartrate, succinate and citrate), as well as cyanide, adipate, gluconate and nitrate. The migration time of nitrate was important since nitric acid was to be used to adjust the BGE pH.

7.3.1 SEPARATION USING SINGLE SURFACTANTS IN THE BGE

Fig. 7.1 shows the separation of diluted Bayer liquor using 2.6 mM TTAB (a) and 2.6 mM DTAB (b) as single surfactants in the BGE. Both separations took under 5 min. to complete. However, the anions of interest were not all fully resolved in either case, although separation using TTAB was generally superior to that obtained using DTAB. The system peak due to the presence of bromide in the BGE was a potential cause for interference and was observed to be larger with TTAB than for DTAB under identical conditions.

In the separation using DTAB as the single surfactant, nitrate migrated between sulfate and oxalate and there was potential for the system peak to interfere with chloride, especially with inadequate dilution of sample. Fluoride, formate, succinate and tartrate were unresolved, and phosphate co-migrated with formate and succinate. For the separation using TTAB as the single surfactant, fluoride was resolved from the usual

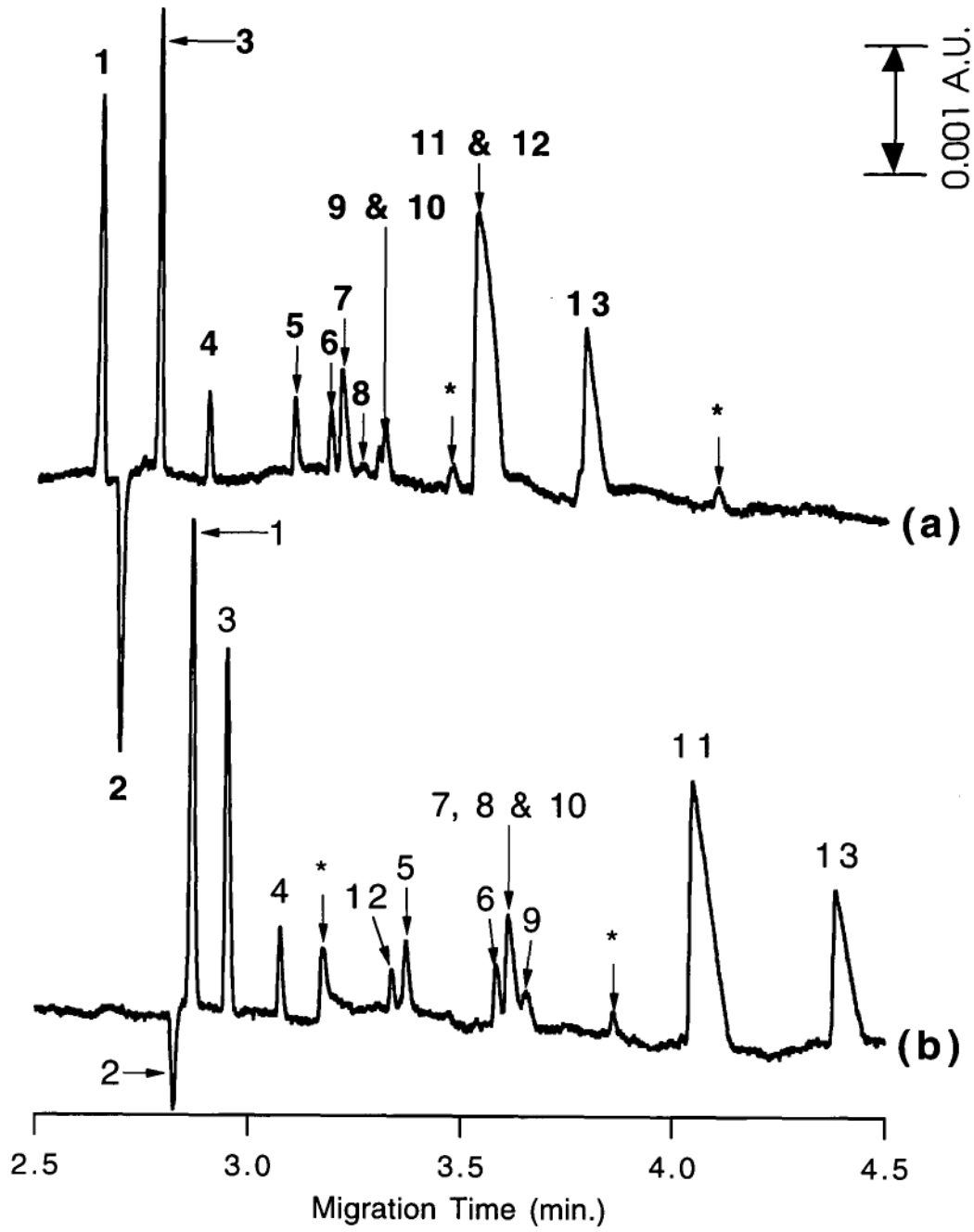

Fig. 7.1: Separation of 1:500 v/v diluted Bayer liquor using BGEs comprising (a) 2.6 mM TTAB and (b) 2.6 mM DTAB. Conditions: both BGEs had 5 mM chromate at pH 9.1. Injection was in the hydrostatic mode (raised 10 cm for 45 s) and detection was in the indirect UV mode at 254 nm. For the separation, -20 kV was applied across a fused silica capillary measuring 60 cm total length (52 cm effective length) and 75 μm I.D. was used. Peak identities: 1 = chloride, 2 = system (bromide), 3 = sulfate, 4 = oxalate, 5 = malonate, 6 = fluoride, 7 = formate, 8 = phosphate, 9 = tartrate, 10 = succinate, 11 = carbonate, 12 = citrate, 13 = acetate and * = unknown.

interfering anions (formate, tartrate, phosphate and succinate) and the separation of chloride, sulfate and oxalate compared well with that achieved by Grocott *et al.* [2]. The disadvantages evident with this BGE were the possible non-resolution of closely migrating adjacent anions (e.g. fluoride and formate), especially where a large disparity in concentration existed, poor resolution of succinate and tartrate, and the likelihood that elevated levels of carbonate would interfere with the determination of citrate. In addition, nitrate co-migrated with oxalate, making any adjustment of BGE pH with nitric acid unsuitable.

7.3.2 Separation Using Binary Surfactant Mixtures in BGE

DTAB and TTAB were used as mixtures because they exhibited different separation characteristics (see Chapter 5). Fig. 7.2 shows the migration order trends of anions using selected equimolar concentrations of DTAB and TTAB in the BGE. A number of selectivity effects were evident, but the general trend was that the migration times were lowered as the total concentration of the surfactant was increased. Binary combinations of these two surfactants at which the separation between fluoride and adjacent anions (e.g. phosphate) were best was viewed as the key in attempting to achieve the fully resolved separation of chloride, oxalate, sulfate, fluoride, formate, malonate, phosphate, carbonate and acetate (nine anions) in Bayer liquor. Based on this premise, the following combinations of TTAB:DTAB (in mM) were chosen; (i) 0.25:0.25, (ii) 1:1, (iii) 3:3 and (iv) 5:0.5-5.

Separation Using 0.25:0.25 & 1:1 TTAB:DTAB Combinations

Fig. 7.3 (a) shows the separation of diluted Bayer liquor using a BGE containing 0.25 mM TTAB and 0.25 mM DTAB. Fig. 7.3 (b) shows the separation of the same sample using a BGE with 1 mM TTAB and 1 mM DTAB. Apart from the change in citrate and

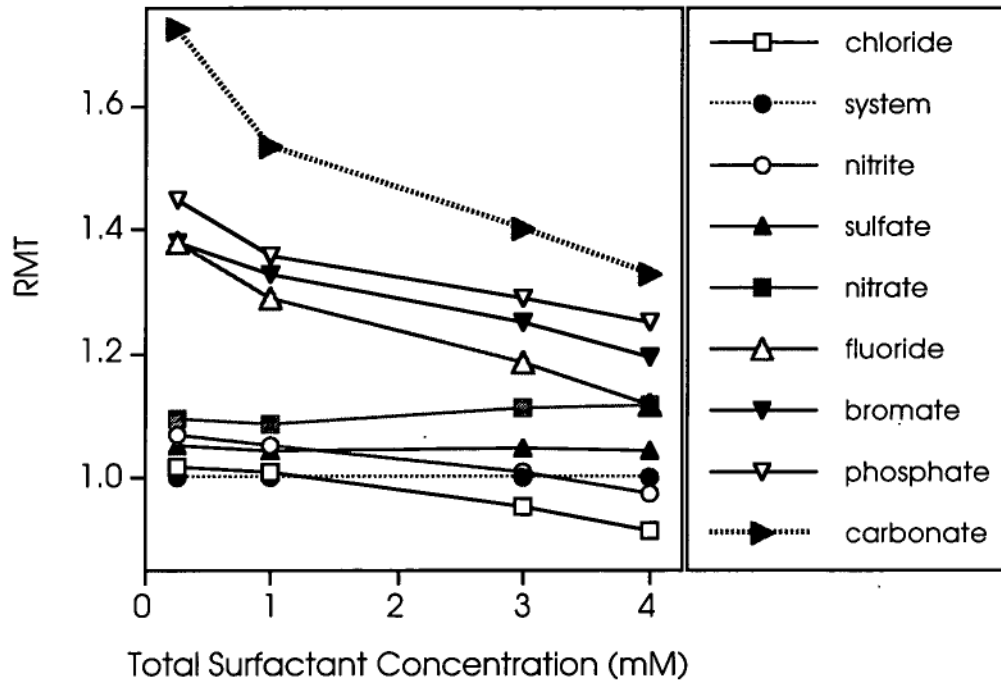

Fig. 7.2: Effect of equimolar combinations in BGE on separation selectivity of inorganic anions. Conditions: Apart from differences in the concentrations of DTAB and TTAB, the other conditions were as in Fig. 7.1. Migration times were normalised to the system peak.

tartrate migration order, the separations were almost identical and both were similar to the separation achieved by Vanderaa and Haddad [10] using single surfactants at typical concentrations. Selectivity with both surfactant combinations was such that nitrate migrated between sulfate and oxalate. Both combinations were rejected due to:

(i) Inadequate resolution of some solutes, e.g. malonate/citrate and fluoride/tartrate/formate/succinate. Phosphate, if detectable, would have migrated within the latter cluster at the formate position.

(ii) Possible detrimental effects on chloride quantification due to splitting by the system peak.

(iii) Noisy baseline.

(iv) Adjustment of BGE pH with nitric acid was unsuitable.

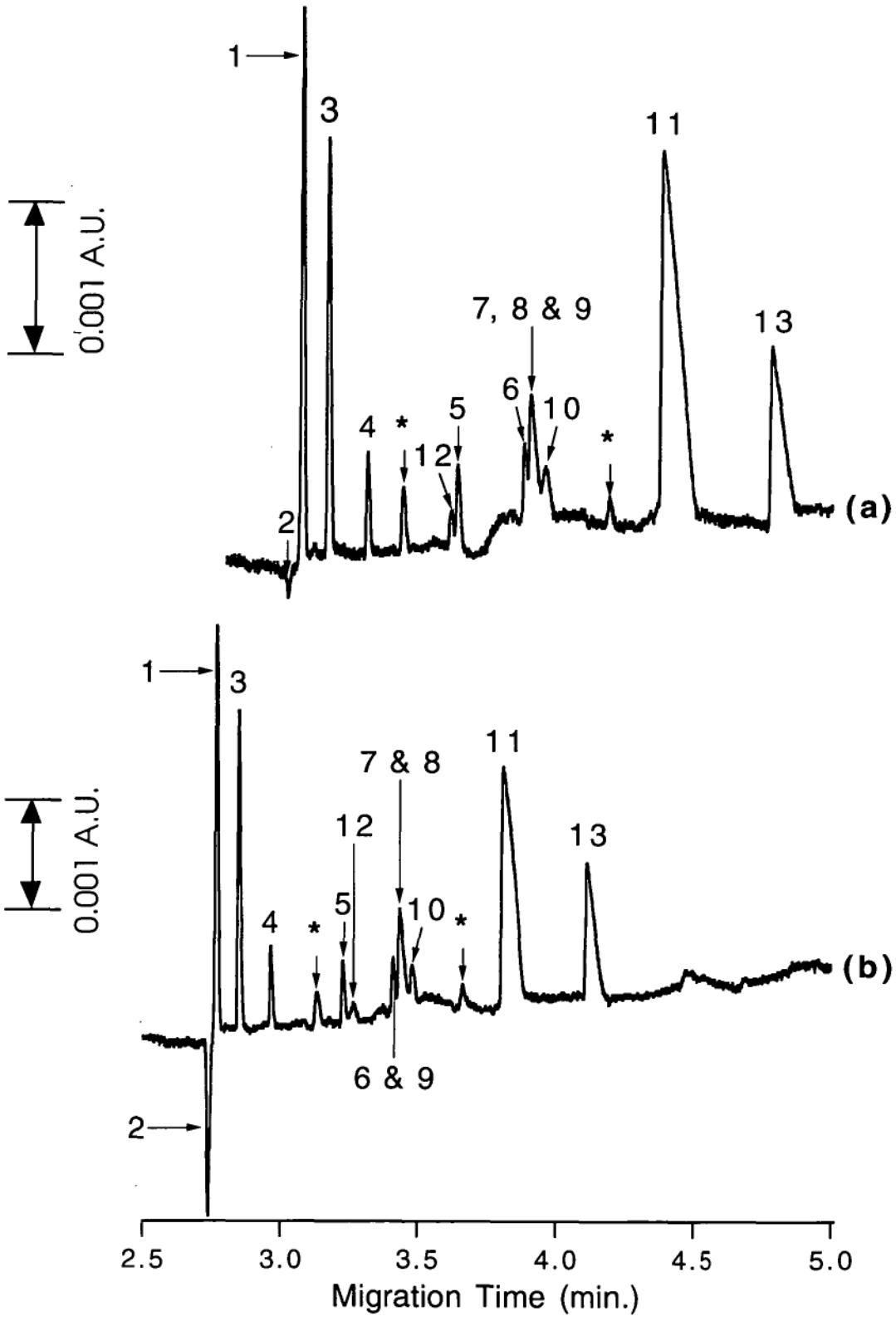

Fig. 7.3: Separation of Bayer liquor using BGEs containing 0.25:0.25 (a) and 1:1 (b) TTAB and DTAB. The other conditions and key were as in Fig. 7.1.

The unidentified peak detected after oxalate using the above combinations as well as the single 2.6 mM DTAB (Fig. 7.1 (b)) was potentially significant. At this stage, spiking with known standards indicated that it was none of the nine anions nor the following; nitrate, iron (III) cyanide, iron (II) cyanide, cyanide, AlF_3, citrate, tartrate, succinate, adipate or gluconate.

Separation Using 3:3 TTAB:DTAB and Optimum 1

Fluoride and malonate were poorly resolved when using a BGE with 3 mM TTAB, 3 mM DTAB and 5 mM chromate. The poor resolution between fluoride and malonate was improved by increasing the chromate concentration to 7.5 mM (Fig. 7.4) - i.e. by EOF reduction. At 7.5 mM chromate, all nine anions were resolved and the conditions used were identified as optimum 1 (Fig. 7.5). With adequate flushing, it was not necessary to use buffering additives in the BGE, e.g. Methyl Z-1 [2] and TRIS. Although not impacting upon resolution and migration order of anions, TRIS offered no real improvement for the separation of the nine anions of interest other than making the carbonate peak depressed but more Gaussian in shape.

Separation Using 5:0.5 - 5:5 TTAB:DTAB Combinations

The last TTAB:DTAB combinations (in mM) considered were 5:0.5, 5:1, 5:2, 5:3, 5:4 and 5:5. The 5:5 combination was disregarded based on possible unsatisfactory resolution between fluoride and sulfate, as suggested by Fig. 5.4 in Chapter 5. Separations were then performed using the remaining combinations of TTAB and DTAB. The 5:4 combination was unsatisfactory due to (i) splitting of the sulfate by the system peak, (ii) migration of nitrate at the fluoride position, (iii) co-migration of oxalate and formate, (iv) co-migration of succinate, tartrate and carbonate and (iv) possible interference of acetate by adipate. It was noted that at the 5:4 combination, there was a further change in the fluoride selectivity, i.e. it migrated before oxalate

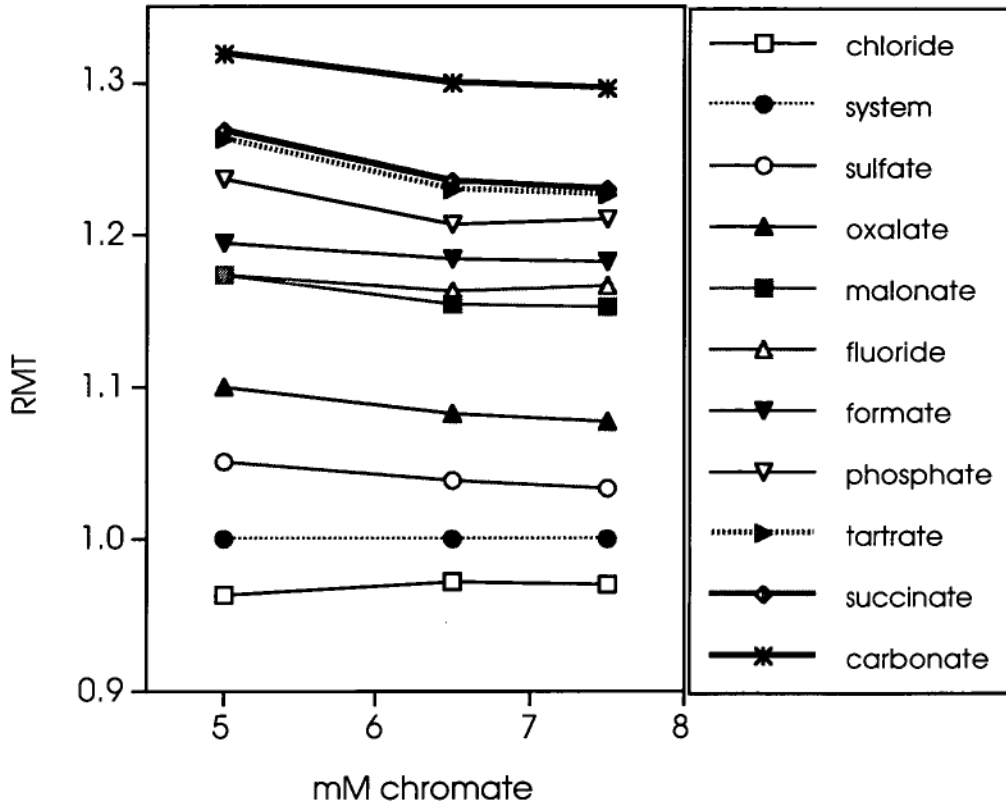

Fig. 7.4: Effect of chromate concentration on resolution using BGEs containing 3:3 mM TTAB and DTAB. Conditions were as in Fig. 7.1 except sampling time was 30 s in the hydrostatic mode and the pH of the BGE was 8.8. Migration times were normalised to the system peak.

The 5:3 combination was rejected due also to reasons (i) - (iv) shown above for the 5:4 combination, whilst the 5:2 combination was unsatisfactory due to the co-migration of fluoride and oxalate.

The more promising combinations of TTAB and DTAB were 5:1 and 5:0.5. The latter was rejected because (i) succinate and tartrate were unresolved, (ii) the baseline was noisy, (iii) formate and malonate were likely to exhibit inadequate resolution at high and disparate concentrations and (iv) nitrate migrated at the fluoride position. The selectivity using the 5:1 combination was such that a large disparity between oxalate

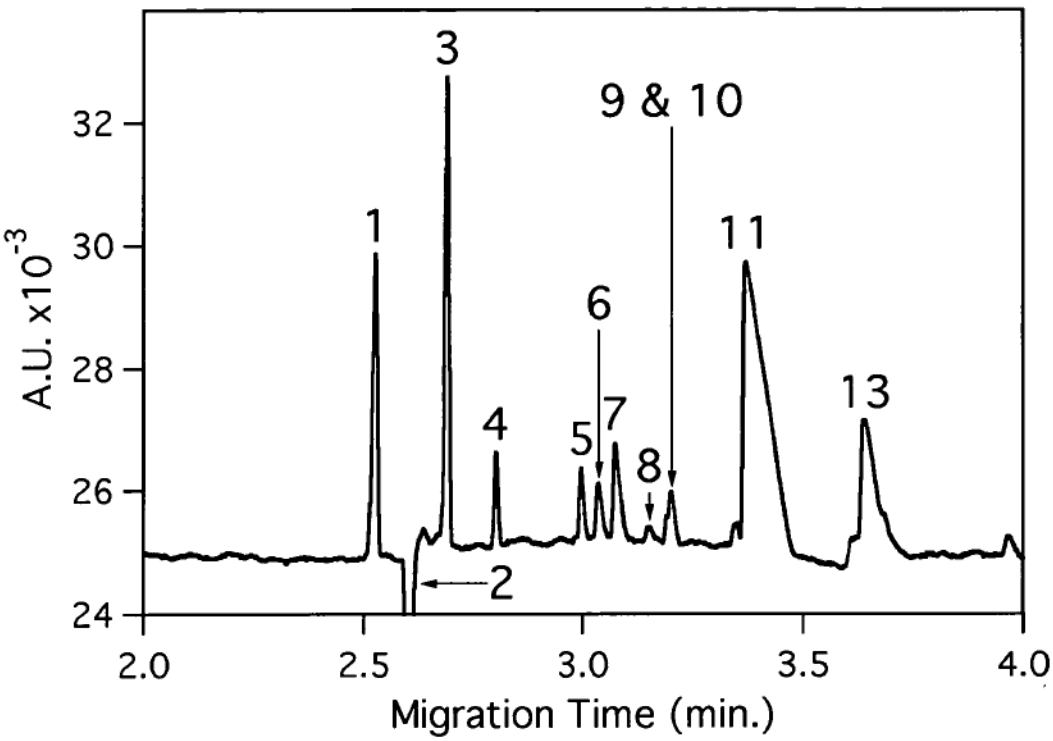

Fig. 7.5: Separation of 1:500 v/v diluted Bayer liquor using BGE with 3 mM each of TTAB and DTAB, 7.5 mM chromate and pH 9 (optimum 1). The other conditions and key were as in Fig. 7.1.

and fluoride was likely to cause resolution problems. The 5:1 TTAB:DTAB combination was therefore optimised in a manner similar to optimum 1 with regard to chromate concentration in order to improve resolution and sensitivity (Fig. 7.6). The conditions used for Fig. 7.6 were designated as optimum 2 and were applicable for the separation of nine anions. It was noted that the selectivities of the two optima were different. (Compare Figs. 7.5 and Fig. 7.6.)

Sensitivity and Quantification

Quantification depends on, amongst other factors, detection sensitivity and the amount of sample injected. As the dynamic reserve (or background noise) governs sensitivity

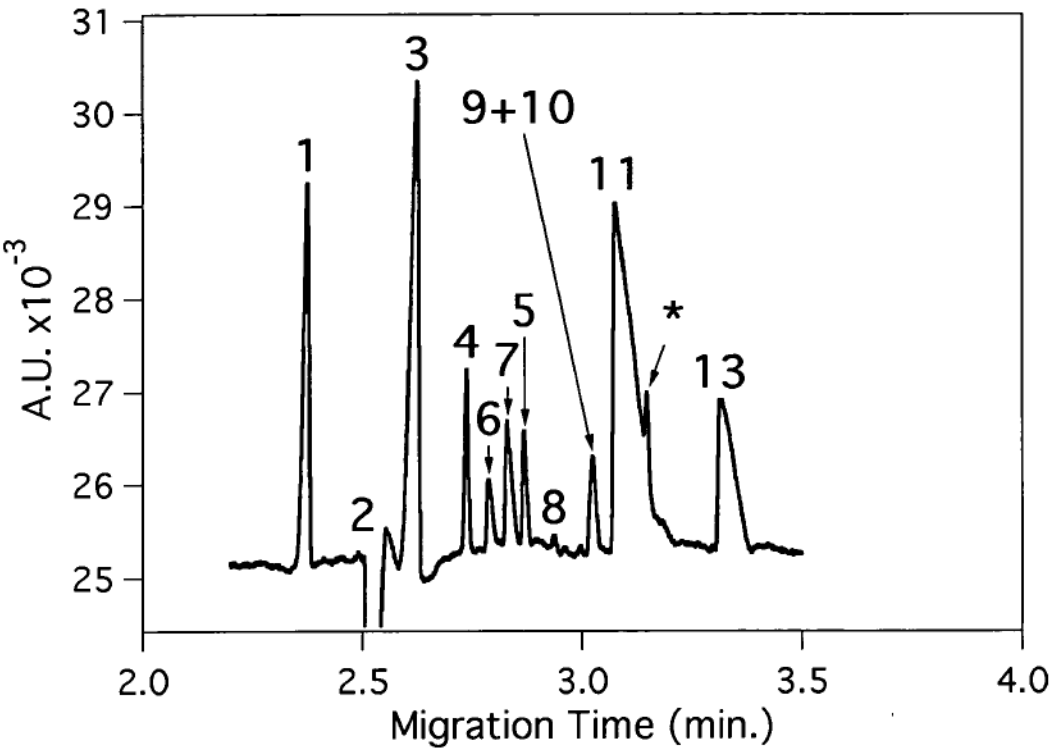

Fig. 7.6: Separation of 2:500 v/v diluted Bayer liquor using BGE with 5 mM each of TTAB and 1 mM DTAB, 5.5 mM chromate and pH 9 (optimum 2). The other conditions and key as in Fig. 7.1 except * = unknown (possibly citrate).

in indirect UV detection, many problems arise when complex samples are separated [11].

With hydrodynamic injection, the sample matrix and viscosity will affect the amount of sample introduced into the capillary. High Na^+ concentration raises the viscosity of Bayer liquor [2] and may affect the amount of sample loaded using hydrostatic injection. High ionic strength also interferes significantly with system peak response. All these factors underline the need for maximum dilution without going below the detection limits of the ions of interest. For this investigation, a dilution of 1:500 was found to be optimal.

Using chloride as a model solute, Fig. 7.7 shows that detectability decreased with increasing (total) surfactant concentration. Optima 1 and 2 were at 3:3 and 5:1 TTAB and DTAB ratios, however the detectability was relatively low at those points. Detectability could be improved easily by increasing the probe concentration. However migration time was increased and resolution was not necessarily improved as expected from the reduction in anion mobility due to the increase in probe concentration. A compromise between the desired selectivity (and resolution) and detectability was more appropriate. For Bayer liquor, selectivity took precedence over detectability as the latter could easily be enhanced by using a less dilute sample.

Analytical Performance

The analytical performance of the two optima was then assessed and the results are summarised in Table 7.1. For optimum 1, peak area precision was better than 6.6% RSD (except for fluoride, phosphate and carbonate). The high (~22%) RSD for carbonate peak area was due to the absorption of carbon dioxide from air whilst fluoride complexed strongly with iron and aluminium (present in Bayer liquor) and low or variable values can be expected [10]. Phosphate was not detected at the 1:500 v/v dilution used. The variable response of phosphate appeared to be a function of capillary conditioning regimes (see Chapter 4). The detection limits (3 x baseline noise) for chloride, sulfate, oxalate, formate, malonate and acetate were ≤ 1 $\mu g.mL^{-1}$. All calibration curves gave linear correlation coefficients (r^2) of $\geq 99.5\%$ except for acetate and phosphate. All recoveries were satisfactory except for phosphate (88%).

For optimum 2, peak area precision was better than 7.8% RSD (except for fluoride and carbonate). Again, carbonate had poor precision with respect to peak area (~22% RSD). Since phosphate was not detected at 1:500 dilution, lower dilutions were used for quantification. Calibration curves of peak area versus concentration for chloride,

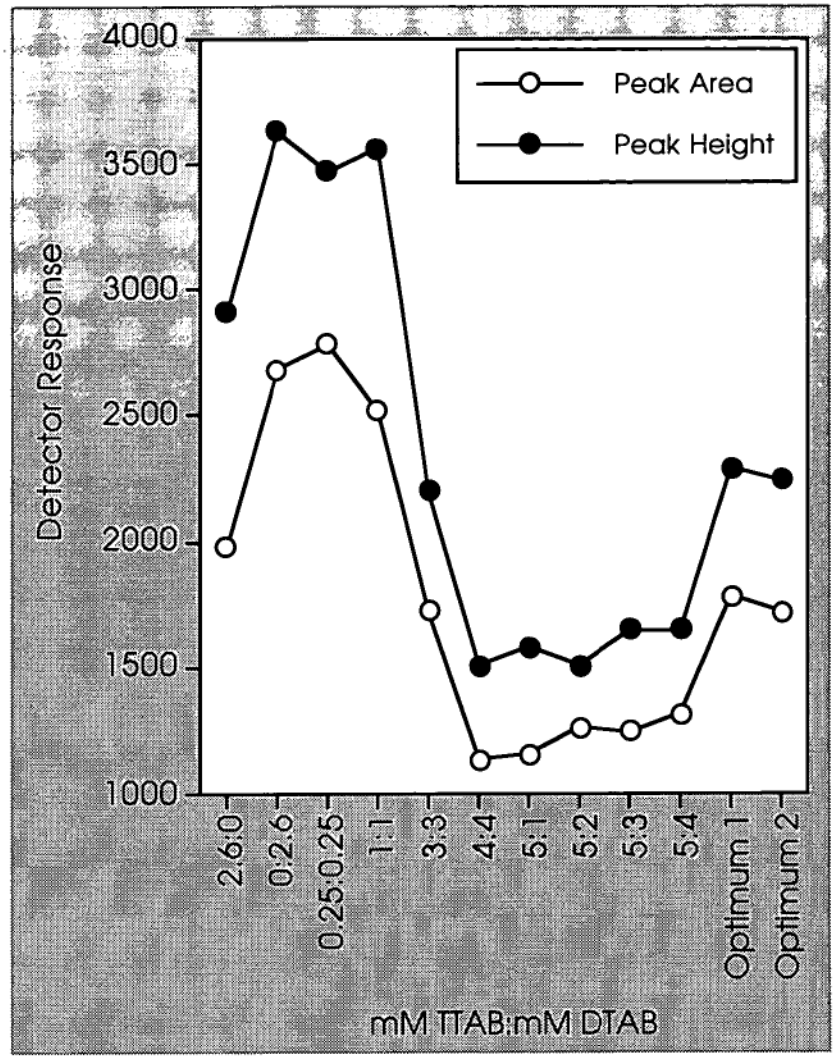

Fig. 7.7: Effect of TTAB and DTAB combinations in the BGE on detector response for chloride. All BGEs contained 5 mM chromate, except for optima 1 and 2. Other experimental conditions were as in Fig. 7.1.

sulfate, oxalate, malonate, formate, phosphate and acetate were linear ($r^2 \geq 99.6\%$) between 1 and 10 $\mu g.mL^{-1}$. The low (71%) recovery of sulfate illustrated the interference of the system peak, especially at low sample dilution.

The two optima shown in Figs. 7.5 and 7.6 allowed for some separation flexibility to be exercised, especially when there were large disparities in concentration between fluoride and adjacent anions. The two optima were also ideal for the separation of

Table 7.1

SUMMARY OF ANALYTICAL PERFORMANCE PARAMETERS USING OPTIMA 1 AND 2

Key: RSD = relative standard deviation, n = number of replicates, 1 = optimum 1, 2 = optimum 2, n.d. = not detected, § = absolute migration time and * = 1:50 dilution used for quantification of phosphate. Detection limit was calculated at 3 x S:N ratio.

ANION	Area % RSD (n)		AMT§ % RSD (n = 10)		Correlation Coefficient (r^2)		Concent-ration ($\mu g.mL^{-1}$)		% Recovery (n)		Limit of Detection ($\mu g.mL^{-1}$)	
	1	2	1	2	1	2	1	2	1	2	1	2
Chloride	1.5 (8)	1.7 (10)	0.27	1.5	99.8	99.6	4.1	4.3	107 (6)	109 (2)	0.14	0.26
System	-	-	0.27	1.6								
Sulfate	2.7 (10)	1.7 (10)	0.27	1.5	100.0	99.8	4.4	5.1	108 (4)	71 (3)	0.11	0.22
Oxalate	3.4 (8)	2.0 (8)	0.30	1.5	99.9	99.9	0.8	0.9	100 (6)	104 (4)	0.11	0.16
Formate	4.8 (6)	4 (10)	0.29	1.6	99.6	99.5	1.4	1.5	99 (6)	98 (4)	0.15	0.29
Fluoride	8.6 (10)	6.4 (8)	0.32	1.7	99.9	100.0	0.4	0.4	106 (3)	109 (3)	0.09	0.17
Malonate	6.6 (10)	5.5 (5)	0.31	1.6	99.8	99.7	0.9	1.0	103 (6)	99 (4)	0.16	0.23
Phosphate*	n.d.	n.d.	n.d.	n.d.	99.6	98.5	0.1	0.4	87.8 (6)	95 (6)	0.07	0.88
Carbonate	21.7 (10)	20.6 (10)	0.33	1.6	-	-	-	-	-	-	-	-
Acetate	4.0 (8)	7.8 (8)	0.38	1.8	99.6	96.4	5.0	4.7	95 (6)	113 (2)	0.34	0.58

additional anions not shown in the two figures (e.g. nitrate, adipate, citrate and gluconate). Variations in the concentration of chromate in the BGE could be used to fine-tune the separation where necessary. The obvious common disadvantage of both optima was the poor resolution of tartrate and succinate.

Precision of Migration Time

As discussed in Chapter 4, the poor precision of migration time is currently a limitation of CZE. The use of binary surfactant mixtures made it possible for excellent precisions of migration time to be achieved for both optima. Fig. 7.8 and Fig. 7.9 show that absolute migration times were stable from the first run. Considering that the data in Figs. 7.8 and 7.9 were obtained using actual Bayer liquor samples, the performance of the two optima can be considered as exemplary.

Interferences and Limitations

The two identified optima were limited by the inability to resolve tartrate and succinate. Furthermore, non-resolution of adjacent anions was a possibility at high and disparate concentrations. Both optima were unsuitable for the determination of free cyanide. Any cyanide present was 'masked' by the large system peak due to bromide since their mobilities were similar. Furthermore, any free cyanide was broken down to formate under the alkaline conditions used, as confirmed by spiking. This also ruled out the possibility of using cyanide as a complexing agent to release bound forms of other anions, e.g. fluoride and chloride present as complexes of iron and aluminium.

7.4 SUMMARY

The aim of this investigation was achieved with the successful simultaneous separation of chloride, sulfate, oxalate, malonate, fluoride, formate, phosphate, carbonate and

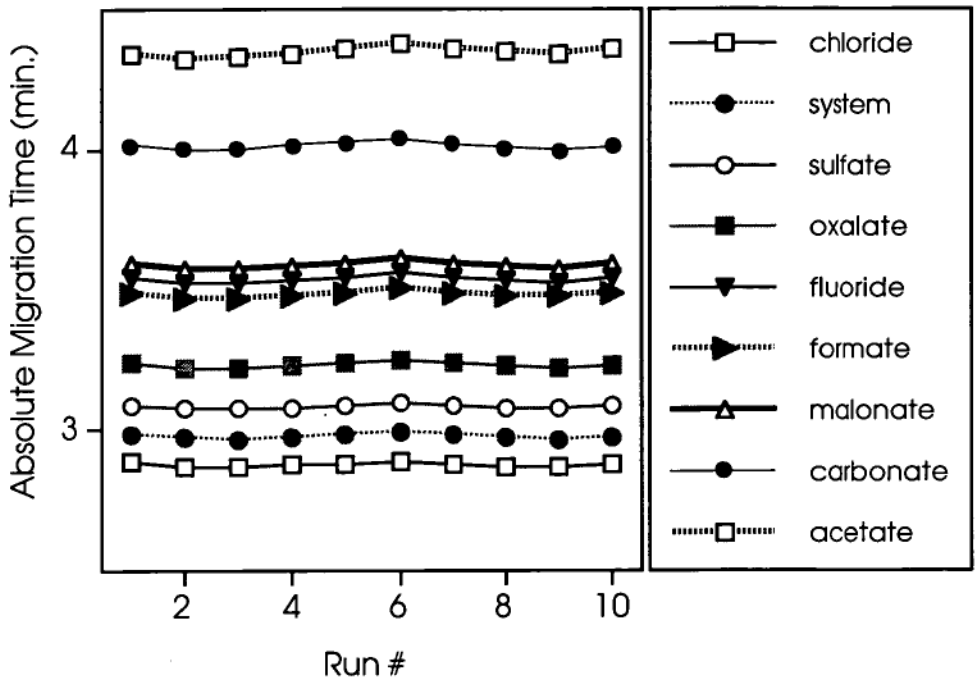

Fig. 7.8: Precision of absolute migration time for anions in Bayer liquor using optimum 1.

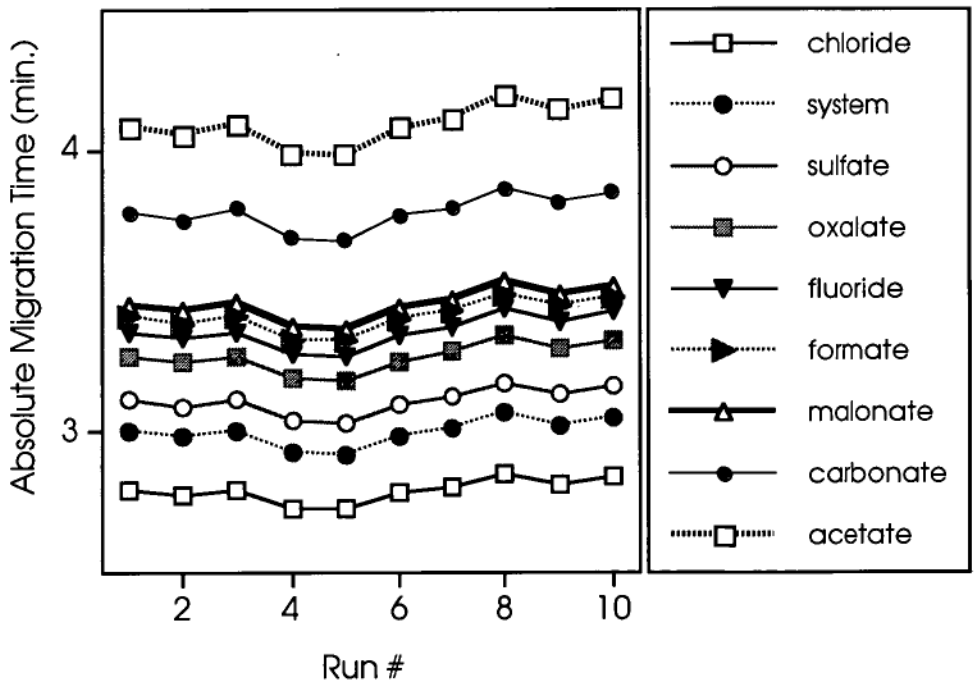

Fig. 7.9: Precision of absolute migration for anions in Bayer liquor using optimum 2.

acetate in Bayer liquor using FRECZE with indirect detection. To achieve this, the use of binary TTAB:DTAB mixtures in the BGE was necessary. Two optimal BGE compositions were identified, namely 3 mM TTAB, 3 mM DTAB and 7.5 mM chromate at pH 9 (optimum 1) and 5 mM TTAB, 1 mM DTAB and 5.5 mM chromate at pH 9 (optimum 2). The two optima exhibited differing selectivities and the choice between them rests on consideration of the relative concentrations of the anions in the sample. Best results were obtained when the Bayer liquor sample was diluted by a factor of 500 before analysis. Linear calibrations were achieved in the working concentration range (1-10 $\mu g.mL^{-1}$) and detection limits fell in the range 0.09-0.34 $\mu g.mL^{-1}$ for optimum 1 and 0.16-0.88 $\mu g.mL^{-1}$ for optimum 2. Recoveries of ions added to the diluted sample were close to quantitative, except for phosphate which showed low and variable recovery, and carbonate which was also variable due to absorption of carbon dioxide by the sample. Tartrate and succinate could not be resolved with either of the optimal BGE compositions.

The methods developed here (optima 1 and 2) were repeatable and have been shown to have the required analytical performance characteristics to be able to be adopted for routine and rapid analysis of anions in Bayer liquor in the alumina and aluminium industries.

7.5 REFERENCES

1 Jarrett, N., "Process Description" in A.R. Burkin, editor, *Production of Aluminium and Alumina* (John Wiley & Sons, Chicester, 1987), p. 3

2 Grocott, S.C., Jeffries, L.P., Bowser, T., Carnevale, J. and Jackson, P.E., *J. Chromatogr.*, **602** (1992) 257.

3 Cardwell, T.J. and Laughton, W.R., 'Analysis of fluoride, acetate and formate in Bayer Liquors by Ion Chromatography', *submitted to J. Chromatogr.* (1994).

4 Haupin, W.E. "Process Control" in A.R. Burkin, editor, *Production of Aluminium and Alumina* (John Wiley & Sons, Chicester, 1987), pp. 168 - 175.

5 Hudson, L.K., "Alumina Production" in A.R. Burkin, editor, *Production of Aluminium and Alumina* (John Wiley & Sons, Chicester, 1987), pp. 11 - 46.

6 Haupin, W.E., "Chemical and Physical Properties of the Electrolyte" in A.R. Burkin, editor, *Production of Aluminium and Alumina* (John Wiley & Sons, Chicester, 1987), pp. 85 - 119.

7 Romano, J., Jandik, P., Jones, W.R. and Jackson, P.E., *J. Chromatogr.*, **546** (1991) 411.

8 Laksana, S. and Haddad, P.R., *J. Chromatogr.*, **602** (1992) 57.

9 Haddad, P.R. and Jackson, P.E., *Ion Chromatography. Principles and Applications*, Journal of Chromatography Library, volume 46 (Elsevier, Amsterdam, 1990).

10 Haddad, P.R. and Vanderaa, S., Poster 509 presented at the *6th International Symposium on High Performance Capillary Electrophoresis*, January 1994, San Diego, CA, USA.

11 Avdalovic, N., Pohl, C.A., Rocklin, R.D. and Stillian, J.R., *Anal. Chem.*, **65** (1993) 1470.

CHAPTER 8

APPLICATIONS USING SELECTIVITY EFFECTS DERIVING MAINLY FROM OTHER PARAMETERS

8.1 INTRODUCTION

A number of applications related to selectivity effects deriving mainly from the parameters studied in Chapter 6 were completed. These included the analysis of phosphocitrate, seawater, toothpaste and concrete. Due to space constraints, the discussion in this chapter will concern the latter two only.

Toothpaste

Fluoride is a vital ingredient in toothpaste and its accurate determination is important. Depending on, for instance, concentration, frequency of use and age of the user, fluoride can be beneficial (prevention of tooth decay) or detrimental (causing dental fluorosis).

Fluoride can be determined using a range of instrumental techniques like ion selective electrodes (ISE), IC and CZE. ISE and IC suffer from relatively long (total) analysis times. CZE, despite its high efficiency, is limited by the poor resolution of fluoride from closely migrating anions, most notably phosphate which is present in most toothpaste. Under the typical conditions employed in CZE, fluoride and phosphate have similar mobilities and are difficult to resolve fully [1-5], particularly when they are present at significantly disparate concentrations. Dilution is usually the first avenue to

improving resolution but will not be suitable for samples containing low levels of the solute anion(s). For such samples manipulation of the separation selectivity is a better approach. The weakly acidic nature of the phosphate suggests that variation of the pH of the background electrolyte (BGE) could be used to maximise the resolution between the fluoride and phosphate.

It was shown in Chapter 6 that the pH of chromate-TTAB BGEs was limited to a range of ≥8 due to precipitation at lower pH. While other UV-absorbing probes and surfactants did not suffer precipitation at pH <8, these are inferior to the chromate-based system in terms of speed, efficiency of separation and detection sensitivity (except for pyromellitic and trimellitic acids [6-8]). Also, it was observed during the work on the effect of 1-butanol in the same chapter that the precipitation could be prevented by incorporation of small amounts of 1-butanol into the BGE. The aim of this study was to maximise the resolution between fluoride and phosphate through exploitation of BGE pH <8 with the aid of 1-butanol.

Concrete

The analysis of concrete for chloride and sulfate is important in the building and construction industry. Both chloride and sulfate have deleterious effects on concrete structures and must be kept at acceptable levels which are ≤0.02% w/w for chloride and ≤0.5% w/w for sulfate. In the case of particularly porous concrete, solutions of these anions can penetrate into the body of the material and if the penetration reaches the reinforcing steel, the chloride will catalyse the corrosion of iron. On the other hand, sulfate attacks two of the components of concrete directly. It can react with calcium hydroxide to form calcium sulfate (gypsum) or with calcium aluminate to form calcium sulfoaluminate (ettringite). Both of these cause an increase in volume which results in cracking and spalling of the concrete. There is a background level (0.3%) of sulfate in

concrete due to the gypsum which is added to the cement to prevent the concrete setting too quickly. Although this reacts with the calcium aluminate, the expansion in volume caused by this reaction is easily accommodated before the concrete has set.

The standard methods of analysis for chloride and sulfate in concrete [9] require the use of classical methods, i.e. a Volhard titration for chloride and a gravimetric determination for sulfate as barium sulfate. The Volhard method is disadvantaged by the use of costly silver nitrate, tediousness and long analysis time, interference from species that may oxidise the thiocyanate titrant, formation of colored complexes by nitrogen oxides from the nitric acid used for digestion and the use of dangerous nitrobenzene may be required. The barium sulfate gravimetric method is affected by a number of positive and negative error sources. Silica and nitrate give high results while heavy metals such as Cr and Fe give low results by interfering in the precipitation of barium sulfate [10]. Errors can also arise from occlusion, adsorption onto the surface of the precipitate and the small but not negligible solubility of the barium sulfate precipitate. Furthermore, results depend on the concentrations of reagents, the rates of reagent addition [11] and the skill level of the analyst.

The normal levels of these anions in concrete are rather low for these standard methods to be applied easily. In a 10 g sample of concrete the chloride is equivalent to 0.6 mL of 0.1 M silver nitrate and the sulfate content produces less than 0.1 g of barium sulfate. Furthermore, the above standard methods do not allow for simultaneous determination of the two anions. A more sensitive and rapid method for the routine determination of the low concentration of anions is required. IC and FRECZE are methods that offer the possibility of simultaneous and rapid analysis of chloride and sulfate. IC however is unsuitable as the low pH (~2) and high ionic strength of the acid-digested sample may adversely affect the suppressor and cause undesirable distortion of the baseline. The

analysis of chloride and sulfate in acid-digested concrete using FRECZE is presently unpublished, and the most likely reasons for this are interferences due to high ionic strength (capillary overloading), low pH of the digested sample and swamping of the chloride and sulfate signals by the high nitrate concentration from the acid used for digestion of the sample.

The high separation speed, quantitative accuracy, low cost, requirement for minimal amount of reagents, etc make CZE an attractive method for the analysis of chloride and sulfate in concrete. The aim of this study was to develop a method that would allow for chloride and sulfate to be separated simultaneously within 10 min.

8.2 EXPERIMENTAL

Chapter 3 lists the details of instrumentation and general separation conditions. All injections were performed in the hydrostatic mode. Detection in the indirect mode was performed at 254 nm (chromate probe, Hg lamp) and at 214 nm (nitrate probe, Zn lamp). For the study of the resolution of fluoride from phosphate, the reagents listed in Chapter 6 were required. For the study of the analysis of acid-digested concrete, the following reagents (AR, BDH, Victoria, Australia) were needed: sodium chloride, sodium sulfate, sodium nitrate, lithium nitrate and concentrated nitric acid (AR, Ajax, Sydney).

As concrete can contain ~0.003% w/w Fe (III) and it is known that iron can form complexes with anionic species[1], the following ligands were tested for their effectiveness in freeing the anions of interest from their metal-complex forms: sodium

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1 This is particularly so for chloride. However, at the pH (~2) of the sample, the Fe-sulfate complex can be formed [12].

tetraborate.$10H_2O$ (AR, BDH, Poole, UK), sodium thiocyanate (GPR, Hopkin & Williams, UK) and sodium fluoride (AR, Rhône-Poulenc, Manchester, UK). To simulate and confirm the effectiveness of freeing the anions of interest, a mixture containing 18 $\mu g.mL^{-1}$ chloride and 0.05% v/v nitric acid was analysed and the results compared with those from another mixture of the same composition but with 0.0003% w/v Fe (III) (undried, as nitrate.$9H_2O$, Research grade, Serva, Feinbiochemica, Heidelberg, NY, USA).

Standards

All stock solutions (1000 $\mu g.mL^{-1}$) were made accurately from salts dried at 100 ^oC overnight except for $Na_3PO_4.12H_2O$. Model solute mixtures were then made as needed by appropriate dilution. For the analysis of fluoride in toothpaste, external working standards in the range 5 - 120 $\mu g.mL^{-1}$ were prepared. Standard additions of 5, 10 and 20 $\mu g.mL^{-1}$ of fluoride were made to the dissolved toothpaste sample for the preparation of the calibration curve and determination of recoveries. For the analysis of concrete, appropriate dilutions were made from 1000 $\mu g.mL^{-1}$ stock solution of chloride and sulfate respectively, to make working external standards in the range 2.5 - 150 $\mu g.mL^{-1}$. Standard additions of 2, 4, 10, 20 and 40 $\mu g.mL^{-1}$ of chloride and 10, 20 and 40 $\mu g.mL^{-1}$ of sulfate were made to a 10% v/v diluted sample.

Preparation of Samples

The toothpaste sample (undried) was prepared by dispersion in water of a weighed amount using ultrasonification, dilution to the required volume and filtration to pass 0.45 μm prior to injection. The comminuted[2] concrete sample was prepared according to British Standard 1881 (Part 6) [13] by dispersing 5.0032g of sample in ~25 mL of water in a 100 mL beaker, adding 1 mL of concentrated nitric acid, mixing well, adding

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2 Sample ground and provided by Dr. Rudi Thomas.

~50 mL of hot water and heating without boiling for at least 15 min. The solution was then filtered through a Whatman 541 filter (pre-washed with hot water), allowed to cool and diluted to 100 mL. For the separation by FRECZE, appropriate dilutions (1 - 10% v/v) of the concrete sample were made to give ~10 $\mu g.mL^{-1}$ of chloride.

Preparation of Background Electrolytes

For the toothpaste analysis, the BGEs were prepared as outlined in Chapter 6. For the concrete analysis, various BGE compositions were investigated and the details of those relevant to this chapter are indicated in the captions to figures. Where the pH of the BGE needed adjusting, dilute NaOH, HNO_3 (AR, BDH, Victoria, Australia) or tris(hydroxymethyl)aminomethane (Aldrich, Milwaukee, WIS, USA) was used.

8.3 Results and Discussion

8.3.1 Toothpaste

Taking advantage of the trends in migration order of anions as a function of the pH of the BGE (Chapter 6, Section 6.1.2) and the fact that phosphate is polyprotic in nature, the use of pH variation as a way to improve the resolution between fluoride and phosphate anions was considered. Experience and common sense suggested that increasing the pH of the BGE would be unsuitable because the baseline would have been noisy and the migration time of the phosphate would need to be increased greatly in order for it to migrate more rapidly than fluoride. Reduction in the pH of the BGE was then considered as a way of maximising the time difference between the two anions. Noting that the pK_{a2} of phosphoric acid is 7.20 [14], reduction of the BGE pH below 8 was a possible means to reduce the migration rate of phosphate.

Practical Limitations of Chromate-TTAB Electrolytes

With BGEs containing 2.5 mM TTAB and 5 mM chromate, the accessible pH range was from 8 to ~13. At pH >13, the baseline was noisy whilst at pH <8, the BGE became opaque due to formation of sparingly soluble chromate-TTAB species. Precipitation did not occur at pH <8 for chromate or TTAB when present individually, suggesting that the observed precipitate involved both species. BGEs containing chromate with cetyltrimethylammonium bromide (CTAB) have been used to separate anions at pH ≤7.5 [3], but the amount of CTAB used was less than 2% of the surfactant concentration used in this study. Preliminary investigations showed that at 2.5 mM, CTAB and dodecyltrimethylammonium bromide (both with 5 mM chromate) also suffer from precipitation problems at pH ~7.

Addition of small amounts of methanol, acetonitrile or 1-butanol to the BGE was found to redissolve the precipitate, even at pH values as low as 7. Of these solvents, 1-butanol was preferred after consideration of UV absorption properties, boiling points, effect on baseline and amount required. It has been shown that relatively small amounts of 1-butanol can generate a stable EOF [15], resulting in more precise migration times for anions. A concentration of 7.5% (v/v) of 1-butanol was found to be optimal for both prevention of the precipitation at pH values as low as 7 and for stabilisation of migration times.

Effect of BGE pH on Resolution of Fluoride and Phosphate

As shown in Fig. 6.6. in Chapter 6, the variation of the pH of the BGE is an effective way of altering the migration order of polyprotic weak acid anions. The above figure considered the pH range 8.1 - 12.7 for BGEs containing TTAB (2.5 mM) and chromate (5 mM) only. There was no change in the migration order of fluoride and phosphate at pH >11 (i.e. in the vicinity of pK_{a3} = 12.36 for H_3PO_4), unlike the

migration order changes noted for these anions elsewhere [6]. This may have been due to errors in the measurement of pH caused by the presence of organic surfactants in the BGE or ion-pairing effects arising from the different amounts of TTAB used [16].

Access to BGE pH values in the range 7-8 created the possibility of manipulation of separation selectivity for phosphate through acid-base dissociation of dihydrogenphosphate at pK_{a2} = 7.20 [14]. Fig. 8.1 shows the effect of BGE pH on resolution between fluoride and phosphate and indicates that the protonation of hydrogenphosphate at pH 7 resulted in slower migration of this species and greatly improved resolution from fluoride. It is notable that the maximum resolution (~11) between fluoride and phosphate demonstrated in this study is the best achieved to date for CZE using chromate-based BGEs. It is also pertinent that the presence of 1-butanol would not have had a direct and significant influence on the improved selectivity observed above for fluoride and phosphate (see Chapter 6). The role of 1-butanol was essentially to prevent precipitation.

The utility of achieving such high resolution and selectivity is demonstrated by the separation of 1 $\mu g.mL^{-1}$ fluoride in the presence of 800 $\mu g.mL^{-1}$ phosphate (Fig. 8.2) and the fully resolved separation of fluoride from phosphate in toothpaste (Fig. 8.3). At pH 8.7 fluoride and phosphate in toothpaste were not resolved (Fig. 8.3 (a)), but were separated at pH 7.5 (Fig. 8.3 (b)). The addition of 1-butanol did not significantly alter the analysis time. Additionally, it can be noted that although maximum resolution between fluoride and phosphate was achieved using a BGE with pH 7 containing 7.5% v/v 1-butanol, the improvement in resolution was such that slightly less 1-butanol (5% v/v) and higher pH (7.5) were sufficient for separation.

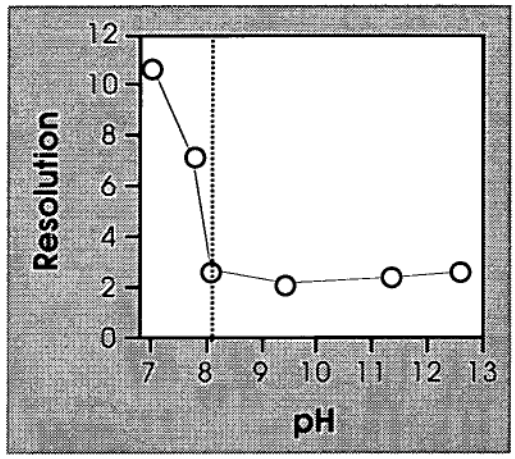

Fig. 8.1: Effect of pH variation on resolution between fluoride and phosphate. Conditions: For BGEs at pH ≥8, the compositions were 2.5 mM TTAB and 5 mM chromate at various pH levels. At pH 7.0 and 7.7 (see dotted line), the BGEs also had 7.5% v/v and 2% v/v 1-butanol as BGE additive. Sampling was in the hydrostatic mode (10 cm for 30 s) and the separation and detection were performed at -20 kV and 254 nm respectively. A fused silica capillary (75 μm I.D. x 52 cm to detector x 60 cm total length) was used. The resolution was calculated according to ref. [17].

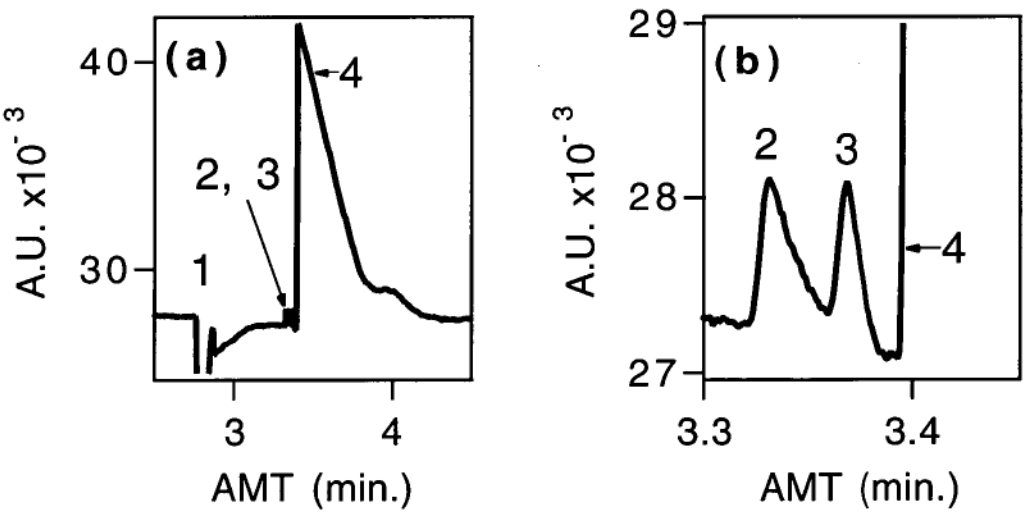

Fig. 8.2: Separation of 1 μg.mL^{-1} fluoride in the presence of 800 μg.mL^{-1} phosphate at normal (a) and expanded (b) scale. Conditions: The BGE had 5 mM chromate, 2.5 mM TTAB and 5% v/v 1-butanol at pH 7. Key: AMT = absolute migration time, 1 = bromide (system peak), 2 = fluoride, 3 = unknown and 4 = hydrogenphosphate.

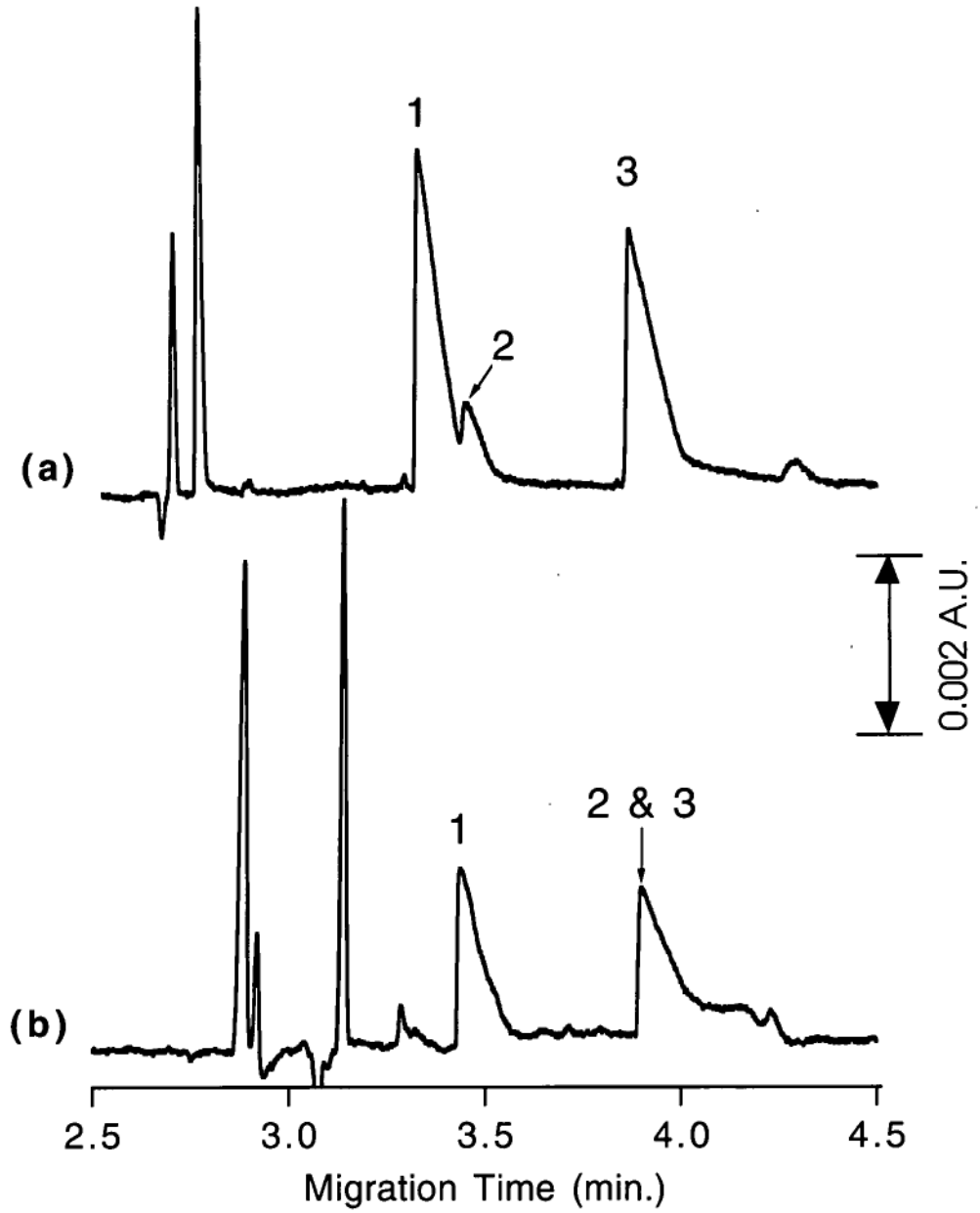

Fig. 8.3: Separation of fluoride from phosphate in toothpaste. Conditions: (a) These were 'typical' conditions and the BGE had 0.5 mM TTAB and 5 mM chromate at pH 8.7. Sampling was in the hydrostatic mode (raised to 10 cm for 30 s) and detection was in the indirect mode at 254 nm. Separation voltage was -20 kV. The capillary was of fused-silica (75 µm x 52 cm to detector x 60 cm total length). (b) The pH of the BGE was 7.5 and it contained 2.5 mM TTAB, 5 mM chromate and 5% v/v 1-butanol as BGE additive. Other conditions were as in (a). The toothpaste (Total gel, Colgate Palmolive, Sydney) sample (1.13g) was prepared by dissolution in water using ultrasonification, followed by dilution to 100 mL. Aliquots of this sample were filtered to pass 0.45 µm (Millex®-HA, Millipore, Bedford, MA, USA) before injection. Anions: 1 = fluoride, 2 = phosphate and 3 = carbonate.

Analytical Performance Characteristics

The analytical performance of the separation shown in Fig. 8.3 was studied in detail and the main characteristics are summarised in Table 8.1. Firstly, it is noted that the quantification of phosphate or carbonate would be unsuitable due to co-migration of these species. However, for the purpose of analysing fluoride as an active ingredient in toothpaste, the separation and resolution shown in Fig. 8.3 (b) was more than adequate.

Precision and quantification

The precision for absolute migration time (AMT) for fluoride was good (1.7% RSD) and compared favourably to values published for synthetic solutions (e.g. 1.5% RSD [18]) and 'real' samples (e.g. ≥2% RSD [19]). There were no day to day variations in precision and this meant that the absolute responses could be used for quantification, i.e. it was not necessary to 'normalise' responses (see Section 2.2.6.8.5 in Chapter 2).

The calibration curves using absolute values of response are shown in Fig. 8.4 (a) for areas and Fig. 8.4 (b) for heights using data for external standards. The peak area response was linear (r^2 = 99.9%) between 5 and 120 $\mu g.mL^{-1}$ of fluoride. On the other hand, the response for peak height was not linear over the above concentration range, and had a 3rd order polynomial fit. However, response was linear (r^2 = 99.5%) over a limited concentration range (5 - 20 $\mu g.mL^{-1}$).

The slopes of the calibration curves using external standards (691 $\mu V.s.mL.\mu g^{-1}$) and standard addition (690 $\mu V.s.mL.\mu g^{-1}$) compared well which meant that there was no significant matrix interference under the conditions used. This also meant that either curve could be used for quantification. For this study, the curve using external

Table 8.1

ANALYTICAL PERFORMANCE CHARACTERISTICS OF CHROMATE-TTAB BGE AT pH 7.5

Key: † = using sample spiked with 20 $\mu g.mL^{-1}$ fluoride and § = using external standard calibration.

Precision (n = 5)		
Absolute migration time (min.):	=	1.7% RSD
Peak area† ($\mu V.s$):	=	0.8% RSD
Peak height† (μV):	=	0.4% RSD
External Standard Calibration (5 - 120 $\mu g.mL^{-1}$)		
Slope ($\mu V.s.mL.\mu g^{-1}$):	=	691
Intercept:	=	-932.666
Correlation coefficient (r^2):	=	0.999
Standard Addition Calibration (+5, +10 and +20 $\mu g.mL^{-1}$)		
Slope ($\mu V.s.mL.\mu g^{-1}$):	=	690
Intercept:	=	6872.417
Correlation coefficient (r^2):	=	0.997
Fluoride Concentration in Sample§		
In solution (n = 2):	=	11.6 $\mu g.mL^{-1}$
In solid (n = 2):	=	0.103% w/w
% Recovery		
+ 5 $\mu g.mL^{-1}$ (n = 3):	=	112%
+10 $\mu g.mL^{-1}$ (n = 3):	=	103%
+20 $\mu g.mL^{-1}$ (n = 4):	=	104%
Limit of Detection (3 x baseline noise)		
Using the sample:	=	0.5 $\mu g.mL^{-1}$

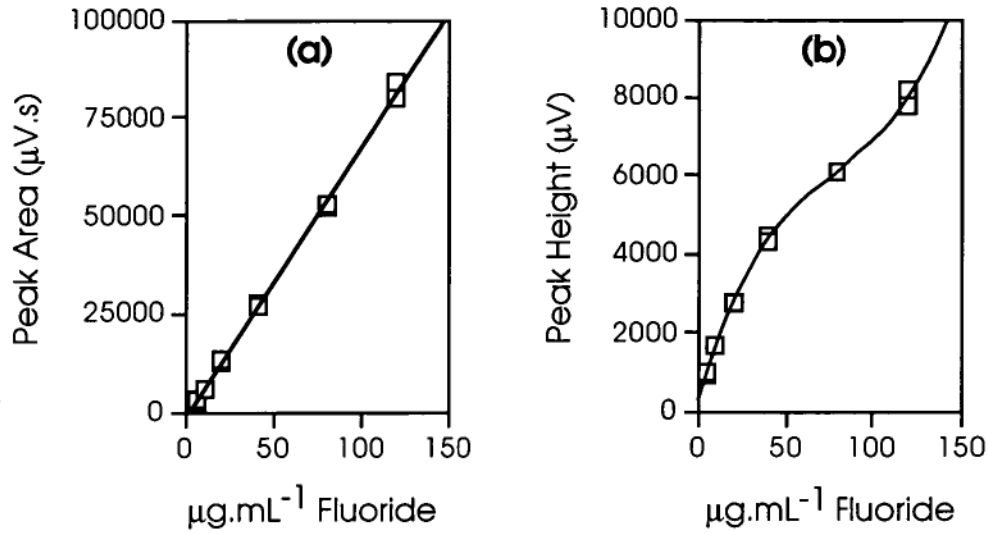

Fig. 8.4: External standard calibration curves using peak area (a) and height (b) responses. Experimental conditions as in Fig. 8.3 (b).

standards was used as it covered a wider concentration range. Thus, it was determined that the concentration of fluoride in the dissolved toothpaste sample was 11.6 $\mu g.mL^{-1}$.

Recovery, sensitivity and speed

The mean recovery was 106% (n = 10) from various amounts of fluoride added to the sample. The limit of detection of 0.5 $\mu g.mL^{-1}$ (at 3 x S:N ratio) was more than satisfactory considering that the sample contained over 10 $\mu g.mL^{-1}$ of fluoride. In addition to the characteristics discussed above, the entire separation was rapid and fluoride as well as phosphate, carbonate and sulfate were separated simultaneously in less than 5 minutes. Where the quantification of phosphate and carbonate is desired, the system at pH 7.5 is unsuitable due to the co-migration of these anions. In this case, a BGE at pH >7.5 (e.g. see Fig. 6.8 (a) in Chapter 6) would be more suitable.

8.3.2 CONCRETE

Omission of Surfactant

The incorporation of surfactants into the BGE is essential to achieve short separation times for anions. For the analysis of samples with high ionic strength such as acid-digested concrete or cement, the choice of surfactant is crucial in order to avoid interferences due to the system peak. The bromide system peak from TTAB is prone to splitting, sign reversal, variation in size, etc as a function of the ionic strength of the sample. Dilution can alleviate the problem but may not be a panacea for all samples. Surfactants in the hydroxide form are not cheaply and readily available and usually have to be converted from the bromide form using ion-exchangers or silver oxide. Further, any carry-over traces of silver can interfere directly with the determination of chloride. Surfactants in the chloride form or the hydrogensulfate form (Chapter 5, Section 5.3.1) will be less prone to system peak interferences but obviously will not allow for simultaneous separation of chloride and sulfate in the concrete. Fig. 8.5 is an example typical of the interference that can be caused by the system peak introduced by the co-ion of surfactants. In this case, the interference was at the sulfate position.

For the above reasons, the analysis was attempted without the use of surfactants in the BGE.

Choice of Probe Type and Counter-ion

Since chloride and sulfate are UV-transparent, it was necessary that their detection had to be performed in the indirect mode. As discussed in Chapter 6, chromate is the probe generally used for the separation of highly mobile anions such as chloride and sulfate. The use of chromate as probe in the separation of chloride and sulfate in nitric acid-digested concrete was unsuitable due to swamping of these peaks by the high level of

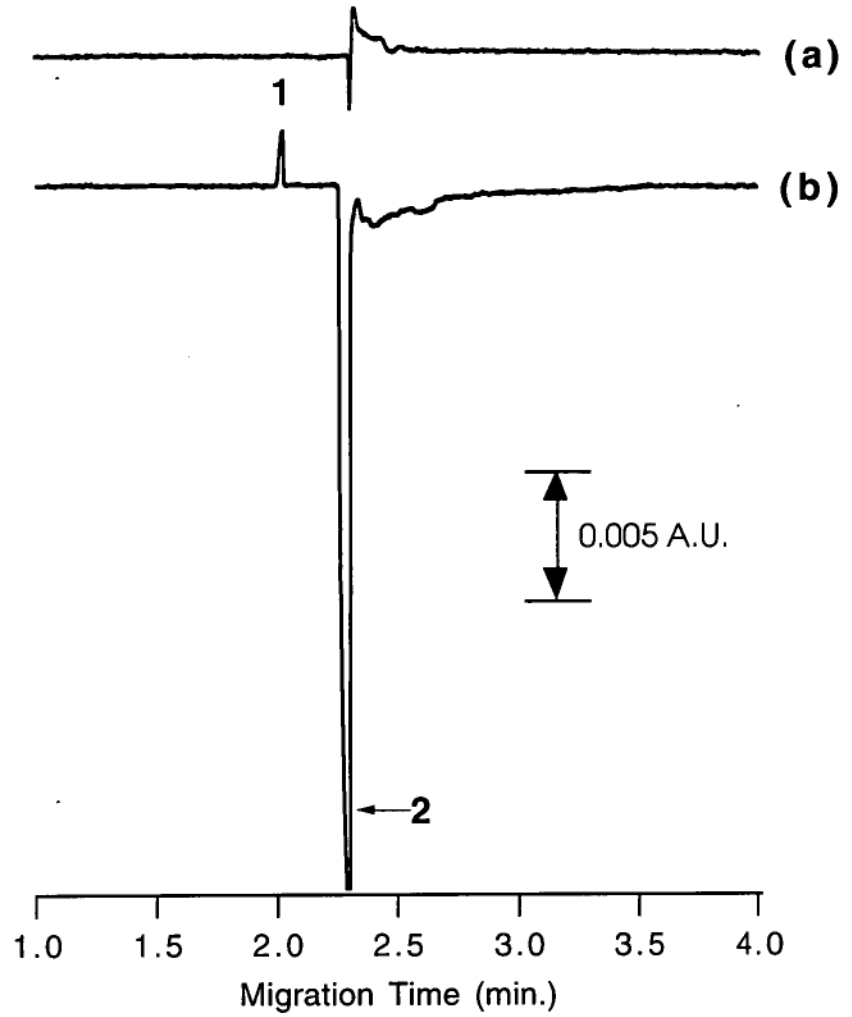

Fig. 8.5: Example of interference due to the system peak. Conditions: The BGE comprised 10 mM nitrate, 2.5 mM cetyltrimethylammonium hydrogensulfate and 0.1% w/v TRIS at pH 7.9. Injection was in the hydrostatic mode (10 cm for 10 s), separation voltage was -20 kV and detection was in the indirect mode at 214 nm. Key: (a) = water blank, (b) = 18 $\mu g.mL^{-1}$ chloride and 0.05% v/v nitric acid spiked with sulfate, 1 = chloride and 2 = system peak (sulfate).

nitrate introduced by the digestion process. Also, there was the risk that the low pH of the sample would cause precipitation (see Chapter 6, Section 6.1.2) and blockage of the capillary, or causing alteration of the effective charge of the chromate probe and thus impacting on the detection (i.e. conversion to dichromate). Considering these, it was decided that nitrate should be used as the probe as its applicability had earlier been

demonstrated (Chapter 6, Section 6.3.1). Apart from acting as the visualising agent, the nitrate would eliminate the interference due to the nitrate matrix of the acid-digested sample.

Initially, sodium nitrate was used as the UV-absorbing probe. The omission of a surfactant from the BGE gave separation times above 10 min. (Fig. 8.6 (a)) and large differences in absolute migration times between the first and subsequent separations, e.g. the RSD difference (~24%) between the two traces in Fig. 8.6 was significantly larger than the 1.5% [18] expected for inorganic anions in synthetic mixtures and 2-14% [19] in real samples. The proclivity towards migration times having poor precision is particularly so with BGEs containing no surfactant as the silanol groups governing the EOF are not 'protected' from the acidic solutions (of both sample and standards). The acidic solution matrix can cause acid-base neutralisation reactions to occur with the silanol groups on the capillary surface, thereby altering the nature of charge, charge density, double-layer size, zeta potential and ultimately the EOF which in turns impacts directly on observed migration time.

The separation time of anions is usually decreased by either using a suitable surfactant or increasing the separation voltage (field strength). Incorporation of surfactants was unsuitable for the reasons discussed earlier (i.e. interference from the system peak). Increasing the voltage was liable to increase the amount of Joule heat generated, to alter baseline behaviour, and increase dispersion. As lithium is relatively less conducting than other cations, the use of nitrate as the lithium salt would produce relatively less Joule heat (and less dispersion) which meant that the separation voltage could be increased to effect a reduction in separation time. Sodium nitrate was thus abandoned in favour of lithium nitrate as it would then be possible to reduce separation time by

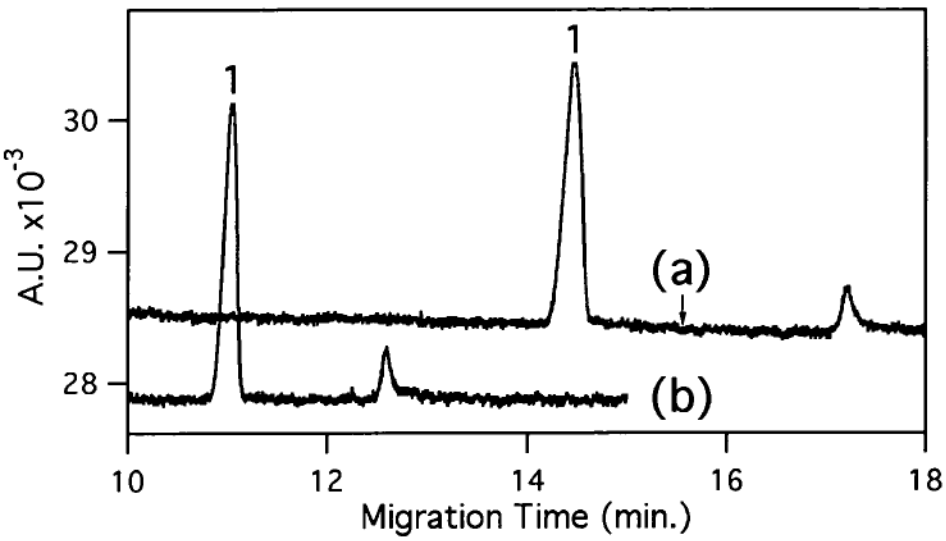

Fig. 8.6: Separation using sodium nitrate BGE without surfactant. Conditions: 10 mM nitrate at pH 6 (unadjusted). The separation conditions were as in Fig. 8.5. Key: (a) = run 1, (b) = run 2 and 1 = 18 $\mu g.mL^{-1}$ chloride with 0.05% v/v nitric acid.

increasing the separation voltage. Fig. 8.7 illustrates the advantage of using lithium nitrate over sodium nitrate as probe. Apart from being relatively more sensitive, the migration time was shorter with lithium as probe counter-ion.

Effect of Nitrate Probe Concentration

An investigation of the effect of the variation of nitrate probe concentration showed that resolution between the chloride and sulfate was better at higher concentration. However, the separation time was increased and the sensitivity did not increase with increasing probe concentration. This is illustrated in Fig. 8.8 using chloride as a model anion. With the intention of developing a method able to separate simultaneously chloride and sulfate in under 10 min., 10 mM nitrate was chosen as the concentration of nitrate probe to be used for the rest of this work. Also, it must be noted that 10 mM nitrate is sufficient to matrix-match the nitrate in the diluted sample introduced as the acid.

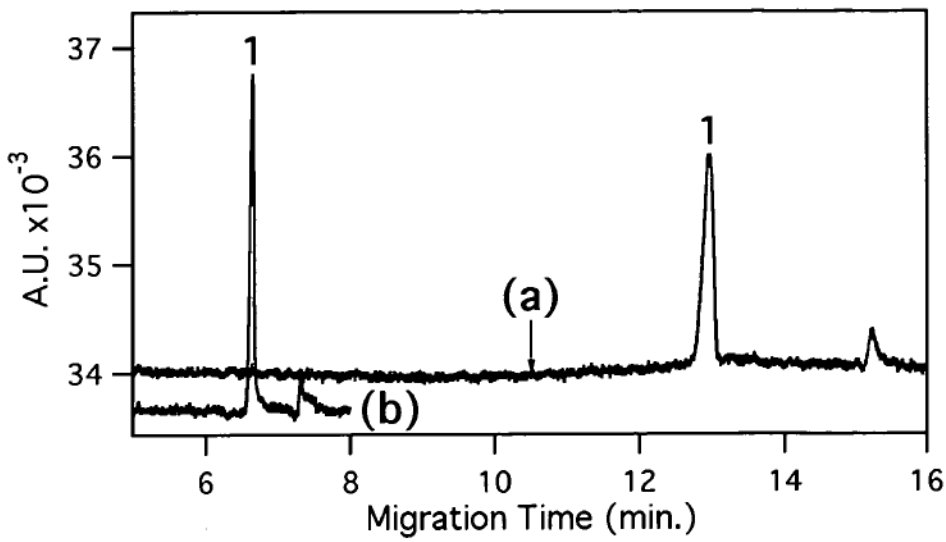

Fig. 8.7: Comparison of separation using nitrate salts of sodium (a) and lithium (b) as UV absorbing probe. Conditions: Apart from the difference in counter-ion, both separations used BGEs with 10 mM nitrate, 1 mM thiocyanate and 0.8% v/v 1-butanol at pH 6. Other experimental conditions were as in Fig. 8.5. Key: 1 = chloride (18 $\mu g.mL^{-1}$ with 0.05% v/v nitric acid).

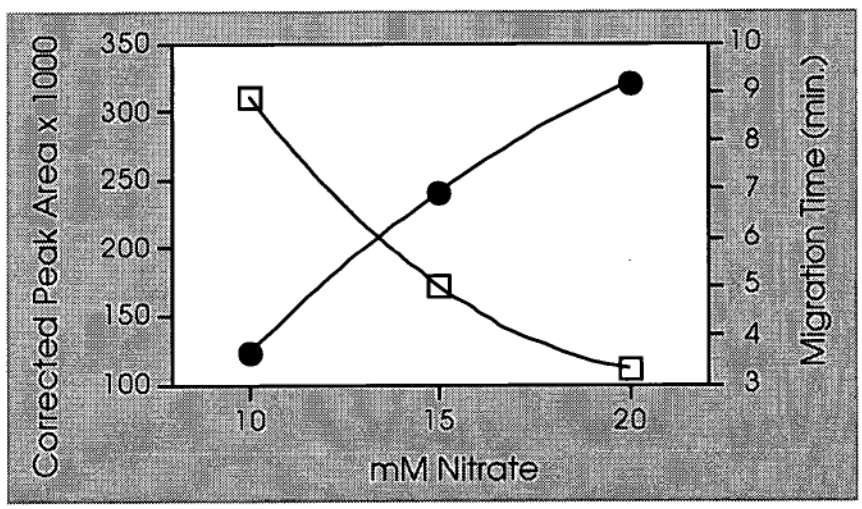

Fig. 8.8: Effect of (lithium) nitrate concentration on the peak area response (□) and migration time (●) of chloride. Conditions: Injection was in the hydrostatic mode (10 cm for 30 s). Data acquisition rate was 20 points/second. The separation voltage was -21 kV. The above responses were for 18 $\mu g.mL^{-1}$ chloride in 0.05% v/v nitric acid.

Interference and Limitations

The iron present in the concrete sample is likely to cause a decrease in the response of the anions of interest, particularly chloride. This is illustrated by the separation of chloride standard in the absence and presence of Fe (III) (Fig. 8.9). Preliminary off-capillary sample treatment (complexation) was performed using a number of ligands as shown in Table 8.2. Tetraborate was the best performed ligand. However, off-capillary treatment was at the cost of time and reagents, and extraneous peaks that have the potential to interfere with the anions of interest can be introduced or produced, e.g. multiple peaks were detected when the sample was treated with cyanide and the chloride peak was masked.

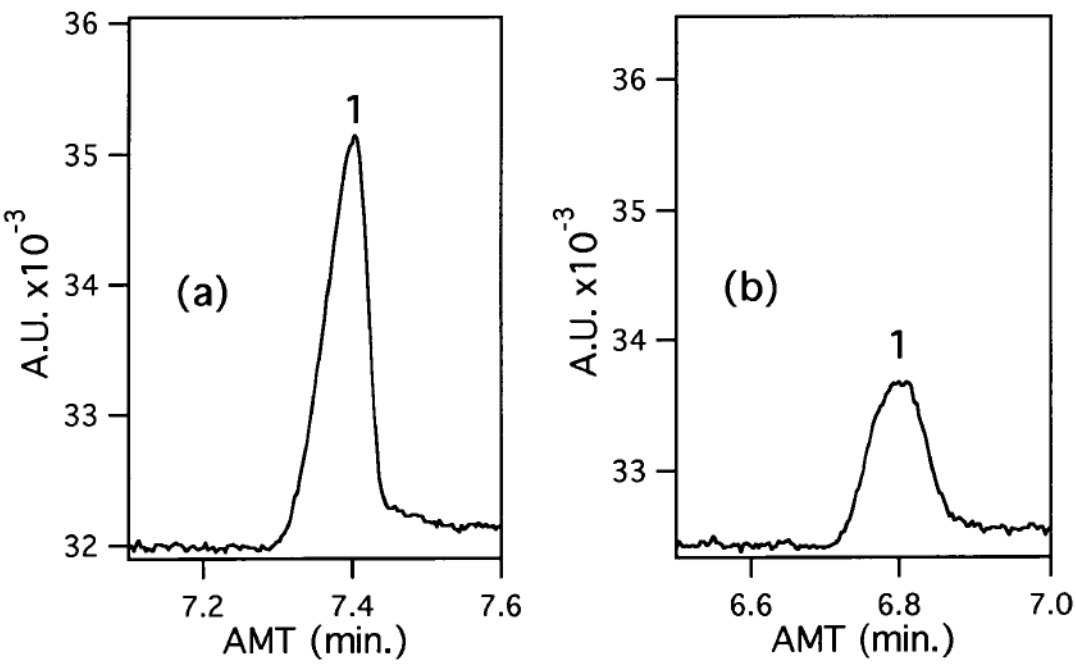

Fig. 8.9: Effect of iron (III) on the response of chloride. Conditions: Electrolyte contained 10 mM lithium nitrate at pH 6. Sampling was for 30s at 10 cm and the separation voltage was -21 kV. Data was acquired at 214 nm using a rate of 5 points/second. Key: AMT = absolute migration time, (a) = 18 $\mu g.mL^{-1}$ chloride in 0.05% v/v nitric acid, (b) = as in (a) but spiked with >0.0003% w/v of Fe (III) and 1 = chloride.

Table 8.2

OFF-CAPILLARY TREATMENT OF SAMPLE

Based on the separation of a 1% v/v diluted sample (1.0084 g per 100 mL dissolved with 1 mL of concentrated nitric acid). The BGE consisted of 10 mM chromate, 2 mM TTAB, 2 mM sodium nitrate and 10 mM TRIS. Sampling was done in the hydrostatic mode (10 cm for 30 s) and separated using -20 kV along a fused silica capillary measuring 75 µm ID x 52 cm to detector and 60 cm total length. Detection was performed at 254 nm. The addition of ligand to the sample was in excess. Thiocyanate was not tested in this preliminary investigation.

LIGAND	% INCREASE IN PEAK AREA	
	Chloride	Sulfate
o-phenanthroline	96	42
cyanide	interference	37
tartrate	176	75
fluoride	negligible	37
tetraborate	218	118

Off-capillary sample treatment therefore was considered unsuitable for rapid and routine analysis of the anions of interest in concrete and cement. Considering the performance (Table 8.2), compatibility with detection mode and wavelength, migration order relative to chloride and sulfate and the fact that metals (e.g. iron and aluminium) are present in concrete, tetraborate, thiocyanate and fluoride were selected and tested for their effectiveness in freeing the complexed anions using on-capillary complexation. Although tetraborate gave best release of chloride, it was deemed unsuitable due to reduced sensitivity, instability of baseline and the separation of chloride and sulfate requiring over 10 min. Fluoride was also unsuitable as it did not give full release of the iron-bound chloride and the sulfate peak was tailed. Thiocyanate was chosen as most

suitable as it enabled full release of chloride from its iron-complex (Fig. 8.10), whilst still allowing for the separation of both chloride and sulfate in under 10 min.

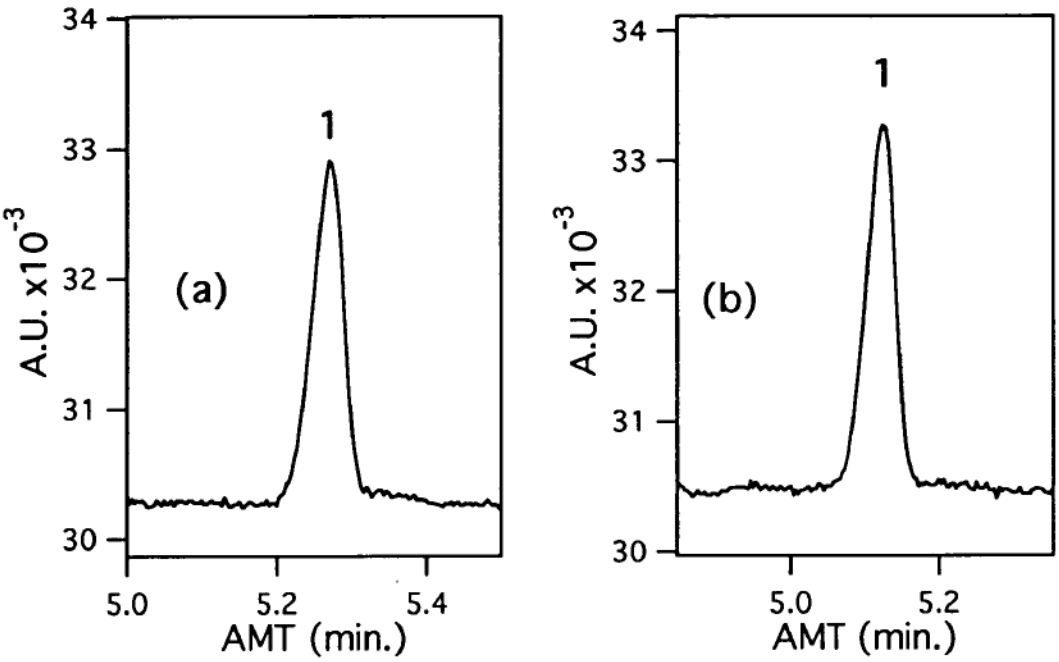

Fig. 8.10: Effect of on-capillary release of iron-complexed chloride using thiocyanate ligand. Conditions: The BGE had 10 mM lithium nitrate, 0.8% v/v 1-butanol and 1 mM thiocyanate at pH 6. Other conditions were as in Fig. 8.9. Key: (a) = 18 μg.mL^{-1} chloride and 0.05% v/v nitric acid and (b) = 18 μg.mL^{-1} chloride, 0.05% v/v nitric acid and 0.0003% w/v of Fe (III).

Separation of Acid-digested Concrete using Optimal Conditions

Fig. 8.11 (a) shows the separation of an acid-digested concrete sample using lithium nitrate as the UV-absorbing probe and thiocyanate as ligand. As a larger than acceptable variation in migration time had been noted earlier (see Fig. 8.6) for BGEs without surfactants, 1-butanol was incorporated in the BGE to aid the stability of migration time [15] and to reduce baseline drift (see Chapter 4, Section 4.3.4). A range of concentrations of 1-butanol was tested and 0.8% v/v was selected for this work. At ≥1% v/v of 1-butanol, the baseline was noted to be unstable. The peak identities in the concrete sample were confirmed by spiking with known standards (Fig. 8.11 (b)).

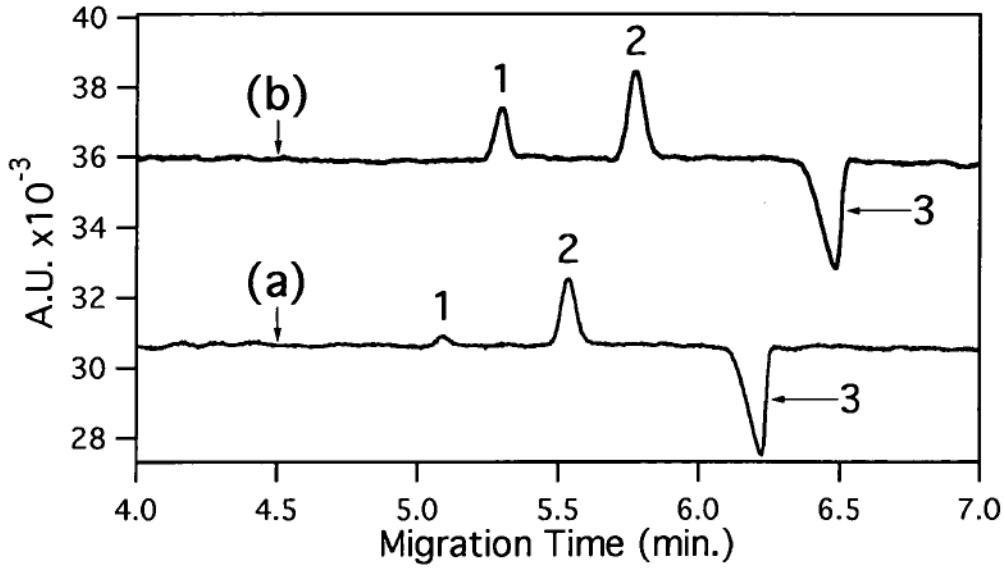

Fig. 8.11: Separation of nitric acid-digested concrete sample using lithium nitrate as UV absorbing probe. Conditions: The BGE contained 10 mM nitrate, 1 mM thiocyanate and 0.8% v/v 1-butanol at pH 6. The separation voltage was -21 kV. Key: (a) = 10% v/v diluted concrete sample, (b) = The sample in (a) spiked with 10 $\mu g.mL^{-1}$ each of chloride and sulfate, 1 = chloride, 2 = sulfate and 3 = system peak (thiocyanate).

Several advantages evident from Fig. 8.11 are that the chloride and sulfate were separated simultaneously, there was no interference from a system peak (originating from a surfactant), the chloride and sulfate were baseline resolved, and the separation time was less than 10 min. (even without the aid of surfactants).

Analytical Performance and Discussion

The analytical performance of the separation shown in Fig. 8.11 was then assessed and the results are summarised in Table 8.3. Before discussing the performance, it must be pointed out that the BGE used to acquire the pherogram in Fig. 8.11 was good for at least three days. This is a decided advantage over other BGEs (e.g. chromate with TTAB) which have to be prepared fresh daily.

Table 8.3

ANALYTICAL PERFORMANCE CHARACTERISTICS

Key: § = as outlined in the discussion, values are based on the separation of a mixture with 10 $\mu g.mL^{-1}$ chloride, 150 $\mu g.mL^{-1}$ sulfate and 0.1% v/v nitric acid, AMT = absolute migration time (min.), * = corrected response (52 x peak response/absolute migration time), n = number of readings, ¶ = calculated from samples spiked with 10 $\mu g.mL^{-1}$ each of chloride and sulfate (n = 3), LOD = limit of detection (3 x baseline noise) using 10 $\mu g.mL^{-1}$ standards. Linearity comparisons are for the concentration range 2.5 - 150 $\mu g.mL^{-1}$ using external standards.

ANION	**% RSD PRECISION§** (n)			**Linearity** (r^2)	**Recovery¶** (%)	**LOD** (µg/mL)
	AMT	Area*	Height*		(n = 3)	(n = 3)
chloride	2.4 (10)	2.9 (7)	4.3 (7)	0.998	103	4
sulfate	2.5 (10)	2.5 (8)	4.5 (9)	0.997	108	6

Precision of absolute migration time and response

As the peak area of the chloride in the injected sample was small, requiring the integration to be performed manually, a mixture comprising 10 $\mu g.mL^{-1}$ of chloride, 150 $\mu g.mL^{-1}$ of sulfate and 0.1% v/v nitric acid was separated in replicates (of up to 10) in order to check the precision of AMT and peak response. The mixture also served to demonstrate that the method developed here had the capacity to resolve significantly disparate levels of chloride and sulfate (Fig. 8.12). Data for the calculation of AMT precision, peak area and peak height were obtained using the conditions in Fig. 8.12.

Precision of AMTs for chloride (2.4% RSD, n = 10) and sulfate (2.5% RSD, n = 10) compared well with those published previously for real samples, e.g. 2 - 14%

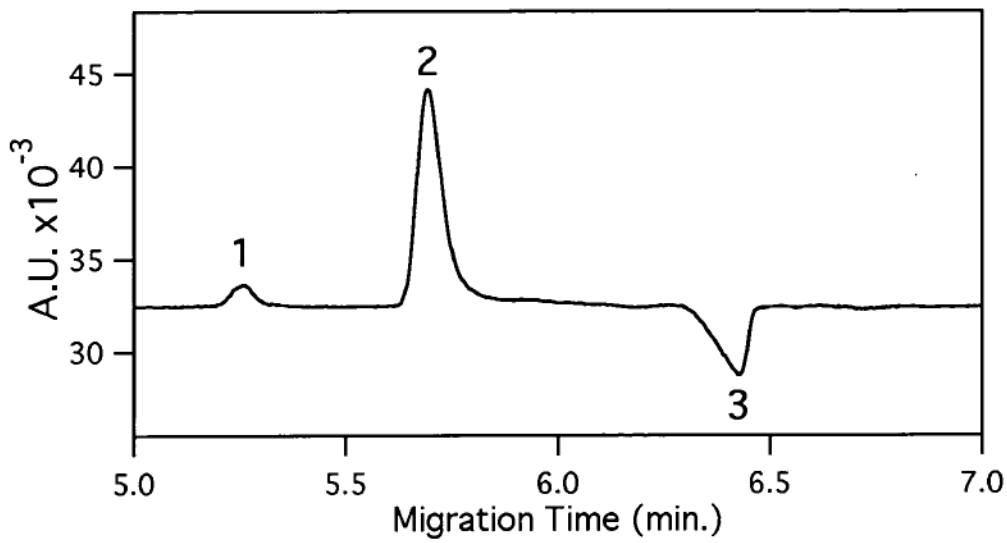

Fig. 8.12: Separation of disparate levels of chloride and sulfate in a standard mixture. Conditions: The conditions were as in Fig. 8.11. The mixture also had 0.1% v/v nitric acid. Key: 1 = 10 $\mu g.mL^{-1}$ chloride, 2 = 150 $\mu g.mL^{-1}$ sulfate and 3 = system peak (1 mM thiocyanate in BGE).

[19]. Even when compared against synthetic mixtures, the AMT precisions were acceptable, e.g. up to 1.5% RSD [18, 20]. Precision of corrected peak areas (i.e. peak area x velocity of anion) for chloride (2.9% RSD, n = 7) and sulfate (2.5% RSD, n = 8) also compared well with those (1.2 - 2.3% RSD) published for anions [21]. On the other hand, precision for corrected peak heights for chloride (4.3% RSD, n = 7) and sulfate (4.5% RSD, n = 9) was inferior to that indicated previously, e.g. 3% RSD [18].

Calibration and linearity

The AMT precision of chloride, sulfate and the thiocyanate system peak showed a trend similar to that discussed in Chapter 4 (see Fig. 4.8), i.e. the AMTs became shorter with repeated separations (Fig. 8.13). Although the overall (n = 10) precisions for the AMT of chloride and sulfate were acceptable (due to a statistical levelling), the AMTs for these anions were also noted to vary with replenishment of the BGE (Fig. 8.13 at run no. 6) in the receiving vial, the pH of the injected solution and the injection

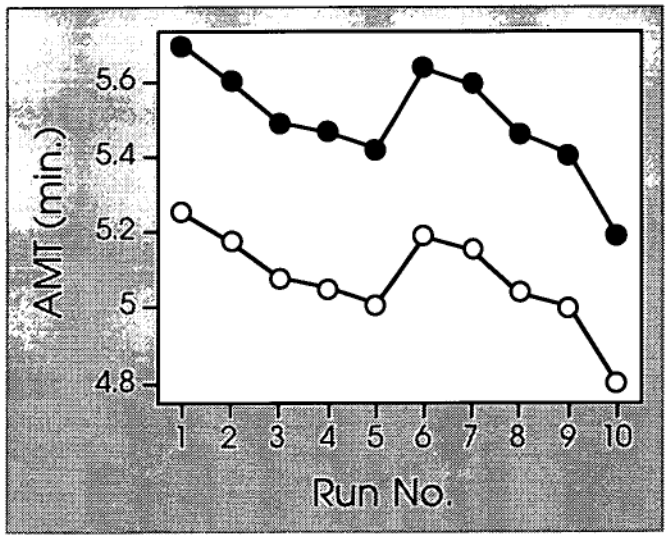

Fig. 8.13: Trends in absolute migration time (AMT) of chloride and sulfate with repeat separations. The conditions were as in Fig. 8.11. The BGE in the source vial (4 mL) was replenished for each run whereas that in the receiving vial was replenished after the fifth run. Key: ● = sulfate and O = chloride.

sequence of solutions. It was therefore decided that corrected responses (e.g. peak area x velocity of anion) would be used for calibration and quantification in order to account for variations due to anion speed [8].

Calibration curves (Fig. 8.14) plotted for chloride and sulfate using external standards and standard addition yielded curves with similar slopes with both modes of calibration for sulfate (Fig. 8.14 (a)), unlike the results reported earlier for chloride. This suggested that at the dilution and conditions used, any interference from the sample matrix on sulfate separation was probably not significant. This meant that either external standards or standard addition could be used for the quantification of sulfate and for this work, the external standard calibration was used. On the other hand, the slopes for chloride using the two modes of calibration were not comparable (Fig. 8.14 (b)). Therefore, for its quantification, the standard addition curve was used.

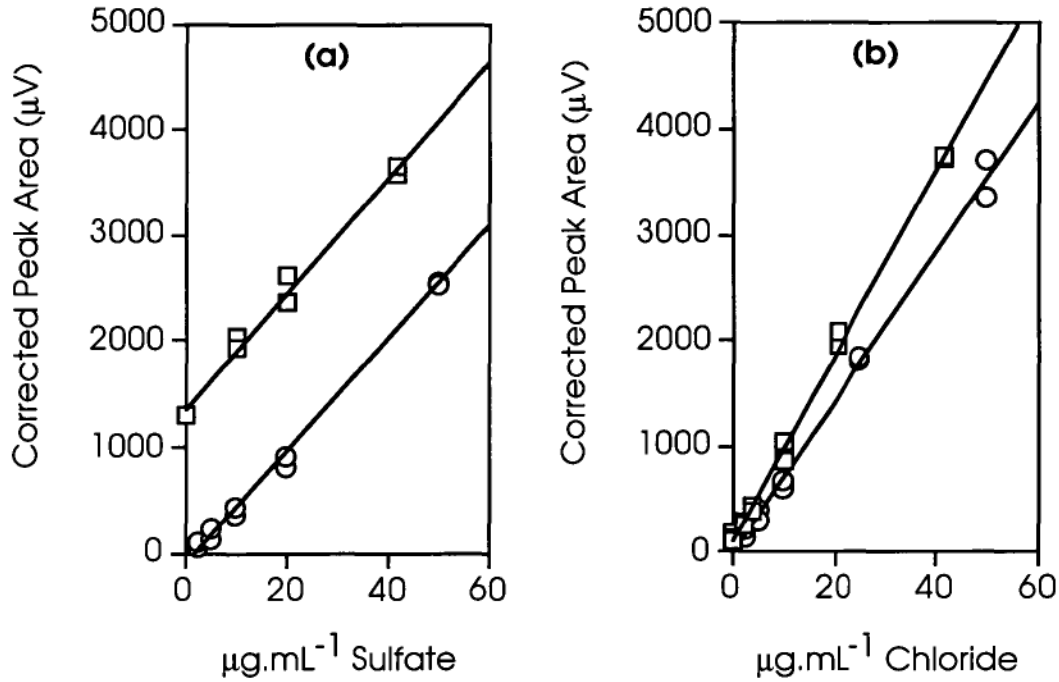

Fig. 8.14: Comparison of calibration curve slopes for sulfate (a) and chloride (b). Key: □ = standard addition and ○ = external standards. For (a), the slopes were 55 and 53 for standard addition and external standards respectively. Likewise, for (b), the slopes were 88 and 71.

Recoveries and limits of detection

The recoveries from 10 $\mu g.mL^{-1}$ standard additions (made to 10% v/v diluted sample) were excellent (Table 8.3). The limits of detection for chloride and sulfate were 4 and 6 $\mu g.mL^{-1}$ respectively. These LODs were more than sufficient for the concrete sample (diluted by 10% v/v) in which a chloride content of >10 $\mu g.mL^{-1}$ and sulfate content of 50 $\mu g.mL^{-1}$ would have been cause for concern.

Using the FRECZE method developed here, the amount of sulfate in the concrete sample was determined to be 0.4% w/w. This compared reasonably well with the value of 0.6% w/w obtained for the same sample using standard barium sulfate gravimetry. On the other hand, the amount of chloride determined using FRECZE (0.02% w/w) did not compare well with the value of 0.18% w/w using the Volhard titration method.

Considering the errors involved with the Volhard method (see Introduction) and the complexity of the sample matrix, the results using FRECZE can not assumed to be incorrect. Separation of a synthetic solution containing 0.0003% Fe (III) and 18 $\mu g.mL^{-1}$ chloride gave responses comparable to 18 $\mu g.mL^{-1}$ chloride without the Fe (III). This suggested that other sources of error may be present with regard to the separation of chloride. Further investigation is required. This should involve using standard reference materials to concurrently check the accuracy of the Volhard and FRECZE methods.

8.4 SUMMARY

The aims of this investigation were achieved with the successful resolution of fluoride from phosphate in toothpaste and the simultaneous and fully resolved separation of chloride and sulfate in acid-digested concrete in under 10 min.

As anticipated, the resolution between fluoride and the interfering phosphate in toothpaste was vastly improved (R_S = 11) due to exploitation of a BGE pH <8 with 1-butanol facilitating the avoidance of precipitation. The accessible pH range was thereby increased by 1 pH unit (20% increase). The excellent resolution demonstrated between fluoride and phosphate using BGEs with pH <8 can also be applied to samples having disparate levels of fluoride and phosphate (e.g. phosphate rock, coca-cola and phosphoric acid).

For the analysis of chloride and sulfate in concrete, the FRECZE method developed here offers significant savings in terms of time and reagents expended compared to the standard classical methods. Its analytical performance was more than adequate for this very acidic and complex sample.

The successful applications described in this chapter have demonstrated the utilisation of the parameters influencing separation selectivity outlined in Chapters 4 and 6 (e.g. pH of BGE, 1-butanol as BGE additive, probe type and probe concentration).

8.5 REFERENCES

1 Ong, C.P., Ng, C.L., Lee, H.K. and Li, S.F.Y., *J. Chromatogr.*, **588** (1991) 335.

2 Chang, H-T. and Yeung, E.S., *J. Chromatogr.*, **608** (1992) 65.

3 Li, K. and Li, S.F.Y., *J. Liq. Chromatogr.*, **17** (1994) 3889.

4 Jimidar, M., Khots, M.S., Hamoir, T.P. and Massart, D.L., *Quimica Analitica*, **12** (1993) 63.

5 Saari-Nordhaus, R. and Anderson, Jr., J.M., *J. Chromatogr. A.*, **706** (1995) 563.

6 Jones, W.R. and Jandik, P., *J. Chromatogr.*, **546** (1991) 445.

7 Harrold, M.P., Wojtusik, M.J., Riviello, J. and Henson, P., *J. Chromatogr.*, **640** (1993) 463.

8 Cousins, S.M., Haddad, P.R. and Buchberger, W., *J. Chromatogr. A.*, **671** (1994) 397.

9 *AS1012.20 - Determination of Chloride and Sulfate in Hardened Concrete and Aggregates*, Standards Australia, Homebush, NSW, 1992.

10 American Public Health Association, Standard Methods for the Examination of Water and Wastewater (APHA/AWWA/WPCF, Washington, 1985).

11 Williams, W.J., Handbook of Anion Determination (Butterworths, London, 1979), p. 119 - 554.

12 Kragten, J., Atlas of Metal-Ligand Equilibria in Aqueous Solution (Ellis Horwood, Chicester, 1978), p. 338.

13 British Standards Institution, *BSI 1881 (Part 6)*, BSI, Milton Keynes, UK, 1971, p. 26.

14 Aylward, G.H. and Findlay, T.J.V., SI Chemical Data, 2nd edition (John Wiley & Sons, Milton, Qld, 1974).

15 Benz, N.J. and Fritz, J.S., *J. Chromatogr.*, **671** (1994) 437.

16 Jimidar, M. and Massart, D.L., *Anal. Chim. Acta.*, **294** (1994) 165.

17 Jones, H.K. and Ballou, N.E., *Anal. Chem.*, **62** (1990) 2484.

18 Altria, K.D. and Simpson, C.F., *Chromatographia*, **24** (1987) 527.

19 Kenney, B.F., *J. Chromatogr.*, **546** (1991) 423.

20 Haddad, P.R., Harakuwe, A.H. and Buchberger, W., *J. Chromatogr. A.*, **706** (1995) 571.

21 Jones, W.R. and Jandik, P., *American Laboratory*, (June, 1990) 51.

CHAPTER 9

GENERAL CONCLUSIONS

Migration Time Stability and Phosphate Response

Imprecision of absolute migration time (AMT) and variability of the response of phosphate are major limitations of FRECZE. It has been demonstrated in this study that precision of AMT can be improved by careful choice of BGE composition, purge time and capillary conditioning. Generally, for better resolution and stability of AMT, the use of dried surfactants is recommended. For improved phosphate detectability, conditioning regime no. 6 developed in this study is recommended.

Selectivity

The following parameters were studied for their influence on separation selectivity of anions using FRECZE; (i) nature of surfactant (counter-ion, alkyl chain length, concentration and binary mixtures), (ii) pH of electrolyte, (iii) 1-butanol as electrolyte additive, (iv) probe type and concentration and (v) separation voltage.

The surfactant influences the separation selectivity of anions through ion pair formation effects. The counter-ion on the surfactant has little effect on separation selectivity. The pH of the BGE influences selectivity through variation of effective charge, and pH effects are pronounced for weak acid anions at or near their pK_a values. 1-Butanol added to the BGE influences selectivity of anions through possible alteration of effective charge and ion pair effects. The selectivity effects due to probe type have been discussed. Probe concentration affects the selectivity of anions through alteration of the effective size (charge:mass ratio) of anions. The use of separation voltage in the

variation of selectivity of anions was demonstrated. It appears to be due to an indirect effect arising from temperature variations and the possible mechanisms are outlined.

Limitations

A practical limitation encountered in this study was the occurrence of precipitation at pH <8 for BGEs containing TTAB and chromate. This restricted the accessible pH range for this widely used electrolyte system. Precipitation was prevented by addition of a small amount of 1-butanol as additive, making feasible the separation of anions at pH ~7 using the chromate-based BGE system. It also allowed for the separation of disparate levels of fluoride and phosphate (i.e. 1:800 in $\mu g.mL^{-1}$).

Applications

The usefulness of the study was demonstrated by the successful application of the information gained in the study to the analysis of complex samples ranging from acidic (e.g. acid-digested concrete) to neutral (e.g. seawater and toothpaste) and alkaline (Bayer liquor). The highlight of the applications was the successful separation of over ten anions in Bayer liquor, a separation not demonstrated previously using IC or even CZE for this complex and difficult sample. This successful application involved taking advantage of selectivity effects derived from the use of binary surfactant mixtures - a selectivity option introduced in this study.

Recommendations

It is suggested that other surfactants and combinations other than binary in nature be studied for their effect on the separation selectivity of anions. It is also suggested that the influence of separation voltage (and temperature) be studied further.

APPENDIX 2A

Table 2A.1

LIST OF SOME REVIEWS IN CAPILLARY ZONE ELECTROPHORESIS

See Chapter 2 for the listing of references.

No.	Year	Review
1	1967	Hjertén [56] reviewed methodology and instrumentation for electrophoresis in small columns and also presented a detailed mathematical treatment for minimising thermally induced band broadening.
2	1983	Jorgenson and Lukacs [5] reviewed band broadening in traditional electrophoresis and CZE. The basic theory, instrumentation and some preliminary results were discussed.
3	1984	Jorgenson [8] discussed the basic theory, instrumentation and some applications demonstrating the use of high voltages. Efficient heat dissipation was also discussed.
4	1985	The basic theory governing the efficiency of separation and the relationship with experimental variables (e.g. separation voltage) was developed by Jorgenson and Lukacs [11].
5	1986	Jorgenson [25] reviewed electrophoresis modes, separation mechanics, detection and instrumental approaches.
6	1988	Gordon, Huang, Pentoney, Jr., and Zare [27] reviewed emerging developments in CE. Selectivity, injection and detection methods were discussed.

Table 2A.1 - *continued.*

7	1988	The instrumentation and application of CE to biochemical analysis was discussed by Karger [44] in a short review. Theory relating to migration time, resolution and efficiency were also outlined. Investigations done by the author and co-workers were highlighted.
8	1988	Snopek, Jelínek and Smoková-Keulemansová [271] have discussed high performance electromigration and the various pseudophases (including micelles) and their effect on migration. The pseudophases were tabulated showing conditions, compounds separated, etc.
9	1989	A 76-page review by Wallingford and Ewing [123] addressed the theory, practical aspects and applications of CE. Sample injection, detection, methodology and the parameters affecting separation efficiency were highlighted.
10	1989	Yeung [150] reviewed indirect detection methods applicable to CE/MECC, outlining the requirements of indirect detection methods.
11	1989	Following the first meeting on CE (in Boston), Widmer [17] briefly reviewed CE modes, theory, separation mechanics and detection. A list of literature concerning CE and a 'who's who' in CE were also included.
12	1989	Ewing, Wallingford and Olefirowicz [14] discussed theory, detection modes and limitations in a brief review. The effect of pH and ionic strength on separation efficiency and resolution were also addressed.

Table 2A.1 - *continued.*

13	1989	Karger, Cohen and Guttman [90] reviewed comprehensively CE instrumentation, principles, detection, formats and applications - particularly in the biological sciences.
14	1990	Hjertén [57] discussed zone broadening in detail and the causes (diffusion, convection, Joule heat, EOF, adsorption and differences in conductivity (pH)). The relevant theory was also given.
15	1990	In the first fundamental review of CE, Kuhr [48] discussed the background to development, separation principles, sample introduction, detection, applications and reviews. Bibliography for the period 1983 to 1989 was also given.
16	1990	Schwer and Kenndler [10] reviewed the principles of CZE (and CITP). Migration, temperature effects (including dispersion), instrumental aspects and applications of CZE were discussed.
17	1991	Jandik and Jones [88] reviewed ways of optimising detection sensitivity for inorganic anions.
18	1991	Yeung and Kuhr [171] reviewed indirect detection methods for separations using capillaries.
19	1992	Burge [61] indicated the relevance of CZE to chemists. This short review also discussed instrumentation, modes, detection methods and some applications.
20	1992	Goodall, Lloyd and Williams [34] reviewed recent developments related to capillary technology and detection methods. Predominantly biochemical applications were discussed.

Table 2A.1 - *continued.*

21	1993	Schöneich *et al.* [30] reviewed CZE, CIEF and CGE as methods for peptide and protein separation and analysis. The same review also considered HPLC and affinity chromatography. Detection methods reviewed included mass spectroscopy and NMR. Problems of protein adsorption onto capillary (cause, remedies. consequence to efficiency, etc) were reviewed.
22	1993	Niessen, Tjaden and van der Greef [272] reviewed CE-mass spectrometry, discussing interfaces, quantitative and qualitative analysis and electrolyte make-up.
23	1993	Xu [273] has reviewed the use of CE in clinical analysis. The modes (including CZE) were discussed.
24	1994	Warner [33] compared CE systems from various makers. Amongst others, operation (e.g. modes, voltage range and polarity reversal), detection, temperature control (of samples and capillary), injection, data management and unit price were tabulated for easy comparison. This review would be useful in choosing a CE system for purchase.
25	1994	Monnig and Kennedy [127] reviewed the literature (books and reviews), fundamentals (e.g. modelling and EOF), instrumentation (e.g. novel sampling techniques) and applications (e.g. inorganic anions).
26	1995	Heiger and Majors [274] reviewed capillaries, capillary coatings and chemistries for selected applications, including inorganic anions.

Appendix 4.3.1.1

Migration Time for Chloride and Sulfate at pH 8.5 with 0.001% v/v Mesityl Oxide Marker

	CHLORIDE (min. MT)		SULFATE (min. MT)		$t_{sulfate} - t_{chloride}$	
	- marker	+ marker	- marker	+ marker	- marker	+ marker
1	2.936	2.980	3.118	3.164	0.182	0.184
2	2.936	2.965	3.117	3.145	0.181	0.18
3	2.950	2.964	3.131	3.143	0.181	0.179
4	2.958	2.964	3.140	3.144	0.182	0.180
5	2.968	2.968	3.151	3.148	0.183	0.180
6	2.972	2.978	3.153	3.159	0.181	0.181
7	2.968	2.983	3.149	3.166	0.181	0.183
8	2.971	2.972	3.152	3.153	0.181	0.181
9	2.978	2.982	3.157	3.164	0.179	0.182
10	2.968	2.996	3.148	3.178	0.180	0.182
$\bar{x}$	2.9605	2.9752	3.1416	3.1564	0.1811	0.1812
SD	0.015035	0.0104754	0.01467	0.011673	0.001101	0.001549

$F = (s^2)/(s^2)$. Using the F-test, the variances for the anions were tested.

Chloride = $0.0150^2/0.0105^2 = 2.04$

Sulfate = $0.0147^2/0.0117^2 = 1.58$

From Table 8a in Johnson[1], $F_{(9,9,\alpha)}$ corresponds to 3.18, 4.03 and 5.35 at the 95% , 97.5% and 99% confidence intervals, respectively.

Therefore it was concluded that the presence of <10 $\mu g.mL^{-1}$ marker in the mixture did not significantly alter the retention times and selectivity. Chloride still migrated before sulfate.

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1 Johnson, R., Elementary Statistics, 4th edition (Duxbury Press, Boston, 1984), p. 524.

Appendix 4.3.1.2

Migration Time for Chloride and Sulfate at pH 11.9 with 0.001% v/v Mesityl Oxide Marker

	CHLORIDE (min. MT)		SULFATE (min. MT)		$t_{sulfate} - t_{chloride}$	
	- marker	+ marker	- marker	+ marker	- marker	+ marker
1	2.840	2.868	3.047	3.077	0.207	0.209
2	2.847	2.872	3.054	3.081	0.207	0.209
3	2.873	2.883	3.083	3.093	0.210	0.210
4	2.873	2.888	3.083	3.100	0.210	0.212
5	2.873	2.892	3.085	3.105	0.212	0.213
6	2.880	2.897	3.092	3.109	0.212	0.212
7	2.901	2.937	3.113	3.153	0.212	0.216
8	2.903	2.929	3.116	3.146	0.213	0.217
9	2.934	2.916	3.150	3.129	0.216	0.213
10	2.933	2.913	3.148	3.126	0.215	0.213
$\bar{x}$	2.8857	2.8995	3.0971	3.1119	0.2114	0.2124
SD	0.03198	0.02344	0.03486	0.02597	0.00299	0.00267

Chloride: $F = 0.03198^2/0.02344^2 = 1.86$

Sulfate: $F = 0.03486^2/0.02597^2 = 1.80$

It can be concluded that the null hypothesis has not been disproved and that the presence of <10 $\mu g.mL^{-1}$ mesityl oxide marker did not significantly alter the migration times and selectivity of the 2 anions under the experimental conditions used.

Appendix 4.3.1.3

Migration Time for Chloride and Sulfate at pH 12.85 with 0.001% v/v Mesityl Oxide Marker

	CHLORIDE (min. MT)		SULFATE (min. MT)		$t_{sulfate} - t_{chloride}$	
	- marker	+ marker	- marker	+ marker	- marker	+ marker
1	2.834	2.833	3.058	3.055	0.224	0.222
2	2.829	2.835	3.051	3.056	0.222	0.221
3	2.828	2.835	3.050	3.056	0.222	0.221
4	2.829	2.834	3.051	3.057	0.222	0.223
5	2.828	2.834	3.052	3.057	0.224	0.223
6	2.828	2.848	3.051	3.073	0.223	0.225
7	2.849	2.862	3.074	3.086	0.225	0.224
8	2.858	2.861	3.086	3.086	0.228	0.225
9	2.856	2.859	3.083	3.084	0.227	0.225
10	2.854	2.866	3.081	3.093	0.227	0.227
$\bar{x}$	2.8393	2.8467	3.0637	3.0703	0.2244	0.2236
SD	0.013174	0.01394	0.01533	0.01563	0.00227	0.00196

Chloride: $F = 0.01394^2/0.01317^2 = 1.1$

Sulfate: $F = 0.01563^2/0.01533^2 = 1.04$

At the 95% confidence limit, null hypothesis was not disproved. Therefore it can be concluded that the effect of the presence of < 10 $\mu g.mL^{-1}$ mesityl oxide marker did not significantly alter the selectivity.

Appendix 4.3.1.4

Migration Time of Chloride and Sulfate at pH 8.5 Using 0.002% v/v Formamide Marker

	CHLORIDE (min. MT)		SULFATE (min. MT)		$t_{sulfate} - t_{chloride}$	
	- marker	+ marker	- marker	+ marker	- marker	+ marker
1	3.044	3.036	3.252	3.243	0.208	0.207
2	3.043	3.035	3.250	3.240	0.207	0.205
3	3.042	3.032	3.248	3.236	0.206	0.204
4	3.041	3.029	3.248	3.234	0.207	0.205
5	3.043	3.029	3.250	3.235	0.207	0.206
6	3.042	3.024	3.248	3.229	0.206	0.205
7	3.041	3.025	3.248	3.228	0.207	0.203
8	3.040	3.025	3.248	3.228	0.208	0.203
9	3.042	3.025	3.250	3.230	0.208	0.205
10	3.042	3.028	3.253	3.233	0.211	0.205
$\bar{x}$	3.042	3.0288	3.2495	3.2336	0.2075	0.2048
SD	0.00115	0.004315	0.00184	0.005103	0.001433	0.001229

Chloride: $F = 0.004315^2/0.00115^2 = 14$

Sulfate: $F = 0.005103^2/0.00184^2 = 7.7$

At the 95% confidence level ($F_{9,9,0.5} = 3.18$), the presence of 0.002% v/v formamide marker did significantly affect the migration time precision and selectivity; i.e. the F-tests with regard to absolute migration times failed. However, the same tests applied to the relative migration times of the above data resulted in the F-test being passed for both chloride and sulfate. The formamide concentration was then reduced and the analysis repeated.

Appendix 4.3.1.5

Migration Time for Chloride and Sulfate at pH 12.15 with 0.0004% v/v Formamide Marker

	CHLORIDE (min. MT)		SULFATE (min. MT)		$t_{sulfate} - t_{chloride}$	
	- marker	+ marker	- marker	+ marker	- marker	+ marker
1	3.058	3.052	3.269	3.264	0.211	0.212
2	3.055	3.049	3.267	3.262	0.212	0.213
3	3.048	3.048	3.260	3.260	0.212	0.212
4	3.046	3.048	3.258	3.261	0.212	0.213
5	3.048	3.049	3.258	3.260	0.210	0.211
6	3.047	3.051	3.258	3.263	0.211	0.212
7	3.052	3.051	3.263	3.264	0.211	0.213
8	3.048	3.043	3.260	3.256	0.212	0.213
9	3.04	3.056	3.252	3.268	0.212	0.212
10	3.039	3.053	3.252	3.266	0.213	0.213
$\bar{x}$	3.048	3.050	3.2597	3.2624	0.2116	0.2124
SD	0.0059	0.003496	0.0056	0.0034	0.00084	0.000699

Chloride: $F = 0.0059^2/0.0035^2 = 2.8$

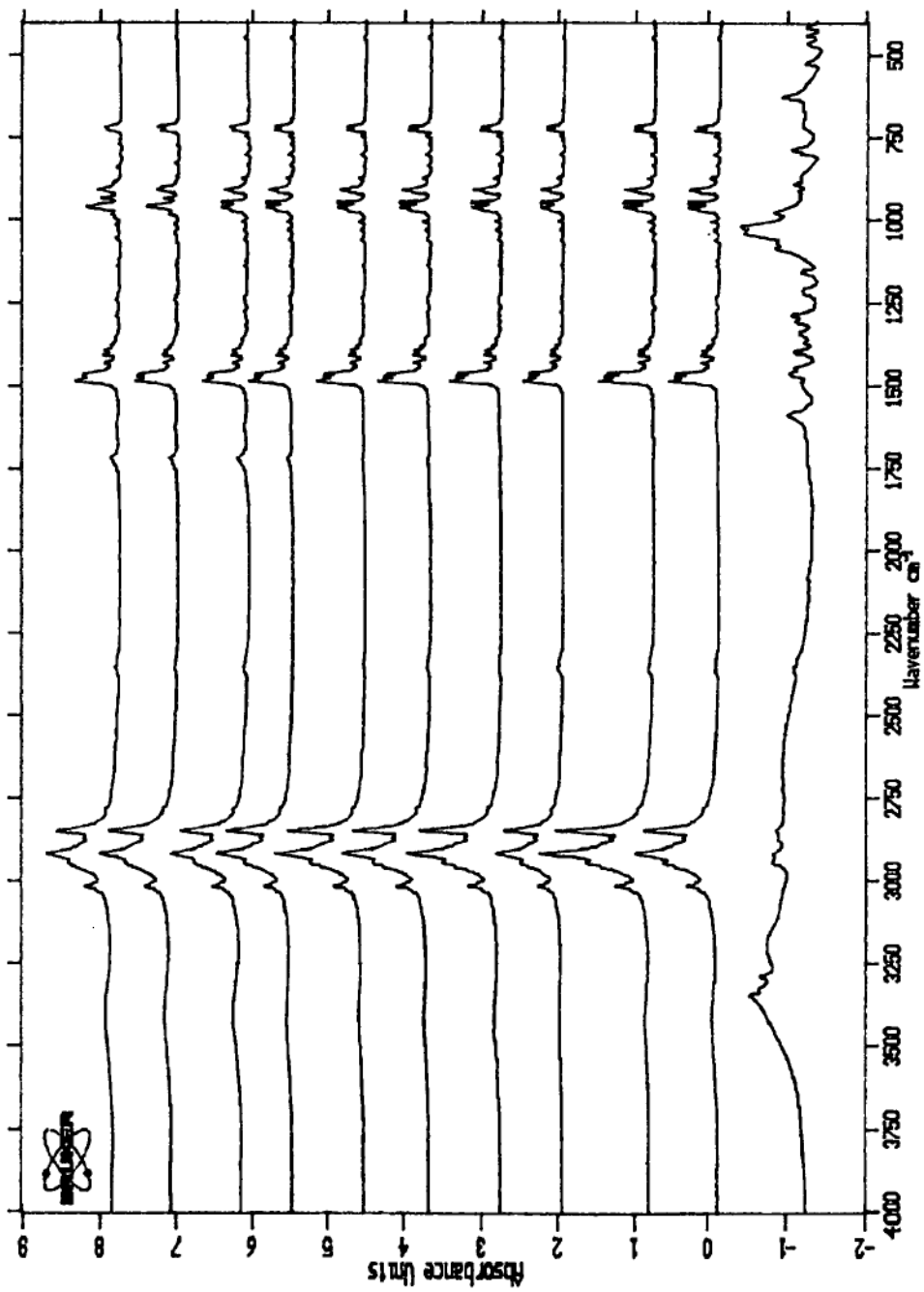
Sulfate: $F = 0.0056^2/0.0034^2 = 2.7$

At the 95% confidence level, the presence of 0.0004% v/v formamide did not significantly alter the selectivity of the test anions.

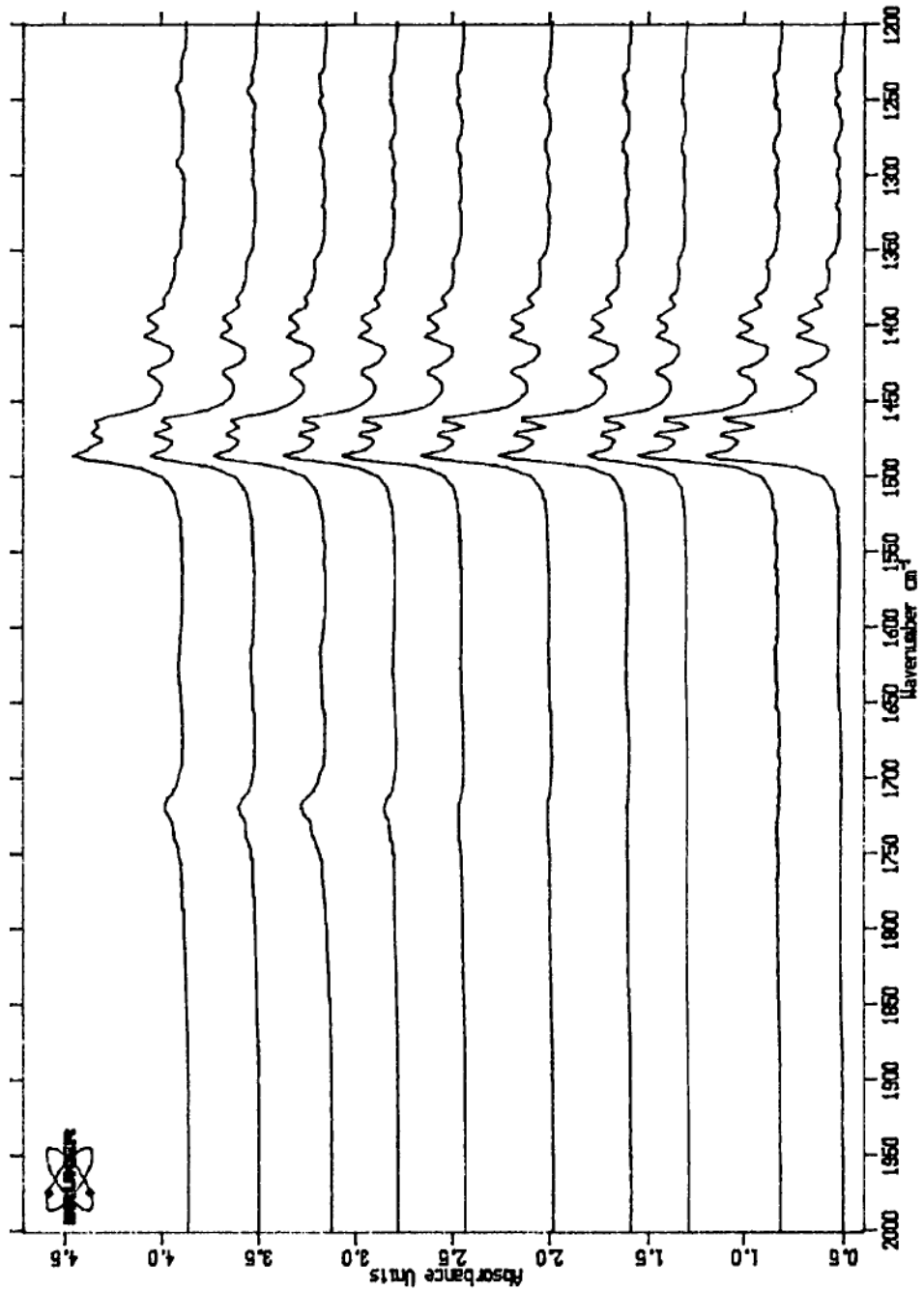
Appendix 4.3.2.1

IR spectra of undried and dried surfactants

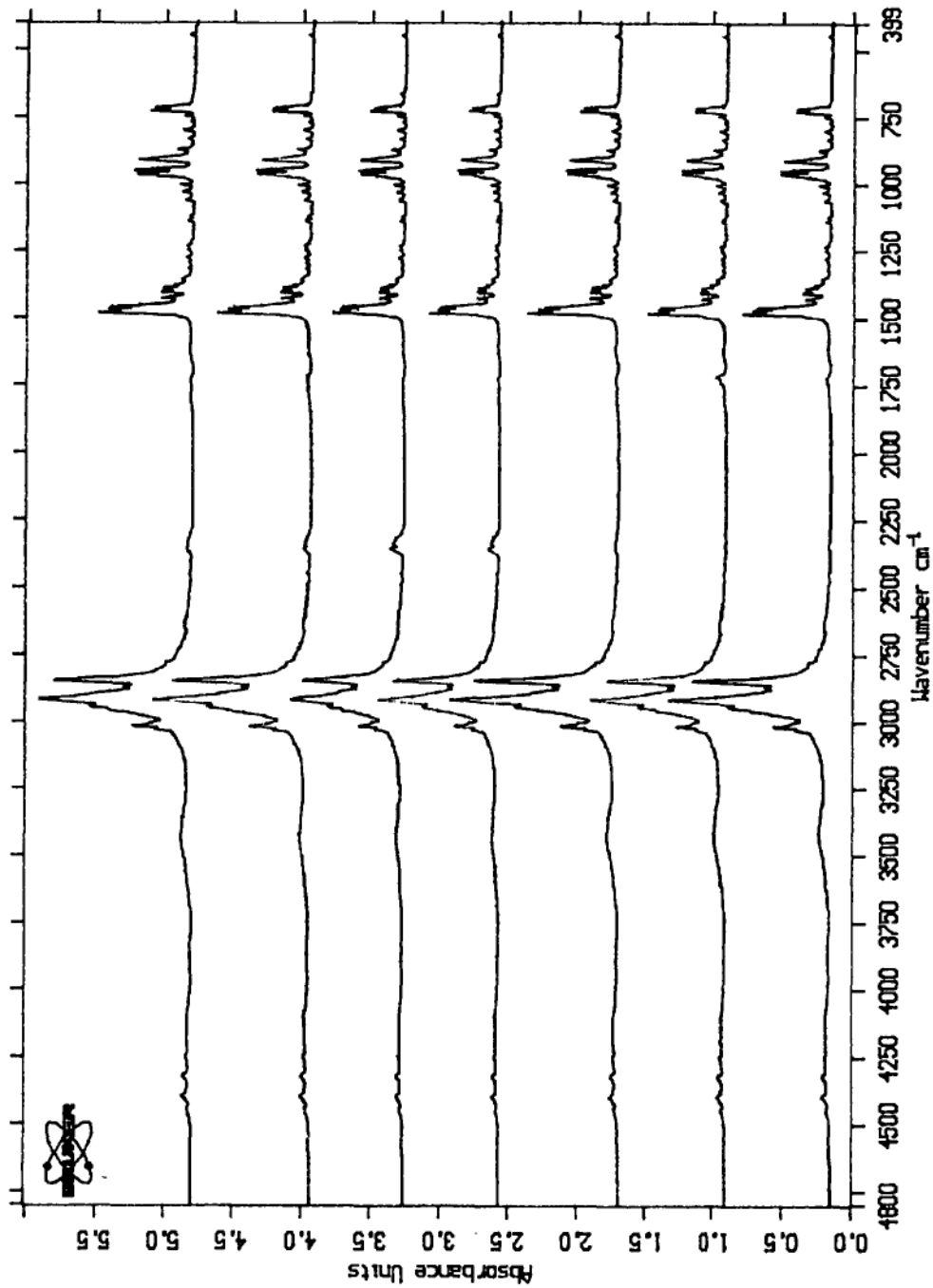
(a) Drying temperature was 100 ^{o}C. Going up from the bottom, the key is (hrs): 1 = TRIS as control (0), 2 = TTAB (0), 3 = TTAB (1), 4 = TTAB (3), 5 = TTAB (6), 6 = TTAB (12), 7 = TTAB (24), 8 = TTAB (48), 9 = TTAB (204), 10 = DTAB (204) and 11 = CTAB (204).



(b) Portion showing appearance of peak at ~1725 cm^{-1}. Key as in (a). TRIS has been omitted.



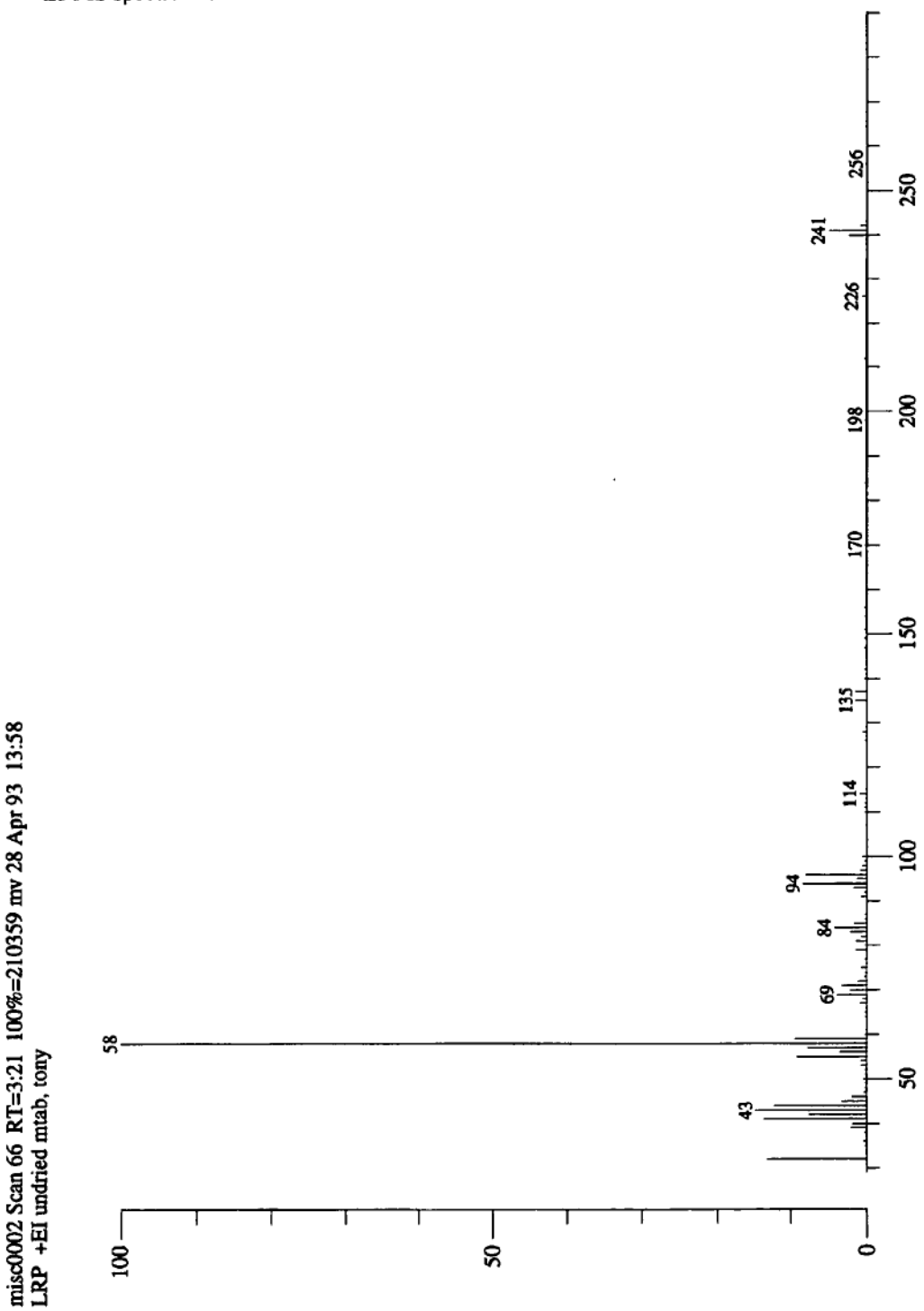
(c) IR spectra of TTAB dried at 100 ^{o}C for various periods. Starting from the top down, the drying periods were 0, 12, 1, 3, 6, 48 and 24 hours, respectively.



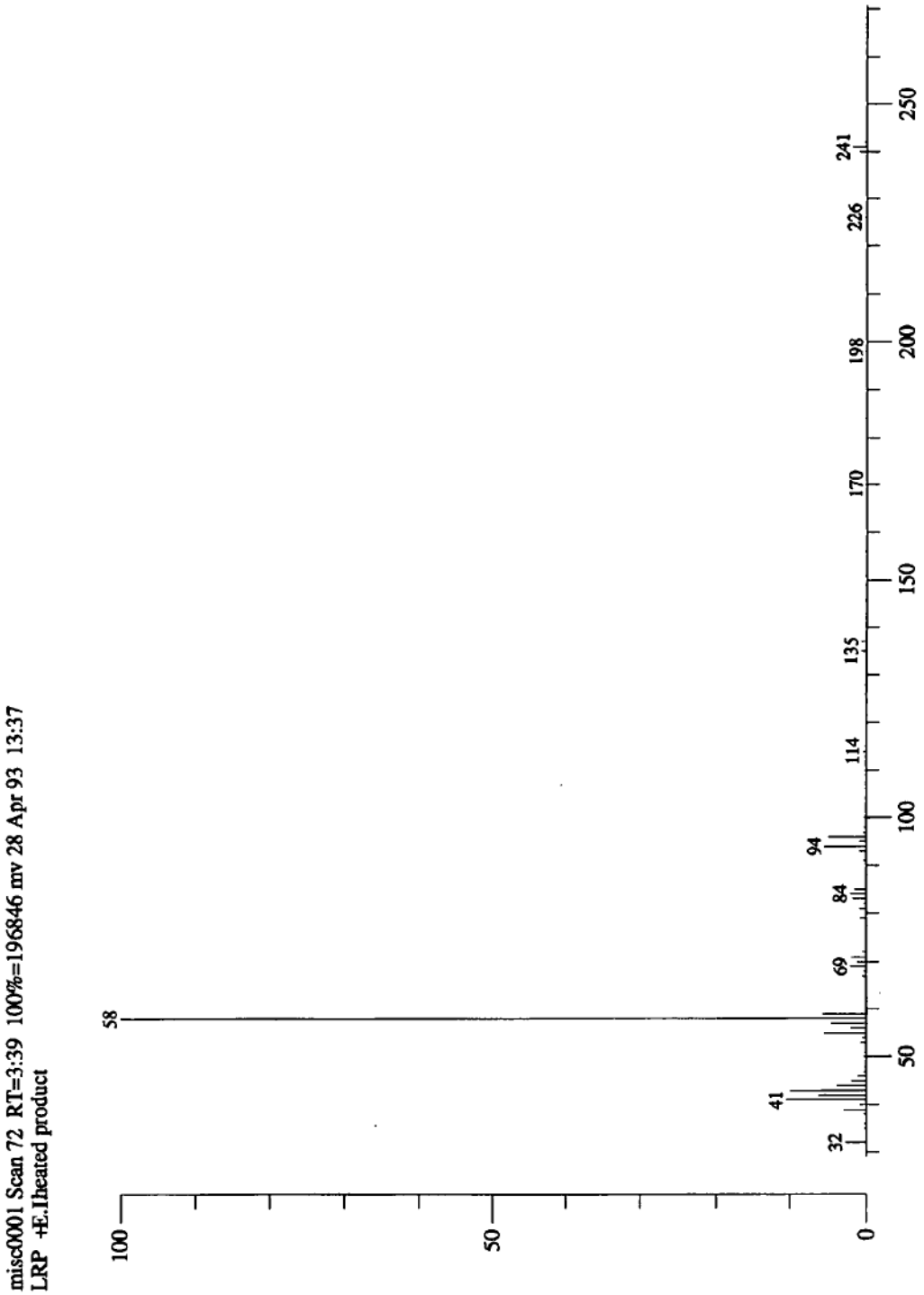
Appendix 4.3.2.2

EI-MS spectra of undried and dried TTAB

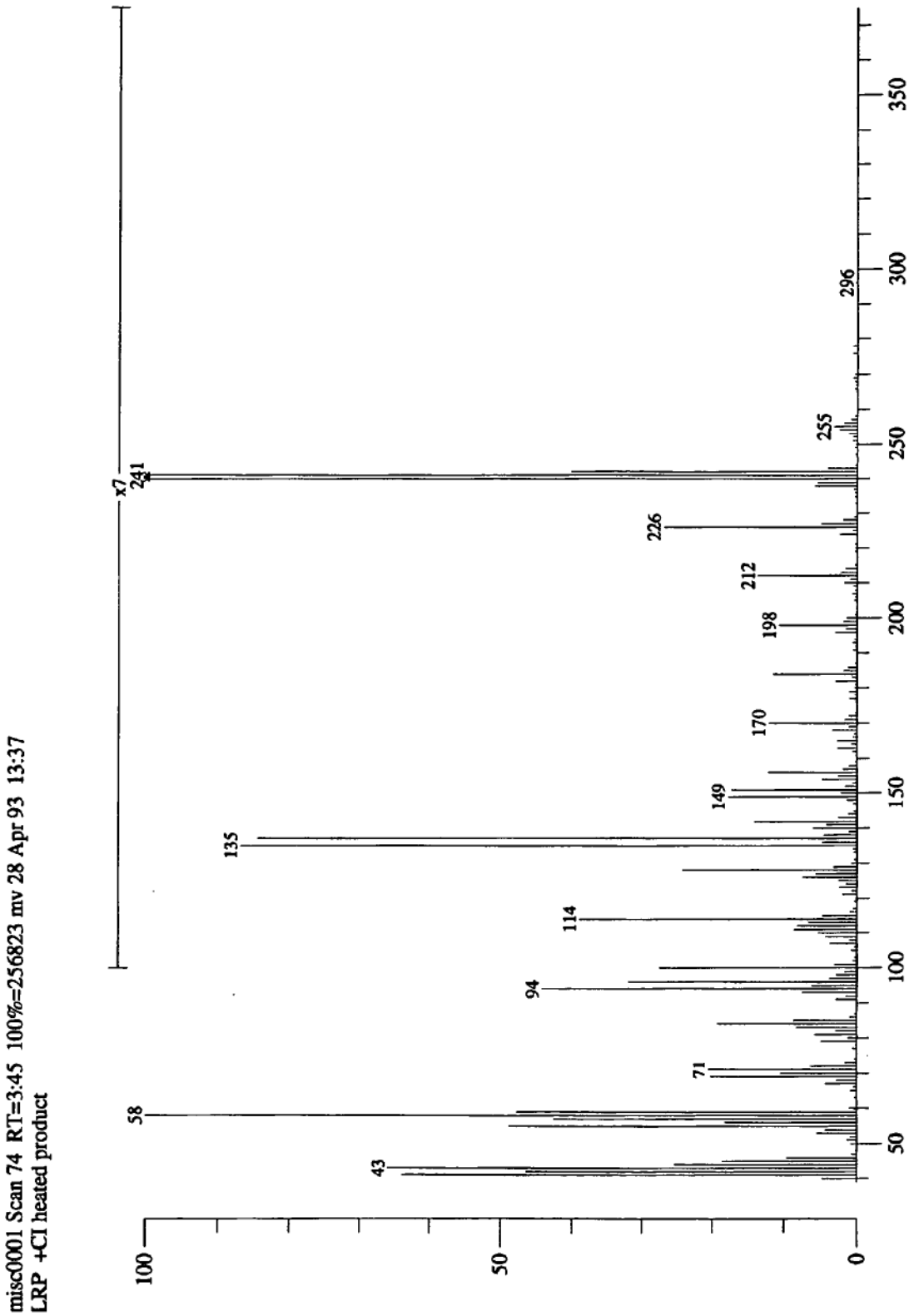
(a) EI-MS spectrum of undried TTAB.



(b) EI-MS spectrum of TTAB dried overnight at 100 °C.



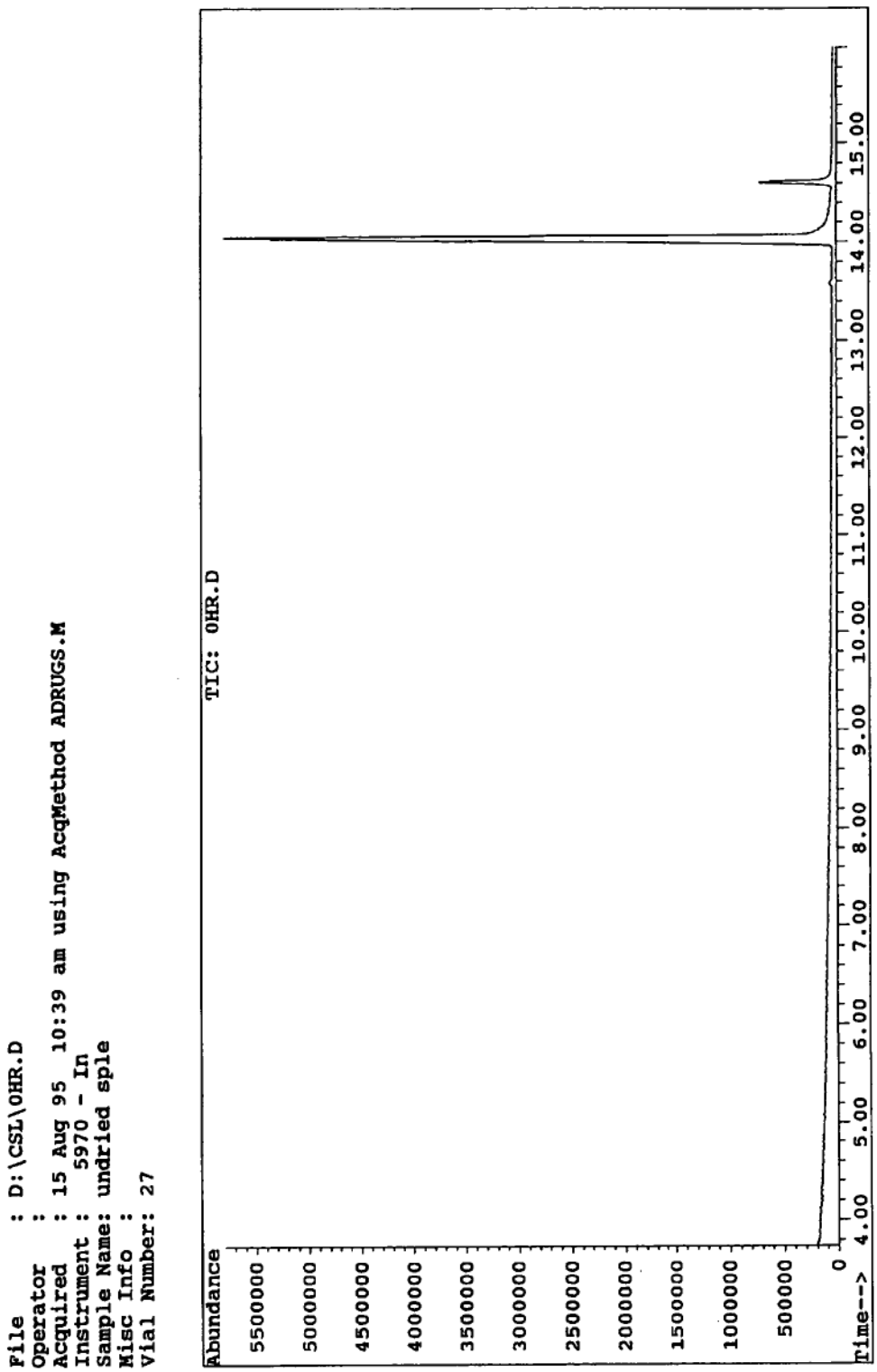
(c) Spectrum in (b) at off-scale.



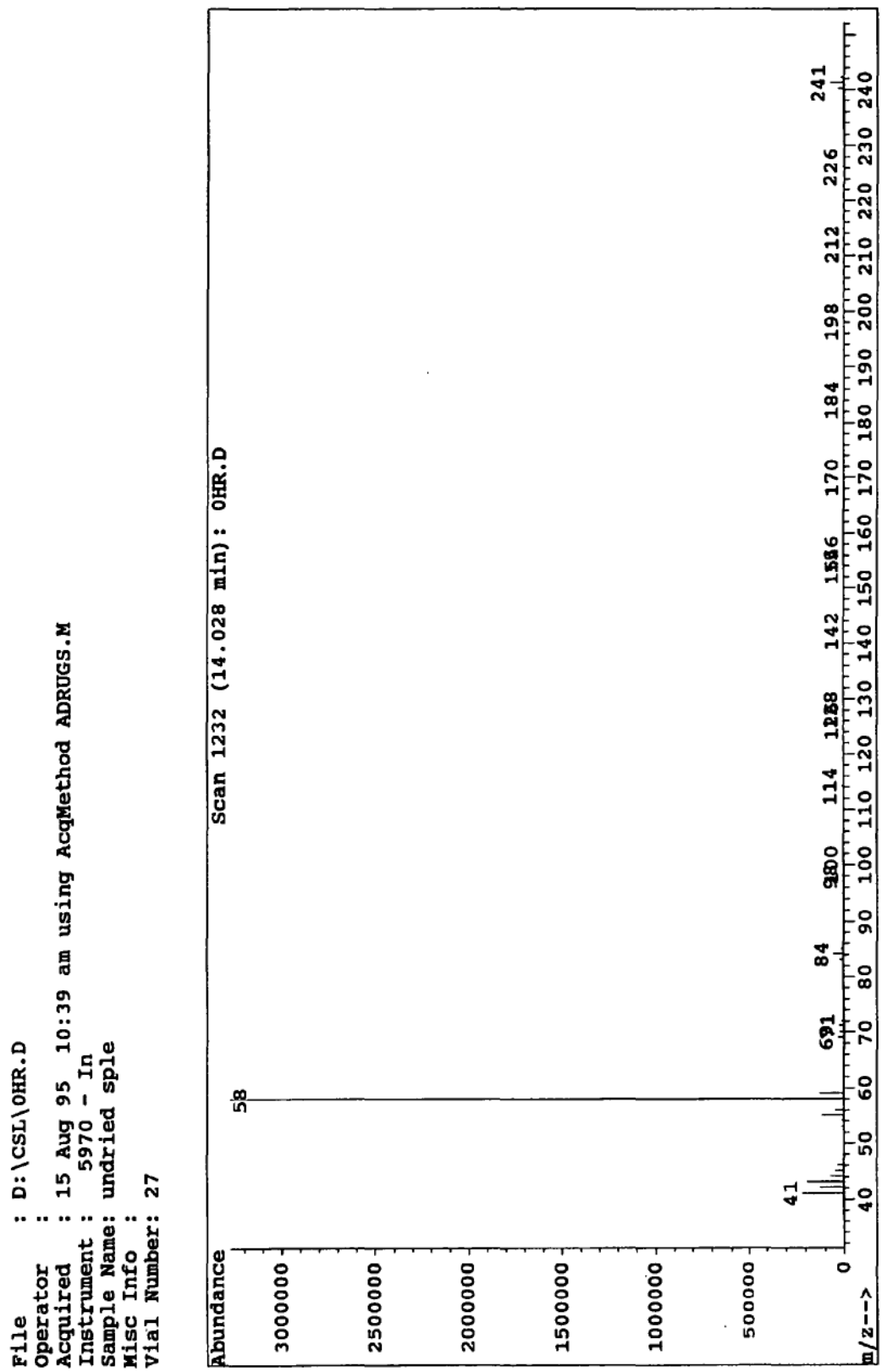
Appendix 4.3.2.3

GC-MS spectra of undried and dried TTAB

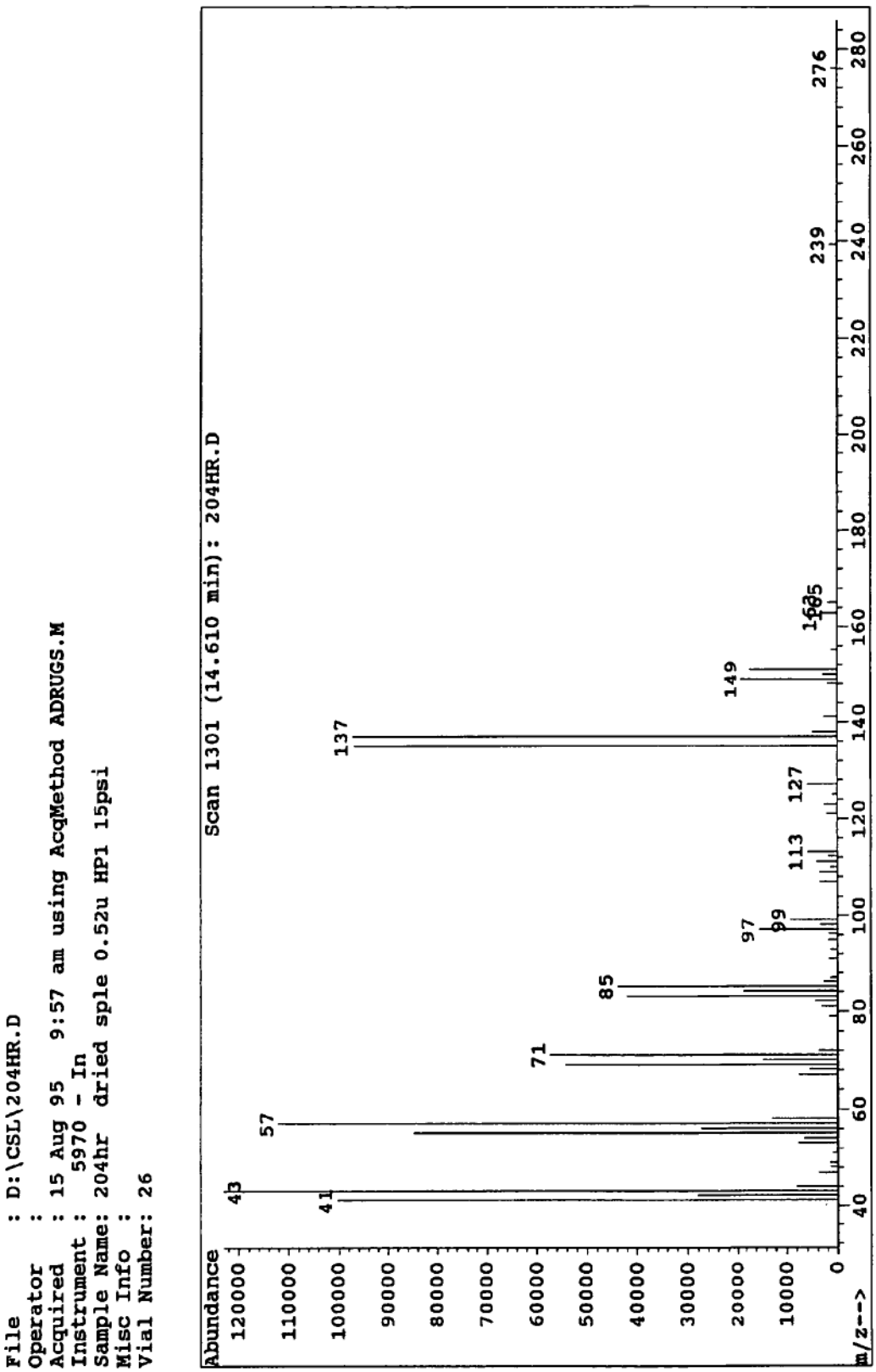
(a) Gas chromatogram showing alkylamine (14.028 min.) and C_{14} alkyl bromide (14.610 min.).



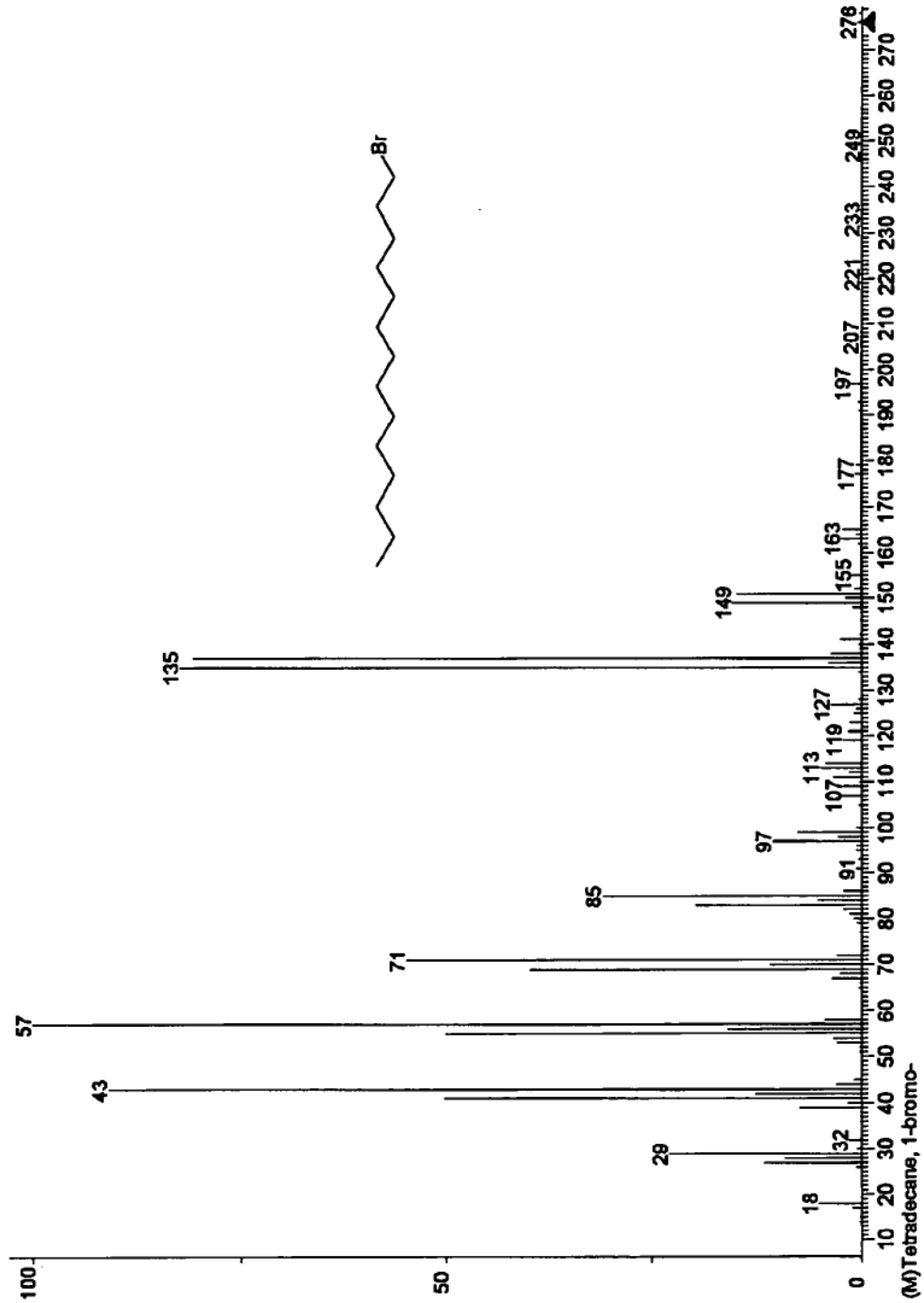
(b) GC-MS spectrum of the peak at 14.028 min. in (a).



(c) GC-MS spectrum of the peak at 14.610 min. in (a).



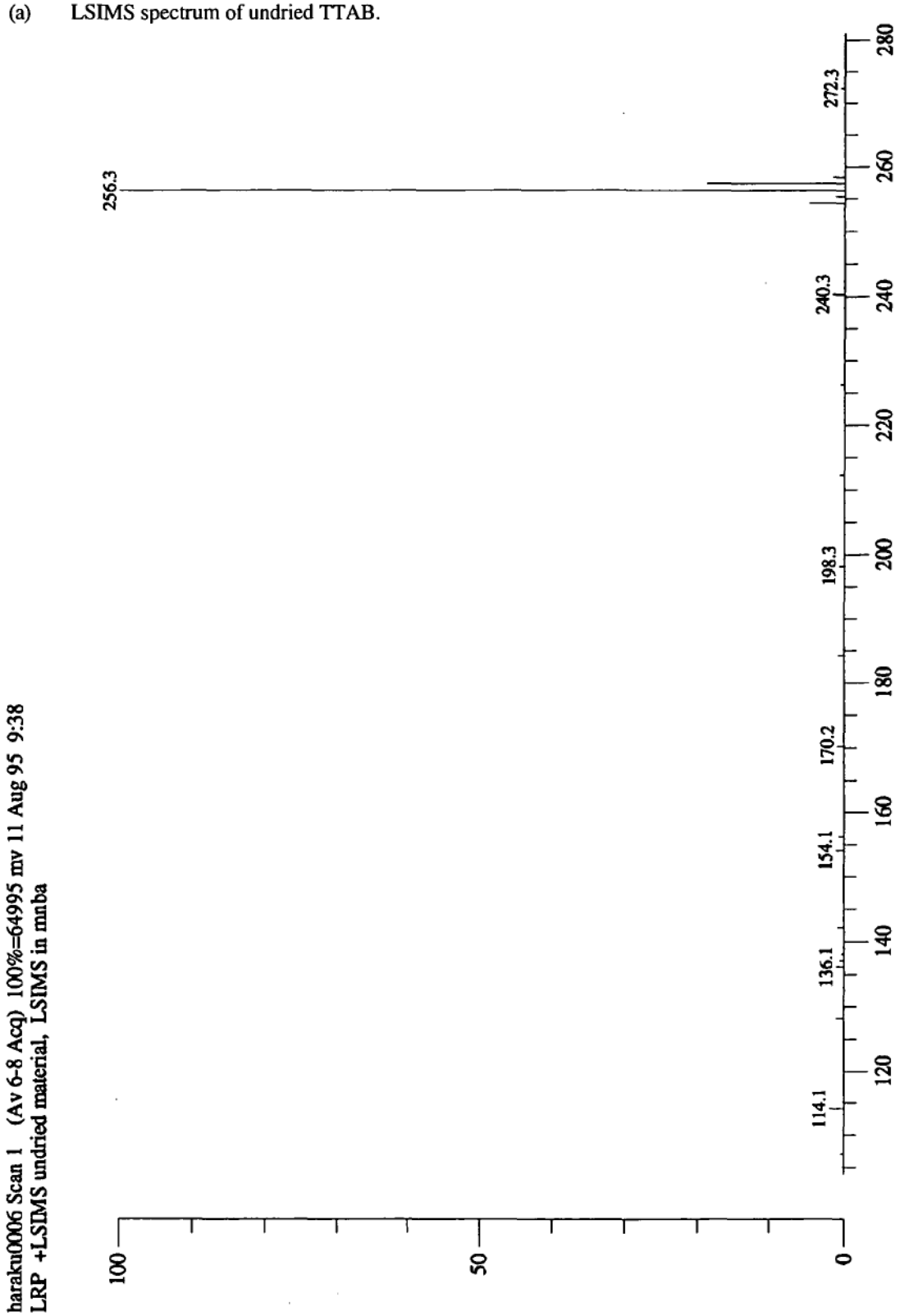
(d) 1-bromo-tetradecane from the library of mass spectra matched well the spectrum from the fragmentation of the TTAB sample.



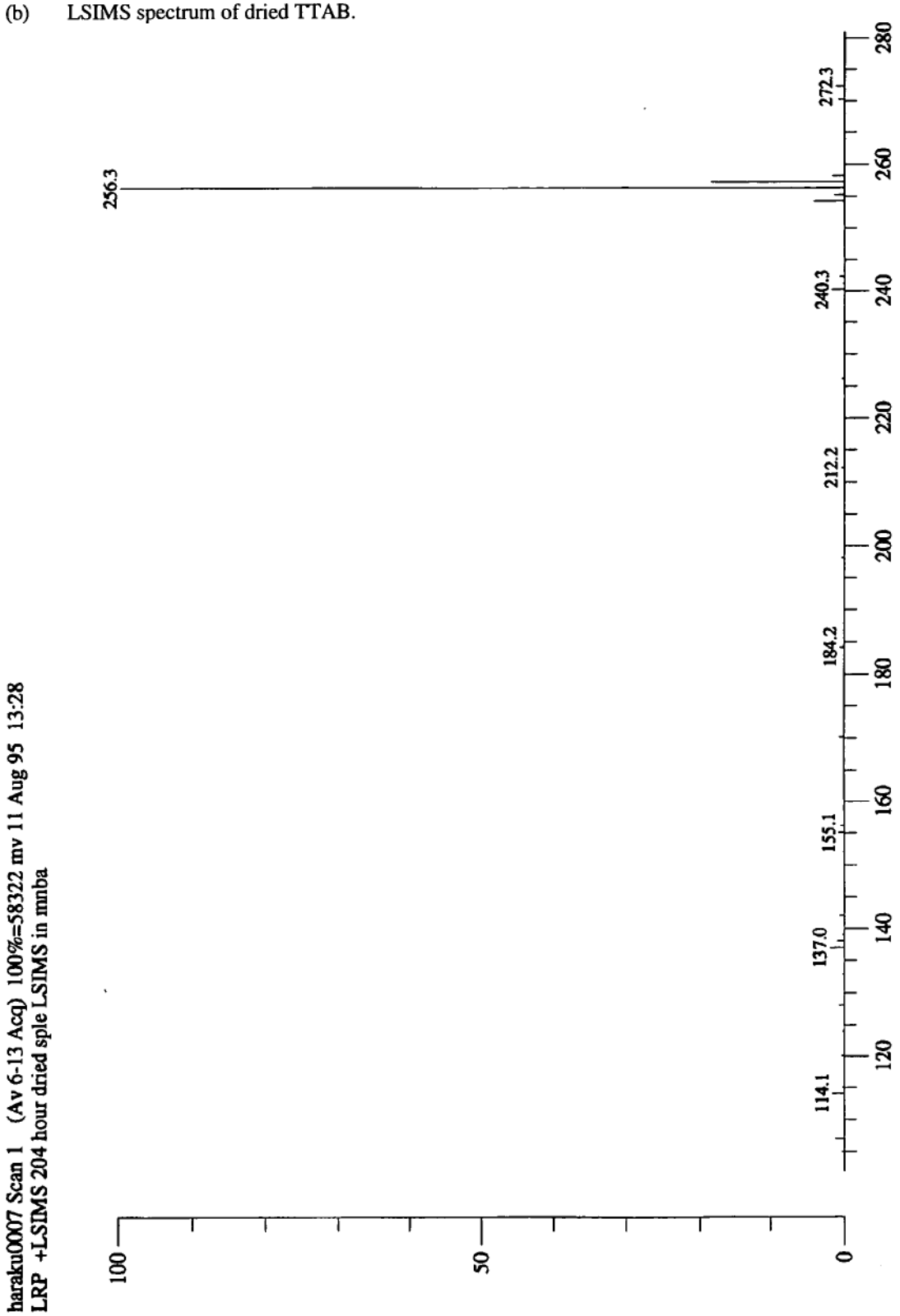
Appendix 4.3.2.4

LSIMS spectra of undried and dried TTAB

(a) LSIMS spectrum of undried TTAB.



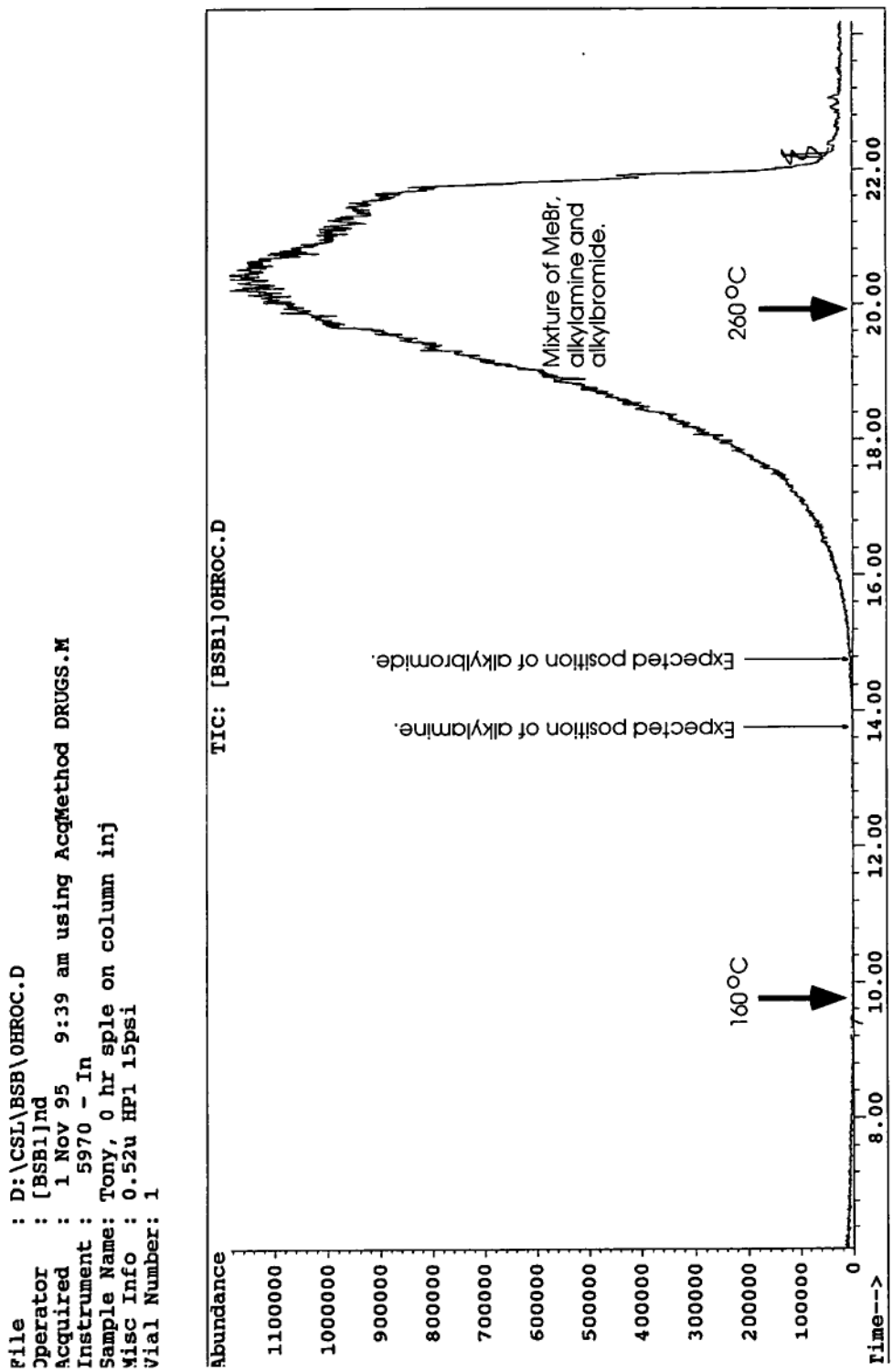
(b) LSIMS spectrum of dried TTAB.



Appendix 4.3.2.5

GC-MS spectra of undried and dried TTAB using on-column injection

(a) GC-MS spectrum of undried TTAB.



(a) GC-MS spectrum of TTAB dried at 100 °C for 8.5 days.