THE DETERMINATION OF TRACE METALS IN CONCENTRATED BRINES
FOR PROCESS CONTROL

HOWARD WILLIAM HANDLEY

A thesis submitted in partial fulfilment of the
requirements of the Council for National Academic Awards
for the degree of Doctor of Philosophy.

Sponsoring Establishment
Polytechnic South West, Drake Circus, Plymouth

Collaborating Establishment
ICI Chemicals and Polymers Ltd, Runcorn

August 1991
ABSTRACT

HOWARD WILLIAM HANDLEY

The determination of the alkaline earths in concentrated feed brines is required for process control purposes in the chlor-alkali industry. Methodology was developed, based on ion-exchange chromatography, for the determination of alkaline earths in 30% sodium chloride. Brine samples were loaded onto a dynamically coated chelating ion-exchange column. The column selectively retained the analytes of interest while allowing the matrix to pass through unchanged. This allowed the simultaneous preconcentration of the analytes and matrix removal. Following the preconcentration step, the retained metals were backflushed from the column, using a lactic acid mobile phase, and separated by cation exchange chromatography. Detection was based upon inverse photometry using a post-column reagent composed of the chelating dye calmagite and a solution of magnesium ethylenediamine tetra-acetic acid. The eluting metals displaced the magnesium which subsequently complexed with the calmagite. The decrease in absorbance, at the absorbance maxima of the dye was monitored. Using a ten ml sample, the following detection limits were obtained: 1 ng ml\(^{-1}\) magnesium, 3 ng ml\(^{-1}\) calcium, 5 ng ml\(^{-1}\) strontium and 9 ng ml\(^{-1}\) barium.

The methodology was successfully applied to the on-line analysis of feed brines used in the chlor-alkali industry. Magnesium, calcium, strontium and barium were determined in the feed brines at 3 ng ml\(^{-1}\), 30 ng ml\(^{-1}\), 80 ng ml\(^{-1}\) and 17 ng ml\(^{-1}\) respectively.

The preconcentration/matrix elimination step was successfully coupled to flame atomic absorption spectroscopy, inductively coupled plasma-atomic emission spectroscopy and inductively coupled plasma-mass spectrometry for the determination of the alkaline earths and transition metals in brine.

Using the expert system shell, CRYSTAL, a trouble-shooting guide was developed for the ion chromatographic system.
I would like to thank my supervisors Les Ebdon and Phil Jones for their continued encouragement and support throughout this work. I would also like to thank my industrial supervisors, of whom there were many. Firstly, Phil Norman, who left me after six months - was it something I said? John Carroll, who suffered me through the summer of '88, both inside and outside of the "Bears Paw", and finally, but by no means least, Neil Barnett, who eventually had to leave for Australia after being introduced to "Purple Bastards". I would also like to thank SERC and ICI Chemicals and Polymers Ltd for their financial support throughout this work.

Finally my thanks go to my typist, Alison Sparkes, for struggling through my lovely handwriting.

Dedicated to Jan
2.3.2 Chromatography 32
2.3.3 Chromatography of the Alkaline Earths using Tartaric Acid 33
2.3.4 Chromatography of the Alkaline Earths, Aluminium and Zinc using Lactic Acid 38
2.3.5 System Calibration and Limit of Detection 43
2.4 Conclusions 51

CHAPTER 3 - PRECONCENTRATION AND MATRIX ELIMINATION TECHNIQUES

3.1 Introduction 55
3.2 Instrumentation and Reagents 57
3.2.1 Instrumentation 57
3.2.2 Reagents 59
3.3 Preconcentration Procedures 59
3.4 Chelex 100 as a Preconcentration Matrix Elimination Column 60
3.4.1 Introduction 60
3.4.2 Preparation of the Chelex 100 Column 61
3.5 Results and Discussion 61
3.5.1 Effect of Washing the Preconcentration Column Upon Residual Sodium Concentration 61
3.5.2 Effect of Sample pH upon Recovery for Chelex 100 65
3.6 The Use of Coated Columns for Preconcentration 68
3.6.1 Introduction 68
3.6.2 Chrome Azurol S 69
3.6.2.1 Coating Procedure for Chrome Azurol S 71
3.6.2.2 Results and Discussion 72
6.3 Application of Preconcentration Techniques to Inductively Coupled Plasma-Atomic Emission Spectroscopy

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.3.1 Instrumentation and Reagents</td>
<td>155</td>
</tr>
<tr>
<td>6.3.2 Principle of Peak Detection by the Perkin Elmer Plasma II</td>
<td>156</td>
</tr>
<tr>
<td>6.3.3 Peak Location using the AutoExec File</td>
<td>158</td>
</tr>
<tr>
<td>6.3.4 Results and Discussion</td>
<td>159</td>
</tr>
</tbody>
</table>

6.4 Application of Preconcentration Techniques to Inductively Coupled Plasma-Mass Spectrometry

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.4.1 Instrumentation and Reagents</td>
<td>165</td>
</tr>
<tr>
<td>6.4.2 Addition of Internal Standard for ICP-MS Studies</td>
<td>167</td>
</tr>
<tr>
<td>6.4.3 Results and Discussion</td>
<td>169</td>
</tr>
<tr>
<td>6.4.3.1 Dionex Metpac CC-1 Preconcentration Column</td>
<td>169</td>
</tr>
<tr>
<td>6.4.3.2 Xylenol Orange Preconcentration Column</td>
<td>170</td>
</tr>
</tbody>
</table>

6.5 Conclusions

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAPTER 7 - CONCLUSIONS AND FUTURE WORK</td>
<td></td>
</tr>
<tr>
<td>7.1 Conclusions</td>
<td>188</td>
</tr>
<tr>
<td>7.2 Future Work</td>
<td>189</td>
</tr>
</tbody>
</table>

References 192
Meetings Attended 200
Conference Presentations 202
Appendix I I
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>Traditional analytical chemistry strategy</td>
<td>4</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Process analytical chemistry strategy</td>
<td>4</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>The elements of process analytical chemistry</td>
<td>8</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>Block diagram of the HPLC system</td>
<td>28</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Structure of the chelating dye Calmagite</td>
<td>31</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Ion-exchange separation of magnesium, calcium, strontium and barium. Eluent 0.2M Tartaric acid (pH 4.2)</td>
<td>34</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Ion-exchange separation of magnesium, calcium, strontium, and barium. Eluent 0.2M Tartaric acid, 0.025M ethylenediamine (pH 4.2)</td>
<td>36</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>Effect of the addition of MgEDTA to the post-column reagent, and ethylenediamine to the mobile phase, on the response obtained for magnesium, calcium, strontium and barium</td>
<td>37</td>
</tr>
<tr>
<td>Figure 2.6</td>
<td>Ion-exchange separation of aluminium. Eluent 0.2M lactic acid (pH 2.8)</td>
<td>39</td>
</tr>
</tbody>
</table>
Figure 2.7  Ion-exchange separation of magnesium, calcium, strontium and barium. Eluent 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8) 41

Figure 2.8  Ion-exchange separation of aluminium, zinc, magnesium, calcium, strontium and barium. Eluent gradient elution 0.2M lactic acid (pH 2.8) stepped to 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8) 3.5 min prior to injection 42

Figure 2.9  Calibration curve obtained for aluminium. Eluent 0.2M lactic acid (pH 2.8) 44

Figure 2.10  Calibration curve obtained for zinc. Eluent 0.2M lactic acid (pH 2.8) 45

Figure 2.11  Calibration curve obtained for magnesium. Eluent 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8) 46

Figure 2.12  Calibration curve obtained for calcium. Eluent 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8) 47

Figure 2.13  Calibration curve obtained for strontium. Eluent 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8) 48
Figure 2.14 Calibration curve obtained for barium. Eluent 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8)

Figure 3.1 Block diagram of the HPLC system

Figure 3.2 Discrete sampler used to interface the outlet of the preconcentration column to the nebulizer tube of the spectrometer

Figure 3.3 Residual sodium concentration with respect to wash volume

Figure 3.4 Relationship between sample pH and magnesium response using the Chelex 100 preconcentration column

Figure 3.5 Structure of the chelating dye Chrom Azurol S

Figure 3.6 Relationship between sample pH and magnesium response using the CAS preconcentration column

Figure 3.7 Relationship between sample pH and calcium response using the CAS preconcentration column
Figure 3.8  Relationship between sample pH and strontium response using the CAS preconcentration column

Figure 3.9  Relationship between sample pH and aluminium response using the CAS preconcentration column

Figure 3.10  Relationship between sample pH and zinc response using the CAS preconcentration column

Figure 3.11  Relationship between sample volume and magnesium response using the CAS preconcentration column

Figure 3.12  Calibration curve obtained for magnesium in 30% m/v sodium chloride using the CAS preconcentration column

Figure 3.13  Calibration curve obtained for aluminium in 30% m/v sodium chloride using the CAS preconcentration column

Figure 3.14  Calibration curve obtained for calcium in 30% m/v sodium chloride using the CAS preconcentration column
Figure 3.15 Calibration curve obtained for strontium in 30% m/v sodium chloride using the CAS preconcentration column

Figure 3.16 Calibration curve obtained for zinc in 30% m/v sodium chloride using the CAS preconcentration column

Figure 3.17 Structure of the chelating dye xylenol orange

Figure 3.18 Relationship between sample pH and aluminium response using the X-O preconcentration column

Figure 3.19 Relationship between sample pH and zinc response using the X-O preconcentration column

Figure 3.20 Relationship between sample pH and magnesium response using the X-O preconcentration column

Figure 3.21 Relationship between sample pH and calcium response using the X-O preconcentration column
Figure 3.22  Relationship between sample pH and strontium response using the X-O preconcentration column

Figure 3.23  Relationship between sample pH and barium response using the X-O preconcentration column

Figure 3.24  Calibration curve obtained for aluminium in 30% m/v sodium chloride using the X-O preconcentration column

Figure 3.25  Calibration curve obtained for zinc in 30% m/v sodium chloride using the X-O preconcentration column

Figure 3.26  Calibration curve obtained for magnesium in 30% m/v sodium chloride using the X-O preconcentration column

Figure 3.27  Calibration curve obtained for calcium in 30% m/v sodium chloride using the X-O preconcentration column

Figure 3.28  Calibration curve obtained for strontium in 30% m/v sodium chloride using the X-O preconcentration column
Figure 3.29  Calibration curve obtained for barium in 30% m/v sodium chloride using the X-O preconcentration column

Figure 4.1  Block diagram showing location of analyser house with respect to the ion-exchange clean-up columns

Figure 4.2  Reservoir normally used for sample collection at the analyser house

Figure 4.3  Block diagram of the HPLC system

Figure 4.4  Block diagram for the switching valves, preconcentration column and sampling loops used in the HPLC system

Figure 4.5  Extract of the chromatogram obtained during the on-line trial. The chromatogram shows three consecutive injections of the retained metals from the preconcentration column, proceeded by a calibration standard

Figure 4.6  Variation in the magnesium concentration with respect to time during the on-line trial

Figure 4.7  Variation in the calcium concentration with respect to time during the on-line trial
Figure 4.8 Variation in the strontium concentration with respect to time during the on-line trial 120

Figure 4.9 Variation in the barium concentration with respect to time during the on-line trial 121

Figure 5.1 Basic elements of artificial intelligence as described by Nilsson 129

Figure 5.2 Initial structure of the knowledge base showing the various levels below the troubleshooting guide 137

Figure 6.1 Discrete sampler used to interface the outlet from the preconcentration column to the nebulizer tube of the spectrometer 147

Figure 6.2 Residual sodium concentration with respect to wash volume obtained during the flame atomic absorption studies 149

Figure 6.3 Calibration curve obtained for magnesium in 30% m/v sodium chloride using the 8HQ pre-concentration column followed by FI-FAAS 150

Figure 6.4 Calibration curve obtained for calcium in 30% m/v sodium chloride using the 8HQ pre-concentration column followed by FI-FAAS 151
Figure 6.5  Calibration curve obtained for strontium in 30% m/v sodium chloride using the 8HQ pre-concentration column followed by FI-FAAS

Figure 6.6  Calibration curve obtained for barium in 30% m/v sodium chloride using the 8HQ pre-concentration column followed by FI-FAAS

Figure 6.7  Transient signals obtained for magnesium, calcium, strontium and aluminium in 30% m/v sodium chloride using the 8HQ preconcentration column followed by FI-FAAS

Figure 6.8  Residual sodium concentration with respect to wash volume obtained during the inductively coupled plasma-atomic emission studies

Figure 6.9  Relationship between sample pH and magnesium response using the metpac CC-1 preconcentration column followed by FI-ICP-AES

Figure 6.10  Relationship between sample pH and calcium response using the metpac CC-1 preconcentration column followed by FI-ICP-AES

Figure 6.11  Relationship between sample pH and strontium response using the metpac CC-1 preconcentration column followed by FI-ICP-AES
Figure 6.12 Block diagram of the switching valves, preconcentration column and sample loops used during the inductively coupled plasma-mass spectrometer studies

Figure 6.13 Residual sodium concentration with respect to wash volume obtained during the inductively coupled plasma-mass spectrometer studies

Figure 6.14 Calibration curve obtained for magnesium in 30% sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

Figure 6.15 Calibration curve obtained for aluminium in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

Figure 6.16 Calibration curve obtained for titanium in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

Figure 6.17 Calibration curve obtained for vanadium in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS
Figure 6.18 Calibration curve obtained for chromium in 30% m/v sodium chloride using the metpac CC-1 concentration column followed by FI-ICP-MS

Figure 6.19 Calibration curve obtained for manganese in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

Figure 6.20 Calibration curve obtained for nickel in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

Figure 6.21 Calibration curve obtained for cobalt in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

Figure 6.22 Calibration curve obtained for zinc in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

Figure 6.23 Calibration curve obtained for copper in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS
Figure 6.24  Calibration curve obtained for strontium in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS 182

Figure 6.25  Calibration curve obtained for magnesium in 30% m/v sodium chloride using the xylenol orange preconcentration column followed by FI-ICP-MS 182

Figure 6.26  Calibration curve obtained for strontium in 30% m/v sodium chloride using the xylenol orange preconcentration column followed by FI-ICP-MS 185

Figure 6.27  Calibration curve obtained for barium in 30% m/v sodium chloride using the xylenol orange preconcentration column followed by FI-ICP-MS 186
CHAPTER 1

INTRODUCTION

1.1 PROCESS ANALYTICAL CHEMISTRY

1.2 PROCESS ANALYTICAL CHEMISTRY IN THE CHLOR-ALKALI INDUSTRY

1.3 DETERMINATION OF TRACE METALS BY LIQUID CHROMATOGRAPHY
   1.3.1 Absorption Chromatography
   1.3.2 Partition Chromatography
   1.3.3 Ion-Exchange Chromatography

1.4 DETECTION SYSTEMS IN LIQUID CHROMATOGRAPHY
   1.4.1 Electrochemical Detection
   1.4.2 Photometric Detection
   1.4.2.1 Post-column reactions in photometric detection

1.5 APPLICATION OF ION CHROMATOGRAPHY TO THE ANALYSIS OF SALINE MEDIA

1.6 THE APPLICATION OF ION CHROMATOGRAPHY TO ATOMIC SPECTROSCOPY

1.7 THE ROLE OF EXPERT SYSTEMS IN ANALYTICAL CHEMISTRY

1.8 AIMS
INTRODUCTION

1.1 PROCESS ANALYTICAL CHEMISTRY

In recent years there have been tremendous advances in the trace analysis of aqueous samples. Laboratory-based procedures are now capable of routine determination of analytes at the low ng ml⁻¹ level. However, with continuous advances in manufacturing and other industries there are now demands for the continuous monitoring of production and effluent streams for process control and environmental purposes. Unfortunately laboratory-based analysis is not usually sufficiently rapid or robust for such process control situations. This has led to the development of rapid, robust on-line methodology for process control. In parallel to these developments there has emerged a new sub-discipline of analytical chemistry, that of Process Analytical Chemistry.

The emergence of Process Analytical Chemistry (PAC) has been driven by a shift in emphasis in manufacturing industries from that of process and product innovation, towards improving existing processes and products to gain a competitive edge (1). In doing so, such words as quality, productivity and waste reduction have become commonplace in these industries. The need for information on products, process streams and effluent streams in real time, in order to maximise profits and reduce waste is continuing to grow.
Traditionally, when an analysis is required on a product a sample is taken, transported to a central laboratory on site, or off-site where it is logged, prioritised and subsequently analysed. The results are then recorded and filed for future reference. The time taken between sample collection and obtaining results can range from several hours to several days. The information obtained is always retrospective and is used mainly in 'post-mortem' analysis after a process has gone wrong. The timescale involved in the process is too great for the data to be useful in process control. In addition to the time factor, each of the steps involved in the process, outlined in figure 1.1, can introduce errors either through contamination, sample losses, poor labelling or poor sample storage.

The philosophy behind PAC is somewhat different to 'traditional' analytical chemistry in that a measurement is made and a decision based on that measurement taken immediately, shown in figure 1.2. Process analytical chemistry tends to be problem driven rather than technique driven, the questions being posed are not "what problems can I solve with this technique?", but rather "what techniques can I use to solve this problem?".

Clearly for PAC to have an impact upon the process it is monitoring, the measurement must be carried out in close proximity to that process. Four areas of PAC have evolved to address this problem. These are at-line, on-line, in-line and non-invasive.
Figure 1.1 Traditional analytical chemistry strategy

SAMPLE \rightarrow TRANSPORT \rightarrow RECORD

DECIDE \leftrightarrow TRANSMIT \leftrightarrow MEASURE

FILE

Figure 1.2 Process analytical chemistry strategy

MEASURE \leftrightarrow DECIDE

-4-
At-line process analysis is similar to the traditional situation, however, in this case a dedicated instrument is situated close to the process to be monitored, samples are taken and analysed immediately. This does, however, usually require the presence of an operator to collect samples and perform the analysis.

On-line process analysers are distinguished from at-line analysers in that an automated sampling system is used to extract the sample from the process itself and introduce it to the analyser, following any sample pre-treatment that may be required. On-line analysis may be divided into two categories, intermittent methods that require injection of a sample stream into the instrument, and continuous methods in which the sample flows continuously through the instrument. In both cases the instrumentation tends to be built into the process itself and allows for unattended operation.

The third type of process analysers are the in-line analysers where the measurement is made in situ. They have the advantage that no sample needs to be taken from the process. Such analysers, however, tend to be difficult to calibrate and are prone to fouling. Lastly there is the non-invasive analysers. These analysers represent the ultimate goal of process analysers in that no sample needs to be taken from the process stream, nor does the instrumentation impinge on the process. Table 1.1 lists the four types of analyser and examples of each (1). Riebe
## Table 1.1

### Four Types of Process Analyser

<table>
<thead>
<tr>
<th>Type of Analyser</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>At Line</td>
<td>Colourimeter</td>
</tr>
<tr>
<td></td>
<td>Flow Injection Analyser</td>
</tr>
<tr>
<td>On-Line</td>
<td>Capillary Gas Chromatography</td>
</tr>
<tr>
<td></td>
<td>Flow Injection Analyser</td>
</tr>
<tr>
<td>In-Line</td>
<td>Conductivity Sensor</td>
</tr>
<tr>
<td></td>
<td>pH Sensor</td>
</tr>
<tr>
<td>Non-Invasive</td>
<td>Diffuse Reflectance Near-IR</td>
</tr>
</tbody>
</table>
and Eustace (1) described process analytical chemistry in terms of the elements that make it up (figure 1.3).

1.2 PROCESS ANALYTICAL CHEMISTRY IN THE CHLOR-ALKALI INDUSTRY

The PAC philosophy is now entering many process industries, one example being the chlor-alkali industry. The electrolysis of brine to produce chlorine and caustic soda is the world's largest electrolytic process (2) producing around 40 million tonnes of caustic soda per annum. New technology in the chlor-alkali industry has led to a shift away from mercury and diaphragm cells, which although tolerant of feed brine quality with respect to trace metal impurities have associated environmental problems, towards membrane cell technology. Membrane cells offer several advantages over the previous two cell designs in that higher strength, purer caustic is produced at lower operating costs. Due to the nature of the membrane, however, trace metal levels in the feed brines must be maintained at the low ng ml$^{-1}$ level. Relatively high concentrations of trace metals such as the alkaline earths cause decreased current efficiencies and physical disruption of the membrane. If economic life times of the membranes are to be achieved therefore, the quality of the feed brines used in the process must be monitored. Due to the tolerances placed upon the purity of the feed brine
Figure 1.3  The elements of process analytical chemistry

- CHEMOMETRICS
- SPECTROSCOPIC METHODS
- CHROMATOGRAPHIC METHODS
- PHYSICAL METHODS
- CONTROL STRATEGIES
- ELECTROCHEMICAL METHODS
- SAMPLING STRATEGIES

PROCESS ANALYSIS PROBLEM
with respect to trace metal levels, the feed brines are passed through ion-exchange columns prior to the electrolysis cells themselves. The ion exchange columns are designed to remove magnesium and calcium from the brines to below 50 ng ml\(^{-1}\), and to a lesser degree remove strontium, barium and the first row transition metals. Typically two such columns are employed, one being used to retain trace metals from the brines whilst the other is regenerated and conditioned. The columns are typically used for seven hours before regeneration. In order to optimise the use of these ion-exchange columns and to ensure that the feed brines entering the electrolysis cells is within the required specification, it is necessary to monitor the concentrations of the trace metals from the outlet of these columns.

For process control purposes, the analysis would need to be made either at-line or on-line. An on-line analyser is preferred as this allows for unattended operation. Any on-line methodology that is developed must be capable of routine, unattended operation, provide data with minimal calibration, meet stringent safety requirements, be robust and function in the presence of a matrix.

Liquid chromatography and flow-injection techniques lend themselves well to on-line analysis. Both techniques utilise flowing streams themselves, so that interfacing with a process stream does not usually require any elaborate coupling. They tend to operate at ambient
temperature and pressure and the instrumentation is robust, requiring minimal maintenance. Flow injection techniques are inexpensive and provide rapid, reproducible analysis allowing for high sample throughput and continuous sampling.

Liquid chromatographic techniques on the other hand provide a means of multi-element determination. Such techniques suffer less from interferences due to the separation step and it is possible to improve sensitivities by incorporating a preconcentration step. These advantages are gained however, at the expense of speed of analysis.

As the feed brines used in the chlor-alkali industry may differ in their composition with respect to the alkaline earths, and as the ion-exchange 'clean up' columns used to purify the brines will have different selectivities for the different metals, it would be useful to obtain qualitative information as well as quantitative information on the feed brines for process control purposes. In view of the requirements, liquid chromatography was chosen as the analytical technique for the determination of trace metals in concentrated brines.

1.3 DETERMINATION OF TRACE METALS BY LIQUID CHROMATOGRAPHY

The basic theory and principles of liquid chromatography are well documented and as such will not be dealt with here. Instead the reader is referred to a text on the
subject (3). The determination of metals by liquid chromatography (LC) may be divided into three areas, adsorption chromatography, partition chromatography and ion exchange chromatography. Adsorption and partition chromatography are primarily used for organic media whilst ion exchange chromatography is applied to aqueous media.

1.3.1 Adsorption Chromatography

Silica gel is the most widely used stationary phase in classical adsorption chromatography. Silica gel is a polar material and is therefore used with low polarity mobile phases. As most metal species are in the ionic form, especially after any sample pre-treatment such as wet oxidation, this presents a problem for the determination of metal species by adsorption chromatography. One method for overcoming this problem is the formation of neutral metal complexes with a suitable organic chelating agent and extracting the complexes into a non-aqueous phase prior to analysis. Such solvent extractions do complicate the analysis although there are advantages to the technique such as removal of the sample matrix and preconcentration of the analytes, both of which lead to reduced interferences and improved sensitivities. Some of the earliest examples of metal separations using adsorption chromatography made use of dithiocarbamate derivatives. Liska et al. (4) separated nine metals as their diethylidithiocarbamates on a silica gel substrate with a mobile phase of chloroform in cyclohexane. Some of the
more important examples of adsorption chromatography for the separation of metals have been reported by Edward-Inatimi (5+6).

The separation of Co, Cu, Hg, Mn, Ni and Pb was reported by Edward-Inatimi and Dalziel (5). The method was also applied to the determination of trace metals in effluents (6). One point which arose from this work was the different sensitivities obtained for the different metals. This is due in part to the difference in the absorption wavelength maximum between the different metal chelates and also to the difference in the molar absorptivities of the different complexes. This illustrates the problems associated with choosing a common wavelength for the determination of several metal complexes.

1.3.2 Partition Chromatography

Partition chromatography is perhaps the most flexible of all the separation systems. Essentially the separation is based on a sorption mechanism where both the mobile phase and the stationary phase are liquids. Its use has been limited due to problems arising from the mutual solubility of the two liquids and mechanical dislodgment of the stationary phase, both of which lead to deterioration in the separation. The classical liquid/liquid systems have now been superseded by bonded phase systems. Bonded phase systems although not strictly partition systems, combine the versatility of partition chromatography with the high
efficiency and rapid mass-transfer of adsorption chromatography. A bonded phase system is typically composed of a silica gel substrate with a range of organic groups bonded to the surface silanol groups. The resulting resin may be used either as a normal phase or reverse phase column depending upon the polarity of the groups bonded to the surface. The most popular method utilizes an alkyl bonded stationary phase with a mobile phase such as methanol or acetonitrile in water. The principal mechanism for separation is based on the formation of stable metal chelates. These chelates are either formed prior to injection of the sample (external) or after injection of the sample (in situ).

Amongst the techniques available for external chelate formation is liquid/liquid extraction, whereby the chelating agent is added to the sample and the metal chelates formed are then extracted into an organic phase. A second method involves the addition of an aliquot of the mobile phase to the sample in the presence of the chelating agent. Thirdly, metal chelates formed in the aqueous phase may be retained on a precolumn and subsequently eluted with a small volume of a non-aqueous solvent prior to injection. Bond and Wallace (7) investigated the above techniques and found, that although the second procedure was less complicated, the other two techniques were applicable to a wider range of metals and, due to the preconcentration step involved, provided improved sensitivities.
If the metal chelates are to be formed \textit{in situ} the aqueous sample is injected directly into the system where the mobile phase contains a low concentration of the complexing ligand.

The separation mechanism in bonded phase systems can be effected by unreacted silanol groups on the surface of the silica gel. Thus the separation mechanism may be due to a combination of partition and adsorption processes.

Although successful separations have been achieved using adsorption and partition chromatography, the most widely studied method is that of ion-exchange chromatography.

1.3.3 Ion-Exchange Chromatography

Although the term "ion-chromatography" was initially applied to the original system developed by Small \textit{et al} (8) for ion-exchange chromatography using conductivity detection and chemically based suppression, it is now used in the broader context of any reasonably efficient separation of ionic species using automated detection of the ions (9). It is hardly surprising that ion chromatography is the most studied method for the separation of metal species as most samples contain metals as either cations or anions and ion-exchange chromatography is performed in aqueous media. Tremendous advances have been made in ion chromatography since the first classical separations such as that by Fritz and Story (10).
presence of complexing agents in the mobile phase improves both the speed and resolution of cationic separations. Chelating agents may be simple monodentate ligands such as halides or polydentate ligands such as poly functional carboxylic acids. The choice of chelating agent is limited somewhat by factors such as corrosion of the HPLC hardware, degradation of the stationary phase, and interferences with detection methods. The most common chelating agents used are carboxylic acids such as oxalic, tartaric, citric and hydroxybutyric acids. The latter was used as a mobile phase for the separation of the lanthanides on Aminex Cation exchange resin by Elchuk and Cassidy (11). Takata and Fujita (12) reported the separation of Cd, Co, Cu, Ni, Pb and Zn using tartaric and oxalic acids. The growth in the number of publications involving ion-exchange chromatography is due in part to improvements in resin materials. Highly cross-linked resins are now available which are tolerant to the high back pressures experienced in some systems, and low capacity columns are available with only the surface of the resin treated with sulphonic acid groups enabling separations to be performed with dilute mobile phases which in turn offer less interferences to detection methods. Such low capacity columns are not, however, suitable for analysis of samples with high salt concentrations.
1.4 DETECTION SYSTEMS IN LIQUID CHROMATOGRAPHY

The most common detectors used in ion chromatography are electrochemical or photometric. The mobile phase used is not always compatible with the sensitive detection of the separated analytes. Depending upon the mobile phase used, the analytes may not be in a form easily detected, or it may contribute to unnecessary interferences. A common technique to overcome these problems is that of post-column reaction, also known as post-column derivitisation.

1.4.1 Electrochemical Detection

Of the electrochemical methods available the most common is conductivity detection, although as reactions take place at the electrodes, it is not strictly electrochemical. Post-column reaction used in conductivity detection is based upon suppression of the conductivity of the mobile phase. Small et al. (8) originally developed suppressed conductivity whereby the mobile phase is converted to non-conducting distilled water. Potentiometric detectors are used mainly for the determination of organic species and inorganic anions (13). Haddad et al (14,15) developed an indirect potentiometric detector based on a copper electrode cell.

There have been many publications on the use of amperometric and coulometric detection systems (7,13,16-20).
1.4.2 Photometric Detection

Most simple compounds do not absorb strongly in the UV or visible regions of the spectrum. Two methods are commonly employed to overcome this problem, pre-column reaction or post-column reaction. Pre-column reactions are generally associated with the formation of dithiocarbamate metal chelates. The pre-formed chelates are then utilized for both the separation and detection and will not be discussed here.

1.4.2.1 Post-column reactions in photometric detection

When performing ion-exchange chromatography with weakly complexing mobile phases, the eluting species have little or no absorbance in the UV or visible region of the spectrum. The colourimetric detection of trace metals is a well known area in analytical chemistry and a wealth of knowledge is available for exploitation (21). The basic principle behind post-column reaction is that the eluent from the analytical column is mixed with a second stream containing a chelating agent. The metal complexes which are subsequently formed are then monitored in the UV/visible region of the spectrum. The choice of chelating agent is limited by several factors. Firstly, both the chelating agent and the metal complex which is formed must be soluble in aqueous media. The formation of the complex should be rapid and non-reversible, and the molar...
absorptivities of the complexes should be high. Finally the wavelength of maximum absorbance of the complex should be well separated from that of the reagent.

The chelating agent 4-(2-pyridyl)azoresorcinol (PAR) satisfies these criteria and reacts with a range of metals including Bi, Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn and the lanthanides. It was first used as a post-column reagent by Kawazu and Fritz (22) and Fritz and Story (10). The PAR reagent has also been used for HPLC separations (23-25).

Other reagents which have been studied for use as post-column reactors are xylenol orange (26) and Arsenazo I and III (10). An example of a specific, rather than a general post-column reagent is bathophenanthroline disulphonic acid (27) which is specific for Fe(II) and Fe(III) only. However, when several eluting species are to be determined there is still a problem of choosing a compromise wavelength to detect the different species, due to the difference in wavelength of maximum absorbance of each. There are two approaches to overcome this. Arguello and Fritz (28) described a method based on a system developed by Takata and Fujita (12) utilizing metal displacement reactions. The method consisted of addition of a Zn EDTA solution to the PAR post-column reagent. When eluting metals mixed with the reagent the Zn was displaced from the EDTA, providing favourable stability constants existed, and the liberated Zn complexed with the PAR reagent. The detector wavelength was set to absorb at the maximum absorbance of the Zn PAR complex. Further studies on
this system were carried out by Jezorek and Freiser (29).

A second method is to monitor the consumption of the reagent rather than the formation of the metal complexes. The detector wavelength is set to the absorbance maxima of the reagent. Any metal which complexes with it will cause a decrease in the absorbance at that wavelength. Dithizone (30) and Erichrome black T (31,32) have been used in this way. The technique is also known as inverse photometry. It has the advantage that only one wavelength needs to be monitored – that of the reagent. Due to an absorbance maxima being monitored however, any pump noise tends to be exaggerated. Metals detected by these systems are essentially the same as those detected by the PAR reagent but with the addition of Mg and Ca. Molecular emission detection, fluorescence, has found fewer applications than absorption. The technique is more sensitive and selective than absorption, however transition metals quench the fluorescence if present in the sample. Beckett and Nelson (33) separated Zn, Cd and Pb as complexes of amino derivatives of EDTA followed by post-column detection with fluorescamine. Jones et al (34) separated Al, Ga and In on a cation exchange resin followed by post-column reaction with 8-hydroxyquinoline-5-sulphonic acid (8HQ5S).
1.5 APPLICATION OF ION-CHROMATOGRAPHY TO THE ANALYSIS OF SALINE MEDIA

While most analytical techniques are readily applicable to the determination of trace elements in simple media, the quantification of these trace elements in complex sample matrices often presents formidable problems. Ion chromatography is no exception to this. The extremely high levels of matrix elements in samples such as sea waters, estuarine water and brines, when passed through an ion-exchange column, swamp the active sites on the resin and upset the equilibrium between the mobile phase and the analytes of interest. This causes serious degradation of the chromatography and detection systems. The most common method of addressing this problem is the use of matrix removal and analyte preconcentration techniques. The most frequently employed method utilizes an iminodiacetic acid chelating resin such as Chelex 100. Chelex 100 is a styrene-divinyl benzene polymer with only light cross-linking (1-2%) onto which is bonded an iminodiacetic acid functionality. There have been numerous reports in the literature on the properties and use of Chelex 100 and its analogue Dowex A-1, for the selective retention of trace metals from saline media (35-40). These methods however, describe the retention of transition metals from mainly sea water samples and subsequent determination of the retained metals is generally carried by atomic spectroscopic methods. There are few papers concerning the determination of the alkaline earths in concentrated brines (30% m/v sodium
chloride) and of these none use ion chromatographic techniques for the determination.

Kehr et al (41) determined calcium and magnesium at the low \( \mu g \ l^{-1} \) level in concentrated brines by direct introduction of the sample into an inductively coupled plasma atomic emission spectrometer (ICP-AES), having first diluted the brine sample 3 + 5 with de-ionised water. Wada et al (42) described the use of the chelating resin Dowex A-1 for the retention of magnesium and calcium from a 2.5M sodium chloride matrix. The combined magnesium and calcium concentration was subsequently determined by flow injection analysis forming a complex with 1-(2-hydroxy-4-diethylamino-1-phenylazo) 2-hydroxynaphthalene-3, 6-disulphonic acid. The magnesium content was subsequently determined in a second sample by masking the calcium by the addition of a ligand buffer containing an excess of barium (II) ethylene glycol tetraacetic acid (EGTA), and the calcium found by difference. Siriraks and Kingston (43) developed an on-line preconcentration ion chromatographic method for the determination of transition metals in sea waters. The method used a more stable form of the iminodiacetic acid chelating resin, with a high degree of cross-linking which allowed its use at elevated pressures. In order to elute the retained metals in as small a volume as possible however, a relatively high acid concentration (0.5 -1.0M H\(^+\)) was required which was not directly compatible with the low capacity cation-exchange resin being used. To overcome this problem a third column was added to concentrate the retained metals from the
chelating resin prior to injection onto the analytical column. The addition of a third column and the associated valves and pumps necessary to deliver additional reagents makes this unattractive for a process environment. This highlights the need for compatibility between the chelation column and the analytical column. More recently Chambaz and Haerdi (44) did describe the on-line preconcentration and elution of trace metals by ion chromatography although in this case no mention was made of the alkaline earths and the determination was carried out in a natural river water sample, rather than a highly saline matrix. Toei (45) described the use of the chelating column TSK-GEL chelate 5-PW for the separation of the alkaline earths using, as a mobile phase, 0.2M potassium chloride with the addition of 0.1m mol 1^-1 0-Cresol-phthaleine complexone. The method was used for the determination of magnesium and calcium in sea water following direct injection of the sample. Although no investigations were made as to the suitability of the column for the preconcentration of the alkaline earths from saline media, the column clearly holds potential for such applications.

1.6 THE APPLICATION OF ION-CROMATOGRAPHY TO ATOMIC SPECTROSCOPY

In recent years there has been an increased interest in the benefits offered by coupling ion chromatography, indeed liquid chromatography in general, to atomic spectroscopic techniques. This is not surprising as although
spectroscopic methods offer rapid elemental determination, they offer no information on the speciation of the elements present. In line with the interest in coupled ion-chromatography-atomic spectroscopy, there is also interest in the applications of matrix removal techniques prior to analysis by atomic spectroscopy. As has already been said, most analytical techniques are not suited to the direct analysis of highly saline media. Although there has been little published work on the applications of chelation preconcentration ion chromatography, this is not the case for the application of such techniques to atomic spectroscopy. The application of the chelating resin chelex 100 to the determination of transition metals in sea waters by flame atomic absorption spectrometry has already been mentioned. Van Berkel et al (46) described the determination of Cd, Co, Cu, Mn, Pb and Zn from sea waters using chelex 100 prior to analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES). When using atomic spectroscopy the choice of chelating agent used in the preconcentration column is not restricted by as many factors as when the determination is carried out using ion chromatography.

For instance, a more strongly chelating agent such as 8-hydroxyquinoline (8HQ) may be used. The use of 8HQ in ion chromatography is limited due to the strength of the mobile phase required to elute the retained metals from the column. Sturgeon et al (47,48) and Willie et al (49) have described the use of immobilized 8HQ for the
preconcentration of Cd, Co, Fe, Mn, Pb and Zn from sea water samples followed by elution with a hydrochloric acid/nitric acid eluent and subsequent determination by graphite furnace atomic absorption spectroscopy (GFAAS). Typically 500 ml aliquots of sample were preconcentrated allowing the determination of the analytes of interest in near-shore sea water at the low ng ml\(^{-1}\) level.

Malamas et al (50) used 8-quinolinol immobilised onto porous glass beads as an on-line preconcentrator column for the extraction of Cd, Co, Cu, Ni, Pb and Zn from aqueous samples followed by flow injection coupled with an atomic absorption spectrometer (FI-AAS). None of the methods cited have described the determination of the alkaline earths and all have been concerned with the analysis of sea water samples rather than concentrated brines (30% m/v sodium chloride).

1.7 THE ROLE OF EXPERT SYSTEMS IN ANALYTICAL CHEMISTRY

During the last decade, expert systems have made a dramatic impact in analytical chemistry. The scope of these systems ranges from decision based systems for use with laboratory instrumentation, laboratory management systems, which, if presented with a sample are capable of suggesting appropriate techniques for the determination of X, Y and Z in that sample. Expert systems are also used in data interpretation for infrared spectra and mass spectra, and used in optimization procedures in liquid chromatography.
Expert systems work well in areas where ideas exist about the solution, but where they have yet to be fully stated, where the knowledge base exists, but additions continue to be made. To either side of this are two areas which are inappropriate. Either insufficient information is available or the problem is too vague, or else the problem is so well defined that it is more convenient to solve the problem with a few lines of BASIC programming. It is not intended to discuss expert systems here, but they are dealt with in Chapter 5.

1.8 AIMS

The aim of this thesis was to develop a method for the determination of the alkaline earth metal cations in concentrated brines (30% m/v sodium chloride) using chelation exchange preconcentration and ion exchange chromatography with inverse photometric detection. The preconcentration techniques developed were subsequently applied to the determination of the alkaline earths and transition metals by FAAS, ICP-AES and ICP-MS. Finally a knowledge based users guide was written for use with the ion chromatography system.
CHAPTER 2
SEPARATION AND DETECTION

2.1 INTRODUCTION

2.2 EXPERIMENTAL
2.2.1 Instrumentation
2.2.2 Reagents

2.3 RESULTS AND DISCUSSION
2.3.1 Detection System
2.3.2 Chromatography
2.3.3 Chromatography of the alkaline earths using tartaric acid
2.3.4 Chromatography of the alkaline earths; aluminium and zinc using lactic acid
2.3.5 System calibration and limit of detection

2.4 CONCLUSIONS
CHAPTER 2
SEPARATION AND DETECTION

2.1 INTRODUCTION

The chromatography of the alkaline earths and first row transition metals have already been reported (51, 12). It was necessary however, to develop a chromatographic separation of the metal ions of interest that was both rapid, robust and did not require gradient elution, so maintaining simplicity of the system. A further requirement was to achieve the separation in a matrix containing an excess of sodium ions. The detection system also had to be reliable, robust, and free from interferences from sodium ions. Photometric detection was favoured as the detection system, and an existing inverse photometric detection system (31) was developed further for use as a universal post-column detection system.

2.2 EXPERIMENTAL

2.2.1 Instrumentation

The basic instrumentation used during the development of the chromatography and detection is outlined in figure 2.1. The system comprises two HPLC pumps (Constametric III, Laboratory Data Control, Riviera Beach, FL., USA) which were used for delivery of the mobile phase and post-column reagent, both were set to 1 ml min⁻¹ unless otherwise stated. A 100 µl sample loop was connected across 2 ports.
Figure 2.1  Block diagram of the HPLC system
of a 6 port switching valve (Rheodyne, 7000 series, Rheodyne, Cotati, CA, USA). The sample loop was filled via suction from a 1 ml syringe, so avoiding possible contamination from the syringe itself. The analytical column was a high capacity strong cation exchange resin (Benson BC-X10, Benson Company, Reno, Nevada, USA). The eluting metals were detected using a uv/vis spectrometer (Shoefel spectroflow monitor, Kratos Ltd., Urmston, Manchester) set to absorb at 610 nm. Both the analytical column and reaction coil were maintained at 60°C in a water bath. Results were collected and plotted on a plotter/integrator (Spectrophysics, Autolab Division, San Jose, C.A.).

2.2.2 Reagents
All reagents were of analytical grade unless stated otherwise. Distilled deionised water was obtained from a milli Q system (Millipore, Bedford, Ma, USA). The mobile phase and post-column reagent were varied throughout this study and will be described when appropriate. Standards were prepared by serial dilution from stock solutions (BDH Chemicals Ltd., Poole, England).
2.3 RESULTS AND DISCUSSION

Initial studies focused on the alkaline earths, magnesium, calcium, strontium and barium.

2.3.1 Detection System

The detection system used throughout this study is based upon inverse photometry. This is a technique whereby the effluent from the analytical column is mixed in a zero dead volume 'T' piece with a post-column reagent, usually a strongly absorbing chelating dye. The principles of inverse photometry have been dealt with in section 1.4.

For this study the chelating dye Calmagite (figure 2.2) was chosen as the post-column reagent. Calmagite is an analogue of erichrome black T (EBT) which has long been used as an indicator in the colorimetric determination of magnesium and calcium (52). Although metal cations such as magnesium, zinc and cobalt complex directly with calmagite thus providing a means of detection, calcium, strontium and barium, however, do not. In order to determine these metals a solution of magnesium ethylenediamine tetra-acetic acid (MgEDTA) is added to the post-column reagent. When the eluting metal cations mix with the post-column reagent the following reaction proceeds:

\[ M^{n+} + MgEDTA \rightarrow Mg^{2+} + [MEDTA]^{n-2+} \]

where \( M^{n+} \) is the eluting cation.

The liberated magnesium ions then complex with the
Figure 2.2 Structure of the chelating dye Calmagite
calmagite so providing a means of detection for metals such as calcium. Clearly any molar excess of EDTA present would negate any detection, as the eluting cations would simply complex with the EDTA without liberating any magnesium ions. Conversely, a large molar excess of magnesium will lead to an increase base line as this magnesium will lower the absorbance of the post-column reagent at the absorbance maxima of the dye. However, for this application it is preferable to have a slight molar excess of magnesium present in the MgEDTA solution. This provides a universal detection system for all the metals of interest. As only one wavelength is monitored no scanning monochromators are required and the system is relatively free from interferences from an excess of sodium ions.

2.3.2 Chromatography

Traditional ion-exchange chromatography is not suitable for the analysis of highly saline samples due to swamping of the exchange sites on the column. To overcome the problems associated with such analysis, techniques were developed to eliminate the matrix (Chapter 3). As it is likely that not all the sodium ions from the matrix will be removed, a high capacity strong cation-exchange resin (Benson BC-X10) was chosen as the analytical column which would provide separation of the metals in the presence of sodium ions. A suitable mobile phase for such a column might be composed of either a 'counter-ion' such as potassium sulphate, a chelating agent or both. Initial studies focused upon
chelating acids due to their different selectivities for different metals thus allowing separations in the presence of the sodium chloride matrix.

2.3.3 Chromatography of the alkaline earths using tartaric acid

Reagents:
Mobile phase: 0.2 M tartaric acid pH adjusted to 4.2 using ammonium hydroxide solution.
Post-column reagent: 2.5 x 10^{-4} M Calmagite 2.5 x 10^{-4} M. MgEDTA solution, prepared in 1M ammonia solution pH 12.

Under these conditions the order of elution of the alkaline earths was Mg, Ca, Sr, Ba. Using this mobile phase however, the retention time for barium was 63 minutes (Figure 2.3). This is too lengthy a separation for process monitoring and the determination of strontium and barium under these conditions becomes difficult due to broadening of the peaks. One way to speed up the chromatography would be to increase the concentration of the mobile phase. However, as the concentration of the mobile phase is increased, so the background noise increases due to impurities present in the tartaric acid, and from the point of view of process monitoring, reagent consumption would be high. An alternative approach is the addition of either a counter ion, or a secondary chelating agent.
Figure 2.3  Ion-exchange separation of magnesium, calcium, strontium and barium. Eluent 0.2M Tartaric acid (pH 4.2)
Ethylenediamine (ene) was chosen as a counter ion. At pH 4.2 ethylenediamine is almost fully protonated and so acts as a competing ion. By the addition of 0.025M ethylene-diamine to the mobile phase, adjusting the pH to 4.2 as before with concentrated nitric acid, the separation of the four metals was accelerated, although some resolution between the magnesium and calcium peaks was lost (Figure 2.4). The retention time of barium was still too long for practical use however. The effect of addition of MgEDTA and ethylenediamine to the post-column reagent and mobile phase respectively was studied.

The pH of the mobile phase and post-column reagent was pH 9. At this pH ethylenediamine is an effective chelating agent and competes for metal ions. Any metal ions which form chelates with ethylenediamine were subsequently not available for chelation with calmagite and so were not detected. The effect was more pronounced with increasing concentration of ethylenediamine.

As magnesium ethylenediamine tetra-acetic acid is added to the post-column reagent, this effect is reduced. Eluting ions chelate with the EDTA so liberating magnesium which is subsequently detected. Further addition of MgEDTA to the post-column reagent resulted in a decrease in signal as the background concentration of magnesium contributes to the background noise (Figure 2.5).
Figure 2.4  Ion-exchange separation of magnesium, calcium, strontium, and barium. Eluent 0.2M Tartaric acid, 0.025M ethylenediamine (pH 4.2)
Figure 2.5  Effect of the addition of MgEDTA to the post-column reagent, and ethylenediamine to the mobile phase, on the response obtained for magnesium, calcium, strontium and barium.
2.3.4 Chromatography of the alkaline earths, aluminium and zinc with lactic acid

Although the chromatography of the alkaline earths has been studied, aluminium and zinc are also of interest. In order to obtain any chromatography of aluminium on a cation exchange column the pH of the mobile phase must be below 3.0 to prevent formation of the hydroxides; Al(OH)$_2^+$, Al(OH)$_2^{2+}$ and Al(OH)$_3^0$. Formation of these hydroxides leads to deterioration of the aluminium peak due to the different charge in each form. The tartaric acid was used as a mobile phase at pH 3.0 for the determination of aluminium. Due to the poor kinetics of ligand exchange between aluminium and tartaric acid, however, the peak shape for aluminium was very poor. The addition of ethylenediamine to the mobile phase caused elution of aluminium on the solvent front.

Evidently tartaric acid is not suitable as an eluent for aluminium in this system. Lactic acid, although a weaker chelating acid than tartaric acid, does have more favourable kinetics associated with ligand exchange. A mobile phase of lactic acid (0.2M pH 2.8) gave improved peak shape for aluminium with a retention time of 3.5 min (Figure 2.6). Chromatography of the alkaline earths was also required if this was to be a suitable mobile phase. By addition of 0.05 M ethylenediamine to the lactic acid at pH 2.8, adjusted with concentrated nitric acid, excellent chromatography was obtained for the four alkaline earths.
Figure 2.6  Ion-exchange separation of aluminium. Eluent
0.2M lactic acid (pH 2.8)

\[ \text{Al.} \]

Time (min)
On addition of ethylenediamine to the mobile phase, however, aluminium eluted on the solvent front, so in order to separate all the metals, a step gradient was required.

The following step gradient was used to elute Al, Zn, Mg, Ca, Sr and Ba. The column was conditioned with the first mobile phase of 0.2 M lactic acid at pH 2.8, because a gradient pump was not available a step gradient was used, whereby the mobile phase was changed at the reservoir of the mobile phase pump. The dead volume from the reservoir to the column in the system was 10 ml. The gradient was stepped from 0.2 M lactic acid at pH 2.8 to 0.2 M lactic acid and 0.05 M ethylenediamine at pH 2.8, 3.5 min prior to injection of the sample. This allowed the Al and Zn to be eluted in the first mobile phase followed by the alkaline earths in the second mobile phase (Figure 2.8).

Following the chromatography of the alkaline earths the column dead volume will contain ethylenediamine. Unless the column is washed free of this ethylenediamine, the aluminium and zinc present in the following samples will elute on the solvent front. To overcome this, the column was washed with a five ml aliquot of 0.1M potassium nitrate following the elution of barium (the last metal of interest) from the column. In this way it was possible to separate all the metals of interest. The separation was too lengthy and too complex for practical use in a process analysis environment. To maintain simplicity of the system.
Figure 2.7  Ion-exchange separation of magnesium, calcium, strontium and barium. Eluent 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8)
Figure 2.8  Ion-exchange separation of aluminium, zinc, magnesium, calcium, strontium and barium.
Eluent gradient elution 0.2M lactic acid (pH 2.8) stepped to 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8) 3.5 min prior to injection.
an isocratic elution is preferred and it was decided to study the metals in two groups.

Aluminium and zinc were studied as one group and the alkaline earths as the other. The mobile phase used in all the preceding experiments for the alkaline earths was 0.2 M lactic acid and 0.05 M ethylenediamine at pH 2.8 and that used for aluminium and zinc was 0.2 M lactic acid at pH 2.8, both pH adjusted using concentrated nitric acid.

2.3.5 System Calibration and Limit of Detection

For routine operation of any instrumentation, whether it be for on-line process control or for use in a laboratory environment, it is preferable that the calibration obtained is linear over the concentration range of interest.

The system was calibrated against multielement aqueous standards. A stock solution of 1000 µg ml⁻¹ Al, Zn, Mg, Ca, Sr and Ba was prepared by dissolution of "Analar" salts, Al₂(SO₄)₃, ZnSO₄, MgSO₄, CaCl₂, SrCl₂ and BaCl₂ in distilled deionised water. A range of multielement standards were prepared by serial dilution of the stock solution for calibration purposes. The calibrations (Figures 2.9 - 2.14) were obtained by injecting 100µl of each standard in triplicate.

The upper limit of the linear range is determined by the concentration of the post-column reagent. Once the metal
Figure 2.9  Calibration curve obtained for aluminium.
Eluent 0.2M lactic acid (pH 2.8)

Correlation coefficient = 0.9967
Slope = 49.97
Y Intercept = -36.96
Figure 2.10 Calibration curve obtained for zinc. Eluent 0.2M lactic acid (pH 2.8)

Correlation coefficient = 0.9972
Slope = 12.38
Y Intercept = 25.60
Figure 2.11 Calibration curve obtained for magnesium.

Eluent 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8)

<table>
<thead>
<tr>
<th>ELEMENTAL CONCENTRATION (ug/ml)</th>
<th>PEAK AREA (ARBITRARY UNITS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>20</td>
<td>400</td>
</tr>
<tr>
<td>30</td>
<td>600</td>
</tr>
<tr>
<td>40</td>
<td>800</td>
</tr>
<tr>
<td>50</td>
<td>1000</td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

Correlation coefficient = 0.9999
Slope = 18.88
Y Intercept = 7.08
Figure 2.12 Calibration curve obtained for calcium.

Eluent 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8)

Correlation coefficient = 0.9995
Slope = 15.70
Y Intercept = 17.07
Figure 2.13 Calibration curve obtained for strontium.

Eluent 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8)

<table>
<thead>
<tr>
<th>ELEMENTAL CONCENTRATION (ug/ml)</th>
<th>PEAK AREA (ARBITRARY UNITS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>30</td>
<td>300</td>
</tr>
<tr>
<td>40</td>
<td>400</td>
</tr>
<tr>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td>60</td>
<td>600</td>
</tr>
</tbody>
</table>

Correlation coefficient = 0.9995
Slope = 11.17
Y Intercept = 18.06
Figure 2.14 Calibration curve obtained for barium. Eluent 0.2M lactic acid, 0.05M ethylenediamine (pH 2.8)

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>0.9997</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>2.92</td>
</tr>
<tr>
<td>Y Intercept</td>
<td>4.29</td>
</tr>
</tbody>
</table>
concentration in the maxima of the elution band exceeds the concentration of the MgEDTA-calmagite system, no further increase in response will be obtained. In the system used, the concentration of both the calmagite and the MgEDTA was $2.5 \times 10^{-4}$ M. Therefore, theoretically, the calibration should be linear up to a maximum concentration of $2.5 \times 10^{-4}$ M for each of the metals. This equates to $6.75 \mu g \ ml^{-1}$ Al, $3.8 \ \mu g \ ml^{-1}$ Zn, $10 \ \mu g \ ml^{-1}$ Mg, $6.25 \ \mu g \ ml^{-1}$ Ca, $3 \ \mu g \ ml^{-1}$ Sr, $2 \ \mu g \ ml^{-1}$ Ba. These concentrations refer to the maxima concentration in the post-column reagent at any one point. As the metals are eluting in up to several millitres of eluent, and the post-column reagent is continually being replenished, these concentrations are only a rough guide. The detection system is dependent upon the rate and degree of chelation between EDTA and the metal ions. These concentrations are only a rough guide.

The lower limit of the linear range, and the limit of detection is dependent upon the sensitivity of the spectrometer used and the selectivity constants for the exchange reaction between the EDTA and the eluting metals. The detection limits for the metals studied, based upon a $100\mu l$ injection volume are listed in table 2.1.

Although the detection system is neither particularly sensitive, nor has a wide linear range, this was not felt to be a problem for the intended application. The metal ion concentration in the feed brines is always within a tight range under normal operating conditions (0 - 50ng
<table>
<thead>
<tr>
<th>Element</th>
<th>Detection Limit $\mu g \text{ ml}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>4</td>
</tr>
<tr>
<td>Zn</td>
<td>3</td>
</tr>
<tr>
<td>Mg</td>
<td>1</td>
</tr>
<tr>
<td>Ca</td>
<td>3</td>
</tr>
<tr>
<td>Sr</td>
<td>5</td>
</tr>
<tr>
<td>Ba</td>
<td>9</td>
</tr>
</tbody>
</table>
ml\(^{-1}\)). Following a suitable preconcentration step this concentration may be increased to the 0 - 50 \(\mu g \text{ ml}^{-1}\) level. If the metal concentration did rise, the upper working limit of the system would not be exceeded as the process would require corrective action before the concentration ever became that high. Thus the detection system is ideal for the application in that it has multielement capability, it is simple and robust, and is free from interferences from the matrix.

2.4 CONCLUSIONS

By using a mobile phase of lactic acid it was possible to separate Al, Zn, Mg, Ca, Sr and Ba with detection based on inverse photometry using calmagite - MgEDTA post-column reagent. In order to elute the alkaline earth metal ions in a realistic time, the addition of a competing ion to the mobile phase was necessary, namely ethylenediamine. In contrast, addition of ethylenediamine caused aluminium and zinc to elute on the solvent front. This was overcome by the use of a gradient system. As an isocratic elution is preferred, however, for a process monitor, the metals were divided into two groups for the rest of the experiments defined by their chromatography, namely aluminium and zinc as one group and the alkaline earths as the other group. Linear and workable calibrations were obtained for all the metals of interest within the concentration range of interest.
CHAPTER 3
PRECONCENTRATION AND MATRIX ELIMINATION TECHNIQUES

3.1 INTRODUCTION

3.2 INSTRUMENTATION AND REAGENTS
3.2.1 Instrumentation
3.2.2 Reagents

3.3 PRECONCENTRATION PROCEDURES

3.4 CHELEX 100 AS A PRECONCENTRATION MATRIX ELIMINATION COLUMN
3.4.1 Introduction
3.4.2 Preparation of the Chelex 100 column

3.5 RESULTS AND DISCUSSION
3.5.1 Effect of wash volume upon residual sodium concentration
3.5.2 Effect of sample pH upon recovery for Chelex 100

3.6 THE USE OF COATED COLUMNS FOR PRECONCENTRATION
3.6.1 Introduction
3.6.2 Chrome Azurol S
   3.6.2.1 Coating procedure for Chrome Azurol S
   3.6.2.2 Results and Discussion
3.6.3 Xylenol Orange
   3.6.3.1 Introduction
3.6.3.2 Coating Procedure for Xylenol Orange

3.6.3.3 Results and Discussion

3.7 Conclusions
CHAPTER 3

PRECONCENTRATION/MATRIX ELIMINATION TECHNIQUES

3.1 INTRODUCTION

Traditional cation-exchange techniques are unsuitable for the determination of trace-metals in a concentrated sodium chloride matrix due to the excess of sodium ions saturating the exchange sites on the column, it is therefore necessary to remove the sodium chloride matrix prior to any chromatography. In addition the detection limits of the post-column reaction system (2.3.5) for the metals of interest are at the low \( \mu g \text{ ml}^{-1} \) level, if trace metal levels at the low ng ml\(^{-1}\) level are to be determined, some form of preconcentration is also required. It would seem prudent therefore, to combine these two objectives and develop a simultaneous preconcentration and matrix elimination step prior to analysis by conventional ion chromatography. As the system is intended for automated, on line analysis, the preconcentration/matrix elimination column will need to be compatible with conventional ion exchange chromatography (IC), in terms of both reagents used (mobile phase) and instrumentation (flow rates and back pressures). Therefore a precolumn, prior to the analytical column, which offers a far greater selectivity for the cations of interest (the alkaline earths) over the ions constituting the matrix (sodium) is required. A chelating, ion exchange column, would appear to be an ideal
choice for such a precolumn, although several criteria severely limit the choice of chelating agent which may be used. Firstly, as has already been mentioned, the precolumn must be compatible with reagents used in conventional IC. The chromatography which has been developed for the alkaline earths, aluminium and zinc (2.3.4) was based upon a chelating mobile phase, namely lactic acid. It is the weakly chelating nature of the lactic acid which offers the separation of the metals of interest. It is important therefore that the chelating agent used in the precolumn does not have a greater affinity for the metals than the lactic acid, or else the metals retained on the precolumn will not be eluted by the mobile phase. Thus the chelating agent chosen must have a lower affinity for the metals at the pH of the mobile phase (pH 2.8) in order for elution to occur in as small a volume as possible. However, in order to retain the metals initially from the sodium chloride matrix, the chelating agent must have a high affinity for these metals at the pH of the sample (pH 11). The higher the affinity, the higher the recovery. The chelating agent must also have a high selectivity for the alkaline earths, aluminium and zinc over other competing ions such as transition metal ions, which may also be present in the sample.

Secondly, the resin backbone, upon which the chelating agent is supported must also be compatible to the IC system. As mentioned above, the pH of the mobile phase and brine sample differ greatly. The resin backbone must be
stable across this pH range. It must also be stable to typical back pressures experienced in IC (approx. 2000 psi) and have a suitable particle size to minimise dead volume in the system. With these constraints in mind, 'strong' chelating agents such as 8-hydroxyquinoline (8-HQ) are not really suitable for this application owing to the need to elute the retained metals from the column with a lactic acid mobile phase. Thus studies focused upon the more 'weakly' chelating agents such as iminodiacetic acid (IDA) and salicylic acid. Three different preconcentration columns were studied, firstly the commercially available chelating resin Chelex 100. Secondly, two chelating exchange resins were prepared in these laboratories using column coating techniques employing the chelating dyes chrome azurol S (CAS) and xylenol orange (XO).

3.2 INSTRUMENTATION AND REAGENTS

3.2.1 Instrumentation

Throughout the study, the instrumentation remained identical for each of the three columns and is shown schematically in figure 3.1.

The instrumentation is essentially the same as that described in section 2.2.1. However, there are now two switching valves for the directional flow of the solvents used. Firstly there is the Rheodyne valve described in section 2.2.1 for the direct introduction of aqueous
Figure 3.1  Block diagram of the HPLC system
standards via the 100 μl sample loop. The preconcentration column is connected across a second, titanium switching valve (Valco, VICE, Valco Europe, CH-6214, Schention, Switzerland). The titanium preconcentration column (100 mm x 42 mm id) was connected such that retained metals would be back-flushed off the column by the mobile phase thereby reducing band-broadening. A PTFE-lined, twin piston reciprocating pump (Eldex AA-94-SF-2, Eldex Laboratories, Menlo Park, California) was used to deliver brine samples to the precolumn. All surfaces which came into contact with the brine samples were either PTFE, PTFE-lined or titanium. The precolumn was maintained at 60°C throughout the study unless otherwise stated, in order to overcome slow ion exchange kinetics for some of the metals. The remainder of the instrumentation is as described in section 2.2.1.

3.2.2 Reagents

The mobile phase and post-column reagent used during the study are described in section 2.4. Additional reagents will be described where appropriate.

3.3 PRECONCENTRATION PROCEDURE

Ten ml aliquots of spiked or unspiked brine were preconcentrated onto the precolumn via the Eldex pump, set to deliver at 2.5 ml min⁻¹. The retained metals were then back-flushed to the analytical column, separated and
subsequently detected as in section 2.3.1. However, it was found that following this procedure, the elution of sodium ions from the pre-column dead volume and connecting tubing was sufficient to disturb the chromatography and so a wash step was introduced prior to the retained metals being back-flushed to the analytical column. During the wash step a 5 ml aliquot of dilute sodium hydroxide \((2.5 \times 10^{-4}\text{M})\) was pumped through the pre-column via the Eldex pump \((2.5\text{ ml min}^{-1})\) in order to wash free the residual sodium chloride from the column dead-volume and connecting tubing. This preconcentration procedure was used throughout the study unless otherwise stated.

### 3.4 CHELEX 100 AS A PRECONCENTRATION MATRIX ELIMINATION COLUMN

#### 3.4.1 Introduction

The basic properties of Chelex 100, a chelating exchange resin with IDA functional groups, have been well documented \((53-55)\). According to the manufacturer \((56)\), the selectivity of the resin is in the order \(\text{Cu} > \text{Ni} > \text{Pb} > \text{Zn} > \text{Co} > \text{Cd} > \text{Mn} \gg \text{Ca} > \text{Mg} \gg \text{Na}\). Although the resin has a greater selectivity for the transition metals over the alkaline earths, this does not present a major problem. Firstly the occurrence of transition metals in the feed brines in question is low, and secondly, by suitable adjustment of the sample pH, the degree of chelation for the alkaline earths can be increased. Various workers have
studied the effect of pH upon the uptake of metals by Chelex 100 (36, 37). Most applications using Chelex 100 as a preconcentration column, involve extraction of transition metals from sea-water, during which magnesium and calcium (in this case the matrix ions) pass through the column and are removed. In this application it is the Mg and Ca which are of interest and the transition metals which are to be removed.

3.4.2 Preparation of the Chelex 100 column

Chelex 100 (BDH Chemicals, Poole, Dorset, England) was weighed out (5 g) and slurried in 50 ml of 0.2 M lactic acid pH adjusted to 2.8 using concentrated nitric acid (the mobile phase to be used). This slurry was subsequently hand-packed into a 100 mm x 4.2 mm id titanium column, and the column was connected across the titanium switching valve as shown in figure 3.1.

The column was then conditioned by alternate washings of the mobile phase, and 1 M sodium chloride solution.

3.5 RESULTS AND DISCUSSION

3.5.1 Effect of washing the preconcentration column upon residual sodium concentration

In order to remove the residual sodium concentration to a level compatible with IC, the precolumn had to be washed
following the preconcentration step. In order to determine
the effectiveness of this step the outlet of the precolumn
was connected to the nebuliser uptake tube of a flame
atomic absorption spectrometer (Perkin Elmer Model 4000).

Due to the large concentration of sodium to be monitored,
the sensitivity of the atomic absorption spectrometer was
reduced. Firstly the less sensitive 303.3 nm line was
selected for sodium, and the burner head was rotated
through 45° to offer a shorter path length. The type of
burner being used did not permit rotation of the head
through a full 90° which would have been preferred.

Brine samples were preconcentrated in the normal fashion.
The column was then washed with various volumes of dilute
(2.5 x 10^{-4}M) sodium hydroxide solution. The column was
then back-flushed with the mobile phase directly into the
flame A.A. via a discrete sampler, (Figure 3.2), which
allowed the flow rate of the mobile phase to be matched to
the uptake rate of the nebuliser flow rate, and the sodium
emission was monitored at 303.3 nm. In this way, the
residual concentration with respect to wash volume could be
calculated and is plotted in figure 3.3. It can be seen
that after a 5 ml wash volume, the residual sodium
concentration is below 0.3% m/v. At this level, it is
still possible to obtain chromatography of the alkaline
earth metals when using a high capacity analytical exchange
column. With increasing wash volume, the residual sodium
concentration was not further reduced significantly. This
Figure 3.2 Discrete sampler used to interface the outlet of the preconcentration column to the nebulizer tube of the spectrometer.
Figure 3.3 Residual sodium concentration with respect to wash volume.
was due to the resin having been converted to the sodium form during the preconcentration procedure. When the lactic acid was passed through the column, the resin was converted back to the hydrogen form again, so releasing the sodium ions. It is not possible to remove this residual level of sodium by the wash step of dilute sodium hydroxide. Nor is this residual sodium concentration solely a result of the wash step, as the sodium hydroxide concentration is $2.5 \times 10^{-4}$ M (0.001%) as compared to the observed 0.3 M residual sodium concentration.

3.5.2 Effect of Sample pH upon Recovery for Chelex 100 Column

With the HPLC system set up as in figure 3.1, initial studies centered upon magnesium. Owing to the pH dependence for the uptake of metals from solution onto the Chelex resin, a range of sample pH's were investigated to obtain an optimum for magnesium recovery.

Molar sodium chloride samples (50 ml) were spiked with the appropriate volume of magnesium standard to yield a final magnesium concentration of 50 ng ml$^{-1}$. The pH of the brine samples was then adjusted in the range 4 - 11 using concentrated nitric acid. Ten ml aliquots of these brine samples were subsequently loaded onto the column as described in section 3.3. Following the chromatography and detection, the peak areas for magnesium were recorded. A plot of magnesium signal against sample pH is shown in
figure 3.4. As expected, the recovery and hence signal increased as the sample pH increased, due to the ionisation of both groups of the iminodiacetic acid. As the pH was increased still further, some formation of magnesium hydroxide occurred, so preventing chelation with the IDA groups. However, a more detailed study of Chelex 100 resin was not possible.

It is known that Chelex 100 undergoes a drastic change in volume from the \( \text{NH}_4^+ \) form to the \( \text{H}^+ \) form (57), which led Sturgeon et al. (58) to abandon a similar column technique. When the resin was exposed to a pH range from pH 2.8 (that of the mobile phase) to pH 11 (that of the brine) it is not surprising that some swelling of the resin took place. As the resin was enclosed within a column this increase in volume of the resin caused an increase in back-pressure (>2500 psi). As Chelex 100 is not stable to such high pressures, the resin collapsed and formed a 'glassy plug' in the centre of the column rendering it useless. It has been reported by Figura (59) that the calcium form of the resin does not undergo such a change in volume as the sodium form. However, this is of little use when the matrix is composed of sodium ions. This led to Chelex 100 being considered unsuitable for our applications, and so further studies on the resin were abandoned.
Figure 3.4  Relationship between sample pH and magnesium response using the chelex 100 preconcentration column.
3.6 THE USE OF COATED COLUMNS FOR PRECONCENTRATION MATRIX ELIMINATION

3.6.1 Introduction

Recently there has been growing interest in the use of coated columns for ion exchange chromatography. Most of these studies involve coating a chelating agent on either a neutral cation or anion exchange HPLC grade resin. The reasoning behind such activity is simple. The selectivity of chelating exchangers is often superior to that of conventional ion exchange, however, due to the complexity normally associated with their synthesis, their wide spread use has, to some extent, been limited. Because of the lack of any suitable commercially available resins, coated column techniques appeared to hold great potential for the present application. The modification of stationary phases by coating them with specific compounds is a well known technique in ion chromatography, although this mainly involves the formation of dynamic ion exchange coatings using quaternary ammonium or alkyl sulphonate based compounds (60).

Chromatographic separations have been achieved using HPLC grade resins coated with chelating dyes (60). Kemula and Brajter (61-63) Brajter et al. (64-67), have made extensive studies of chelate-forming resins prepared by modification of common anion exchange resins with sulphonated aromatic compounds. These studies, however, have involved first row
transition metals, some precious metals and aluminium. None have reported the use of such columns for the retention of the alkaline earths. A wide range of such sulphonated aromatic compounds exist, and many have been studied in depth. It was not within the scope of this project to assess the suitability of all of these compounds for the retention of the alkaline earths from saturated brines, instead two such compounds were studied. Firstly Chrome Azural S (CAS) and secondly Xylenol Orange (XO), each of which were immobilised onto a range of resin backbones.

3.6.2 Chrome Azurol S (CAS)

The chelating dye Chrome Azurol S (figure 3.5) has been previously coated onto HPLC grade resins and used for the preconcentration and separation of Al, Cu, Mg, Mn and Zn, and also the separation of Al, In and Ga (60).

The chelating group of the dye is essentially an analogue of salicylic acid. It is a weaker chelating group than that of 8-hydroxyquinoline and also that of IDA. It is also known to be stable across a wide pH range being resistant to oxidation. In studies carried out by Jones and Schwedt (60) Cu, Mg, Mn and Zn were preconcentrated onto a CAS loaded column at pH 8.7 from a 1 M potassium nitrate solution prior to elution with lactic acid. Following these results, CAS was considered a suitable dye for the present studies.
Figure 3.5  Structure of the chelating dye Chrom Azurol S
A similar coating procedure to that of Jones and Schwedt was used. Three hundred milligrams of CAS (Kodak) was dissolved in 50 ml of 20% v/v methanol water solution, acidified with a 1ml aliquot of molar acetic acid. This solution was then pumped through a 100 mm x 4.2 mm id titanium column packed with a neutral HPLC grade resin (PLRPS) at 1 ml min⁻¹, noting visually when breakthrough was observed. Following breakthrough a further 10 ml of the CAS solution was passed through the column. The column was subsequently flushed for 30 mins with a 0.1 M acetic acid solution of pH 3.0. A further 50 ml of 0.1 M acetic acid pH 5.7 (adjusted using potassium hydroxide) was passed through the column, noting any bleeding of the dye from the column. Following this procedure a total loading of 30 mg CAS was obtained (10 mg/g resin). The column was then conditioned using alternate washings of a 1.0 M sodium chloride solution, and the mobile phase of lactic acid. The resin beads swell and contract slightly as they are exposed to high and low ionic strength media, and this results in some further bleeding of the dye from the resin.

The conditioning was repeated until no more dye was visually observed to bleed from the column. Although no in depth study was made as to the nature of the immobilisation of the dye onto the resin, it is thought to be due to a combination of two processes. Firstly non-polar, non-polar interaction between the benzene ring of the dye and the
phenolic rings of the resin and secondly, physical trapping of the dye in the pores of the resin beads. This second mechanism would explain the initial bleeding of the dye from the resin during conditioning. The column, referred to as the CAS column, was then connected to the HPLC system in figure 3.1.

3.6.2.2 Results and Discussion

One hundred millilitre aliquots of 1M sodium chloride samples were spiked with the appropriate amount of metal standard to yield a final concentration of 50 ng ml$^{-1}$ for each of the metals, and the pH adjusted in the range 6 - 11 using concentrated nitric acid. Following the preconcentration procedure described in section 3.3, ten ml aliquots of each of these samples were loaded onto the CAS column. Following the chromatography of the metals the peak areas were recorded. Figures 3.6 - 3.10 show the variation in signal against sample pH for magnesium, calcium, strontium, aluminium and zinc respectively. Again, the results follow what would be expected in that an increase in pH results in an increase in signal up to the point where the pH is high enough to cause the formation of the respective hydroxides. Following this, a more in depth study of Mg uptake was performed.

In order to optimise the preconcentration procedure outlined in section 3.3, a molar sodium chloride sample was spiked with 50 ng ml$^{-1}$ magnesium and the pH adjusted to
Figure 3.6 Relationship between sample pH and magnesium response using the CAS preconcentration column.

![Graph showing the relationship between sample pH and magnesium response using the CAS preconcentration column.](image-url)
Figure 3.7 Relationship between sample pH and calcium response using the CAS preconcentration column
Figure 3.8 Relationship between sample pH and strontium response using the CAS preconcentration column.
Figure 3.9 Relationship between sample pH and aluminium response using the CAS preconcentration column
Figure 3.10 Relationship between sample pH and zinc response using the CAS preconcentration column
9.5. A range of sample volumes from 0 to 20 ml were then loaded onto the CAS column and the subsequent peak areas for Mg recorded. A plot of Mg signal against sample volume is shown in figure 3.11. It can be seen that no preconcentration took place whilst the first 2 ml aliquot of sample was loaded onto the column. Prior to the preconcentration of any sample, the pH of the CAS column was 2.8 (that of the mobile phase) hence, no chelation could take place until the pH of the column had reached at least pH 7. From figure 3.11 it is clear this did not take place until 2 ml of sample had been passed through the column. It might be expected that a plot of sample volume preconcentrated against signal obtained would be linear for the concentration range of interest. The total loading of the dye on the column was 30 mg, and the concentration of magnesium present was 50 ng ml$^{-1}$. Therefore 600 litres of a molar sodium chloride solution spiked with 50 ng ml$^{-1}$ Mg should be loaded onto the column before breakthrough occurs. From figure 3.11 breakthrough occurred after only 15 ml. This may be explained by the fact that the chelating group on CAS is salicylic acid, and as such is only weakly chelating. A probable explanation therefore, for the early breakthrough observed, is due to migration of the magnesium ions along the CAS column. Thus, in order to remain in the linear section of the curve for sample volume preconcentrated against signal, a ten ml aliquot of brine sample was preconcentrated in all preceding studies. In addition, the preconcentration procedure was modified to include a 5 ml aliquot of 2.5 x 10$^{-4}$ M sodium hydroxide.
Figure 3.11 Relationship between sample volume and magnesium response using the CAS preconcentration column.
prior to the brine sample, to adjust the pH of the CAS column.

Using this modified preconcentration procedure, ten ml aliquots of 1M sodium chloride were spiked with appropriate amounts of magnesium to yield final concentrations in the range 0 - 100 ng ml$^{-1}$. The pH of each was adjusted to 9.5. These brine samples were then loaded onto the CAS column, and following the chromatography a calibration curve was plotted, shown in figure 3.12. However, following these initial, promising results, the CAS column apparently degenerated. The CAS coating appeared to have been washed from the resin. A second column was prepared, although the CAS used on this occasion was obtained from an alternative source (Sigma Chemicals, Poole, England).

Calibrations for Al, Ca, Sr and Zn were obtained in the same manner as that for Mg, using the new CAS column (Figures 3.13 - 3.16). From these plots it was clear that the column was not performing as well as expected. The calibration curve for Al (3.13) levelled off at the 50 ng ml$^{-1}$ level indicating early breakthrough of aluminium. That of calcium (3.14) was very flat suggesting poor recovery for calcium from the brine by the column. The curve for Zn may partially be explained by the formation of Zn(OH)$_4^{2-}$. The column has a low efficiency for zinc and so the recoveries at low concentration of the metal are poor. When the experiments were repeated using 30% m/v brine samples the results were even more disappointing. It would appear,
Figure 3.12 Calibration curve obtained for magnesium in 30% m/v sodium chloride using the CAS preconcentration column.

Correlation coefficient = 0.9980
Slope = 4.8
Y Intercept = 67.23
Figure 3.13 Calibration curve obtained for aluminium in 30% m/v sodium chloride using the CAS preconcentration column

Correlation coefficient = 0.9975
Slope = 4.91
Y Intercept = 18.52
Figure 3.14 Calibration curve obtained for calcium in 30\% m/v sodium chloride using the CAS preconcentration column.

<table>
<thead>
<tr>
<th>Peak Area (arbitrary units)</th>
<th>Element Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>150</td>
<td>75</td>
</tr>
<tr>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

Correlation coefficient = 0.9971
Slope = 1.56
Y Intercept = -1.24
Figure 3.15 Calibration curve obtained for strontium in 30% m/v sodium chloride using the CAS preconcentration column.

Correlation coefficient = 0.9979
Slope = 0.81
Y Intercept = -0.61
Figure 3.16 Calibration curve obtained for zinc in 30% m/v sodium chloride using the CAS preconcentration column.
that the salicylic acid functional group of the CAS dye was not sufficiently strong enough to retain the metals of interest in such a high ionic strength media. Problems were also associated with coating the dye onto the resin, and the coatings obtained were not reproducible. These factors, in addition to the wide range in purity of the dye from various suppliers, led to the decision to abandon the dye for this specific application.

3.6.3 Xylenol Orange

3.6.3.1 Introduction

The chelating dye xylenol orange (XO) figure 3.17 has been used previously to modify conventional ion exchange resins. Brajter (66) immobilised XO onto the anion exchange resin Amberlyst A-26 and used it for the determination of mainly first row transition metals. The functional group of XO is iminodiacetic acid (IDA) which although being a weaker chelating agent than 8-hydroxyquinoline, is a stronger chelating agent than salicylic acid, for the metals of interest. The large size of the XO molecule, having three cyclic rings similar to CAS, increases the strength of interaction with the sorption matrix. Finally, the presence of the $\text{SO}_3^-$ group on the dye makes it water soluble. Xylenol orange therefore, was considered to be a suitable dye for the current application.
Figure 3.17 Structure of the chelating dye xylenol orange
3.6.3.2 Coating procedure for Xylenol Orange

The xylenol orange column was prepared in a batch process, rather than coating the dye onto the resin in the column, as was the case for CAS. It was considered that a batch process would result in a deeper, more stable coating of the dye on the resin. Dowex 1 X8 (3 g) was slurried in 25 ml of a 40% v/v methanol water solution. Xylenol orange (Sigma Chemicals, Poole, England) (100 mg) was added and the solution was stirred on a hot plate at 50°C for 48 hours, additional methanol solution being added when necessary due to evaporation. The coated resin was then packed into a 100 mm x 4.2 mm i.d. titanium column and the column connected to the HPLC system shown in figure 3.1. The column was conditioned with alternate washings of saturated brine and the mobile phase until no more dye was visually observed to bleed from the column. The immobilisation of the dye on the resin is thought to be similar to that of the CAS on the neutral resin. In addition to nonpolar-nonpolar attraction and physical trapping however, a third mechanism now operates, that of the strong ionic interaction between the tertiary ammonium group of the resin and the sulphonate group of the dye. The loading of the dye on the resin was not calculated owing to difficulties in measuring the amount of the dye bleeding from the column, but was thought to be a slightly higher loading than on the CAS column.
3.6.3.3 Results and Discussion

The modified preconcentration procedure described in section 3.6.2.2 was used for all the studies of the XO column. The pH dependance of uptake for each of the metals was studied. A 30% m/v brine sample was spiked with a known concentration of metal (50 ng ml\(^{-1}\)) and the pH adjusted in the range 6 - 12 using concentrated nitric acid. Ten ml aliquots of these samples were loaded onto the XO column and following the chromatography the peak areas were collected. Figures 3.18 - 3.23 show the pH dependance for Al, Zn, Mg, Ca, Sr and Ba respectively. It can be seen that the metals studied may be divided into two groups based on their optimum sample pH for preconcentration. Aluminium and zinc have optimum pH around 7 - 8, while the alkaline earths all have optimum pH's above 10. As the functional group of the dye is iminodiacetic acid, strong chelation will not place until both groups are in the anionic form (36). This typically occurs at pH 7. In the case of aluminium, an optimum pH for recovery is observed at pH 8. This is due to a combination of the small ionic radius (0.045 nm) and +3 charge for aluminium forcing the formation of the stable two ring chelate at a lower pH than the alkaline earths. As the pH is raised still further we see a sharp fall in the response for aluminium. This may be explained by the formation of the hydroxide species Al(OH)\(_4^−\), so leaving the aluminium unavailable for chelation. Similarly for zinc there is an optimum at pH 7.5. Again this was due in part
Figure 3.18 Relationship between sample pH and aluminium response using the xylenol orange preconcentration column
Figure 3.19 Relationship between sample pH and zinc response using the X-O preconcentration column.
Figure 3.20 Relationship between sample pH and magnesium response using the xylenol orange preconcentration column.
Figure 3.21 Relationship between sample pH and calcium response using the xylenol orange preconcentration column.
Figure 3.22 Relationship between sample pH and strontium response using the xylenol orange preconcentration column.
Figure 3.23 Relationship between sample pH and barium response using the xylenol orange preconcentration column.
to the small ionic radius (0.069 nm) of zinc. At higher pH's the stable Zn(OH)$_4^{2-}$ is formed which again prevented chelation with the IDA groups of the dye. In the case of the alkaline earths, no appreciable chelation occurred until pH 9. Magnesium does not appear to have reached an optimum in the pH range investigated. Calcium on the other hand showed an optimum between pH 9 and pH 10.8. This may be explained by virtue of the stability order of the alkaline earth metal cation and the anion of the aminocarboxylic acid being: Mg < Ca > Sr > Ba (68). Thus an optimum is reached for calcium while not for magnesium. Both strontium and barium showed optimum signal in the pH range 10 – 11.

The reproducibility of the system was tested by repeated calibration of the system on a day-to-day basis, each calibration point being repeated in triplicate. The precision for each of the metals on both a day-to-day basis, and on the same day are listed in table 3.1.

The recoveries for each of the metals were calculated at the optimum pH for that metal. The recoveries were based on the signal obtained by preconcentrating a 10 ml aliquot of 30% m/v sodium chloride sample containing 50 ng ml$^{-1}$ of the metal (based on three such analyses) against the signal obtained by injecting 100 μl of a 5 μg ml$^{-1}$ aqueous standard of the same element (based on three such analyses) both signals having been blank subtracted. The recoveries for the metals are listed in table 3.2.
Table 3.1
Precision of analysis for Al, Zn, Mg, Ca, Sr and Ba on an same-day and day-to-day basis.

<table>
<thead>
<tr>
<th>Element</th>
<th>Same Day % RSD n = 3</th>
<th>Day-to-Day % RSD n = 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>2.7</td>
<td>3.2</td>
</tr>
<tr>
<td>Zn</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Mg</td>
<td>0.9</td>
<td>4.0</td>
</tr>
<tr>
<td>Ca</td>
<td>2.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Sr</td>
<td>4.0</td>
<td>4.2</td>
</tr>
<tr>
<td>Ba</td>
<td>3.8</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Table 3.2
Recovery for Al, Zn, Mg, Ca, Sr and Ba from 30% m/v sodium chloride solution.

<table>
<thead>
<tr>
<th>Element</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>68</td>
</tr>
<tr>
<td>Zn</td>
<td>69</td>
</tr>
<tr>
<td>Mg</td>
<td>80</td>
</tr>
<tr>
<td>Ca</td>
<td>70</td>
</tr>
<tr>
<td>Sr</td>
<td>50</td>
</tr>
<tr>
<td>Ba</td>
<td>55</td>
</tr>
</tbody>
</table>
Although the recoveries were less than 100%, the reproducibility of the system was good and the usual problems associated with low recoveries being shifted by sample matrix are not encountered as the matrix remains fixed.

Standard addition calibrations were obtained for each of the metals by spiking the brine samples with known concentrations of the analyte in the range 0 - 100 ng ml\(^{-1}\) at the optimum pH for that analyte. Ten ml aliquots of these brine samples were loaded onto the column and following the chromatography, peak areas were collected. Linear calibrations (Figures 3.24 - 3.29) were obtained for all of the metals within the concentration range of interest with the exception of strontium. The calibration curve for strontium (Figure 3.28) tends to curve towards the axis above 50 ng ml\(^{-1}\) although the precision is such that this portion of the calibration curve may still be used. All the calibrations intercept on the y axis as they are all, standard addition curves. Hence the concentration of each of the analytes in the brine may be determined (Table 3.3).
Figure 3.24 Calibration curve obtained for aluminium in 30% m/v sodium chloride using the X-O preconcentration column

![Graph showing calibration curve with peak area vs. elemental concentration]

- Correlation coefficient = 0.9930
- Slope = 5.56
- Y Intercept = 9.04
Figure 3.25 Calibration curve obtained for zinc in 30% m/v sodium chloride using the X-O preconcentration column

![Graph showing a linear relationship between peak area and elemental concentration. The x-axis represents elemental concentration (ng/ml) ranging from 0 to 200, and the y-axis represents peak area (arbitrary units) ranging from 0 to 50. There are data points at concentration levels of 50, 100, and 150 ng/ml, with corresponding peak areas of approximately 10, 20, and 40 units, respectively.]
Figure 3.26 Calibration curve obtained for magnesium in 30% m/v sodium chloride using the X-O preconcentration column.

Correlation coefficient = 0.9993
Slope = 1.89
Y Intercept = 1.25
Figure 3.27 Calibration curve obtained for calcium in 30% m/v sodium chloride using the X-0 preconcentration column

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>0.9945</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>2.95</td>
</tr>
<tr>
<td>Y Intercept</td>
<td>13.13</td>
</tr>
</tbody>
</table>
Figure 3.28 Calibration curve obtained for strontium in 30% m/v sodium chloride using the X-0 preconcentration column.
Figure 3.29 Calibration curve obtained for barium in 30% m/v sodium chloride using the X-0 preconcentration column

Correlation coefficient = 0.9987
Slope = 2.16
Y Intercept = 0.81
Table 3.3
Concentration of analyte present in 30% m/v sodium chloride samples determined by standard addition curves.

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration ng ml$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>4.0</td>
</tr>
<tr>
<td>Zn</td>
<td>4.0</td>
</tr>
<tr>
<td>Mg</td>
<td>12.0</td>
</tr>
<tr>
<td>Ca</td>
<td>8.0</td>
</tr>
<tr>
<td>Sr</td>
<td>36.0</td>
</tr>
<tr>
<td>Ba</td>
<td>0.0</td>
</tr>
</tbody>
</table>
3.7 CONCLUSIONS

The chelating resin Chelex 100 was found to be unsuitable for the preconcentration of the alkaline earths from sodium chloride matrices. This was due entirely to the fact that Chelex 100 is not a HPLC grade resin. The swelling associated with the change in ionic strength and pH between the different solvents used caused an increase in backpressure on the column, for which the resin was not designed.

Coated column techniques have now been used for several years and offer advantages over conventional ion-exchange columns for reasons discussed earlier. In this application too, coated columns have a significant advantage in that they are relatively simple to manufacture, and with a suitable choice of the dye and resin back bone may be 'tailor made' for specific applications. Of the two dyes studied, xylenol orange offered the greater stability to variations in pH and ionic strength and did not degrade in the manner that the CAS column did. The XO column offered superior recoveries and reproducibilities over the CAS column, due in part to the stronger chelating function of xylenol orange (IDA) over Chrome Azurol S (salicylic acid) and in part to the more stable coating of xylenol orange. Using the xylenol orange coated column aluminium, zinc, magnesium, calcium, strontium and barium were preconcentrated quantitatively from saturated brines at the low ng ml\(^{-1}\) level.
CHAPTER 4

APPLICATION OF PRECONCENTRATION - HPLC SYSTEM TO
ON-LINE MONITORING OF FEED BRINES FOR PROCESS CONTROL

4.1 INTRODUCTION

4.2 INSTRUMENTATION

4.3 RESULTS AND DISCUSSION

4.4 CONCLUSIONS
CHAPTER 4

APPLICATION OF THE PRECONCENTRATION - HPLC SYSTEM TO
ON-LINE MONITORING OF FEED BRINES FOR PROCESS CONTROL

4.1 INTRODUCTION

The need for process monitoring in the chlor-alkali industry has already been dealt with in Chapter 1. The subsequent development of the HPLC system for the determination of low ng ml$^{-1}$ levels of specific cations has been described in Chapters 2 and 3. There is, however, a large difference between operating a manual system in the controlled environment of the laboratory, and applying that system to run continuously, unattended on an industrial site. This chapter will deal with the application of the methodology described in Chapters 2 and 3 to an automated, on-line monitoring system for the determination of trace-metals in concentrated feed brines.

Because both the preconcentration conditions and chromatographic conditions for aluminium and zinc differed from those of the alkaline earths, (2.3.4 and 3.6.3.3), this study only deals with the determination of the alkaline earths to maintain simplicity of the system.

The reason for the on-line trial was to test the long-term stability of the system, stability of the reagents used, reliability of the instrumentation and robustness of the
technique. A four week trial was arranged for the study at a chlorine plant (ICI Chemicals and Polymers Ltd., Lostock site, Cheshire) during May 1990. During the trial methodology developed at Plymouth was transferred to a HPLC system on loan from ICI Chemicals and Polymers Ltd. The equipment was housed in an existing analyser house located next to the ion exchange clean-up columns (figure 4.1).

Under normal operating conditions, brine samples are collected from a sample reservoir situated alongside the analyser house shown in figure 4.2.

During the trial, however, extensive conversion work was being carried out on the site. During this time brine was sampled directly from a reservoir filled from the outlet of the ion-exchange clean-up columns.

4.2 INSTRUMENTATION

In order to automate the HPLC system to allow collection, preconcentration, and injection of samples the HPLC system needed to be re-designed. A schematic of the layout is shown in figure 4.3.

The system essentially comprises five pumps. Two HPLC pumps (Knauer HPLC Pump 64, Roth Scientific, Alpha House, Alexandra Road, Farnborough, U.K.) were used to deliver the mobile phase \((1.0 \text{ ml min}^{-1})\) and a sodium hydroxide solution \((2.5 \times 10^{-3}\text{M})\) \((2.5 \text{ ml min}^{-1})\). A pressure vessel
Figure 4.1 Block diagram showing location of analyser house with respect to the ion-exchange clean-up columns
Figure 4.2  Reservoir normally used for sample collection at the analyser house

BRINE FROM
ION EXCHANGE COLUMNS

TO
ANALYSER HOUSE

TO
WASTE
Figure 4.3  Block diagram of the HPLC system

HPLC PUMP
MOBILE PHASE

HPLC PUMP
NaOH

PRESSURE VESSEL
PCR

CONTROLLER

SWITCHING VALVES AND COLUMNS

BRINE

AQU. STANDARD

DETECTOR

112
(Dionex Corporation, Albany Park, Camberley, Surrey, U.K.) was used as a third pump to deliver the post-column reagent (1 ml min$^{-1}$). A twin channel peristaltic pump (Minipulse, Gilson, Luton, Bedfordshire), was used to deliver brine samples (3.3 ml min$^{-1}$) and aqueous standards for calibration (0.1 ml min$^{-1}$). The preconcentration column was a 100 mm x 4.2 mm i.d. titanium column packed with Dowex 1-X8 coated with xylenol orange and the analytical column was a 50 mm x 4.2 mm i.d. titanium column packed with a strong cation-exchange resin, (Benson BC-X10, Benson Company, Reno, Nevada, USA.). Both columns and the reaction coil were maintained at 60°C in a water bath.

Although no automation of the pumps was necessary, as they were required to operate continuously, automation of the switching valves was required. The arrangement of the switching valves and columns is shown in figure 4.4.

Three switching valves (Dionex Corporation), were housed in a Dionex advanced chromatography module. Valve switching was controlled via a Dionex auto ion controller. The valves were configured so that a brine sample could be preconcentrated while a chromatogram of the previous sample was being obtained. To avoid having to pump concentrated brine through the HPLC pump, a ten ml sample loop was filled with brine via the peristaltic pump.

While the sample loop was filling with brine, the dilute (2.5 x 10$^{-3}$ M) sodium hydroxide solution was pumped through
Figure 4.4 Block diagram for the switching valves, preconcentration column and sampling loops used in the HPLC system
the preconcentration column to condition it prior to analysis. The contents of the sample loop were then flushed onto the preconcentration column using the sodium hydroxide stream as a carrier stream. Once the sample loop had emptied the valve was switched back to the original position so that the loop could be re-filled with brine. A further five ml aliquot of sodium hydroxide was pumped through the preconcentration column to remove the residual sodium chloride (Chapter 3) before the retained metals were back-flushed off the column with the mobile phase onto the analytical column. In this way brine samples may be preconcentrated reproducibly without pumping brine through the HPLC pump. Brine samples could not be pumped directly onto the preconcentration column by the peristaltic pump because the back-pressure across the preconcentration column (approx 700 psi) was too high for a peristaltic pump to operate against.

The elution of the retained metals from the preconcentration column was complete within two minutes. Hence, after two minutes the valve may be returned to the original position to allow conditioning of the column for the next brine sample, while the previous chromatogram developed. The time taken to elute the four ions of interest was ten minutes. The elution of the retained metals from the preconcentration column took two minutes. The preconcentration cycle was designed to take eight minutes so allowing six replicate analyses an hour. In practice, the system also needed to be calibrated. This
was achieved using the third switching valve which had a 100 μl sample loop placed across it. Aqueous standards were delivered to the loop via the peristaltic pump. Once an hour, the aqueous standards were injected onto the analytical column by the mobile phase. Using this instrumentation, five brine samples were analysed an hour plus one calibration standard.

4.3 RESULTS AND DISCUSSION

An extract from the chromatogram obtained during the on-line trial is shown in figure 4.5. It shows three replicate brine samples followed by one of the calibration points. Even though real samples are being analysed on line continuously, good chromatography of the metals has been retained with good reproducibility and low background noise. The reproducible chromatography is testimony to the stability of the reagents used. It can be seen that the retention times for the metals in the aqueous calibration standard differ slightly from the retention times of the metals in the brine samples. This is due to the standard solution being injected directly onto the analytical column rather than through both the preconcentration column and analytical column. During the earlier studies (3.4.2), it was shown that aqueous standard calibrations could be used to determine concentrations of analytes in saturated brines, provided that the recovery of each metal on the xylenol orange column was known. In so far as the on-line trial was concerned, aqueous standard calibrations directly
Figure 4.5 Extract of the chromatogram obtained during the on-line trial. The chromatogram shows three consecutive injections of the retained metals from the preconcentration column, preceded by a calibration standard.
onto the analytical column were preferred over spiked brine calibrations, through both the preconcentration column and analytical column, to maintain simplicity of the system. The latter calibration procedure would have required a fourth switching valve, an extra pump and more complicated plumbing. The brine feed was monitored for a total of 36 hours. Figures 4.6 - 4.9 show the concentration with respect to time for magnesium, calcium, strontium and barium respectively. The calibration points for each element have been omitted for clarity.

Magnesium was present at 3.5 ng ml$^{-1}$ with an RSD of 7%. This is an encouragingly small spread of results when considering this level is close to the detection limit of the system. The precision across the calibration points for magnesium was 3.2%.

Calcium shows three low results at the start of the trial. This was caused by loss of prime on the HPLC pump delivering the sodium hydroxide solution, and as this acts as a carrier stream for the brine sample, this prevented all the sample being preconcentrated. Other than these first three points, the results indicate that there was 30 ng ml$^{-1}$ of calcium present in the feed brines with an RSD across these results of 8.3%. Precision across the calibration points for calcium was 4%.

Strontium also exhibited low values at the start of the trial for the same reasons as calcium. The subsequently
Figure 4.6  Variation in the magnesium concentration with respect to time during the on-line trial.
Figure 4.7 Variation in the calcium concentration with respect to time during the on-line trial.
Figure 4.8  Variation in the strontium concentration with respect to time during the on-line trial
Figure 4.9 Variation in the barium concentration with respect to time during the on-line trial
higher concentration of strontium found reflects the high concentration of strontium naturally occurring in the salt deposits used to make up the feed brines used at Lostock. Precision for the reported results was 15%.

Finally barium showed a concentration in the brines of approximately 17 ng ml\(^{-1}\). Although barium was included during the on-line trial and the earlier studies, it was not expected that barium would be detected in the feed brines. Prior to these analyses, the only on-line determinations carried out at Lostock were for magnesium and calcium by Flow Injection Analysis. All other metal ion determinations were performed off-line by inductively coupled plasma - atomic emission spectrometry following a five-fold dilution of the brine. During these analyses barium had never been detected. However, during experimental trials on the membrane cells, barium had been found in the membranes themselves after use with these feed brines. As there is no other possible source of barium, other than the feed brines, it is reasonable to assume that the feed brines do contain barium at the low ng ml\(^{-1}\) level. Following the five-fold dilution of the brine prior to analysis by ICP-AES, the barium concentration was below the detection limit of the system at the time the analysis was performed.

The large spread in the results obtained for barium was not due to any instrumental variation, but rather the means of recording the data. Due to the location of the analyser
house, a plotter/integrator which would have provided peak areas could not be used because of electrical interference from other installations. Thus data had to be collected using a chart recorder. The fact that barium was being determined close to the detection limit of the system, and that it is the last metal to elute led to very small peak heights being obtained for barium, and it was peak heights which were used for measurement. The smallest unit of height that could be measured accurately was 0.5 mm. In the case of barium, changes in peak height of 0.5 mm equated to a change in concentration of 7.5 ng ml$^{-1}$. Thus a change in peak height equivalent to the width of the recorder pen yield large changes in the apparent concentration. If an integrator could have been used to collect peak areas, a more accurate picture may have emerged. It can be said, however, that the barium concentration was approximately 20 ng ml$^{-1}$ in the brine.

The on-line trial took place over four weeks during May 1990, although results were only obtained for a 36 hour period. This was caused by instrumental problems rather than poor methodology or unstable chemistry. The instrumentation used during the trial was designed for use in the laboratory, rather than on a chemical site. The operation of an HPLC pump continuously, in the harsh environment of a chemical plant, is far more problematic than its operation for a few hours a day in the controlled environment of a laboratory. The problems encountered were mainly due to piston seals and check valve seals on the
pumps failing. This would not normally present a problem. However, in an industrial environment, this kind of instrumental failure presents a much larger problem. Apart from the pumps, the other part of the instrumentation that caused problems were the switching valves and connections. The Dionex valves used were rated to a maximum operating pressure of 2000 psi. When both the preconcentration and analytical columns were in series, the back pressure was typically 1800 psi. Although this was slightly below the maximum operating pressure, the sudden increase in back pressure when switching both columns in series was enough to cause fittings to disconnect and valves to leak. Due to these problems, the HPLC system was not operational until three and a half weeks into the trial. However, during this time the occasional chromatogram was obtained, usually with reagents (mobile phase and post-column reagent) that had been prepared for some days. In all cases neither the chromatography nor the detection had suffered in any way due to the use of old reagents. Once the system was running no operator attention was necessary and the system was still operating when the trial was ended. Due to the conversion work being carried out at the site, it was not possible to extend the trial.

4.4 CONCLUSIONS

During the on-line trial the stability of reagents, chromatography, detection and overall methodology was proved. The preconcentration column used had been prepared
nine months prior to the trial and had been used extensively during those nine months, and still performed well during the trial. During the trial the alkaline earths were determined on-line at the low ng ml\(^{-1}\) in concentrated feed brines using a fully automated system without the use of any sample pretreatment. Barium was determined in the feed brines at the 20 ng ml\(^{-1}\) level, whereas previous determinations had failed to detect its presence despite the fact that barium had been determined in the ion-exchange membranes used in the electrolytic cells.

Finally, the on-line trial, once again proved that laboratory-based instrumentation is not suited to on-line process analysis. Clearly pumps and switching gear need to be made more reliable and robust for a viable on-line instrument.
CHAPTER 5

AN EXPERT SYSTEM BASED USERS GUIDE
FOR AN ION CHROMATOGRAPH PROCESS MONITOR

5.1 INTRODUCTION

5.2 DEVELOPMENT OF AN EXPERT SYSTEM FOR CONTROL OF AN
ON-LINE MONITORING SYSTEM

5.3 STRUCTURE OF THE KNOWLEDGE BASE

5.4 CONCLUSIONS
CHAPTER 5

AN EXPERT SYSTEM BASED USERS GUIDE
FOR AN ION CHROMATOGRAPH PROCESS MONITOR

5.1 INTRODUCTION

Artificial intelligence (A.I) is generally regarded as being the development of computational approaches to intelligent behaviour, or to put it another way, how to make computers 'smart'. There are many areas to which AI has been applied, all of which are linked by one common aspect. No algorithmic solutions exist for the problem being tackled. Scientific and engineering calculations are primarily numeric, algorithms for such problems are well known and solutions are clearly defined. However, the problems to which AI systems are usually applied rarely have a numeric solution. The problems deal with words and concepts and more often than not some form of search is required. The solutions produced cannot be easily defined into TRUE or FALSE categories, and the systems cannot always guarantee a correct solution. Nilsson (69) described AI in terms of its basic elements (figure 5.1). One of the elements of AI is expert systems. As the name implies, expert systems are designed to mimic the decision making process of a human expert. Feigenbaum (70) defined an expert system as an intelligent computer program that uses knowledge and inference procedures to solve problems that are difficult enough to require significant human
Figure 5.1 Basic elements of artificial intelligence as described by Nilsson
expertise for their solution. The knowledge of an expert system consists of facts and heuristics. The "facts" constitute a body of information that is widely shared, publicly available and generally agreed upon by experts in a field. The heuristics are mostly private, rules of good judgement, rules of plausible reasoning that characterise expert-level decision making in the field. The performance level of an expert system is primarily a function of the size and quality of the knowledge base it possesses. In recent years it has become fashionable to characterise any AI system that uses substantial domain knowledge as an expert system. This has lead to nearly all application of AI to real-world problems being considered as expert systems, although the term knowledge based systems may more accurately describe such applications.

Expert systems are generally recognised as consisting of three main modules. The knowledge base, or rule base, the inference engine or rule interpreter and the user interface.

The knowledge base contains all the domain facts and heuristics necessary for the decision making process. As Feigenbaum stated, it is the knowledge base that primarily determines the performance of the expert system. A common way of constructing a knowledge base is through the use if IF-THEN commands. When the IF portion of the command is found to be TRUE, the THEN portion of the rule is considered logically true.
By chaining such commands together it is possible to construct a simple inference chain. If we consider the rule base as a tree in which there is the starting state, the goal state and many different branches and possible routes between the two, represented by the IF-THEN commands, then in order that the goal state be reached, it is important that the correct questions be asked, and in the correct sequence, so as not to terminate a valid path of reasoning. The are two basic methods of searching the knowledge base, either forward chaining (goal driven) or backward chaining (data driven).

In forward chaining, the established facts are considered, and where these facts lead to in the inference chain. In backward chaining, a goal state is selected and the facts interrogated to try and justify that goal state. Once a sizeable knowledge base has been developed it becomes difficult and tedious to keep track of the structure of the inference chain, and the current position within it. This task is carried out by the inference engine.

As the inference engine progresses through the rule base so the subsequent application of rules changes the system status and therefore the data base. This in turn enables new rules and disables others thus leading ideally, to the goal state. It is the inference engine that drives the search through the rule base using the heuristics to confine and direct the search. Once written, the inference
engine becomes a fairly rigid set of rules in its own right, rarely amended but constantly used. This is in contrast to the knowledge base itself which, ideally, should constantly be changing with new rules added and old rules deleted. This structure highlights the difference between expert systems and conventional computer programmes. In a conventional computer programme the information required for solving the problem is built into the operating structure of the program itself. This makes modification to the information difficult, as a change of information at one point will effect a change elsewhere in the program.

In the expert system however, the modularity of the system allows for easy modification. Common languages for the inference engine are LISP and PROLOG. LISP (List Processing Language) was the first to be developed in the late 50's by J. McCarthy (in Gevarter, 71).

The interface allows a dialogue between the program and the user, allows for explanations to decisions made by the system and for interfacing to other programs such as spreadsheets, or data from scientific instruments. For an expert system to function, it is necessary to establish all of these modules. This process is not only time consuming but also requires considerable computing expertise as well as the domain knowledge required. Due to the modular nature of expert systems however, it is possible to remove the knowledge base whilst retaining both the inference
engine and the user interface.

In doing so, what is left is an empty shell containing a well developed inference engine and user interface into which can be added the knowledge base required, thereby creating a new expert system. Such expert system shells are commercially available and reduce development time for an expert system by up to an order of magnitude.

These AI tools are programming languages in their own right. Although such tools may appear to fit an application perfectly, their efficient use requires training and practice as in all programming languages. The expert system shell still requires programming although it contains a well defined inference engine and user interface, it does not contain any knowledge on which to act, however the programming that is required, allows the user to concentrate on the problem at hand.

Expert systems work well in areas where ideas exist about solutions, but have not been fully stated, where the knowledge base is firmly established but additions will continue to be made. To either side of this are areas which are inappropriate for expert systems, either insufficient information is available, or the problem is too vague and experts are not available, or else the problems are so well defined that the task could be carried out more effectively with conventional computer programmes. Expert systems have been applied to a wide range of areas
including diagnostics, repair, monitoring, analysis, interpretation, consulting, planning, designing, instructing, explaining and learning. In the field of analytical chemistry expert systems are used for interpretation on mass spectral and nuclear magnetic resonance data, as well as in x-ray powder defraction. Optimisation routines for chromatography have been developed and peak deconvolution techniques built on expert systems.

5.2 DEVELOPMENT OF AN EXPERT SYSTEM FOR CONTROL OF AN ON-LINE MONITORING SYSTEM

It was initially proposed to build an expert system for control of and interpretation of data from an on-line process monitor based on ion-chromatography. Such a system would control all operations of the system, collect and monitor data and give advice or warnings if and when necessary. It would also monitor the status of the instrumentation and advise on maintenance when required. In order to do so, domain knowledge on the chromatography and preconcentration conditions would be required. It would also require knowledge of the post-column reagent system. This would allow the system to identify the peaks obtained from the chromatography and identify any problems associated with either the separation or detection. Knowledge would also be required for the process itself, such as maximum tolerance levels for the metals of interest, identification of trends, rejection of 'fliers'
and history of post data trends. In addition to this knowledge base, information would have to be supplied on the status of the instrumentation, such as back pressures, valve configuration and column temperature as well as from the data itself. In view of the fact that the instrumentation available for the on-line trial was not suitable for interfacing to a computer, and that no hardware was made available for the expert system during the on-line trial, in addition to the length of time required to build such a knowledge base, it was decided instead to build a trouble-shooting guide for the ion-chromatography system and post column reagent system, that is, to complete the first objective of the expert system. It was intended that the user guide would identify problems with the chromatography and detection using information from the user, rather than directly from the instrumentation itself. The data acquisition and interpretation and the knowledge base for the process will not be tackled.

The system would be forward chaining and menu driven. The user is prompted for a reply to given menu options to move through the rule base and ideally to a goal state. Rather than being an expert system, it may be more accurately described as a knowledge based system.

The expert system shell CRYSTAL (International Business Machine Corporation: Microsoft Corporation) was used to write the knowledge base, the shell itself was loaded onto
the hard disc drive of an OPUS PC III personal computer.

5.3 STRUCTURE OF THE KNOWLEDGE BASE

The knowledge base was divided into three main areas, the trouble-shooting guide itself, general information on the instrumentation and general information on the reagents. From this starting point the user can travel through the knowledge base by replying to prompts from the system. None of the prompts require the input of any text, merely an appropriate selection from a list of possible options. For example, if the user had selected the trouble-shooting guide the next prompt would ask whether the problem being encountered was related to either the chromatography, detection or instrumentation. The chromatography itself can be effected by the instrumentation or reagents, and so the whole knowledge base is interlinked. Figure 5.2 shows the initial structure of the knowledge base.

The system comprised thirty nine separate knowledge bases. Each separate knowledge base was composed of text files which were displayed to the user, and as the user replied to the prompts within the text files, so he or she progressed through that knowledge base and into other knowledge bases. It would be inappropriate to include all the text files in the expert system here. Instead, the rule base, which runs the expert system by loading the relevant text files following inputs by the user, is given in appendix 1. The rule base is given in alphabetical
Figure 5.2  Initial structure of the knowledge base showing the various levels below the troubleshooting guide
order of the knowledge base. There is no particular order to the knowledge bases as they are all interlinked.

5.4 CONCLUSIONS

Using the trouble-shooting guide it was possible to obtain information on any part of the ion chromatography system, whether it be on reagent or instrumentation. Problems associated with base-line noise, base-line drift and lack of signal were tackled and solutions to these problems were given in the system. Given the nature of the knowledge base it is impractical to display it graphically in its entirety. Further work is clearly required on the system if it is to be used in conjunction with an on-line process monitor.
CHAPTER 6

6.1 INTRODUCTION

6.2 APPLICATION OF PRECONCENTRATION TECHNIQUES TO FLAME ATOMIC ABSORPTION SPECTROSCOPY

6.2.1 Instrumentation and Reagents
6.2.2 Results and Discussion

6.3 APPLICATION OF PRECONCENTRATION TECHNIQUES TO INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROSCOPY

6.3.1 Instrumentation and Reagents
6.3.2 Principle of peak detection by Perkin Elmer plasma II
6.3.3 Peak location using the autoexec file
6.3.4 Results and discussion

6.4 APPLICATION OF PRECONCENTRATION TECHNIQUES TO INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

6.4.1 Instrumentation and Reagents
6.4.2 Addition of internal standard for ICP-MS Studies
6.4.3 Results and Discussion

-139-
6.4.3.1 Dionex metpac CC-1 preconcentration column
6.4.3.2 Xylenol Orange preconcentration column

6.5 CONCLUSIONS
6.1 INTRODUCTION

Since its introduction by Walsh (72) atomic absorption spectrometry (AAS) has become an extremely valuable and popular technique for the determination of trace amounts of metals. During this time the method has gone through a number of developmental stages aimed at improving reliability, ease of operation, reproducibility and limit of detection. To this end, flame atomisation has, to some extent been superseded by graphite furnace atomic absorption spectrometry (GFAAS) and new techniques have emerged such as inductively coupled plasma atomic emission spectrometry (ICP-AES) and in more recent years inductively coupled plasma-mass spectrometry (ICP-MS), the latter two being developments of flameless atomic emission spectroscopy (FAES). In general, all of these techniques have been developed for the analysis of aqueous solutions, and all operate well in a low ionic strength medium. In the presence of a large excess of a matrix ion the same is not always true. Although many of the interferences encountered in atomic spectroscopy may be minimised or removed by either suitable adjustment of the operating parameters used or sample pretreatment, the analysis of concentrated media, such as brines, still remains a
difficult and time consuming process. When highly saline matrices are aspirated in either FAA or ICP, blockage of the nebuliser may occur. In GFAAS, excessive build up of salt will occur in the furnace tube and in ICP-MS, blockage of the sampling cone can occur with saline concentrations in excess of 0.3% m/v, if continuously nebulised.

In addition to these physical interferences, there are also many interferences experienced in the absorption, emission, or mass spectra obtained. This has lead to considerable interest in techniques to help eliminate such interferences. Much work has been carried out in the past using chelating exchange resins for the extraction of transition metals and heavy metals from sea waters prior to either FAA or GFAAS and indeed ICP-AES/MS (46-50, 57, 73-79). However, in general, these procedures tend to be time consuming and require large sample volumes. In recent years flow injection (FI) has repeatedly proved itself to be a powerful technique for overcoming these problems for FAAS and ICP.

Because of the advantages of FI over direct nebulisation for certain sample types, it was a logical step to combine the 'Chelex type' procedures with FI, and recently there has been wide interest in on-line preconcentration - flow injection with spectroscopic detection. The advantages of such a coupling are numerous and include:
1) reduced reagent consumption;
2) rapid analysis;
3) ease of operation;
4) improvement in precision;
5) reduced contamination;
6) lowered detection limits;
7) analysis of saline matrices.

It has been reported that if such preconcentration techniques are to be effective then efficient use of the spectrometer must also be made (80-81). However, the emphasis in this particular application is not placed on high sample throughput, but on the ability to carry out analysis in saline media.

Most applications of preconcentration flow injection for atomic spectroscopy are concerned with the determination of heavy metals and first row transition metals in sea water matrices and polluted waters. This requires a preconcentration column which selects against the alkaline earth metals in favour of the first row transition metals (magnesium and calcium being the matrix ions in sea water) rather than one which will selectively preconcentrate the alkaline earths in favour of the first row transition metals, sodium in each case passing through the column unchanged. In light of the problems associated with the analysis of trace metals in saline media, and the few applications concerning the determination of the alkaline earths, the preconcentration/matrix elimination procedures
developed for ion chromatography (Chapter 3) have been applied to FAA, ICP-AES, and ICP-MS. Various preconcentration columns were used and provided a means of not only determining the elements of interest, but also a source of comparison for those results obtained by ion chromatography. The work was not concerned with fundamental studies of the processes of atomisation and ionisation within the flame/plasma, nor the effects of an excess of sodium ions upon these parameters. The emphasis was on the applicability of the preconcentration techniques already discussed, to the determination of trace metals in saline media by atomic spectroscopy. The following combinations of preconcentration columns (listed as their functional groups) and spectroscopic techniques were studied:

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Spectroscopic Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>8HQ</td>
<td>FAA</td>
</tr>
<tr>
<td>IDA</td>
<td>ICP-AES</td>
</tr>
<tr>
<td>IDA</td>
<td>ICP-MS</td>
</tr>
<tr>
<td>IDA Coated Column</td>
<td>ICP-MS</td>
</tr>
</tbody>
</table>

6.2 APPLICATION OF PRECONCENTRATION TECHNIQUES TO FLAME ATOMIC ABSORPTION SPECTROSCOPY

The preconcentration column used in these studies was comprised of 8-hydroxyquinoline immobilised onto control pore glass beads which were packed into a 5 cm titanium column.
6.2.1 Instrumentation and Reagents

A model 4000 flame atomic absorption spectrometer (Perkin Elmer, Beaconsfield, Bucks), was used throughout the study. The operating parameters for each of the elements are listed in table 6.1. The preconcentration column was connected across a six port switching valve (VALCO VICI, Valco Europe, CH-6214, Schenkon, Switzerland), and the outlet connected to the nebuliser tube of the spectrometer. A dual channel peristaltic pump (Minipulse Gilson, Luton Beds), was used to deliver the mobile phase and brine samples at 2.5 ml min\(^{-1}\). In order to match the flow-rate from the column (2.5 ml min\(^{-1}\)) to the uptake rate of the nebuliser (7 ml min\(^{-1}\)) a discrete sample interface was used (figure 6.1). Analytical grade reagents were used throughout unless stated otherwise. Double distilled, deionised water (DDW) was obtained from a Milli Q system (Millipore, Bedford, Ma, USA).

6.2.2 Results and Discussion

Prior to any studies of the metals of interest, it was necessary to establish the effect of wash volume upon the concentration of residual sodium. If the wash volume was too little then excess sodium would be eluted to the spectrometer, and too large a wash volume would not only lead to lengthy analysis times but would also contribute to the blank values.
<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Lamp current (mA)</th>
<th>Band Pass (nm)</th>
<th>Flame Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td>285.2</td>
<td>3.0</td>
<td>1.0</td>
<td>Air-acetylene</td>
</tr>
<tr>
<td>Calcium</td>
<td>422.7</td>
<td>7.0</td>
<td>1.0</td>
<td>Air-acetylene</td>
</tr>
<tr>
<td>Strontium</td>
<td>460.7</td>
<td>12.0</td>
<td>0.5</td>
<td>Nitrous oxide - acetylene</td>
</tr>
<tr>
<td>Aluminium</td>
<td>309.3</td>
<td>8.0</td>
<td>1.0</td>
<td>Nitrous oxide - acetylene</td>
</tr>
</tbody>
</table>
Figure 6.1 Discrete sampler used to interface the outlet from the preconcentration column to the nebulizer tube of the spectrometer.
Ten millilitre aliquots of saturated brine were loaded onto the preconcentration column. The column was subsequently washed with differing volumes of DDW. The column was then back-flushed with 1M nitric acid to the flame spectrometer, and the sodium emission monitored at 303.3 nm, with the burner head rotated through 45°. Figure 6.2 shows the residual sodium concentration with respect to wash volume.

It can be seen that following a wash volume of 7 ml the residual sodium concentration was below 0.35% m/v. It is felt that the large wash volume required, and the background concentration which was left was related to the structure of the column material. The porous glass beads, on which the SHQ was immobilised were 170μm in size, which in itself led to a large dead volume in the column. This was in addition to the honeycomb structure of each bead, which increased the column dead volume further. This requires a large wash volume to remove the sodium chloride matrix. All subsequent analyses were carried out using a 10 ml aliquot of sample followed by a 7 ml aliquot of distilled deionised water.

The work performed by FAAS was limited to the four metals listed in table 6.1. Calibrations were obtained for each of the metals in turn by spiking 30% m/v sodium chloride samples with the appropriate concentration of analyte to yield final concentrations in the range 1 - 50 μg l⁻¹. These calibrations are shown in figures 6.3 - 6.6 for magnesium, calcium, strontium and aluminium respectively.
Figure 6.2 Residual sodium concentration with respect to wash volume obtained during the flame atomic absorption studies.
Figure 6.3 Calibration curve obtained for magnesium in 30% m/v sodium chloride using the 8HQ pre-concentration column followed by FI-FAAS.
Figure 6.4 Calibration curve obtained for calcium in 30% m/v sodium chloride using the 8HQ pre-concentration column followed by FI-FAAS.

Correlation coefficient = 0.9999
Slope = 29.95
Y Intercept = 522
Figure 6.5  Calibration curve obtained for strontium in 30% m/v sodium chloride using the 8HQ pre-concentration column followed by FI-FAAS:

Correlation coefficient = 0.9994
Slope = 3.59
Y Intercept = 6.25
Figure 6.6  Calibration curve obtained for barium in 30% m/v sodium chloride using the 8HQ pre-concentration column followed by FI-FAAS.

Correlation coefficient = 0.9988
Slope = 7.22
Y Intercept = 37.39
Figure 6.7 shows the transient signals obtained for magnesium, calcium, strontium and aluminium at the 50 µg l⁻¹ level. The pH of the brine was adjusted to 10 using concentrated nitric acid for the alkaline earths and pH 7 using concentrated nitric acid for aluminium, following results obtained in section 3.6.

From the calibrations it can be seen that although the column has successfully retained the metals, the precision is very poor. However, initial results were promising and allowed the determination of the metals studied at the low µg l⁻¹ level by FAAS. Following this brief study, the work was transferred to inductively coupled plasma-atomic emission spectrometry, as it was felt this would provide improved precision and stability.

6.3 APPLICATION OF PRECONCENTRATION TECHNIQUES TO INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROSCOPY

There are several advantages in coupling preconcentration techniques to ICP-AES as opposed to FAA, including ease of operation, improved precision and lower detection limits.

6.3.1 Instrumentation and Reagents

An inductively coupled plasma atomic emission spectrometer (Plasma II, Perkin Elmer Ltd., Beaconsfield, Bucks), was used throughout the study. Data was collected directly
Figure 6.7 Transient signals obtained for magnesium, calcium, strontium and aluminium in 30% m/v sodium chloride using the 8HQ preconcentration column followed by FI-FAAS.
onto a plotter integrator (spectra physics Auto Lab Division, Ma, USA), from a serial port on the spectrometer, although all instrumental parameters were still controlled via the manufacturer's software.

A Dionex advanced chromatography module (Dionex Corp., Albany Park, Camberley, Surrey, U.K), was used to house two inert switching valves (Dionex Corp.) and was controlled via a Dionex auto ion controller. The preconcentration column (Metpac CC-1, Dionex Corp.), was connected across one of the Dionex switching valves. A dual channel peristaltic pump (Mini pulse Gilson, Luton, Beds.), was used to deliver the brine samples and mobile phase, set to 2.5 ml min$^{-1}$ unless otherwise stated. The outlet from the injection manifold was connected directly to the nebuliser tube of the ICP. The peristaltic pump on the instrument was also set to deliver at 2.5 ml min$^{-1}$ to further regulate the flow from the preconcentration column. All reagents were of analytical grade unless stated otherwise, and doubly distilled, deionised water was obtained from a milli Q water system (Millipore, Bedford, Ma, USA).

6.3.2 Principle of Peak Detection by Plasma Spectrometer

The software of the ICP instrument used during this study did not allow the measurement of transient signals. The instrument was designed to measure intensity with respect to wavelength, whereas a measurement of intensity with respect to time was required. When a wavelength was...
selected on the instrument, the monochromator scanned to that wavelength, and then stepped back by up to 0.1 nm from the selected wavelength. Once a run sequence was initialised the monochromator scanned across the peak window. The peak window was the area around the elemental line used for curve fitting, and was set by the operator. The wider the peak window, the longer the scan took, however, if the window is set too small, then the peak maximum may be missed.

If the data was collected in this fashion by repeatedly scanning the monochromator across the elemental emission line of interest then it would be highly unlikely that the maximum concentration of the elution band from the column would be measured, as the delay time between replicate scans was of the order of one second. However, it was not possible to collect data without the monochromator scanning. In addition to this, if the monochromator was disabled, due to the set up procedure described above, the monochromator would not have been at the correct wavelength for measurement. With these points in mind two modifications were carried out on the instrument to allow for measurement of transient signals.

Firstly a program was written in the autoexec file which, when run, allowed the monochromator to be stepped in 0.005 and 0.001 nm increments. This allowed for the peak maximum to be located. Secondly a device was installed to disable the monochromator, but allowed the software to continue as
if the monochromator was still scanning.

6.3.3 Peak Location using the Autoexec File

The wavelength for the element of interest was selected. A 5 \( \mu g \, l^{-1} \) standard of the element was then directly aspirated into the ICP. The signal was monitored and collected on the plotter/integrator, and the autoexec file executed so that the monochromator stepped across the line in 0.005 nm increments, with a dwell time of five seconds at each step. The wavelength of maximum signal was then noted. The monochromator was then driven to this wavelength and the process repeated, stepping the monochromator in 0.001 nm increments to locate accurately the wavelength of maximum emission. Once this had been located the monochromator was then disabled. The peak window, which may be set by the operator (typically 0.05 nm) effectively determined the time taken for the scan. If this peak window is set artificially wide then the scan time became long enough to measure the transient signal. If a peak window of 0.1 nm was selected, with a dwell time of 100 nsec at each step, the total scan time was over two minutes.

Once the monochromator had been disabled, and the run sequence initialised, the software allowed for collection of data over this time and hence it was possible to collect intensity data with respect to time. In doing so it was possible to use the calibration and standardisation routines present in the software. Operating conditions for
the spectrometer are given in Table 6.2.

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength /nm</th>
<th>Height* (mm)</th>
<th>Pmt (V)</th>
<th>Pw (nm)</th>
<th>Dwell time (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>279.553</td>
<td>15</td>
<td>450</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>Ca</td>
<td>393.366</td>
<td>15</td>
<td>500</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>Sr</td>
<td>407.771</td>
<td>16</td>
<td>350</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>Al</td>
<td>396.152</td>
<td>12</td>
<td>750</td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td>Na</td>
<td>288.144</td>
<td>15</td>
<td>600</td>
<td>0.1</td>
<td>100</td>
</tr>
</tbody>
</table>

* above the load coil

Pmt = Photomultiplier voltage

Pw = Peak Width

6.3.4 Results and Discussion

The residual sodium concentration was determined by preconcentrating 5 ml aliquots of saturated brine, and washing the column with various volumes of distilled deionised water prior to elution to the ICP at a flow rate of 2.5 ml min

The results are shown in Figure 6.8. A five millilitre
Figure 6.8  Residual sodium concentration with respect to wash volume obtained during the inductively coupled plasma-atomic emission studies.
wash volume was sufficient to reduce the sodium concentration to an acceptable level.

The effect of sample pH upon recovery was determined for magnesium, calcium and strontium. The pH of 100 ml aliquots of brine spiked with the appropriate concentration of analyte to yield a final concentration of 50 µg l⁻¹ was adjusted in the range 7 - 12 with concentrated nitric acid. Five ml aliquots were then preconcentrated onto the metpac cc-1 preconcentration column and subsequently eluted to the ICP. The results are shown in figures 6.9 to 6.11. The resin behaved in a similar manner to the coated resin prepared in 3.6.3.2, which was as expected due to the functional groups being IDA in both cases. At this time during the studies the inductively coupled plasma-mass spectrometer became available and the studies were continued using this instrument.

6.4 Application of Preconcentration Techniques to Inductively Coupled Plasma-Mass Spectrometry.

The main advantage of ICP-MS over FAA and rapid sequential ICP-AES is in its multielement capability. Preconcentration techniques have been shown to be of great potential in the analysis of saline matrices, however, only one element may be determined in each injection, when all the elements of interest may be present in that injected sample. Inductively coupled plasma-mass spectrometry also has the advantage of lower limits of detection for many of
Figure 6.9  Relationship between sample pH and magnesium response using the metpac CC-1 preconcentration column followed by FI-ICP-AES
Figure 6.10 Relationship between sample pH and calcium response using the metpac CC-1 preconcentration column followed by FI-ICP-AES
Figure 6.11 Relationship between sample pH and strontium response using the metpac CC-1 preconcentration column followed by FI-ICP-AES.
the elements studied. However, the technique does suffer from several disadvantages with respect to saline matrices. The technique is far less tolerant with respect to dissolved solids requiring levels of less than 0.3% dissolved solids, if the sample cone is to remain unblocked. In addition, the presence of chlorine in samples causes several interferences from 'isobaric' chloride ions which effect the determination of some important elements.

Given these interference problems, and recognising the improved limits of detection of the technique compared to ICP-AES, the emphasis in this work was placed not so much on the ability to preconcentrate the analyte, but to eliminate the matrix interference.

Until now, attention has been focused on the alkaline earths, however, due to the flexibility of the technique, the first row transition metals were also studied.

6.4.1 Instrumentation and Reagents

A Plasmaquad II (VG Elemental, Winford, Cheshire), was used throughout the study. Typical conditions used for the ICP and the quadrupole mass analyser are listed in table 6.3.
TABLE 6.3  ICP-MS Operating Conditions

Mass Analyser

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of channels</td>
<td>2048</td>
</tr>
<tr>
<td>No. of scan sweeps</td>
<td>100</td>
</tr>
<tr>
<td>Dwell time / μs</td>
<td>320</td>
</tr>
<tr>
<td>Points per peak</td>
<td>5</td>
</tr>
</tbody>
</table>

Skipped Mass Regions/mass units

<table>
<thead>
<tr>
<th>from</th>
<th>to</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.00</td>
<td>23.50</td>
</tr>
<tr>
<td>27.50</td>
<td>41.50</td>
</tr>
<tr>
<td>70.00</td>
<td>85.00</td>
</tr>
</tbody>
</table>

Isotopes Selected

<table>
<thead>
<tr>
<th>Mass/units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
</tr>
<tr>
<td>Al</td>
</tr>
<tr>
<td>Ca</td>
</tr>
<tr>
<td>Ti</td>
</tr>
<tr>
<td>V</td>
</tr>
<tr>
<td>Cr</td>
</tr>
<tr>
<td>Cr</td>
</tr>
<tr>
<td>Mn</td>
</tr>
<tr>
<td>Fe</td>
</tr>
<tr>
<td>Ni</td>
</tr>
<tr>
<td>Co</td>
</tr>
<tr>
<td>Zn</td>
</tr>
<tr>
<td>Cu</td>
</tr>
<tr>
<td>Sr</td>
</tr>
<tr>
<td>In</td>
</tr>
<tr>
<td>Ba</td>
</tr>
</tbody>
</table>
The injection manifold used is shown schematically in figure 6.12. It was basically the same as that described in section 6.3.1. However, a third switching valve was added, which allowed the addition of an internal standard as discussed in section 6.4.2. A four channel peristaltic pump was used to deliver brine samples and eluent. All reagents were of analytical grade unless otherwise stated, doubly distilled, deionised water was obtained from a milliQ water system. Two preconcentration columns were used, a Dionex metpac cc-1 column and the xylenol orange column prepared in 3.6.3.2.

6.4.2 Addition of internal standard for ICP-MS studies

It is preferable to include an internal standard in studies involving ICP-MS to compensate not only for effects encountered in the plasma, but also in the mass analyser, as ions of different mass tend to behave differently.

In the present application then, when there is a large excess of sodium, such standardisation is critical. Indium was chosen as an internal standard as its mass (115) lay within the range of metals to be studied. Indium is also mono isotopic and not likely to be present in the samples to any significant extent. If the indium was added to the brine samples themselves it may be expected that it would preferentially complex on the column, at worst causing decreased recoveries of the analytes of interest and certainly not likely to behave in a similar manner to those
Figure 6.12  Block diagram of the switching valves, preconcentration column and sample loops used during the inductively coupled plasma-mass spectrometer studies.
analytes being determined. If, on the other hand the indium was added to the eluent which in the previous studies was continuously aspirated, then a ratio would be made between a transient signal from the analyte and a constant signal from the indium. To overcome these problems the injection manifold was modified and is shown in figure 6.12. In this arrangement doubly distilled, deionised water was continuously pumped to the nebuliser of the ICP via the peristaltic pump. A 2.5 ml loop was filled with the eluent (1M HNO₃) spiked with 50 µg l⁻¹ indium. Following the preconcentration procedure already outlined in section 3.6.2.2, the distilled deionised water flushes the acid from the loop to the preconcentration column where the retained metals are eluted to the ICP-MS. In this way, both the analytes of interest and the internal standard are measured as transient signals and both experience the same environment within the plasma with respect to sodium concentration. This procedure does not however, compensate for any drift in the preconcentration, it will only compensate for effects in the ICP-MS instrument.

6.4.3 Results and Discussion

6.4.3.1 Dionex metpac cc-1 preconcentration column

The preconcentration manifold was connected to a Perkin Elmer 4000 flame absorption spectrometer, and the residual sodium concentration from the preconcentration column was monitored, with respect to wash volume, at 303.3 nm, with
the burner head rotated through 45°. Figure 6.13 shows the residual sodium concentration with respect to wash volume, and, as in previous cases, a five millilitre aliquot was sufficient to remove the sodium level to approximately 0.3% m/v. Calibrations for the metals of interest are shown in figures 6.14 - 6.24. These were obtained by preconcentrating 1.2 ml aliquots of brine spiked with the appropriate concentration of metal to yield final concentrations in the range 1 - 100 µg ml⁻¹. Limits of detection for the elements of interest were not determined as the net effect of the matrix elimination procedure was to dilute the sample 1:1.

It was not possible to determine calcium in any of the samples due to the major isotope being at 40 m/z which suffers from isobaric interferences from argon in the plasma. The abundance of the isotopes at m/z 42 and m/z 44 were too low for determination of calcium at this low concentration. The determination of iron at m/z 56 was not possible due to the formation of ArO⁺. However, the Cl³⁵O¹⁶⁺ and Cl³⁷O¹⁶⁺ interferences were eliminated thus allowing the determination of vanadium at m/z 51 and chromium at m/z 53.

6.4.3.2 Xylenol Orange Preconcentration column

The xylenol orange preconcentration column prepared in section 3.6.3.2 was used as a matrix elimination column for analysis of brine samples obtained during the on-line trial
Figure 6.13 Residual sodium concentration with respect to wash volume obtained during the inductively coupled plasma-mass spectrometer studies.
Figure 6.14 Calibration curve obtained for magnesium in 30% sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

Correlation coefficient = 0.9977
Slope = 2.42
Y Intercept = 72.46
Figure 6.15 Calibration curve obtained for aluminium in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS.
Figure 6.16  Calibration curve obtained for titanium in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS
Figure 6.17 Calibration curve obtained for vanadium in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

Correlation coefficient = 0.9989
Slope = 3.44
Y Intercept = 14.26
Figure 6.18 Calibration curve obtained for chromium in 30% m/v sodium chloride using the metpac CC-1 concentration column followed by FI-ICP-MS

Correlation coefficient = 0.9383
Slope = 2.65
Y Intercept = 30.17
Figure 6.19 Calibration curve obtained for manganese in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS.

Correlation coefficient = 0.9991
Slope = 6.58
Y Intercept = 21.03
Figure 6.20 Calibration curve obtained for nickel in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

Correlation coefficient = 0.9947
Slope = 2.80
Y Intercept = 12.39
Figure 6.21 Calibration curve obtained for cobalt in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

Correlation coefficient = 0.9971
Slope = 5.85
Y Intercept = -1.59
Figure 6.22. Calibration curve obtained for zinc in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

![Graph showing the calibration curve with the following data:

- Correlation coefficient: 0.9988
- Slope: 2.49
- Y Intercept: 23.46

The x-axis represents Elemental Concentration (ng/ml), and the y-axis represents Intensity (arbitrary units).]
Figure 6.23 Calibration curve obtained for copper in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS

![Graph showing the calibration curve with the following data:
- Correlation coefficient = 0.9935
- Slope = 1.74
- Y Intercept = 29.11]
Figure 6.24  Calibration curve obtained for strontium in 30% m/v sodium chloride using the metpac CC-1 preconcentration column followed by FI-ICP-MS.

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>0.9935</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>0.98</td>
</tr>
<tr>
<td>Y Intercept</td>
<td>212.4</td>
</tr>
</tbody>
</table>
The preconcentration manifold remained the same as for the Dionex column. Brine samples obtained during the on-line trial were analysed by standard additions using 1.2 ml aliquots of sample. Calcium could not be determined for the reasons mentioned in 6.4.3.1.

The calibrations obtained by standard additions for magnesium, strontium and barium are shown in figures 6.25-6.27.

Table 6.4 shows the comparison of results obtained by preconcentration ion chromatography and preconcentration ICP-MS for the brine samples.

<table>
<thead>
<tr>
<th>Element</th>
<th>Ion Chromatography $\mu g , l^{-1}$</th>
<th>ICP-MS $\mu g , l^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>3.5</td>
<td>9</td>
</tr>
<tr>
<td>Ca</td>
<td>30</td>
<td>not determined</td>
</tr>
<tr>
<td>Sr</td>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>Ba</td>
<td>18</td>
<td>9</td>
</tr>
</tbody>
</table>

The discrepancy between results may be due largely to the brine samples being stored for two months prior to analysis by ICP-MS.
Figure 6.25 Calibration curve obtained for magnesium in 30% m/v sodium chloride using the xylenol orange preconcentration column followed by FI-ICP-MS.
Figure 6.26 Calibration curve obtained for strontium in 30% m/v sodium chloride using the xylenol orange preconcentration column followed by FI-ICP-MS
Figure 6.27 Calibration curve obtained for barium in 30% m/v sodium chloride using the xylenol orange preconcentration column followed by FI-ICP-MS
6.5 Conclusions

The applicability of these preconcentration techniques to atomic spectroscopy has been proved. Detection limits of less than 100 $\mu g \ l^{-1}$ for magnesium, calcium, strontium and aluminium were obtained by flame atomic absorption using such techniques.

ICP-MS has great potential for such analysis with detection limits of 2 orders of magnitude lower, and multielement capability. The Dionex preconcentration column was successfully used for the determination of the alkaline earths and first row transition metals in brine samples. Although the study of such techniques was not comprehensive, it does prove the applicability, especially to ICP-MS, for the removal of ClO interferences.
7.1 CONCLUSIONS

Chromatography of the alkaline earths (magnesium, calcium, strontium and barium) and aluminium and zinc was achieved using a mobile phase of 0.2 M lactic acid in conjunction with a strong cation exchange resin, although the addition of a competing ion, ethylendiamine, was required in the case of the alkaline earths to effect the separation in a realistic time. A universal detection system was developed based on inverse photometry using the chelating dye calmagite with the addition of a magnesium ethylene diamine tetra-acetic acid solution. This allowed detection of all the metals studied using one fixed wavelength. Coated column techniques were successfully employed for the preconcentration of the metals from saturated brines, whilst leaving the brine matrix to pass through the column unchanged. The chelating dye xylenol orange was found to be the most suitable chelating agent for these studies. Commercially available resins such as Chelex 100 were found to be unsuitable for this application due to the swelling of the resin between the high and low ionic strength media employed. The stability of the reagents used and the methodology was proved during an on-line trial during which the system ran unattended, sampling continuously from a saturated brine feed tank. During the analysis, one
hundred and eighty determinations of each of the four metals were carried out at the low ng ml\(^{-1}\) level. Significantly Barium was determined at the 20 ng ml\(^{-1}\) level. Prior to the on-line trial barium had not been detected in the saturated brines using existing methodology. The presence of barium in the feed brines was known however from previous studies on the membrane cells themselves.

The applicability of the preconcentration techniques developed to atomic spectroscopy was shown especially when coupled to ICP-MS. The Dionex metpac cc-1 chelating exchange column was used to retain titanium, vanadium, chromium, manganese, cobalt, nickel, copper, zinc and aluminium from saturated brines prior to determination by ICP-MS. The methodology employed removed sodium levels to below 0.3% m/v and completely removed ClO\(^-\) interference allowing analysis of sea water samples without the need for dilution. Finally a knowledge based user guide was built in the expert system shell CRYSTAL.

7.2 FUTURE WORK

Although the initial aims of the study were met in that on-line determination of trace metals in saturated brine was achieved, rather than being completed, the work has really just begun. In order to simplify the ion-chromatography system developed it may be possible to preconcentrate the trace metals of interest, and separate
them using one column. Since each metal studied was shown to have different pH optima for retention on the preconcentration column it may be possible with suitable choice of chelating agent to selectively elute the metals using a pH gradient, each metal being eluted as the pH falls below the optimum for that metal. Further development is evidently needed in the instrumentation to improve the reliability of the pumps and switching valves for use as a process analyser.

Further development is required on the expert system. This would include sending data from the chromatogram, such as peak areas and retention times, directly to a spreadsheet. This information could then be interrogated by the expert system. Retention times would not only identify the peaks, but also provide information on the chromatography itself, on the condition of the column and/or the reagents used. Information gained from peak areas would then enable trends in the concentration of the elements to be plotted and, with a suitable knowledge base on the process itself, the system would be able to maintain efficient use of the ion-exchange clean-up columns. Identification of 'fliers' would also be possible.

Only a brief study was carried out on the use of preconcentration/matrix elimination techniques for ICP-MS, although clearly the number of applications for such techniques are limitless. Not only does the routine analysis of sea waters, waste waters become possible, but
also a variety of effluent and process stream. Clearly there is a wide range of applications for such techniques.
REFERENCES


<table>
<thead>
<tr>
<th></th>
<th>Reference</th>
</tr>
</thead>
</table>


Appendix 1

Rule structure to the knowledge base.

The separate knowledge bases are listed in alphabetical order

(Choice)
If
and :assign Choice$:="1"
and :menu Choice$
and :test Choice$="1"
and :test load("tshoot")
Or
and :test Choice$="2"
and :test load("two")
Or
and :test Choice$="3"
and :display form.

(Chrom)
If
and :assign Chrom$="1"
and :menu Chrom$
and :test Chrom$="1"
and :test Speak
and :test load("choice")
Or
and :test Chrom$="2"
and :menu Tpeak
and :test load("choice")
Or
and :test Chrom$="3"
and :menu Ppeak
and :test load("choice")
Or
and :test Chrom$="4"
and :test load("Rtlong")
Or
and :test Chrom$="5"
and :test load("Rtshort")
Or
and :test Chrom$="6"
and :test load("Pres")
Or
and :test Chrom$="7"
and :test load("two")
Or
and :test Chrom$="8"
and :display form.

(Column)
If
and :menu column$ 1.
and :test  load("chrom")

(Column2)
If :menu  column
and :test  load("MM")

(Creagents)
If :assign Which$="1"
and :menu  Which$
and :test  Which$="1"
and :test  load("MobileP")
Or :test  Which$="2"
and :test  load("PCR")
Or :test  Which$="3"
and :test  load("STD")
Or :test  Which$="4"
and :test  load("sample")
Or :test  Which$="5"
and :test  load("two")

(Det)
If :assign Det$="1"
and :menu  Det$
and :test  Det$="1"
and :test  load("PCR")
Or :test  Det$="2"
and :test  load("Dpower")
Or :test  Det$="3"
and :test  load("Positive")
Or :test  Det$="4"
and :test  load("Negative")
Or :test  Det$="5"
and :test  load("noisy")

(Dirty)
If :menu  Dirty$="press resturn to continue"
and :test  load("Punit")

(Dpower)
If :menu Dpower$ and :display form and :assign Samcal$="1" and :menu Samcal$ and :test Samcal$="1" and :test load("Sigcal")

Or :test Samcal$="2" and :test load("Sigsam")

Or :test Samcal$="3" and :display form.

 legislators
If :menu detector and :test load("MM")

(Ginstru) If :assign Ginstru$="1" and :menu Ginstru$ and :test Ginstru$="1" and :test load(Gpump)

Or :test Ginstru$="2" and :test load(Valve)

Or :test Ginstru$="3" and :test load(Column2)

Or :test Ginstru$="4" and :test load(Gdet)

Or :test Ginstru$="5" and :display form

(Gpump) If :menu pump$ and :test load("MM")

(Instru) If :assign Module$="1" and :menu Module$ and :test Module$="1" and :test load("Punit")

Or :test Module$="2" and :test load("Valves")

Or :test Module$="3" and :test load("Column")

Or :test Module$="4" and :test load("Dpower")

3.
Or
    :test  Module$="5"
    and :test  load("Recorder")
Or
    :test  Module$="6"
    and :display form.

(Loose)
If
    :menu  Loose$
    and :test  loose$="press return to continue"
    and :test  load("Punit")

(MM)
If
    :assign  MM$="1"
    and :menu  MM$
    and :test  MM$="1"
    and :test  load("Ginstru")
Or
    :test  MM$="2"
    and :test  load("Two")
Or
    :test  MM$="3"
    and :display form

(MobileP)
If
    :menu  Composition$
    and :test  Composition$="press return to continue."
    and :menu  pH$
    and :test  pH$="press return to continue."
    and :test  load("Choice")

(Negative)
If
    :menu  Negative$
    and :assign  choice3$="1"
    and :menu  choice3$
    and :test  choice3="1"
    and :test  load(MobileP)
Or
    :test  choice3$="2"
    and :menu  prime$
    and :test  load("Choice")
Or
    :test  choice3$="3"
    and :test  load("Tshoot")
Or
    :test  choice3$="4"
    and :test  load("two")
Or
    :test  choice3$="5"
    and :display form

(Noisy)
If
    :menu  Noise$
and :test load("choice")

(Nosig)
If
   assign Nosig$="1"
and :menu Nosig$
and :test Nosig$="1"
and :test load("Instru")
Or
   :test Nosig$="2"
and :test load("Creagents")
Or
   :test Nosig$="3"
and :test load("Chrom")
Or
   :test Nosig$="4"
and :test load("Cal")
Or
   :test Nosig$="5"
and :display form

(One)
If
   :Menu Welcome$
and :test welcome$="press return to continue"
and :menu welcome
and :test load("Two")

(PCR)
If
   :menu PCR$
and :test PCR$="press return to continue"
and :menu PCRpH$
and :test load("Choice")

(Positive)
If
   :menu Positive$
and :assign choice2$="1"
and :menu choice2$
and :test choice2$="1"
and :test load("PCR")
Or
   :test choice2$="2"
and :menu prime$
and :test load("Choice")
Or
   :test choice2$="3"
and :test load("Tshoot")
Or
   :test choice2$="4"
and :test load("Two")
Or
   :test choice2$="5"
and :display form

(Power)

5.
If :menu Power$ and :test Power$="press return to continue." and :test load("Punit")

(Pres)
If :assign Peakpres$="1"
and :menu Peakpres$
and :test Peakpres$="1"
and :menu overlap$
and :test load("MobileP")
Or :test Peakpres$="2"
and :test load("Column")
Or :test Peakpres$="3"
and :test load("Sample")

(Pressure)
If :menu Pressure$
and :test Pressure$="press return to continue."
and :test load("Punit")

(Prime)
If :menu Prime$
and :test prime$="return"
and :test load("Punit")
Or :test Prime$="C"
and :menu C$
and :test C$="press return to continue"
and :test load("Punit")

(Punit)
If :assign Symptom$="A"
and :menu Symptom$
and :test Symptom$="A"
and :test load("Power")
Or :test Symptom$="B"
and :test load("Pressure")
Or :test Symptom$="C"
and :test load("Prime")
Or :test Symptom$="D"
and :test load("Loose")
Or :test Symptom$="E"
and :test load("Worn")
Or :test Symptom$="F"
and :test load("Dirty")

Or :test Symptom$="G"
and :test load("Prime")

Or :test Symptom$="H"
and :test load("Tshout")

Or :test Symptom$="I"
and :test load("Two")

(Recorder)
If :menu Recorder$
and :test load("Choice")

(Rtlong)
If :assign Rtlong$="1"
and :menu Rtlong$
and :test Rtlong$="1"
and :test load(MobileP")
Or :test Rtlong$="2"
and :test load("Column")

(Rtshort)
If :menu Rtshort$
and :test Rtshort$="1"
and :test load("MobileP")
Or :test Rtshort$="2"
and :test load("Column")

(Sample)
If :menu Sam$
and :test sam$="press return to continue."
and :test load("Choice")

(Samples)
If :menu sam$
and :test "Press return to continue"
and :test load("Choice")

(Sigcal)
If :assign Sigcal$="1"
and :menu Sigcal$
and :test Sigcal$="1"
and :display form

7.
and :display form
and :test load("Choice")

Or
and :test Sigcal$="2"
and :display form.
and :test load("Choice")

Or
and :test Sigcal$="3"
and :display form.

(Sigsam)
If
:assign
Sigsam$="1"
and :menu Sigsam$
and :test Sigsam$="1"
and :display form.
and :display form.
and :test load("Choice")

Or
and :test Sigcal$="2"
and :display form.
and :test load("Choice")

Or
and :test Sigcal$="3"
and :display from.
and :test load("Choice")

Or
and :test Sigcal$="4"
and :display form.

(STD)
If
:menu STD$
and :test STD$="press return to continue"
and :test load("Choice")

(Tshoot)
If
:assign
T$="1"
and :menu T$
and :test T$="1"
and :test load("Chrom")

Or
and :test T$="2"
and :test load("Det")

Or
and :test T$="3"
and :test load("Instru")

Or
and :test T$="4"
and :display form.

8.
(Two)
If :assign choice$="1"
   and :menu choice$
   and :test choice$="1"
   and :test load("Tshoot")
Or :test choice$="2"
   and :test load("Ginstru")
Or :test choice$="3"
   and :test load("Creagents")
Or :test choice$="4"
   and :display form.

(Valve)
If :menu valve$
   and :test load("MM")

(Valves)
If :menu Dionex
   and :menu Valve
   and :test load("choice")

(Worn)
If :menu Worn$
   and :test Worn$="press return to continue"
   and :test load("Punit")