Aspects of flow injection sample introduction for atomic absorption spectrometry

This item was submitted to Loughborough University's Institutional Repository by the/an author.

Additional Information:


Metadata Record: https://dspace.lboro.ac.uk/2134/33155

Publisher: © Ahyar bin Idris

Rights: This work is made available according to the conditions of the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) licence. Full details of this licence are available at: https://creativecommons.org/licenses/by-nc-nd/4.0/

Please cite the published version.
ASPECTS OF FLOW INJECTION SAMPLE INTRODUCTION

FOR ATOMIC ABSORPTION SPECTROMETRY.

BY

AHYAR BIN IDRIS, B.Sc. (Hons.), M.Sc.

A Doctoral Thesis
Submitted in partial fulfilment of the requirements
for the award of
Doctor of Philosophy
of the Loughborough University of Technology.

October 1983.

Supervisor: Dr. J. F. Tyson, B.Sc., Ph.D., D. I. C.,
C. Chem., M. R. S. C.

ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude and appreciation to my supervisor, Dr Julian F. Tyson, for his excellent contributions, conscientious assistance and advice during the course of the research and during the preparation of the manuscript of this thesis.

Thanks are due to laboratory technicians of the Chemistry Department, especially to Mr J. J. Swithinbank and Mr M. K. Patel who have provided technical assistance and advice.

The invaluable assistance from Ms Barbara A. Byrne for helping me preparing the proofs is appreciated, and the assistance of Mr John M. H. Appleton for reading the proofs is also acknowledged.

The friendly cooperation given by the other research workers in the laboratory is acknowledged.

I would also like to express my sincere gratitude and appreciation to the Public Services Department of Malaysia and to the Universiti Kebangsaan Malaysia for awarding me scholarship and study leave to undertake this research.

Finally to all my family whose constant encouragement has made this thesis possible.

Ahyar Bin Idris.

Loughborough, United Kingdom.
October, 1983.
## CONTENTS

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRONTISPIECE</td>
</tr>
<tr>
<td>DECLARATION OF ORIGINALITY</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
</tr>
<tr>
<td>ABSTRACT</td>
</tr>
</tbody>
</table>

### Chapter 1 - INTRODUCTION

1. Continuous Flow Analysis
   - 1.1. Air-segmented Flow Analysis
   - 1.2. Non-segmented Flow Analysis
   - 1.3. Flow Injection Analysis
     - 1.3.1. Basic Principles of FIA
     - 1.3.2. Controlled Dispersion
     - 1.3.3. Components of FIA System
     - 1.3.4. Techniques in FIA
     - 1.3.5. Applications
   - 1.4. Flow Injection Analysis with Atomic Absorption Spectrometry
     - 1.4.1. FIA-AAS Manifold
     - 1.4.2. FIA-AAS Applications

### REFERENCES

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>13</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>19</td>
</tr>
<tr>
<td>24</td>
</tr>
<tr>
<td>28</td>
</tr>
<tr>
<td>29</td>
</tr>
<tr>
<td>31</td>
</tr>
<tr>
<td>34</td>
</tr>
<tr>
<td>44</td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>51</td>
</tr>
<tr>
<td>51</td>
</tr>
</tbody>
</table>
2.2.2. Effect of Capillary Length at Mixer Block Assembly 52
2.2.3. Effect of Capillary Tubing 54
2.2.4. Linear Range 55
2.3. Flow Injection Analysis and Atomic Absorption Spectrometry 59
   2.3.1. Effect of Pumping Rate 59
   2.3.2. Effect of Tube Dimension 63
   2.3.3. Effect of Sample Volume 63
   2.3.4. Shapes of Calibration Curve 66
   2.3.5. Effect of Water and Air as Carrier Stream 69
2.4. Discussion and Conclusions 72

REFERENCES 76

Chapter 3 - SIMPLIFIED MODEL FOR DISPERSION IN FIA-AAS 78
3. Introduction 78
   3.1. Basic Model for Dispersion 84
      3.1.1. Exponential Gradient for a Single Mixing Chamber 84
      3.1.2. Exponential Gradient for Dual Mixing Chambers of Unequal Volume 87
   3.2. Apparatus, Standards and Reagents 91
   3.3. Measuring the Volume of Hypothetical Mixing Chamber 91
   3.4. Factors Affecting on the Volume of the Hypothetical Mixing Chamber 94
      3.4.1. Flow Rate 94
      3.4.2. Tube Length 95
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4.3. Volume of Injection</td>
<td>96</td>
</tr>
<tr>
<td>3.5. Use of Hypothetical Mixing Chamber for Prediction Purposes</td>
<td>98</td>
</tr>
<tr>
<td>3.6. Use of Hypothetical Mixing Chamber for Calibration Purposes</td>
<td>101</td>
</tr>
</tbody>
</table>

REFERENCES

Chapter 4 - APPLICATION OF A SIMPLIFIED MODEL FOR DISPERSION -
DETERMINATION OF CALCIUM IN IRON ORES

4. Introduction

4.1. Flow Injection Analogue of Standard Additions Method

4.2. Basic Model for Dispersion

4.3. Apparatus, Standards and Reagents

4.4. Interference Effects

4.4.1. Phosphate

4.4.2. Aluminium

4.5. Use of Releasing Agents

4.6. Procedure

4.6.1. Sample Dissolution

4.6.2. Measurements

4.7. Results and Discussion

4.8. Conclusions

REFERENCES

Chapter 5 - APPLICATION OF A SIMPLIFIED MODEL FOR DISPERSION -
DETERMINATION OF CHROMIUM IN STEELS

5. Introduction
5.1. Basic Model for Dispersion
   5.1.1. Reagent Additions Method
   5.1.2. Analogue of Standard Additions Method
5.2. Apparatus, Standards and Reagents
5.3. Procedure
   5.3.1. Dissolution Procedure for British Chemicals Standards Samples (BCS)
   5.3.2. Absorbance Measurements
   5.3.3. Calculations of Reagent for Standard Additions and Reagent Additions Methods
5.4. Results and Discussion
5.5. Conclusion

REFERENCES

Chapter 6 - METHODS OF CALIBRATION
6. Introduction
   6.1. Standard Working Curve Method
      6.1.1. Least-Squares Method
   6.2. Standard Additions Method
   6.3. Internal Standards Method
   6.4. Flow Injection Analogue of the Standard Additions Method
   6.5. Variable Dispersion Calibration Method
      6.5.1. Injection of Variable Volumes at Constant Concentration
      6.5.2. Injection of Constant Volume at Constant Concentration
      6.5.3. Concentration Gradient Formation


REFERENCES

Chapter 7 - FLOW INJECTION - SOLVENT EXTRACTION TECHNIQUE

7. A Brief Introduction

7.1. Description of the System

7.2. Apparatus, Standards and Reagents

7.3. Procedure

7.4. Results and Discussion

REFERENCES

CONCLUSIONS

SUGGESTIONS FOR FUTURE WORK
ABSTRACT

The various factors affecting the optimum performance of an atomic absorption spectrometer with flow injection techniques are described. These include the study of dispersion processes in the flowing stream by manipulation of the carrier tube dimensions (diameter and length), the flow rate, the volume of sample and physical properties of the solution such as viscosity, chemical nature and level of dissolved solids. Investigation of the peak shape produced by FIA-AAS techniques leads to a simple exponential model proposed to account for the absorbance-time behaviour. By determining the volume of the hypothetical mixing chamber, the model can be used for calibration purposes and prediction of some of the experimental parameters. The use of this model in calibration techniques is shown in the analogues of reagent addition methods and standard additions methods and in a gradient concentration method. The applicability of the model and associated calibration methods is described and discussed in detail for the determination of calcium in iron ore in which interference effects from aluminium and phosphate are encountered and are overcome by the use of releasing agents, and in the determination of chromic in steel where the major interferences are from iron and acids and are 'overcome' by addition of interferents. A brief description of solvent extraction with FIA-AAS is given. Various factors affecting the sensitivity of this technique are investigated for the system by using copper, ammonium pyrrolidine dithiocarbamate (APDC) and 4-methylpentan-2-one (MIBK). The potential use of this technique for separation methods and analysis is discussed.
Chapter 1

INTRODUCTION

1. Continuous Flow Analysis (1,2)

Continuous flow analysis (CFA) may be referred to as any process in which the concentrations of single or multiple analytes are measured, uninterrupted, in a moving stream of gases, liquids or solids. The sample is converted into a flowing stream by a pump system and the necessary reagent additions are made by continuous pumping and merging of the sample and reagent streams. Finally, the treated sample is pumped to a flow-through detector and thence to waste.

A discrete sampling method, each sample is maintained as a separate entity and placed in a separate receptacle. Dilution, reagent additions and mixing are performed separately by mechanically transporting the sample to dispensing units where controlled additions are made to each sample individually, and each treated sample is presented in turn to the detection unit.

Although the discrete method has the advantage in high sampling rate and cross-contamination is almost entirely eliminated, the discrete analysers are appreciably more expensive than the continuous analysers, and the continuous analysers are mechanically simpler than the discrete ones. This is because in
the discrete method each analytical operation requires a separate group of moving parts whereas in continuous flow method, only a few moving parts are required to control the entire sequence of operation. For example, in CFA, only one suitable peristaltic pump is required for sample transport and reagent addition.

The concept of CFA has been used extensively in clinical chemistry, chromatographic separations and manufacturing plant process monitoring systems. CFA may be classified mainly into three categories, namely, air–segmented flow analysis, non–segmented flow analysis and flow injection analysis.

1.1 Air–segmented Flow Analysis (1,2)

Based on the concept of steady-state signal and air segmentation, introduced by Skaggs, an automatic modular instrument known as the AutoAnalyzer was developed in 1957. Its widespread use in analytical chemistry has led to the introduction of additional modules to extend its range and flexibility, and more specific configurations have also been developed for specific analyses such as in biomedical analysis and process control analysis. The AutoAnalyzer has been developed and expanded from a single-channel analyser in 1957 to the sequential multiple analyser (SMA) with 12 sample channels. This was introduced in 1967. The SMA was replaced later in 1974 with the new computer-controlled sequential multiple analyser (SMAC) with 20 channels which is capable of 40 simultaneous assays at a rate of 200 samples per hour (3).
Basically, the AutoAnalyser modules consists of the following: sampler, proportioning peristaltic pump, dialyser, heating unit and detection system. A schematic diagram of a single-channel AutoAnalyser is shown in Figure 1.1. In the sampling process, samples are removed automatically and successively from the container by means of a probe and are mixed with diluent and reagents, and are separated by air bubbles. The proportioning pump propels the appropriate sample and reagents volume into the analyser and transport the solutions and air bubbles through the system at a fixed speed. Separation of the species of interest from unwanted material is performed by diffusion through a semi-permeable membrane. In the dialyser unit the segmented sample and reagent streams flow concurrently through two specially-grooved plates separated by the membrane materials. A fixed period heating unit is provided in the analyser consisting of a glass mixing coil immersed in a temperature-controlled bath. The detection system may comprise a colorimeter, flame photometer, fluorimeter or uv spectrophotometer or other types of detector.

The AutoAnalyser is designed so that successive samples which pass through the system are isolated from one another by the introduction of air bubbles into the tubing to avoid cross-contamination as well as to promote mixing between sample, diluent and reagents. The movement of sample, reagents and diluent through the tubing is accomplished by means of a peristaltic pump. The diluted sample then passes into the dialyser which contains membranes through which the small analyte ions or molecules are free to diffuse into the
reagent stream. The residual molecules remain in the diluent system and pass from the system to waste. The analyte and the reagent are pumped into the heating unit where reaction occurs in the constant temperature heating bath. Air bubbles are removed to waste from the final solution before it flows to the detector where a signal is monitored and measured.

Figure 1.1. A schematic diagram of a single-channel AutoAnalyzer.

Another important function of air segmentation is also to reduce longitudinal dispersion of sample along the flow line, which in turn decreases sample interaction (carryover) and increases analysis rate. Sample dispersion in the flow of air-segmented liquid stream through open tubing for ideal and non-ideal models has been described by Snyder and Adler (4,5). The models of dispersion developed allow the prediction of sample dispersion as a function of all important experimental parameters such as
tube length, liquid viscosity, surface tension, liquid and air segments volume and liquid velocity.

1.2. Non-segmented Flow Analysis

The use of CFA without air segmentation or similar method has probably been introduced since the end of 19th century by Stewart (6) in measuring the mean speed of flow in small blood vessels. Danckwerts (7) in his theoretical investigation of the distribution of residence times in continuous flow systems and in trying to explain how distribution functions for residence times can be defined and measured for actual system of open and packed tubes, has brought up the idea that a radioactive tracer could be injected into the flowing stream of liquid and the concentration of the injected material in the exit stream could be measured at variable time. Taylor (8) injected a conducting potassium permanganate solution by means of a needle valve and a stop-watch to measure the volume of the solution, into the tube through which water was flowing. At a fixed point the conductivity was measured and the conductivity-time curve was plotted. The same method has also been applied in the investigation of matter in turbulent flow where brine was injected into a straight pipe containing water and the conductivity was recorded at a fixed point downstream (9).

A non-segmented flow has also been used by Levenspiel and Smith (10) in their study of diffusion model for the longitudinal mixing
of fluids in flow. A volume of potassium permanganate solution was injected into the flowing water in the pyrex tube and the concentration of potassium permanganate in water was measured downstream by an emission type photoelectric cell. Bate et al. (11) have filled the tube with labelled iodide ions and then displaced it by the steady flow of unlabelled fluid from a reservoir in their study of dispersion of diffusible ions in fluid flow through a cylindrical tube. Nagy, Feher and Pungor (12) have injected a small volume of active solution into the stream of supporting electrolyte by means of a syringe through a rubber tube inserted into the system. A hydrostatic method was employed to assist the flow of supporting electrolyte and a magnetic stirrer was placed between the injection point and the measuring cell to ensure the reproducibility of the measurement.

Stewart et al. (13) have described the prototype instrument of non-segmented CFA, its principle of operation and its application. The instrument consists of two separate flow systems: the sample flow system which carries the sample solution and the wash solution, and the reaction flow system in which the sample is analysed. These two flow systems are connected by a stream sampling valve, which removes a fixed portion of the sample without air contamination from the sample flow system and introduces it into the reaction flow system for subsequent analysis. These flow systems are shown schematically in Figure 1.2. The sample flow system consists of a sampler connected to the stream sampling valve and a peristaltic withdrawal pump. The reaction flow system
consists of a pressurized substrate reservoir connected to a line filter, a flowmeter, the stream sampling valve, a reaction coil, a cooling coil, a colorimeter and a waste tank.

The sample solution is withdrawn by suction from a sample cup alternately with the wash solution. A stream sampling valve timed to the sampler is used to remove a portion of the sample from the sample stream and injects it into the reaction flow system.

![Diagram](Figure 1.2. A schematic diagram of continuous flow analyser by Stewart et al (13).)

This avoids the introduction of air bubbles in the reaction flow system which is introduced into the sample stream when the probe moves from the sample cup to the wash cup and from the wash cup to the sample cup. The portion of sample flows into the reagent stream and reacts with it in the reaction coil, cooled and detected by the colorimeter before flowing to waste.
1.3. Flow Injection Analysis

Flow injection analysis (FIA) has been introduced by Ruzicka and Hansen (14) in 1975 as a new analytical technique for the analysis of samples in a non-segmented flowing stream. The similarity of this technique with non-segmented flow analysis can be found in the literatures long before this as has been described above. Although the basic concept of FIA may be similar to the work of previous workers on continuous flow analysis and possibly to the concept of liquid chromatography, the precise requirements for FIA such as unsegmented stream, reproducible sample injection and controlled dispersion differentiate the FIA concept from the concepts of HPLC and air-segmented continuous flow analysis.

The difference between air-segmented CFA and FIA is that, in air-segmented CFA, the sample is usually added in alternation with wash solution to a reagent line, usually as a broad slug; the combined stream after sample introduction is then segmented with air bubbles and the segmented stream flows through the incubation coil and into the detector. The final output sensed by the detector and each sample curve shows a plateau and the height of this plateau is proportional to the analyte concentration. Although the use of air bubbles decreases the intermixing of the samples in the flow system, and allows greater throughput rates, it adds a further level of complexity of the system in terms of equipment, operator interaction with the system and hydraulic
performance. The approach to be selected depends on the throughput rate required, the degree of dispersion associated with a given assay and the importance of the system simplicity. In FIA, the sample is injected as a plug into the reagent stream and flows through the coil and into the detector. A series of peaks are obtained with peak height being proportional to the analyte concentration. FIA has the purpose of analysing the maximum number of discrete samples using the minimum amount of time, reagent and sample solution when the sample zone dispersion is controlled exactly to suit the detection method and chemistry associated with it. A comparison of the basic theory of sample dispersion in CFA and FIA has been given by Snyder (15).

Although basically, the FIA system may look similar to the high-pressure liquid chromatography (HPLC) system, they are different techniques altogether because their principles and applications are not the same. HPLC involves separation of sample components based on their distribution between two immiscible phases with minimum band broadening in order to obtain adequate resolution of several components.

These three techniques, namely, air-segmented CFA, HPLC and FIA, are shown schematically in Figure 1.3. for comparison of their principles. The most important common concept for all three techniques is that the sample is treated in a closed, continuous system. The methods of transportation for liquids in the system for these techniques are also very similar.
Figure 1.3. Schematic depiction of principles for (a) liquid chromatography, (b) air-segmented continuous flow analysis, (c) flow injection analysis.
Since its introduction, FIA has been developed extensively and expanded very rapidly. At present, there have been at least six reviews and reports (16,17, 18, 19, 20, 21) on this subject and it has also appeared in textbooks (22,23). The increasing interest in FIA has been shown by the two International Conferences on Flow Analysis in Amsterdam (24) in 1979 and in Lund, Sweden (25) in 1982. Recently, fourteen papers on FIA were presented at SAC 83 - an International Conference on Analytical Chemistry at Edinburgh University (26).

1.3.1. Basic Principles of FIA (27,28,29)

A simple flow injection setup may comprise a peristaltic pump to propel a carrier or a reagent at a constant rate, an injection valve, a mixing coil and a detector. This is shown in Figure 1.4.

![Diagram of FIA system](image)

**Figure 1.4. A simple FIA system.**

The sample is introduced through the injection valve as a plug in a carrier or a reagent stream, it reacts with the reagent to
form a compound which can be detected as it passes through the
detector. The sample and the reagent disperse within
each other and this process influences peak height and also the
overall change in peak shape. Dispersion, $D$, has been defined
as $D = \frac{C_m^s}{C_p^s}$, where $C_m^s$ is the original concentration of the
sample and $C_p^s$ is the concentration at peak maximum at the time
it is detected. Thus, the value of $D$ reflects how many times
the sample has been diluted during its transport from the injection
point to the detector. The peak obtained at a steady state value
when the sample is pumped into the stream, $H_m^s$, is assumed to
be proportional to original concentration of the sample, $C_m^s$, and the peak height, $H_p^s$, obtained when a volume of sample is
injected into the carrier stream (e.g. water) is proportional
to $C_p^s$. Thus, the dispersion, $D$, is the ratio of the two peaks,
i.e., $D = \frac{H_m^s}{H_p^s}$. The dispersion can be controlled and the
manifold of an FIA system can be designed to obtain a suitable
dispersion for a specific analytical application.

In FIA, the residence time is always constant because the carrier
or reagent is free from air bubbles and the flow is constant.

During the residence period in the system, the analyte in the
sample is supposed to react with a reagent so that a detectable
compound is formed. Since the residence time is constant, it is
not necessary that this reaction reaches completion. The yield of
the reaction can be improved either by increasing the residence
time or by increasing the temperature. The residence time can
be increased either by using a longer mixing coil so that the distance between injection site and the detector is increased or by decreasing the flow rate or even stopping the flow.

1.3.2. Controlled Dispersion

If a sample is injected into the reagent stream at dispersion of 2, equal dilution of the reagent and the sample is obtained. The dispersion value can be obtained by varying the sample volume, the coil length, the flow rate of the reagent and the geometry of the system. The residence time of the sample in the system must be long enough so that the chemical reaction can be allowed to take place.

A defined dispersion can be obtained by using two streams in an FIA system - one carrier stream and one reagent stream. The injection valve is situated in the carrier stream and if the flow rates are equal, a dispersion value of 2 is obtained at the merging point.

If a sample plug is introduced in a tube through the injection valve, it will be subjected to change due to the injection itself as the plug is accelerated from zero velocity up to the flow rate of the propelling stream almost instantaneously. The injection valve and the flow rate cause the change of the concentration profile of the sample plug. In the stream, the sample portion is extended by the convection process. The elements of fluid
situated in the centre move faster than those situated much closer to the walls, resulting in a parabolic flow profile producing axial dispersion and peak broadening.

The flow can be characterized in terms of the Reynolds number, either laminar or turbulent (30). The Reynolds number, \( \text{Re} \), is given by

\[
\text{Re} = \frac{4\rho Q}{\pi \mu d^3}
\]

where \( \rho \) is the density of the fluid in \( \text{g/cm}^3 \), \( Q \) is the fluid flow rate in \( \text{cm}^3/\text{s} \), \( d \) is the diameter of the tube in cm, and \( \mu \) is the fluid viscosity in poise. For water or diluted solutions, \( \text{Re} = 127 Q / d \). An unsegmented flow such as FIA is considered to be laminar when \( \text{Re} \leq 2000 \) and turbulent when \( \text{Re} \geq 3000 \), with a transition region when \( \text{Re} \) is between 2000 and 3000. Tube curvature and tube imperfections can contribute to transition at lower \( \text{Re} \).

Dispersion of the sample zone can be varied by changing the inner diameter of the tube, the length of the tube, the pumping rate of the carrier and the sample volume injected. An effective way to decrease the dispersion is to use small-bore tubes but there are limitations such as particles are easily stuck in very narrow tubes and the hydrodynamic pressure increases so that peristaltic pump cannot be used. The dispersion increases with the square root of the tube length, thus, lengthening the reaction coil is an inefficient way of promoting mixing in FIA system. The pumping rate influences the dispersion only slightly for a given setup and an increase of the pumping rate leads to increased dispersion. An efficient way of changing the dispersion is to change the
sample volume.

If the viscosity of the sample solution differs significantly from that of the reagent solutions the dispersion process may be seriously affected and erroneous results will be obtained. This happens especially when the matrix of the sample and the reagent solution are not the same. One way of avoiding this effect is to use the matrix itself as a carrier solution.

Another simple and efficient way of increasing the dispersion is by merging diluent streams with the stream carrying the sample.

A more detailed discussion on the dispersion will be presented in Chapter 3 of this thesis.

1.3.3. Components of FIA System

a) The delivery system - The most frequently used device is a multichannel peristaltic pump. By using these pumps the delivery rate can often be varied easily. The disadvantages are that it is not completely pulse free and that the pump tubes deteriorate so that the flow rate changes. The peristaltic pump cannot be used when the hydrodynamic resistance of the system is large due to the presence of narrow tubes or closed-packed reaction columns in the manifold.

Although most FIA methods now consume less than 2 ml/min of
carrier or reagent, FIA methods with atomic absorption spectrometry (AAS) requires at least a total of 4 ml min⁻¹ in order to obtain optimum performance of the nebulizer.

b) The injection system - A simple injection technique, namely, direct penetration of a rubber tube with a syringe needle followed by manual emptying of the syringe has been described by Ruzicka and Hansen (14). This approach was later refined, leading to the construction of a flap valve (16). Stewart et. al (13) used a slider valve of the type usually employed for liquid chromatography. Some of these valves are usually not provided with a bypass, which means that the carrier stream is obstructed for a short period during injection. Another valve is a rotary valve which has been described by Ruzicka and Hansen (28). This valve is equipped with a bypass coil so that the carrier stream flows continuously via the bypass to the manifold while the sample solution is filled into the sample loop. When the sample is injected into the carrier it is carried to the manifold.

c) The manifold - The manifold is usually constructed from polytetrafluoroethylene (PTFE) or polypropylene tubes which are flanged so that standard connectors can be used. The inner diameters are about 0.4 - 1.0 mm in most cases. Confluence and divergence of streams can be effected by using T connectors.

d) The detector system - The detector system may comprise any sensor suitable for use in a flowing stream. The most common
detection system used has been spectrophotometry.

The simplest FIA manifold has been shown in Figure 1.4 with a single-line system. This manifold is normally used for simple and fast determination where no reagent or only one reagent is used in the system. It can be used as a sample introduction method in AAS by injecting a small volume of sample into the carrier stream of water and the sample is propelled by the pump to the nebulizer and the flame. If a reaction is required in the manifold, the reagent or the sample can be used as a carrier stream and either one of them is then injected into the stream.

If more than one reagents or reactions are required in the system, a more complex manifold has to be designed. As an example, in the determination of phosphate, two reagents are needed and thus, two lines are constructed for the system. The molybdate reagent is first mixed with the ascorbic acid reagent, and then, when the sample is injected, it reacts in the mixing coil and the detection is made after a few seconds. The manifold for phosphate determination is shown in Figure 1.5.

Manifolds for stopped-flow, intermittent pumping, merging zones and solvent extraction techniques are shown schematically in Figure 1.6, 1.7, 1.8, and 1.9 respectively. The first three techniques will be described later in the next section of this chapter, and the FIA with solvent extraction technique by using AAS as detection system will be discussed later in Chapter 7.
Figure 1.5. FIA manifold for determination of phosphate.

Figure 1.6. FIA manifold for stopped-flow determination of SO$_2$ in wine.

Figure 1.7. FIA manifold for intermittent pumping.
1.3.4. Techniques in FIA

a) Stopped-flow - The idea of stopping a stream for a fixed period of time is to increase the residence time of the sample in the system so that the sensitivity of the measurement is increased, and to save the use of the reagent. The stop can be made as soon as the sample and the reagent solutions have been mixed to a sufficient extent but at a fixed time after the injection. When the carrier stream ceases to move, the dispersion of the sample zone will stop (except for a negligible contribution from molecular diffusion) and dispersion will become independent of time. Thus by an appropriate choice of stop period the reaction time can be gained when the carrier stream does not move. After the stop period, the carrier stream is restarted and the sample is flushed out so that the peak can be recorded (see Figure 1.6).

The use of this technique has been described in some of the FIA literature (e.g. see references 103 and 105).

b) Intermittent pumping - It is used in order to enhance the sample throughput by increasing the washout speed from the coils and from the flow cell and to save reagent. The manifold consists of two pumps which can operate independently (see Figure 1.7). One pump is used to propel the carrier stream of the reagent and the second pump is used to flush the system with wash solution (water).
Merging zones principle - In this principle the sample is injected and the reagents are introduced in such a way that the sample zone meets the selected section of the reagent stream in a controlled manner, while the rest of the system is filled with wash solution. By using this principle, the consumption of reagent is greatly reduced. This principle can be achieved in two different ways: by intermittent pumping and by use of a multiple injection valve. In the system with intermittent pumping, the sample zone is injected into a flowing inert carrier solution while the pump controlling the reagent solution is stopped. When the sample zone has reached the merging point, the pump controlling the reagent is activated, delivering the reagent for a preset period of time, whereupon it is stopped again. In the second way, equal sample and reagent volumes are injected into identical flow rates of carrier streams, merge at the merging point after passing through equal lengths of tubing, and continue downstream while being mixed and dispersed into the stream. Figure 1.8a and 1.8b show the manifold of merging zones principle by intermittent pumping and by use of a multiple injection valve respectively. The use of this approach can be found in references (83, 104, 106, 107, and 108).

d) Zone-sampling process - This process utilizes the introduction of well-defined aliquots of a processed zone into a second carrier stream. The flow system composed of at least two almost independent parts, each of which behaves as a single flow injection system (Fig. 1.10). The sample is injected into the first carrier stream and, after a
Figure 1.8a. FIA manifold for merging zones system based on intermittent pumping.

Figure 1.8b. FIA manifold for the synchronous merging of two zones in a symmetrical system.
Figure 1.9. FIA manifold for solvent extraction.

Figure 1.10. Schematic representation of the zone-sampling process.
time interval an aliquot of the dispersed zone is taken and introduced into the second carrier stream. A second zone is established, processed and measured in a flow-through detector before going to waste. The selection of the portion of the aliquot of dispersed zone depends on the time interval which in turn depends on the pumping rate and the size of the manifold.

This process provides an efficient way of achieving a high degree of dispersion than the use of very small injected volumes, long coils or more complex systems. This approach is also particularly useful when the required sample dispersions are very different for two simultaneous different determinations. By employing suitable values of time interval, a calibration graph with only one standard can be obtained because each portion of the zone corresponds to a different concentration. A detailed study of the boundary of the sample zone and the carrier stream can be performed when a small fraction of the sample zone is injected into the second carrier stream.

The use of zone-sampling approach has been demonstrated in the simultaneous determination of aluminium and iron (107), and potassium (110) in plant digests.
1.3.5. Applications

The applications of FIA principles in analytical methods are mostly based on the types of samples to be analysed, the manifold design and the detection system. The development of the FIA methods depend on whether any reaction or reactions will take place or whether any other analytical techniques such as separation or solvent extraction involved. The detector to be used with FIA system depends on how the analyte or species to be determined is monitored. Some of the most important detection techniques in FIA are spectrophotometry, fluorescence, chemiluminescence, atomic absorption, flame photometry, potentiometry with ion-selective electrodes and voltammetry.

Some of the examples of the applications of FIA are summarised in Table 1.1 based on detection techniques and species determined.

Table 1.1. Species determined by FIA.

<table>
<thead>
<tr>
<th>Detection Techniques</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometry</td>
<td>Acids</td>
<td>136, 140</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>113, 129</td>
</tr>
<tr>
<td></td>
<td>Aluminium</td>
<td>54, 106, 107, 157</td>
</tr>
<tr>
<td></td>
<td>Amino acids</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Ammonia-ammonium</td>
<td>52, 137</td>
</tr>
<tr>
<td></td>
<td>Aromatic sulphonyl</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>Halocaines</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.1 (continued)

<table>
<thead>
<tr>
<th>Detection Techniques</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometry</td>
<td>Boron</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Calcium</td>
<td>66, 108, 133, 134, 140,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>144, 145, 158</td>
</tr>
<tr>
<td></td>
<td>Carbon dioxide</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>Chloride-chlorine</td>
<td>43, 45, 57, 59, 131, 136,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Chromium</td>
<td>49, 50, 140</td>
</tr>
<tr>
<td></td>
<td>Cobalt</td>
<td>42, 44</td>
</tr>
<tr>
<td></td>
<td>Copper</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Corticosteroids</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>105, 119, 124, 143</td>
</tr>
<tr>
<td></td>
<td>Glycerol</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>54, 107</td>
</tr>
<tr>
<td></td>
<td>Isoprenaline</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Ketones</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Lead</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>Magnesium</td>
<td>48, 132, 133, 134, 144</td>
</tr>
<tr>
<td></td>
<td>Manganese</td>
<td>46, 51</td>
</tr>
<tr>
<td></td>
<td>Molybdenum</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td>Nickel</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Nitrate-nitrite</td>
<td>31, 32, 38, 109, 112, 115, 131</td>
</tr>
<tr>
<td></td>
<td>Nitrogen</td>
<td>56, 58, 61, 135, 158</td>
</tr>
<tr>
<td></td>
<td>Oxygen</td>
<td>39, 141</td>
</tr>
</tbody>
</table>
### Table 1.1 (continued)

<table>
<thead>
<tr>
<th>Detection Techniques</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectrophotometry</td>
<td>Phosphate-phosphorus</td>
<td>37, 53, 55, 58, 59, 61, 104, 126, 136, 145, 158</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Strontium</td>
<td>132, 134</td>
</tr>
<tr>
<td></td>
<td>Sulphate</td>
<td>102, 111, 130, 131, 145, 147, 156, 160</td>
</tr>
<tr>
<td></td>
<td>Terbutaline sulphate</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Uranium</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Vanadium</td>
<td>36, 101</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Acids</td>
<td>69, 127, 128</td>
</tr>
<tr>
<td></td>
<td>Amines and amino acids</td>
<td>72, 76</td>
</tr>
<tr>
<td></td>
<td>Arsenic</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Ascorbic acid</td>
<td>64, 65</td>
</tr>
<tr>
<td></td>
<td>Cadmium</td>
<td>71, 74, 80, 82, 84</td>
</tr>
<tr>
<td></td>
<td>Calcium</td>
<td>66, 69</td>
</tr>
<tr>
<td></td>
<td>Chloride-chlorine</td>
<td>73, 77</td>
</tr>
<tr>
<td></td>
<td>Copper</td>
<td>71, 74, 80, 82</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Fluoride</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Hydrogen peroxide</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>Iodide</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>Iron</td>
<td>67</td>
</tr>
<tr>
<td>Electrochemical</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Detection Techniques</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemical</td>
<td>Lead</td>
<td>71, 78</td>
</tr>
<tr>
<td></td>
<td>Nitrate-nitrite</td>
<td>62, 63, 168</td>
</tr>
<tr>
<td></td>
<td>Nitrogen</td>
<td>56, 61</td>
</tr>
<tr>
<td></td>
<td>Nitrophenol and nitrobenzene</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Organic compounds</td>
<td>78, 154</td>
</tr>
<tr>
<td></td>
<td>Penicilloic acid</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Potassium</td>
<td>61, 62, 69, 77</td>
</tr>
<tr>
<td></td>
<td>Sodium</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Sulphite</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>71, 82</td>
</tr>
<tr>
<td>Fluorimetry</td>
<td>Albumin</td>
<td>103, 117, 139</td>
</tr>
<tr>
<td></td>
<td>Drug-protein binding</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td>Gallium</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>117, 139</td>
</tr>
<tr>
<td></td>
<td>Lactate</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>Quinine sulphate</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
<td>123, 124</td>
</tr>
<tr>
<td>Chemiluminescence</td>
<td>Cadmium</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>Cobalt</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>Fluorescein</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>Sulphide</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>Triethylamine</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>Zinc</td>
<td>122</td>
</tr>
</tbody>
</table>
1.4. Flow Injection Analysis with Atomic Absorption Spectrometry

There have been some papers published on flow injection analysis methods with atomic absorption spectrometry (FIA-AAS) as a detection system. Flow injection methods are much more recognized as a discrete method of sample introduction system to the atomic absorption instrument, especially when a very limited dispersion is required. There are several advantages in using FIA-AAS methods. When samples are in high viscosity or high solids content, a small volume of sample can be injected into the carrier stream and it will be diluted by the carrier before it reaches the nebulizer and the flame. When reaction or reactions are required in the system, such as in the use of releasing agents, or separation of the analyte from other materials or complexation in solvent extraction, a variable degree of dispersion can be selected for the particular analysis.

A high dispersion is required in the FIA-AAS system when it is to be used for calibration methods (see Chapter 6). In order to construct a particular calibration graph a wide range of dispersion values is required. If the calibration graph to be constructed is based on the formation of a concentration gradient of the analyte along the stream, a high dispersion of the analyte in the system is needed. If the calibration graph to be constructed is based on the volume injected, any suitable dispersion value in the system can be selected. When the calibration graph is plotted, i.e., absorbance against time or absorbance against concentration, the linear relationship between absorbance and concentration is
Dispersion in FIA-AAS can be controlled in the same way as in other FIA systems. This includes controlling of the tube dimensions, i.e., tube length and internal tube diameter, volume injected, flow rate and the use of a real mixing chamber between the injection port and the detector. While other factors mentioned above can be used easily to control the dispersion of the sample, the flow rate cannot be used to vary the dispersion of the sample to suit the requirements without affecting the optimum performance of the atomic absorption instrument. Furthermore, the variation of the flow rate does not change the dispersion of the sample very much, thus, it may not be able to give the correct dilution or dispersion of the sample for a specific determination, especially when high dispersion becomes necessary. A real mixing chamber which can be located between the injection point and the nebulizer, can be used when high dispersion or high dilution of sample is required in the system.

1.4.1. FIA-AAS Manifold

A simple FIA-AAS system may consist of a single-line manifold as shown in Figure 1.11. The system may comprise a water carrier stream or a reagent in the polytetrafluoroethylene (PTFE) tubing with a suitable internal diameter, a pump to propel the carrier, a sample injection valve, and a dispersion coil connected to the nebulizer of the instrument. This setup is normally used for very simple and very rapid determinations where no or only one reagent
is used. A more complex of FIA-AAS manifold has to be designed for the determinations that need more than one reagent or that need other analytical techniques such as solvent extraction (see Chapter 7) to be linked with it.

An FIA-AAS manifold used with merging zones principle has been described by Zagatto et. al (83) in the determination of calcium, magnesium and potassium in plant material. The sample and the reagent were injected into water carrier stream and the corresponding sample and reagent zones merged at the confluence point and were directed to a dispersion coil before they reached the flame.

![Diagram of FIA-AAS system](image)

Figure 1.1. Simple system for FIA-AAS

Yozu et. al (84) have described a manifold for FIA-AAS by use of air-compensation method for the introduction of the carrier stream into the nebulizer. By using a three-way connector, the carrier is aspirated through one branch into the instrument and at the same time another carrier is flowing into the nebulizer from the FIA manifold. Wolf and Stewart (85) have used the flow injection
principle for the automation of sample introduction in flame AAS. Discrete micro-volumes of liquid sample were inserted into a flowing solvent stream which was pumped directly into the nebulizer. Fukamachi and Ishibashi (86) have connected the teflon tubing from the sample injector to the nebulizer without the use of peristaltic pump to propel the carrier. The carrier flow rate depended on the aspiration rate of the nebulizer which was controlled by adjustment of the valve regulating the air flow to the nebulizer.

The use of FIA-AAS techniques as a sample pretreatment system where liquid samples can be processed automatically in order to bring the concentration of the analyte and the interfering substances to the most suitable level for analysis has been described by Mindel and Karlberg (87). Several lines were added in the manifold so that various steps of water streams or diluents can be added into the sample. Layman, Crock and Lichte (88) have described the use of multichannel peristaltic pump with a pneumatic nebulizer for AAS and emission spectrometry. The pump simultaneously delivered the sample, releasing agent and diluent to the nebulizer and thus, reducing sample preparation time.

1.4.2. FIA-AAS Applications

Most of the FIA-AAS applications described in the literature are based on a single-line manifold. A discrete volume of sample is injected into the carrier stream, usually water, propelled by the peristaltic pump and flows through a length of tube to the nebulizer. The more complicated manifolds are used when other
FIA techniques or analytical techniques are to be applied. A very useful review on the applications of FIA-AAS based on these techniques and the manifold designs has been given by Tyson (89).

Some examples of the applications of FIA-AAS techniques are presented here in order to show the versatility and flexibility of the techniques in solving analytical problems.

An automated procedure for simultaneous determination of sodium, potassium, magnesium and calcium in surface, ground and domestic water has been described by Basson and Van Staden (90). Organic solvents have been used by Fukamachi and Ishibashi (86) as carrier streams in the determination of trace elements. The use of FIA-AAS techniques for studying metal-ligand binding in clinical samples has also been described by Renee et. al (91), where FIA was used as an interface between HPLC and AAS. Astrom (92) has determined bismuth by using FIA-AAS methods with hydride generation technique, whereas Rocks et. al (93) have described the method for direct determination of lithium in serum.

FIA-AAS methods with solvent extraction technique have been described by Nord and Karlberg (94), where a flow injection manifold for automatic extraction of metal ions in aqueous samples into methyl-isobutylketone (MIBK) with ammonium pyrrolidinedithiocarbamate (APDC) as an extracting agent was constructed. The organic extract was led into the loop of an injector situated in an integrated feed system of an AAS. Olsen et. al (95) have developed a technique
for screening of a large number of sea water samples while determining their lead, cadmium, zinc and copper contents. An on-line preconcentration method has also been developed by using a microcolumn of chelex-100 resin (95).

Jacintho et. al (96) have proposed the use of flow injection system with inductively-coupled argon plasma atomic emission spectrometry. The use of inductively-coupled plasma - atomic emission spectrometry (ICP-AES) with FIA has also been described by Greenfield (97, 98) and a method for an introduction system for liquid microsamples in ICP-AES has been described by Alexander et. al (99).

A generalized standard additions method has been used with ICP-AES to overcome matrix and spectral interferences with merging zones approach to demonstrate the method for analyses of nickel, copper and zinc in alloys (100).
REFERENCES


2. Methods of Sample Introduction

Methods of sample introduction into flame atomic absorption instruments have become among the most important factors in the development of flame atomic absorption spectrometry. Conventional methods of nebulization seem to have many disadvantages. The lack of speed and uneconomical use of sample in conventional nebulization methods have led to a search for alternative methods. At present most of the methods of sample introduction into the flame are based on discrete nebulization, microsampling-cup and continuous nebulization. With the exception of continuous nebulization, the major advantages of these techniques are that they are very economical in sample consumption which is normally less than 100 μl of sample volume, comparable in sensitivity and precision with conventional nebulization, simple and rapid in analysis.

The discrete nebulization technique has been used by Thompson and Godden (1) in order to reduce the possibility of blockage of the nebulizer system and the burner slot in the determination of aluminium, arsenic and tin in a very high content of iron. The need for separation of the analyte from the matrix was avoided by pipetting about 200 μl of sample into a 20 ml disposable polystyrene beaker prior to nebulization into the flame.
This technique has also been used successfully for the analysis of solutions with a high dissolved solids content (2). A propylene disposable pipette tip, which had a volume of 200 μl was inserted into the end of the silicone rubber nebulizer uptake tube. By inserting the end of the pipette tip below the surface of the sample solution and removing it immediately as the sample reached the start of the silicone rubber uptake tube, it was possible to nebulize discrete aliquots of approximately 200 μl. The similarity of this technique with the one that has been developed by Fry et al (3) is obvious. Instead of a pipette tip, Fry et al have used a vertically orientated microsampling cone (apex downwards) which was connected to the nebulizer via the small bore plastic nebulizer tubing. A sample volume of up to 10 μl could be delivered into the cone and nebulized into the flame without any problems from high salt or solids content or high viscosity. Another microsampling cone has also been developed for smaller sample size and for ease in microstandard additions and microdilution. Tsalev and Petrov (4) have introduced chlorinated solvents and other organic extractants into the flame by injecting a sample volume between 10 and 50 μl via a teflon sampling cup which was attached to the end of the nebulizer capillary tubing. This method could overcome the unstable and noisy flame and also could reduce the toxic products released, such as hydrogen chloride and phosgene, as a result of introducing the organic solvents.

A specially designed sampling cup has been developed for the delivery between 50 and 200 μl sample solution into the flame (5). The cup has four shallow holes of 200 μl volume, made by
drilling the plane surface of a teflon rod. The droplet of the sample solution placed in a hole with a micropipette was completely aspirated through a capillary tube. It was claimed that in practice, this method was superior to electrothermal atomization in reproducibility, ease of use and rapidity of measurement, although the smaller the sample volume introduced into the flame, the worse the shape of the calibration graph observed. Uchida et al (6) have also developed a small teflon funnel coupled directly to the nebulizer capillary for the delivery of a small sample volume. By injecting about 60 μl of sample, the steady state signal was achieved similar to that obtained by conventional nebulization.

A system for introducing the solid sample directly into the flame without the requirement of prior dissolution has also been developed (7). The sample chamber which was built specifically for the nebulization of powder sample was connected to the nebulizer of the instrument by polythene tubing. The sample powder was agitated with a solvent by vibration and a low oxidant flow rate was adequate to carry the material to the flame. Although the disadvantage of the discrete nebulization technique is that the flow rate of the sample solution depends on the oxidant flow rate and the reduction in the oxidant flow rate would disturb the optimum fuel-oxidant ratio and thus, reduces the signal, it has been used widely in microdeterminations.

A comprehensive review on discrete sample nebulization in atomic spectroscopy has been presented by Cresser (8).
The introduction of a microsampling cup method by Delves (9) in his determination of lead in blood by flame AAS is another new development of sample introduction system into flame atomic absorption. Since the introduction of the injection method (10, 11) as a way of introducing small sample into the flame, the microsampling cup technique has been developed to achieve the same aim. A sample volume of up to 5 µl has been used successfully in the microsampling cup technique for the determination of elements which were easily volatized and atomized (12). Methods of increasing the volatilization and atomization rates of sample and reducing the possible interference effects of the matrix have also been studied (13). The performance of the microsampling cup technique has been evaluated by using different types of microsampling cup and different types of flame.

The microsampling cup technique has been used successfully in the determination of cadmium (14) and lead (15) in biological material which has required minimal contamination, sample pretreatment, speed of analysis and simplicity. The sample which was homogenized with deionized water was pipetted into nickel microsampling cups, dried at 105°C and introduced into the flame directly without any reagents added. Automation of the microsampling cup technique has been designed to eliminate errors associated with manual injection and to reduce the overall time of analysis without loss of precision (16, 17). The disadvantages of dry ashing in electrothermal atomization and wet oxidation in conventional flame AAS, namely the time involved and the risk of contamination have been completely overcome by this technique.
Although the use of this technique has been limited to the determination of more volatile metals, its advantages of giving higher sensitivity than conventional nebulization, small sample size, no reagents required, simplicity, rapidity and providing a more representative sample has made it very convenient to use with flame AAS (18).

Gomisek and Span (19) have used the technique of continuous nebulization in introducing metal chelates in chlorinated organic solvents into the flame. A large glass vessel was connected to the nebulizer capillary and the solution in the vessel was forced continuously into the nebulizer by pressurising the air above the liquid surface. The toxic products released as a result of introducing the chlorinated organic solvents into the flame and the low absorption signal caused by the chlorinated hydrocarbons have made this technique inconvenient to use with flame AAS.

The advent of FIA which was introduced by Ruzicka and Hansen (20) has widened the scope of methods of introducing samples into the flame. Its capabilities as a sample introduction system to many instruments has made it a very important tool for analytical chemistry at present. This technique could well supersede other techniques of sample introduction into flame AAS because of its versatility and flexibility. The tubing which is connected between the sample injection valve and the nebulizer makes it very efficient in introducing sample solution either aqueous or
organic. Continuous nebulization of distilled water as a carrier stream makes the cleaning and washing of the nebulizer from the residue of the previous solution possible. With the injection valve located very close to the nebulizer, practically, the dispersion of the sample solution in the carrier stream is very limited, thus, the sensitivity can be achieved as that obtained in conventional nebulisation or discrete nebulisation. Since a separate pump is used to carry the sample solution in flow injection, the optimum flow rate of the sample can be easily controlled without disturbing the fuel-oxidant ratio of the flame. In a very concentrated or viscous sample solution, the dilution can be easily performed by increasing the tube length between the injection valve and the nebulizer. The arrangement of the apparatus is simple, the sample size used is very small with rapid sample injection and high reproducibility. This technique has been applied successfully with AAS (e.g. 21, 22, 23, 24).

In the present chapter, the systems of AAS and FIA-AAS are optimised with calcium as a test element. Factors such as lamp current, adjustment of concentric nebulizer capillary tip, and the length of tubing are investigated for AAS. Factors that affect the peak shape and calcium signal in FIA-AAS such as pumping rate, tube dimensions and sample volume are also studied. The performance of FIA-AAS and discrete nebulisation are compared. The effects of carrier stream and physical properties of the
solution are described.

2.1. **Apparatus, Standards and Reagents**

a) **Apparatus**

A Shandon Southern AJ300 Atomic Absorption Spectrometer.

A Gilson minipuls 2 peristaltic pump.

An Altex type 201-25, 8 port injection valve with two external sample loops (manually operated).

An HPLC 8 port injection valve with an external sample loop (pneumatically operated), model 201-06.

Anaches FTFE 0.53 and 1.14 mm internal diameter tubing as the basis for the flow injection manifolds.

Connectors.

Recorder.

b) **Standards and Reagents**

Calcium and magnesium solutions were prepared from 1000 ppm of stock solution (BDH Chemical Limited).

Sodium chloride, GR 99.5% pure.

Glycerol, 98% pure.

**Note**

1) "Steady state" signal was measured either by pumping the standard solution direct to the flame or by injecting a very
large volume of standard solution (e.g. 1000 μl) into the
carrier stream so that the central portion of the plug was
not mixed with the carrier stream.

ii) "Normal nebulization" signal was measured by aspirating the
solution into the nebulizer by the action of the oxidant
flow rate.

iii) Measurement of absorbance - each solution was measured at
least 5 times and in some cases 10.

iv) Tubing - most of the tubing used was 0.58 mm internal diameter
(including sample loop), unless otherwise stated.

2.2. Optimization of Instrument

The instrument was optimised with 10 ppm calcium solution
according to the instruction manual. The effects of lamp current,
nepulizer adjustment and the length of the tubing connected to
the nebulizer were investigated.

2.2.1. Effect of Lamp Current

A 10 ppm calcium solution was nebulised directly to the flame
alternately with tridistilled water at various operating currents.

The effect of current on calcium absorbance is shown in Figure
2.1a. The absorbance is a linear function to the current but
due to high noise to signal ratio, the use of low current is
not recommended. The current chosen for the experimental work
was 5.0 mA which had low noise to signal ratio and gave reason-
ably high absorbance of calcium.

![Graph](image)

**Figure 2.1a. Effect of lamp current on calcium absorbance.**

### 2.2.2. Effect of Capillary Length at Mixer Block Assembly

A 10 ppm of calcium solution was nebulised to the flame alternately with tridistilled water at 5.0 mA lamp current at various capillary lengths which were measured from outside to the face of the nebulizer (see Figure 2.1b). This was performed to avoid the unnecessary extinguishing of flame when changing the capillary length.
Total length of capillary = 79.5 mm.

Length of nebuliser from face to face = 70.0 mm.

Figure 2.1b. Mixer block assembly for AAS.

Figure 2.1c. Effect of capillary length on calcium absorbance.
Table 2.1. Effect of various capillary lengths on the absorbance of calcium.

<table>
<thead>
<tr>
<th>Capillary length, mm (measured from outside)</th>
<th>Capillary length, mm (measured from inside)</th>
<th>Absorbance, A</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>4.5</td>
<td>0.436</td>
</tr>
<tr>
<td>6.0</td>
<td>3.5</td>
<td>0.532</td>
</tr>
<tr>
<td>7.0</td>
<td>2.5</td>
<td>0.585</td>
</tr>
<tr>
<td>8.0</td>
<td>1.5</td>
<td>0.578</td>
</tr>
<tr>
<td>9.0</td>
<td>0.5</td>
<td>0.628</td>
</tr>
<tr>
<td>10.0</td>
<td>-0.5</td>
<td>0.570</td>
</tr>
</tbody>
</table>

Figure 2.1c shows the effect of capillary length on calcium absorbance. About 0.5 mm of the capillary projecting away from the face of the nebuliser gave a maximum absorbance of calcium. It was thought that at this length, a fine dispersion of the solution was achieved and the efficiency of the nebuliser was at its highest.

2.2.3. Effect of Capillary Tubing

A 10 ppm calcium solution was nebulized at 2.5 kgc⁻² air pressure with maximum flowmeter reading at various lengths of capillary tubing.

The uptake rate of the solution was at optimum when the length
of the tubing was between 2.5 and 5.0 cm as shown in Figure 2.2. It was suggested that for use in normal nebulisation, the length of the tubing must not be longer than 10 cm in order to get the manufacturer's uptake rate between 3.0 and 5.0 ml min⁻¹ for distilled water. Above this length the absorbance of the calcium was decreased and high noise level was observed. This was probably due to low uptake rate and erratic performance of nebuliser.

The uptake rate for tridistilled water at 7.5 cm tube length and at maximum flowmeter reading was 3.60 ml min⁻¹.

The following optimum parameters were used throughout the experimental work on calcium.

Air pressure = 2.5 kgc⁻² (maximum flowmeter reading).
Acetylene pressure = 0.5 kgc⁻² at 3.7 l min⁻¹.
Lamp current = 5.0 mA.
Slit width (band pass) = position 2 (0.3 mm).
Wavelength = 422.7 nm.
Burner height = position 4.
Capillary length = 9.0 cm (measured from outside).
Tube length = 7.5 cm (for normal nebulisation).

2.2.4. Linear Range

A series of calcium solutions was nebulized and the absorbance obtained was plotted against the concentration of calcium.
Figure 2.2. Effect of length of capillary tubing on the absorbance of calcium.
Figure 2.3. Linear range of calcium from 0.0 to 1.0 ppm.
Figure 2.4. Linear range of calcium for three concentration ranges, 

\[(0 - 5 \text{ ppm.}), (0 - 10 \text{ ppm.}) \text{ and } (0 - 25 \text{ ppm.})\].
Figure 2.3 and 2.4 show four concentration ranges and their linearity.

2.3. Flow Injection Analysis and Atomic Absorption Spectrometry

Figure 2.5 shows the manifold of flow injection analysis with atomic absorption spectrometry. The carrier solution is pumped through one of the sample loops of the injection valve (see Figure 2.6) while the sample solution is injected into another loop. By using the slider of the valve the sample solution is positioned between the carrier solutions and is swept into the system. The dispersion process occurs between the injection valve and the nebuliser of the atomic absorption spectrometer.

Figure 2.6 shows how the Altex type 201-25 injection valve works in the system.

2.3.1. Effect of Pumping Rate

The peristaltic pump was calibrated with tridistilled water to find the pumping rate for various scale readings of the pump water by measuring the volume of the water at a fixed period of time. The calibration graph is shown in Figure 2.7.

A series of 100 µl of 5 and 10 ppm calcium solutions was injected by use of the HPLC valve, into the water carrier stream, at various pumping rates at 2.3 cm tube length. For comparison, the steady state signal was also measured by pumping.
Figure 2.5. Manifold of flow injection analysis with atomic absorption spectrometry.
Figure 2.6. Schematic drawing of a FIA injection valve (Altex type 201-25); a) Sampling position and b) Inject position.
Figure 2.7. The calibration graph for peristaltic pump for various scale readings of the pump meter.
direct to the flame.

Figure 2.8 shows the effect of pumping rate on the absorbance of calcium. The optimum pumping rate was between 4.0 and 6.0 ml/min⁻¹. At higher range than this pumping rate, the efficiency of the nebulization process was decreased and thus, a lower signal was observed. The decrease in efficiency of the nebulization process was probably due to the formation of much larger droplets in the spray chamber.

2.3.2. Effect of Tube Dimension

A series of 100 µl of 10 ppm calcium solution was injected into water carrier stream at various tube lengths (internal diameter 0.58 mm and 1.14 mm) from the injection valve to the nebuliser at pumping rate of 4.93 ml/min⁻¹.

Figure 2.9 shows the effects of tube length and tube diameter on the absorbance of calcium. The longer the tube length and the larger the diameter of the tubing the lower the absorbance.

2.3.3. Effect of Sample Volume

A series of volumes of 10 ppm calcium solution was injected into the water carrier stream at pumping rate of 4.93 ml/min⁻¹ and tube length of 2.3 cm. The absorbance for steady state and normal nebulization were measured for comparison.
Figure 2.8. Effect of flow rate on the absorbance of calcium; a) 10 ppm., and b) 5 ppm.
Figure 2.9 Effects of tube length and tube diameter.

Figure 2.10. Effect of sample volume.
The steady state signals obtained were 0.433 absorbance for tubing with i.d. 0.58 mm and 0.489 for tubing with 1.14 mm i.d. Figure 2.10 shows that about 200 µl of calcium solution was needed to reach 95.5% of the steady state signal for tubing with 0.58 mm i.d. and about 300 µl was needed to reach the same percentage of the steady state signal for tubing 1.14 mm i.d. This indicates that as the dispersion increases the volume needed to reach the steady state signal also increases.

The variation of dispersion as a function of volume injected and tube length is shown in Figure 2.11 for selected values of these parameters. Construction of such graphs enables appropriate values to be chosen so as to achieve a desired dispersion.

2.3.4. Shapes of Calibration Curve

100 µl of a series of concentrations of calcium was injected into the water carrier stream at pumping rate of 4.93 ml/min⁻¹ and at tube length of 2.3 cm. Measurements were also made for the steady state and normal nebulization absorbance.

The shapes of the calibration curve for direct injection of 100 µl solution, steady state and normal nebulization are shown in Figure 2.12. About 90% of the normal nebulization signal was achieved when a 100 µl of 5 ppm calcium solution was injected. The percentage of the signal obtained decreased as the concentration of the solution was increased. When a 100 µl of 25 ppm of calcium solution was injected only 80% of the normal nebulization signal was obtained. The dispersion has increased from 1.08 for 5 ppm calcium to 1.11 for 25 ppm calcium.
Figure 2.11. Variation of dispersion, $D$, measured as the ratio of steady state absorbance to peak absorbance with tube length, $L$, and volume injected, $V_i$. Volume injected: A, 13; B, 50; and C, 200 µl. Tube length: 1, 200; 2, 100; and 3, 2.3 cm.
Figure 2.12. Shapes of calibration curves for calcium for: A, normal nebulization; B, steady state; and C, 100 μl injection.
2.3.5. Effect of Water and Air as Carrier Streams

In comparing the sensitivity of flow injection with limited dispersion, with non-dispersion sample introduction, a series of volumes of 10 ppm calcium solution was injected by the HPLC valve at flow rate of 4.93 ml/min\(^{-1}\) and at tube length of 2.3 cm in water and in air as carrier stream.

Figure 2.13 shows that the absorbance was only reduced by 1% in the water carrier stream as compared in the stream of air. Therefore, the efficiency of the flow injection with limited dispersion is as good as "normal" discrete nebulization.

2.3.6. Effects of Physical Properties of Solution

The effect of high dissolved solids was investigated by injecting various volumes of 0.5 ppm magnesium with increasing amount of sodium chloride at 200 cm tube length and at 5.53 ml/min\(^{-1}\) of flow rate. A comparison was made by measuring the absorbance of the solutions by normal nebulization.

Figure 2.14 shows the effect of injecting a small volume of high dissolved solid into the carrier stream of water. The signal gradually increases as the percentage of sodium chloride increases. By normal nebulization, the presence of sodium chloride did not seem to help to increase the magnesium signal. Although there was no burner blockage observed when magnesium in 2.5% w/v of sodium chloride solution was nebulized normally in this experiment,
Figure 2.13. Effect of carrier on the dispersion of calcium solution; A, in air stream, and B, in water stream.
Figure 2.14. Effect of sample volume of 0.5 ppm Mg in increasing amount of sodium chloride.
the possibility of burner blockage when higher percentage of sodium chloride solution is nebulised normally, or when it is nebulised continuously into the flame can be avoided by injecting a small volume of the solution into the carrier stream.

The study of viscosity of the solution was carried out by injecting various volumes of 0.5 ppm magnesium solution in the increasing percentage of glycerol at 200 cm of tube length and 5.53 ml/min⁻¹ of flow rate. Measurements by normal nebulization were also made for comparison.

Figures 2.15 and 2.16 show the effects of sample volume and the amount of glycerol on the absorbance of magnesium respectively. Figure 2.15 shows how normal nebulization method could not cope with increasing viscosity of the solution and Figure 2.16 shows how the volume of injection can be chosen to suit the requirement for a particular sample solution. For example, if the sample solution is too viscous, a small volume of sample solution can be injected into the carrier stream so that a more diluted sample solution can be obtained in the system.

2.4. Discussion and Conclusions

The use of limited dispersion in flow injection sample introduction provides the same advantages as the discrete nebulization technique. As additional features, the effects of sample viscosity is greatly reduced and the nebulizer is continuously washed with carrier stream. The sensitivity can be achieved similar to that
Figure 2.15. Effects of sample volume and the amount of glycerol on the absorbance of 0.5 ppm magnesium.
Figure 2.16. Effect of sample volume in reducing the viscosity of sample solution; A, 0% of glycerol; B, 2% of glycerol; and C, 50% of glycerol in 0.5 ppm magnesium.
obtained in discrete nebulization if a very short tube length is used between the injection valve and the nebulizer, and similar to that obtained in normal nebulization if a reasonably large volume of sample solution is injected into the carrier stream at that tube length. The precision was found about 1% relative standard deviation based on peak height. The size of the sample can be easily varied by changing the sample loop, and the flow rate of the sample does not depend on the oxidant flow rate. The uptake rate can be easily optimised by adjusting the pumping rate of the carrier stream.

If the sample is too concentrated, it can be diluted by controlling the dispersion. This is achieved by varying the volume injected and the tube length. The injection of sample solution is simple and rapid, and this increases the sampling frequency in the analysis.
REFERENCES


Chapter 3

SIMPLIFIED MODEL FOR DISPERSION IN FIA-AAS

3. Introduction

The distinctive feature of flow injection analysis is that when a volume of sample is injected into the carrier solution, the difference in the initial concentrations of an analyte in the carrier stream and in the sample solutions causes concentration gradients to be established across the interfacial region between the sample plug and the carrier during residence time in the flow line. Theoretically, whether the concentration profile of the sample plug in the carrier solution is Gaussian or (slightly) skewed depends on the flow rates, dimensions of the tubing and methods of injection and detection. For example, at low flow rates and within specified dimensions of tubing, the dispersion of the sample into the carrier stream is mainly by diffusion and the concentration-time profile of the sample plug downstream is Gaussian.

The dispersion process in laminar and turbulent flows in the tube has been described and discussed by Taylor (1, 2), theoretically and experimentally. Taylor suggested that the dispersion of a soluble matter into the stream of solvent in a capillary tube was due to the combined action of convection parallel to the axis and molecular diffusion in the radial direction which was described by a virtual coefficient of diffusion, thus, the
concentration gradients along the tube was governed by this coefficient of diffusion. Danckwerts (3) has suggested that in laminar flow, there is a variation in velocity from the axis to the wall of the tube, the central stream line of the fluid moving faster than the mean velocity, while the fluid near the wall lags behind. In turbulent flow, the velocity is more uniform across the tube. In a perfect mixing situation, Danckwerts suggested that the equation of the curve was represented as "F-diagram", i.e., \( F(\theta) = 1 - e^{-\theta/V} \), where \( \theta \) is the time taken from injection to detection, \( V \) is the flow rate of the fluid and \( V \) is the volume of the vessel occupied by the fluid. The shape of the F-diagram depends on the distribution of residence times and will give a good deal of information about the behaviour of the fluid flowing through the vessel.

An alternative treatment on the dispersion of a solute in a fluid flowing through a tube has been given by Aris (4). Although the discussions were purely mathematical, the results have shown that the distribution of solute ultimately tends to Normal or Gaussian. Rate et al. (5) have shown that the combined effects of molecular diffusion and mass transport and the rate of change of concentration could be represented in the differential equation form as \( \partial c/\partial t = D \nabla^2 c - \nabla (\rho c) \), where \( c \) is the concentration of solute, \( \rho \) is the convection velocity and \( D \) is the Diffusion Coefficient.

Levenspiel and Smith (6) have suggested that in the streamline flow of fluids through pipes, the longitudinal mixing is mainly
due to the fluid velocity gradients while lateral mixing is a result of molecular diffusion. The concentration distribution is a function of time and length of the pipe used and is given as

$$CV/V_1 = \frac{1}{2\sqrt{\pi (ut/V)(D_1/UL)}} \exp\left(-\frac{(1 - ut/V)^2}{4(ut/V)(D_1/UL)}\right),$$

where $V_1$ is the volume injected, $L$ is the length of the pipe, $V$ is the volume of the pipe, $t$ is time, $D_1$ is longitudinal dispersion coefficient and $U$ is the average velocity ($= uL/V$, where $u$ is fluid flow rate in $\text{ft}^3/\text{sec}$) in $\text{ft/sec}$ and $x = Ut$.

The skewness of the concentration profile increases with $D_1/UL$ and for very small values of $D_1/UL$, the profile approaches to Normal or Gaussian curve. When a longer pipe is used, the longitudinal mixing effect will be smaller relative to the total amount of fluid but lateral mixing will be greater with respect to longitudinal mixing and the model will become more reliable.

A simple model of the variation of the concentration as a result of solute injected into the stirred tank reactor has been described (7, 8). The mathematical model for this system has been developed by Van De Vusse (7). Chase (9) has constructed the multiple gradient instrument consisting of ten airtight mixing chambers connected in series in such a manner that the rate of fluid transfer between chambers is practically independent of solution density. The individual chamber gradients were theoretically derived and experimentally verified. The derived curves show that the concentration of the effluent is a function of effluent volume and initial chamber solution concentration. The general equation of
The multiple gradient device has been given as

$$C_E = C_R \left[ 1 - \exp^{-V_E/V_H} \sum_{n=1}^{\infty} \frac{1}{(n-1)!} \left( \frac{V_E}{V_H} \right)^{n-1} \right] + \exp^{-V_E/V_H} \sum_{n=1}^{\infty} \frac{C_{Hn}}{(n-1)!} \left( \frac{V_E}{V_H} \right)^{n-1}$$

where $C_E$ is the concentration of the effluent, $C_R$ is the concentration of the reservoir solution, $V_E$ is the volume of effluent, $V_H$ is the volume of each mixing chamber and $C_{Hn}$ is the initial concentration of the $n$th mixing chamber. The solution for any individual mixing chamber and for the contribution from the reservoir were also given.

Nagy et al. (8, 10) have achieved a fairly good approximation to the measured real conditions with theoretical concentration variations based on the following assumptions: the rate of injection was constant, i.e., no concentration gradient was formed in the direction of flow in the plug of liquid, the mixing of the solution in the reactor was instantaneous and the concentration of the solution emerging from the reactor was always equal to that prevailing inside the reactor. The concentration variation has been given as

$$C = (N/uT) \left[ 1 - \exp^{-ut/V_H} \right] \text{ for the rising peak, and}$$

$$C = (N/uT) \left[ 1 - \exp^{-uT/V_H} \right] \exp \left[ -u(t-T)/V_H \right] \text{ for the falling peak, where } N \text{ is the amount of sample injected into the system, } u \text{ is the flow rate, } T \text{ is the time required by the portion of liquid containing the injected material to traverse the cross-section of the tube preceding the stirring unit, } V_H \text{ is the volume}$$
of the stirring unit and \( t \) is the time interval.

The linear relationship between the logarithm of the concentration of the solution and the time has also been shown (10) from a simple mathematical model for the concentration-time profiles when a small volume of sample or reagent is injected into the carrier solution streaming at a constant flow rate into a stirred tank reactor. The concentration of the sample changes continuously as a function of time, i.e.,

\[
C = C_0^s \exp\left(-\frac{ut}{V_H}\right) \quad \text{or} \quad \log C = \log C_0^s - \frac{ut}{V_H} \log e
\]

when the stirred tank is filled up with a sample solution of concentration \( C_0^s \). \( u \) is the flow rate, \( V_H \) is the volume of stirred tank reactor and \( t \) is time.

Symmetrical gradients profiles, which can be described by a Gaussian curve, will be obtained with tubes of large length and small diameter at low flow rate, while at shorter length and larger diameter of tubes, asymmetrical gradients will be produced with an increasing degree of dispersion and decreasing skewness as the length of the tube is increased (11). This system can be described by a tank-in-series model (12) as follows

\[
c/c_0^s = (\frac{t}{\bar{t}_1})^{N-1} \frac{1}{(N-1)!} \cdot \exp\left(-\frac{t}{\bar{t}_1}\right)
\]

where \( \bar{t}_1 \) is the mean residence time of the sample in one hypothetical tank, \( N \) is the number of these tanks containing the sample some, \( c_0^s \) is the original concentration of the injected
sample solution and \( t \) is the gradient chamber holding time. For
\( N = 1 \), the well-mixed gradient chamber yields a pure exponential
concentration gradient,
\[
C = C_0 \exp^{-t/\ell}.
\]
Thus, by varying the diameter
and length of tube which accommodates the carrier stream and serves
as the chemical reactor for the injected samples and by appropriate
selection of the linear velocity of the reagent stream and sample
volume, a wide range of concentration gradients of sample within
the carrier solution can be obtained (11).

The simple models to describe the concentration profiles with and
without the insertion of a mixing chamber have also been given (13,
14, 15). The concentration profiles have also been influenced by
viscosity, specific gravity and temperature of the solution as
well as the detection systems used (13). Some of the theoretical
aspects of the dispersion phenomena in describing the concentration
profiles in flow injection analysis have been described and discussed
in detail (16, 17, 18, 19, 20, 21). Both straight and coiled tubes
have been compared (16, 17) and simple expressions based on
laminar flow have been given. The influence of injection, trans-
portation of sample solution, detection system and volume of the
sample on the response of curves have been explained theoretically
and experimentally (18, 19). Pardu and Fields (22, 23) have used
a variable time kinetic model to evaluate a single-channel and
dual-channel flow injection systems which include a gradient
chamber. The systems were evaluated subjectively and semi-quantitatively by using the physical model, mathematical equations,
concentration-time profiles, experimental data and formal definition.
The dispersion model is largely used in electrochemistry (8,10, 13, 14, 15), e.g., amperometry (24) and coulometry (25) and in titrimetry (e.g., 11, 26) and in stopped-flow enzymatic assay of substrates (27). Stewart and Rosenfeld (28) have used gradient concentration technique with colorimetric, fluorometric, conductometric and flame emission detector to demonstrate the usefulness of this system.

3.1. Basic Model for Dispersion

3.1.1. Exponential Gradient for a Single Mixing Chamber

The dispersion effects due to injection, carrier tubing and detector of the instrument are considered to be due to a single well-stirred mixing chamber of volume V that produces a concentration gradient (see Figure 3.1).

![Diagram of a single mixing chamber](image_url)

Figure 3.1. Dispersion effects are considered to be due to a single well-stirred mixing chamber of volume V that produces a concentration gradient.
If a volume of \( V_1 \) of concentration \( C^s_m \) is injected into the carrier stream of concentration \( C^R_m \) flowing at a rate of \( u \), then,

\[
\text{rate of mass inflow} = C^s_m u
\]

and if the concentration in the mixing chamber is \( c \), then,

\[
\text{rate of mass outflow} = c u
\]

\[
\frac{dc}{dt} = \frac{C^s_m u}{V} - \frac{c u}{V}.
\]  

(3.1)

\[
\frac{dc}{dt} = \frac{u}{V} \left( \frac{C^s_m - c}{c^s_m - c} \right)
\]

\[
v/u \int \frac{dc}{(c^s_m - c)} = \int dt
\]

\[
- \frac{v}{u} \ln \left( \frac{C^s_m - c}{c} \right) = t + k
\]

When \( t = 0 \), \( c = C^R_m \)

\[
- \frac{v}{u} \ln \left( \frac{C^s_m - c^R_m}{c^s_m - c} \right) = k
\]  

(3.2)

\[
- \ln \left( \frac{C^s_m - c}{c^s_m - c^R_m} \right) + \ln \left( \frac{C^s_m - c^R_m}{c^s_m - c} \right) = \frac{ut}{V}
\]

\[
\ln \left( \frac{c^s_m - c^R_m}{c^s_m - c} \right) = \frac{ut}{V}
\]

\[
\frac{c^s_m - c}{c^s_m - c^R_m} = \exp(-\frac{ut}{V})
\]
\[ C = C_m^a - (C_m^a - C_m^R) \exp(-ut/V) \]  

(3.3)

When the concentration of the carrier stream is zero, i.e., \( C_m^R = 0 \), equation (3.3) becomes

\[ C = C_m^a \left[ 1 - \exp(-ut/V) \right] \]  

(3.4)

At peak maximum, \( V_1 = ut \), thus equation (3.4) becomes

\[ C_p^s = C_m^a \left[ 1 - \exp(-V_1/V) \right] \]  

(3.5)

Dispersion is defined as the ratio of concentration injected to the concentration at peak maximum, i.e., \( D = C_m^s/C_p^s \). On arrangement of equation (3.5), Dispersion is given as

\[ D = \left[ 1 - \exp(V_1/V) \right]^{-1} \]  

(3.6)

From equation (3.6), it is clearly shown that dispersion is a function of volume injected, \( V \), and volume of mixing chamber, \( V \). Equation (3.5) can be written in the form of

\[ \ln \frac{C_p^s}{C_m^a} = \ln \frac{C_m^s}{C_p^s} - \frac{V}{V_{\text{max}}} \ln C_m^a \]

Thus, a plot of \( \ln \frac{C_p^s}{C_m^a} \) vs. \( t_{\text{max}} \) will give a linear graph with slope equal to \( u \ln C_m^s \), and intercept at concentration axis at \( \ln C_m^a \). Once the volume of mixing chamber is known, the dispersion and time to peak maximum can be easily predicted for various volumes injected.
3.1.2. Exponential Gradient for Dual Mixing Chamber of Unequal Volume

The concentration gradient produced as a result of using two mixing chambers is derived below (see Figure 3.2).

When a volume of solution flows out from the mixing chamber \( M_2 \), the same volume will enter \( M_2 \) from reservoir, \( R \), so that the change \( dC_y \) in concentration \( C_y \) with time, at a flow rate \( u \), in the mixing chamber \( M_2 \) will be

\[
-dC_y = (C_3 - C_y)\frac{udt}{V_2} \tag{3.7}
\]

By separating the variables and integrating, we obtain

\[
-\ln (C_3 - C_y) = \frac{ut}{V_2} + K \tag{3.8}
\]
or

\[
C_3 - C_y = K' \exp(-ut/V_2)
\]

To determine the integration constant, boundary conditions \( t = 0 \) is used, therefore,

\[
\exp(-ut/V_2) = 1 \quad \text{and} \quad C_y = C_2 \quad \text{and} \quad K' = C_3 - C_2
\]
On substitution in equation (3.8) gives

\[ c_y = c_3 - (c_3 - c_2)\exp(-ut/V_2) \]  

(3.9)

For the next mixing chamber, i.e., \( M_1 \), the same principle is also applied, i.e.,

\[ \frac{dC_x}{udt} = (c_y - c_x)\frac{udt}{V_1} \]  

(3.10)

\[ \frac{dC_x}{udt} + \frac{C_x}{V_1} = \frac{c_y}{V_1} \]

But \( c_y = c_3 - (c_3 - c_2)\exp(-ut/V_2) \).

Thus, equation (3.10) becomes

\[ \frac{dC_x}{udt} + \frac{C_x}{V_1} = \frac{c_3 - (c_3 - c_2)\exp(-ut/V_2)}{V_1} \]  

(3.11)

The integrating factor for equation (3.11) is \( \exp\int u\cdot dt/V_1 = \exp ut/V_1 \).

By multiplying equation (3.11) with \( \exp ut/V_1 \), we get

\[ \frac{d(C_x\exp ut/V_1)}{udt} + \frac{C_x\exp ut/V_1}{V_1} = \frac{c_3\exp ut/V_1 - (c_3 - c_2)\exp(ut/V_1 - ut/V_2)}{V_1} \]

i.e.,

\[ \frac{d(C_x\exp ut/V_1)}{udt} = \frac{c_3\exp ut/V_1 - (c_3 - c_2)\exp(ut/V_1 - ut/V_2)}{V_1} \]

\[ c_x\exp ut/V_1 = \int\frac{c_3\exp ut/V_1}{V_1} - \int\frac{(c_3 - c_2)\exp(ut/V_1 - ut/V_2)}{V_1} \]
\[ c_x \exp \frac{ut}{V_1} = c_3 \exp \frac{ut}{V_1} - \frac{(c_3 - c_2)V_1V_2 \exp (1/V_1 - 1/V_2)}{V_1(V_2 - V_1)} + k'' 
\]

\[ c_x = c_3 - \frac{V_2(c_3 - c_2) \exp (-ut/V_2) + k'' \exp (-ut/V_1)}{(V_2 - V_1)} \quad (3.12) \]

By using boundary conditions, \( t = 0 \),

\[ \exp (-ut/V_2) = 1, \quad \exp (-ut/V_1) = 1, \quad \text{and} \quad c_x = c_1, \quad \text{thus}, \]

\[ k'' = c_1 - c_3 + \frac{V_2(c_3 - c_2)}{(V_2 - V_1)} \quad (3.13) \]

On substitution in equation (3.12), this gives,

\[ c_x = c_3 - \frac{V_2(c_3 - c_2) \exp (-ut/V_2)}{(V_2 - V_1)} + (c_1 - c_3) \exp \left( \frac{ut}{V_1} \right) + \frac{V_2(c_3 - c_2) \exp (-ut/V_1)}{(V_2 - V_1)} \]

\[ c_x = c_3(V_2 - V_1) - \frac{V_2(c_3 - c_2) \exp (-ut/V_2) + (c_1 - c_3)(V_2 - V_1) \exp (-ut/V_1) + \frac{V_2(c_3 - c_2) \exp (-ut/V_1)}{(V_2 - V_1)}}{(V_2 - V_1)} \]

When \( c_3 = c_0 \), that is the initial concentration and the carrier concentration is equal to zero, i.e., \( c_2 = c_1 = 0 \),

\[ c_x = \frac{c_0 V_2 \left[ 1 - \exp(-ut/V_2) \right] - c_0 V_1 \left[ 1 - \exp(-ut/V_1) \right]}{(V_2 - V_1)} \quad (3.14) \]
or

\[ C_x = \frac{C_0 V_2 [1 - \exp\left(-\frac{ut}{V_2}\right)]}{(V_2 - V_1)} - \frac{C_0 V_1 [1 - \exp\left(-\frac{ut}{V_1}\right)]}{(V_2 - V_1)} \]

\( V_1 \) is the sample volume injected into the carrier stream, and at peak maximum, \( t_{\text{max}} = V_1/u \), thus,

\[ C_p = \frac{C_0 V_2 [1 - \exp\left(-\frac{V_1}{V_2}\right)]}{(V_2 - V_1)} - \frac{C_0 V_1 [1 - \exp\left(-\frac{V_1}{V_1}\right)]}{(V_2 - V_1)} \] \hspace{1cm} (3.15)

If concentration is a linear function to absorbance, equation (3.15) becomes

\[ A_p = \frac{A_0 V_2 [1 - \exp\left(-\frac{V_1}{V_2}\right)]}{(V_2 - V_1)} - \frac{A_0 V_1 [1 - \exp\left(-\frac{V_1}{V_1}\right)]}{(V_2 - V_1)} \] \hspace{1cm} (3.16)

For two mixing chambers of equal volumes, \( V_m \), the concentration at the outlet is given as (29):

\[ C = C_3 - (C_3 - C_2) \exp\left(-\frac{V_1}{V_m}\right) - \left[C_2 - C_1 \frac{V_2}{V_m} \exp\left(-\frac{V_1}{V_m}\right)\right] \] \hspace{1cm} (3.17)

and for \( n \) mixing chambers:

\[ C = C_{n+1} - \exp\left(-\frac{V_1}{V_m}\right) \sum_{i=1}^{n} \left[C_{n+1} - C_i \right] \frac{(V_i/V_m)^{n-i}}{(n-i)!} \] \hspace{1cm} (3.18)
3.2. Apparatus, Standards and Reagents

a) Apparatus

A Shandon Southern A3300 Atomic Absorption Spectrometer.
A Gilson minipuls 2 peristaltic pump.
An Altex type 201-25, 8 port injection valve with two external sample loops (manually operated).
Anachem PTFE tubing as the basis for the flow injection manifolds with 0.58 mm internal diameter.
Connectors.
Recorder.

b) Standards and Reagents

Calcium solutions were prepared from 1000 ppm of stock solution (BDH Chemical Limited).

3.3. Measuring the Volume of Hypothetical Mixing Chamber

A chart recording for the steady state conditions of 10 ppm calcium at flow rate 82.2 µl/sec., tube length 2.3 cm, was obtained at 1 cm/sec. chart speed. A chart recording for 100 µl of 10 ppm calcium, injected into water carrier stream, at the same conditions as above was also obtained. Typical chart recordings observed are shown in Figure 3.3.

From equation (3.4),
Figure 3.3. Typical chart recordings for a) a steady state signal and b) a 100 µl of 10 ppm calcium.
If absorbance is a linear function of concentration,

\[ A = A_s^\infty \left[ 1 - \exp\left(-ut/V\right) \right] \quad (3.19) \]

where \( A \) is the absorbance at time \( t \), \( A_s^\infty \) is the absorbance at steady state, \( u \) is the flow rate, \( t \) is the time at absorbance \( A \) and \( V \) is the volume of the hypothetical mixing chamber. By rearranging equation (3.19), we get

\[ t = V \frac{\ln \frac{A_s^\infty}{(A_s^\infty - A)}}{u} \]

When \( A = A_s^\infty /2 \), \( t = V \ln 2/u \) or \( V = ut/\ln 2 \),

When \( A = 3A_s^\infty /4 \), \( t = (2V \ln 2)/u \) or \( V = ut/(2 \ln 2) \) and so on.

Therefore, the volume of mixing chamber can be calculated for the various values of absorbance from the steady state. Table (3.1) shows the volume of \( V \) obtained for various values of absorbance.

**Table 3.1. Volumes of Hypothetical Mixing Chamber for various values of absorbance.**

<table>
<thead>
<tr>
<th>( A )</th>
<th>( A_s^\infty /2 )</th>
<th>( 3A_s^\infty /4 )</th>
<th>( 7A_s^\infty /8 )</th>
<th>( 15A_s^\infty /16 )</th>
<th>( 31A_s^\infty /32 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t ) (sec)</td>
<td>0.4</td>
<td>0.6</td>
<td>0.9</td>
<td>1.4</td>
<td>2.0</td>
</tr>
<tr>
<td>( V ) (μl)</td>
<td>47.5</td>
<td>35.6</td>
<td>35.6</td>
<td>41.5</td>
<td>47.5</td>
</tr>
</tbody>
</table>
The average value of \( V \) is 41.5 \( \mu l \).

On substitution of equation (3.5),

\[ \frac{A^e}{A^p} = \left[ 1 - \exp(-V_1/V) \right]^{-1} = D, \text{ where } D \text{ is dispersion.} \]

By using the average value of \( V \),

\[ D = \left[ 1 - \exp(-100/41.5) \right]^{-1} = 1.10 \]

Compare with the experimental value in Figure 3.3,

\[ D = \frac{A^e}{A^p} = \frac{0.605}{0.560} = 1.08. \]

3.4. Factors Affecting on the Volume of the Hypothetical Mixing Chamber

3.4.1. Flow Rate

Since the optimum flow rate has to be maintained in AAS during the experiment for obtaining high sensitivity, the effect of flow rate on the mixing chamber volume was not studied in great detail. However, an experiment was carried out with 10 ppm calcium solution at various flow rates with a tube length of 2.3 cm and an injection volume of 100 \( \mu l \). It was found that as the flow rate was increased, the volume of mixing chamber slightly increased. This was because the slope of the rising part of the peaks increased when the flow rate was increased. Table (3.2)
shows the effect of flow rate on the volume of mixing chamber.

Table 3.2. Effect of flow rate on the volume of mixing chamber.

<table>
<thead>
<tr>
<th>Flow rate, ( u, \mu l/sec. )</th>
<th>89.7</th>
<th>107.8</th>
<th>125.8</th>
<th>147.8</th>
<th>165.8</th>
<th>187.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V, \mu l )</td>
<td>59.3</td>
<td>63.0</td>
<td>67.1</td>
<td>65.9</td>
<td>72.2</td>
<td>61.2</td>
</tr>
</tbody>
</table>

3.4.2. Tube Length

In studying the effect of tube length on the hypothetical mixing chamber, chart recordings of the steady state signal for 10 ppm calcium solution for various tube lengths at constant flow rate, i.e., 89.3 \( \mu l/sec. \), were obtained at 1 cm/sec. chart speed. Analysis of individual curve for each tube length gave the results shown in Table (3.3). Theoretically, when the tube length is increased, the slope of the rising part of the curve will decrease due to the dispersion processes and thus, the volume of the mixing chamber increases.

Table 3.3. Effect of tube length on the volume of mixing chamber,

<table>
<thead>
<tr>
<th>Tube length, ( cm. )</th>
<th>2.3</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
<th>150</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V, \mu l )</td>
<td>44.0</td>
<td>63.9</td>
<td>64.8</td>
<td>84.5</td>
<td>75.5</td>
<td>92.2</td>
<td>89.1</td>
<td>104.2</td>
</tr>
</tbody>
</table>
3.4.3. Volume of Injection

The volume of mixing chamber can also be measured from a series of injected volumes and the corresponding peak absorbance. A plot of $t_{\text{max}}$ against $\ln \left[ A_m^S / (A_m^S - A_p^S) \right]$, has the slope equals to $V/u$.

In this experiment a series of volumes of 10 ppm calcium solution was injected into a water carrier stream at constant flow rate (i.e., 87.0 µl/sec.) at several tube lengths. There was a slight change in flow rate due to length of the sample loop tubing. The flow rate was slightly increased when longer tube length (i.e., large volume injected) of sample loop was used. Table (3.4), (3.5) and (3.6) show the volumes of mixing chamber obtained for a series of tube lengths (i.e., 2.3, 100 and 200 cm) using a series of volumes injected.

Different values of volume of mixing chamber were obtained when different methods of obtaining time were used. In the first method, a steady state signal was obtained at fast chart speed and the time taken to reach a certain peak absorbance was plotted against $\ln \left[ A_m^S / (A_m^S - A) \right]$. In the second method, a series of volumes was injected and the time to reach the peak absorbance for each volume was measured and plotted against $\ln \left[ A_m^S / (A_m^S - A_p^S) \right]$. The difference in volumes of the mixing chamber were more pronounced at longer tube lengths. This was probably because the rising part of the peak absorbance obtained for a series of volumes injected was slightly deviated from the rising part of the absorbance of the steady state signal due to dispersion processes and simultaneously reduced the peak absorbance. Figure 3.4 shows this effect for a
Figure 3.4. Signals obtained for a series of volumes injected into a water carrier stream at tube length of 200 cm., and at flow rate of 5.22 ml/min.
series of volumes injected.

3.5. Use of Hypothetical Mixing Chamber for Prediction Purposes

The experimental results for several parameters in flow injection analysis were compared with the theoretical calculations by using the volume of hypothetical mixing chamber measured for three different tube lengths. The volume of hypothetical mixing chamber was calculated from a step change in concentration from zero to a steady state signal. Based on this value the volume of injection, dispersion value and \( t_{\text{max}} \) were calculated as follows:

a) The volume of injection, \( V_i \), was calculated from equation

\[
V_i = V \ln \left[ \frac{A_p^S}{(A_m^S - A_p^S)} \right],
\]

where \( A_p^S \) was the absorbance at peak maximum when \( V_i \) was injected.

b) The dispersion value, \( A_m^S/A_p^S \), was calculated from equation

\[
A_m^S/A_p^S = \left[ 1 - \exp\left(-V_i/V\right) \right]^{-1}.
\]

c) \( t_{\text{max}} \) was calculated from equation

\[
t_{\text{max}} = V \ln \left[ \frac{A_p^S}{(A_m^S - A_p^S)} \right]/u,
\]

where \( A_p^S \) and \( A_m^S \) were the absorbance of volume injected and the steady state signal.

Tables 3.4, 3.5 and 3.6 show the results obtained for the calculation of these parameters.
Table 3.4. Comparison between calculated values and experimental values of volume injection, dispersion and $t_{\text{max}}$, for tube length 2.3 cm.

Volume of mixing chamber, $V$, measured = 57.0 µl.

| $V$, µl | $D = A_m/A_p$ | $t_{\text{max}}$, sec. |
|---------|----------------|----------------|-----|-----|-----|
| 13.3    | 16.1           | 3.57            | 4.81 | 0.4 | 0.2 |
| 24.8    | 23.3           | 2.98            | 2.83 | 0.5 | 0.3 |
| 49.9    | 54.6           | 1.62            | 1.71 | 0.6 | 0.6 |
| 101.3   | 104.5          | 1.19            | 1.20 | 1.1 | 1.1 |
| 149.5   | 144.1          | 1.09            | 1.08 | 1.2 | 1.6 |
| 205.5   | 183.6          | 1.06            | 1.03 | 1.8 | 1.8 |
| 298.4   | -              | -               | -    | -   | -   |
| 501.5   | 262.6          | 1.02            | 1.01 | 2.2 | 2.3 |
| 1001.3  | -              | 1.00            | 1.00 | 4.2 | -   |

+ Exptl.= Experimental.
+ Calcn.= Calculation.

Table 3.5. Comparison between calculated values and experimental values of volume injected, dispersion and $t_{\text{max}}$, for tube length 100 cm.

Volume of mixing chamber, $V$, measured = 93.6 µl.
<table>
<thead>
<tr>
<th>$V, \mu l$</th>
<th>$D = \frac{A^S_N}{A^S_p}$</th>
<th>$t_{\text{max.}}, \text{sec.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.3</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>24.8</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>49.9</td>
<td>36.3</td>
<td></td>
</tr>
<tr>
<td>101.3</td>
<td>72.6</td>
<td></td>
</tr>
<tr>
<td>149.5</td>
<td>108.2</td>
<td></td>
</tr>
<tr>
<td>205.5</td>
<td>184.5</td>
<td></td>
</tr>
<tr>
<td>298.4</td>
<td>214.5</td>
<td></td>
</tr>
<tr>
<td>501.5</td>
<td>388.8</td>
<td></td>
</tr>
<tr>
<td>1001.3</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.6. Comparison between calculated values and experimental values of volume injected, dispersion and $t_{\text{max.}}$ for tube length 200cm.

Volume of mixing chamber, $V$, measured = 111.2 $\mu l$.

<table>
<thead>
<tr>
<th>$V, \mu l$</th>
<th>$D = \frac{A^S_N}{A^S_p}$</th>
<th>$t_{\text{max.}}, \text{sec.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.3</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>24.8</td>
<td>17.3</td>
<td></td>
</tr>
<tr>
<td>49.9</td>
<td>34.8</td>
<td></td>
</tr>
<tr>
<td>101.3</td>
<td>66.9</td>
<td></td>
</tr>
<tr>
<td>149.5</td>
<td>109.0</td>
<td></td>
</tr>
<tr>
<td>205.5</td>
<td>158.6</td>
<td></td>
</tr>
<tr>
<td>298.4</td>
<td>249.5</td>
<td></td>
</tr>
<tr>
<td>501.5</td>
<td>512.0</td>
<td></td>
</tr>
<tr>
<td>1001.3</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
As shown in the tables, when the tube length is increased, the agreement between the calculated values of all parameters above and the experimental (measured) values gets poorer. This is probably due to the dispersion processes which has been described earlier which deviates the rising part of the peak and reduces the peak absorbance. As the tube length gets longer, the dispersion gets larger and the peak shape tends towards Gaussian. Thus, the exponential equation which has been derived cannot be applied. An alternative equation between exponential and Gaussian needs to be derived and verified.

The breakdown of the model between exponential and Gaussian for longer tube length or high dispersion was not studied. It is suggested that at longer tube length, the volume of mixing chamber comprise two parts; the volume of mixing chamber of the nebulizer of the instrument and the volume of mixing chamber of the flow injection manifold. A suitable equation for this model may be derived based on the above suggestion and may be verified with the experimental results.

3.6. Use of Hypothetical Mixing Chamber for Calibration Purposes

The volume of hypothetical mixing chamber can be used as the basis for calculating the concentration of a series of volumes injected into the carrier stream. By plotting the absorbance of each peak produced from a series of volumes injected against the calculated concentration, the calibration graph can be constructed from a single concentration solution. Below is a procedure to construct
a calibration graph from a single concentration of solution by using the volume of hypothetical mixing chamber as the basis of calculation.

a) Obtain a value of the hypothetical mixing chamber, V, from the analysis of the step concentration change to the steady state curve.

b) Use the value of V to calculate \( t \) from equation

\[
t = V \cdot \ln \left( \frac{A^s_m}{(A^s_m - A)} \right) / u,
\]

where \( A \) and \( A^s_m \) are obtained from experimental values.

c) Use the value of \( t \) and \( V \) to calculate concentration, \( C \), from equation

\[
C = C^s_m \cdot \left[ 1 - \exp(-ut/V) \right],
\]

where \( C^s_m \) is the initial concentration of standard and \( u \) is the flow rate of the carrier.

d) Plot a graph of absorbance, \( A \), against concentration, \( C \), for a series of volumes injected.

For example, a series of volumes of 10 ppm calcium solution was injected into water carrier stream at tube length 2.3 cm and at flow rate 90.0 \( \mu l/sec \). (See Table 3.7). Figure 3.5 shows the calibration graph obtained from various volumes of injection with the concentration range from zero to 10 ppm calcium (see Chapter 6, Section 6.5).
Table 3.7. Concentration range obtained from a single 10 ppm calcium standard.

<table>
<thead>
<tr>
<th>Volume, $V_1$, $\mu$l</th>
<th>Absorbance, $A_{std.}$</th>
<th>Concentration, $C_{std.}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.3</td>
<td>0.140</td>
<td>2.8</td>
</tr>
<tr>
<td>24.8</td>
<td>0.168</td>
<td>3.4</td>
</tr>
<tr>
<td>49.9</td>
<td>0.310</td>
<td>6.2</td>
</tr>
<tr>
<td>101.3</td>
<td>0.420</td>
<td>8.4</td>
</tr>
<tr>
<td>149.5</td>
<td>0.460</td>
<td>9.2</td>
</tr>
<tr>
<td>205.5</td>
<td>0.470</td>
<td>9.4</td>
</tr>
<tr>
<td>298.4</td>
<td>0.479</td>
<td>9.6</td>
</tr>
<tr>
<td>501.4</td>
<td>0.488</td>
<td>9.8</td>
</tr>
<tr>
<td>1001.3</td>
<td>0.500</td>
<td>10.0</td>
</tr>
</tbody>
</table>
Figure 3.5. A calibration graph for calcium obtained from various volumes of injection based on a single concentration of calcium (10 ppm).
REFERENCES

4. Introduction

The determination of calcium in iron ores is always subject to various interference effects from other components present in them. The interference effects on calcium can be divided into two general categories; depressive effects and enhancing effects. Depressive effects are usually related to the formation of involatile or thermally stable compounds such as calcium-aluminium, calcium-phosphate and calcium-sulphate compounds, in which the production of free calcium atoms in the flame is greatly reduced, thus, decreasing the signal from calcium. The high salinity of the solution due to high content of sodium chloride also depresses the calcium signal because of the reduction in nebulization efficiency.

Enhancing effects are normally due to the releasing action of the reagents on calcium or the suppression of ionization of calcium by the reagents. Lanthanum is a typical example of an enhancing agent due to its releasing agent action. It also acts as an ionization buffer. Lanthanum which is more easily ionized than calcium, suppresses the ionization of calcium and causes the ionization equilibrium for calcium to be shifted towards the
formation of neutral atoms, thus, increasing the production of free calcium atoms in the flame and therefore, enhancing the calcium signal. Although enhancing effects are always favourable in the determination of calcium, their presence in the sample solution has to be matched with the standards used, or otherwise, inaccurate results would be obtained.

Both types of interference effects have been investigated extensively and methods of overcoming them have also been suggested.

Rocchiccioli and Townshend (1) have shown that most of the interferences on calcium, such as sodium, magnesium, lanthanum, strontium and barium reached a maximum effect and formed a plateau region when more of them were added into the calcium solution, except silicon, aluminium and iron. They have also suggested how the rate of atom production accounted for the bending of the calibration curve of calcium towards the concentration axis. The efficiency of various cations in removing anionic interferences has been explained and the mechanisms of various enhancing and depressive effects have also been proposed (1). The behaviour of calcium in interfering systems such as hydrochloric acid, sodium chloride, acetic acid, aluminium and phosphate has been investigated and possible mechanisms have also been suggested (2). The mechanism of the formation of the thermally stable calcium phosphate compound has also been studied by Stojanovic et. al (3) by titrating calcium chloride solution (acts as a releasing element) with magnesium chloride (metal solution) and phosphoric acid (acts as an anion inhibitor) solution, and the titram was aspirated simultaneously into the flame (i.e.,
the changes in magnesium signal were recorded). The releasing element, i.e., calcium, will form a stable compound with the anion, i.e., phosphate, during the vaporization of the particle, leaving the metal, i.e., magnesium, unbound. The reaction can be generally represented by $3\text{Ca}^{2+} + (3\text{Mg}^{2+} + 2\text{PO}_4^{2-}) \rightarrow \text{Ca}_3(\text{PO}_4)_2 + 3\text{Mg}^{2+}$. The magnesium signal measured increases during the titration until the total amount of calcium has reacted stoichiometrically with the anion, then decreases as it reached the titration end-point where the inhibition effect ($2\text{PO}_4^{2-} + 3\text{Mg}^{2+} \rightarrow \text{Mg}_3(\text{PO}_4)_2$), is again present. The reaction between calcium and phosphate was found to be complete and the equilibrium was established with the formation of tricalcium phosphate. David (4) has explained the mechanism of magnesium chloride and sulphuric acid in controlling the interference from phosphorus, aluminium and silicon on calcium signal. He suggested that, since both an anion and a cation are essential, two solid phases may be formed when water was evaporated from droplet of sample solution; phosphorus, aluminium and silicon entering one solid phase and calcium the other. When combined in different solid phases, phosphorus, aluminium and silicon may not be able to interact with calcium, even in the vapour phase after volatilization.

Several methods of overcoming these interference effects have been proposed. Solvent extraction techniques have been used to remove iron, chromium, aluminium and other cations before determining calcium by flame AAS (5, 6). Atsuya and Goto (7) have used plasma jet spectrometry to eliminate the interference effects of aluminium,
silicon and iron, and to reduce the fluctuation of the curve for calcium in the determination of calcium in iron ores. Hwang and Sandonato (8) have investigated the effect of fuel-oxidant ratio in minimizing the chemical interference on calcium. They suggested that the use of lean air-acetylene flame was preferable to fuel-rich air-acetylene flame in order to reduce the interferences although the latter flame gave better sensitivity. Attempt has also been made to eliminate some of the well-known interferences by using strontium chloride, ethylenediaminetetraacetic acid (EDTA), ammonium fluoride and mixtures of these reagents as suppressing or releasing agents with air-acetylene and nitrous oxide-acetylene flames (9, 10). Taylor and Belcher (11) have suggested that the use of the higher temperature nitrous oxide-acetylene flame was necessary to overcome the interferences, however, sodium and strontium were required as well in order to suppress ionization effects. The interfering effect of the magnesium matrix on calcium has also been eliminated by the addition of hydroiodic acid and hydrogen peroxide to generate iodine in the calcium solution (12). The mechanism of the effect of iodine on calcium was not discussed in this paper.

A comparative study has been made for strontium chloride and lanthanum chloride as releasing and suppressing agents for the interferences of phosphate, aluminate, silicate and sulphate (13). 5-sulphosalicylic acid and potassium chloride have also been used effectively as protector and ionization inhibitors using air-acetylene and nitrous oxide-acetylene flames (14). A series of reagents has been studied to minimize the effect of aluminium on
calcium (15). Some of the alkali metals have helped to alleviate the depressive effect of aluminium. Interferences from vanadium, sodium, barium and phosphorus have been effectively overcome by using lanthanum chloride (16).

A standard method for sample dissolution for the determination of calcium in iron ores by flame AAS has been described (17) and the sample preparation techniques have also been reviewed extensively (18). A method of sample dissolution in the present of high content of silica was also suggested.

4.1. Flow Injection Analogue of Standard Additions Method

The interference effects of phosphate and aluminium on calcium have been successfully overcome by using a flow injection analogue of the standard additions method. In this method, a sample carrier stream is pumped through the flame while a series of standards is injected into it. The signal recorded would be the difference in concentration between the concentration of standard and the concentration of sample. The method works perfectly well with phosphate as an interferent which exerts a constant depressive effect on calcium above a certain phosphate to calcium ratio. Aluminium which does not give a constant depression on calcium has to be overcome by addition of a releasing agent into the sample solution. The workability of this method depends on the dispersion of the reagent or standards in the flowing stream, and the amount of interferent or reagent present in the sample.
solution. Thus, the system must be designed so that when the standard solution is dispersed in the flowing stream the interferent to analyte ratio never falls below the value required to give a constant depression. The same is also applied to the reagent used, where the reagent must not fall below the amount required to completely release calcium from the interaction of interferent (aluminium).

4.2. Basic Model for Dispersion

If a step concentration change from $C_m^R$ to $C_m^S$ is made immediately prior to a mixing chamber of volume $V$ in a stream flowing with velocity $u$, then the resulting concentration ($C$)-time ($t$) profile, is given by the following equation (see Section 3.1.1.),

$$C = C_m^S - (C_m^S - C_m^R)\exp(-ut/V) \quad (4.1)$$

If the concentration in the flowing stream, $C_m^R$, is zero, this reduces to

$$C = C_m^S \left[1 - \exp(-ut/V)\right] \quad (4.2)$$

If a volume, $V_i$, is injected into the flowing stream then the time for the absorbance maximum to be reached will be $V_i/u$; substituting this in equation (4.2) gives

$$C_p = C_m^S \left[1 - \exp(-V_i/V)\right] \quad (4.3)$$
Dispersion, $D$, is defined as the ratio of the injected concentration to the concentration at peak maximum, i.e., $c_m^s/c_p^s$, thus, from equation (4.3)

$$D = \frac{c_m^s}{c_p^s} = \left[1 - \exp(-V_f/V)\right]^{-1} \quad (4.4)$$

If a series of standards is injected into the continuously flowing sample stream, then, this system provides the analogue of standard additions method. At the concentration of peak maximum, $c_p^s$, equation (4.1) applies and the maximum change in concentration, $\Delta C$, is given by $\Delta C = c_p^s - c_m^s$, or

$$\Delta C = c_p^s - c_m^s = (c_{\text{std}} - c_m^s) \exp(-V_f/V) \quad (4.5)$$

Combining equations (4.4) and (4.5), this gives

$$\Delta C = (c_{\text{std}} - c_m^s)/D \quad (4.6)$$

By assuming that absorbance is a linear function to concentration, then,

$$\Delta A = k(c_{\text{std}} - c_m^s)/D \quad (4.7)$$

Thus, a plot of $\Delta A$ against $c_{\text{std}}$ would give a straight line and would intercept the $c_{\text{std}}$ axis at concentration $c_m^s$, i.e., when $\Delta A = 0; c_{\text{std}} = c_m^s$. 

4.3. **Apparatus, Standards and Reagents**

a) **Apparatus**

A Shandon Southern A3300 Atomic Absorption Spectrometer.

A Gilson minipuls 2 peristaltic pump.

An Altex type 201-25, 8 port injection valve with two external sample loops (manually operated).

An HPLC, 8 port injection valve with an external sample loop (pneumatically operated), model 201-06.

Anachem PTFE 0.58 mm internal diameter tubing as the basis for flow injection manifolds.

Connectors.

Recorder.

b) **Standards and Reagents**

Calcium stock solution, 1000 ppm (BDH Chemical Limited), diluted as necessary.

Lanthanum chloride solution, LaCl₃·7H₂O, 10% w/v La (BDH Chemical Limited).

5-Sulphosalicylic acid, C₆H₃(OH)(COOH)SO₃H·2H₂O.

Potassium chloride, 99.8% pure (Fisons, AR).

Strontium chloride, SrCl₂·6H₂O, 98% pure (BDH Chemical Limited).

Hydrochloric acid, 32% w/w, (sp. gr. 1.16).

Nitric acid, 69-71% w/w, (sp. gr. 1.42).

Perchloric acid, 71-73% w/w, (sp. gr. 1.70).

Orthophosphoric acid, 88% w/w, (sp. gr. 1.75).
4.4. Interference Effects

4.4.1. Phosphate

The effect of phosphate on the calcium signal was investigated by injecting 50 μl of 20 ppm standard calcium solution containing increasing amount of phosphate in the form of phosphoric acid, into a stream of tridistilled water and into a carrier stream of 10 ppm calcium solution, at pumping rate 4.93 ml/min and tube length of 40 cm. For comparison, measurements were also performed by the normal nebulization method.

The effect of phosphate on the calcium signal with a carrier stream of tridistilled water is shown in Figure 4.1a. Figure 4.1b shows the effect on the calcium signal when calcium standards in the presence of phosphate are injected into a carrier stream containing calcium. The steady state signal for 10 ppm calcium was 0.498 absorbance and the dispersion was 2.08. The curve shows that the carrier signal has been reduced by about two fifths in the presence of phosphate.

The effect of the dispersion in the flowing stream on the shapes of the calibration curve was studied by injecting a series of standards calcium into the carrier stream of 20 ppm calcium and 40 ppm phosphate at various tube length. Figure 4.2 shows the...
Figure 4.1a. Effect of phosphate on calcium signal.
Figure 4.1b. Effect of phosphate on calcium when injected into 10 ppm calcium carrier stream.
Figure 4.2. Effect of dispersion on calcium signal when calcium standards are injected into 20ppm calcium + 40 ppm phosphate.
effect of the dispersion on the calcium signal. As the dispersion is increased, the shape of the calibration curve becomes more linear. Although the dispersion has been increased up to 4.64, the curve does not intercept the concentration axis at the concentration of the flowing stream. This is because the amount of phosphate present in the flowing stream was not enough to give a constant depression on the calcium signal and therefore, as the standards were injected into the carrier stream, the phosphate to calcium ratio fluctuated along the curve of the interference graph of phosphate on calcium as shown in Figure 4.1a and 4.1b. If the amount of phosphate in the flowing stream was on the plateau region of the graph, and when the standards were injected into the carrier stream would give the same interference effect on calcium, a constant depressive effect was obtained.

Figure 4.3 shows how a flow injection system can be used to compensate for the interference of phosphate on calcium when a constant depression from phosphate is achieved. A series of standards was injected into the flowing stream containing 10 ppm calcium and 500 ppm phosphate at various dispersions. The graph intercepts at the concentration of the flowing stream when a critical value of the dispersion is reached. The critical value of the dispersion is achieved when the standards injected experience the same constant depressive effect as does the flowing stream. At a very limited dispersion, the calibration graph has an S-shape because the standard solutions do not receive the same depressive effect from phosphate. Therefore, when the concentration of the standard
Figure 4.3. Effect of dispersion in overcoming the interference of phosphate on calcium (carrier stream containing 10 ppm calcium + 500 ppm phosphate).
is increased, the interference effect (i.e., due to the phosphate to calcium ratio) varies according to the concentration of standard, thus, the same effect of interference is not achieved for a series of standards.

Figure 4.4 shows the effect of the amount of phosphate in the carrier stream on the calcium signal as the tube length is varied. The curves were obtained by injecting a series of 100 \( \mu l \) of 10 ppm calcium solution into the carrier streams containing increasing amount of phosphate. At a very limited dispersion, the interference effect from phosphate in nearly insignificant although the amount of phosphate has been increased. This is probably due to the reasons of incomplete mixing between phosphate and calcium because of brief mixing time and insufficient amount of phosphate to react with calcium. This type of injection shows another method of determining samples by flow injection techniques, i.e., by injecting a series of standards and sample solutions under the influence of an interferent or reagent in the carrier stream. This is analogous to matching the standards to the samples with respect to this interferent. The method will be discussed further in Chapter 5.

4.4.2. Aluminium

The interference effect of aluminium on calcium was studied by injecting a series of 50 \( \mu l \) of 20 ppm calcium containing increasing concentration of aluminium into a carrier stream of water or 10 ppm of calcium solution. For comparison, measurements by normal nebulization were also carried out.
Figure 4.4. Effect of phosphate as a carrier stream on calcium absorbance for two different tube lengths.
Figure 4.5 shows the effects of aluminium on calcium signal when calcium in the increasing concentrations of aluminium were nebulized conventionally and when they were directly injected into the carrier stream of tridistilled water. The interference from aluminium does not produce a constant depression or plateau region on calcium signal. The calcium signal drops practically to zero absorbance when the concentration of aluminium is increased up to 150 ppm.

Figure 4.6 shows the shape of the curve when calcium in varying concentration of aluminium was injected into the flowing stream of calcium solution. The steady state signal was 0.551 absorbance and the dispersion was 2.01. The calcium signal drops to zero absorbance (i.e., completely depressed by the aluminium) when the concentration of aluminium is above than 150 ppm although the initial total calcium concentration used was 30 ppm.

The possibility of using a bracketing standard method in a flow injection system is shown in Figure 4.7a. A series of 50 μL of 40 ppm aluminium with increasing concentrations of calcium was injected into a carrier stream of 20 ppm calcium and 40 ppm aluminium. Since both the standards and the carrier stream contained the same concentration of aluminium, the concentration of calcium in the flowing stream was accurately recovered. The slight initial curvature observed is due to complete depression of the calcium signal by aluminium. This is further illustrated in Figure 4.7b, when a series of calcium concentrations in a fixed amount of
Figure 4.5. Effect of aluminium on calcium absorbance, (a) by normal nebulization and (b) when 50 μl of the solution was injected into water stream.
Absorbance, A

0.30

0.20

0.10

0.00

-0.10

-0.20

-0.30

-0.40

-0.50

-0.60

Concentration of aluminium in 20 ppm calcium, ppm.

Figure 4.6. Effect of aluminium on calcium absorbance when injected into 10 ppm calcium carrier stream. (Tube length = 40 cm., flow rate = 4.93 ml/min., volume = 50 μl).
Figure 4.7a. A bracketing standard method in flow injection system. (Carrier stream containing 20 ppm calcium + 40 ppm aluminium).
Figure 4.7b. Effect of aluminium on calcium absorbance;
(a) by normal nebulization, and (b) when 50 µl of solutions were injected into water carrier stream.

Absorbance, A

Tube length = 40 cm.
Flow rate = 4.93 ml/min.
Volume injected = 50 µl.

Concentration of calcium in 40 ppm aluminium, ppm.
aluminium (i.e., 40 ppm) was injected into a water carrier stream, and when it was nebulized normally.

The interference effects caused by aluminium and other elements which do not produce a constant depression or plateau region have to be overcome by either matching the standards and the samples, or by the addition of releasing agents before the flow injection sample introduction system can be used. The addition of reagent by a flow injection technique is achieved by using the reagent as the carrier stream as opposed to the normal reagent addition method where the reagent is added to the standards and the samples. The advantages of this technique are, of course, as well as saving chemical reagent, it also makes it possible to use the standards for the analysis of other sets of samples.

4.5. Use of Releasing Agents

Several types of releasing agents have been used to overcome the depressive effects of aluminium. Before the reagent can be used for real samples, its effect on the analyte to be determined has to be examined.

Figure 4.8 shows the effect of lanthanum chloride on the absorbance of 10 ppm calcium and 10 ppm calcium with 20 ppm aluminium. The graphs show that about 30% enhancement of the calcium signal when lanthanum is added into the calcium solution is obtained. The interference effect of aluminium was completely eliminated when
Figure 4.8. Effect of lanthanum chloride on the absorbance of calcium,

(a) 10 ppm calcium + 20 ppm aluminium, (b) 10 ppm calcium,
by normal nebulization.
0.2% w/v of lanthanum was added into the solution.

Figure 4.9 shows four calibration graphs obtained by using lanthanum chloride as a releasing agent to overcome the influence of aluminium on calcium signal by using flow injection technique. Graph (a) was obtained when a series of calcium standards in 0.2% w/v lanthanum was injected into a carrier stream of 10 ppm calcium, 20 ppm aluminium and 0.2% w/v lanthanum. This is similar to normal reagent additions method where reagent is added to both standards and samples. Graph (b) was obtained when a series of calcium standards was injected into the carrier stream of 10 ppm of calcium and 20 ppm of aluminium. Since no reagent was added into these solutions, the calibration graph does not intercept at the concentration of calcium of the flowing stream, but intercepts at about 2.5 ppm at the concentration axis. This means that, only about 2.5 ppm of calcium was available free when it was nebulized into the flame. The rest of the calcium has interacted with aluminium and formed stable compounds. Graph (c) was obtained when a series of calcium standards was injected into the flowing stream of 10 ppm calcium, 20 ppm aluminium and 0.2% w/v lanthanum. The interference effect from aluminium was completely eliminated and the amount of lanthanum used was greatly reduced.

For comparison, graph (d) was obtained when a series of calcium standards was injected into the carrier stream of 10 ppm calcium.

Figure 4.10 shows the effect of 1% w/v 5-sulphosalicylic acid and and 0.10% w/v lanthanum as protective and releasing agents for 10
Figure 4.9. Effect of lanthanum as a releasing agent to overcome the aluminium interference on calcium: Carrier; (a) and (c) 10 ppm Ca + 20 ppm Al + 0.25% w/v La, (b) 10 ppm Ca + 20 ppm Al, and (d) 10 ppm Ca; Standards; (a) Calcium (aqueous) + 0.25% w/v La, (b), (c), and (d) Calcium (aqueous).
Figure 4.10. Effects of 5-sulphosalicylic acid and lanthanum in overcoming the aluminium interference on calcium signal for various dispersions. (Carrier containing 10 ppm calcium + 1% w/v 5-sulphosalicylic acid + 0.1% w/v lanthanum + 20 ppm aluminium).
ppm calcium in 20 ppm aluminium solution at various dispersions. The addition of 5-sulphosalicylic acid to the sample solution has reduced the amount of lanthanum used by half compared with the amount of lanthanum needed in the absence of 5-sulphosalicylic acid. It is interesting to note that even at a dispersion of less than 2.0, the concentration of calcium in the flowing stream can still be 'recovered' by using these reagents, although the shape of the calibration graph is slightly curved. A mixture of 0.2% w/v of 5-sulphosalicylic acid and 0.2% w/v of strontium chloride was used in the determination of calcium in iron ores and satisfactory results were obtained.

4.6. Procedure

4.6.1. Sample Dissolution

a) For British Chemical Standards 303 (BCS 303) - Iron Ore Sinter.

The sample was weighed accurately (between 0.01 to 0.02 g) into a 100 ml beaker and 5 ml of concentrated hydrochloric acid and 1 ml of concentrated nitric acid were added. The beaker, covered with a watch glass, was heated gently for 30 to 60 minutes until about 1 or 2 ml of the solution was left. The solution was transferred quantitatively to a 250 ml volumetric flask containing 0.2% w/v of strontium chloride and 0.2% w/v of 5-sulphosalicylic acid in the final solution. The solution was diluted to/the mark.
About 0.20 g of sample was weighed accurately into a 250 ml teflon beaker and 10 ml of concentrated hydrochloric acid, 5 ml of concentrated nitric acid and 5 ml of hydrofluoric acid were added. The beaker, covered with a teflon lid, was heated for 1 to 2 hours until about 1 or 2 ml of the solution was left. The solution was diluted to 100 ml in a volumetric flask; 10 ml was transferred to a 100 ml volumetric flask. The solution was diluted to mark containing 0.3% w/v of lanthanum in the final solution.

4.6.2. Measurements

The sample solution was pumped continuously to the nebuliser of the atomic absorption spectrometer while the standards were injected into the flowing stream and the signals were obtained on a chart recorder. At first, both of the valves (i.e., model 201-06 and model 201-25) were used, but because of the valve model 201-06 proved not to be resistant to the mixture of acids used, the use of it was abandoned.
The following flow injection parameters were employed for the determination of calcium in iron ores:

Tube length = 200 cm; i.d. = 0.58 mm.
Volume injected = 50 and 100 μl.
Pumping rate = 5.30 ml/min.
Dispersion = 4

The flame conditions were employed as described in the Preliminary Studies (see Chapter 2, Section 2.2.3.).

4.7. Results and Discussion

The results obtained for the analyses of some of the BCS iron ores are given in Table 4.1. All of the results obtained for the samples were analysed at least four times and were calculated with 95% confidence interval.

The slope of the calibration graph obtained for the samples was slightly curved (like an S shape), and this may give an erroneous result if the graph is not interpolated carefully. The slightly curved shape of the graph was probably due to the disproportionate of the enhancing effects of the reagents used between the sample and the standard solutions. The sample which was mixed with the reagents experienced the maximum enhancing effects from the reagents and as a consequence, the sample signal was greatly enhanced. The standards which were injected into the sample containing reagents stream, did not experience the maximum enhancing
effects from reagents in the carrier stream, especially when the residence times of the standards in the carrier stream were short, or when the dispersion of the standards was limited. This problem could be overcome by increasing the dispersion value to 4 or more, where the standards would be four times diluted and mixed with the reagents and the sample.

Table 4.1. Determination of calcium in some BCS iron ores by flow injection analogue of standard additions method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Calcium present, %</th>
<th>Calcium found, %</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS 303</td>
<td>14.0</td>
<td>14.0 ± 0.3</td>
<td>5</td>
</tr>
<tr>
<td>BCS 378</td>
<td>4.7</td>
<td>4.7 ± 0.1</td>
<td>5</td>
</tr>
<tr>
<td>BCS 302/1</td>
<td>2.89</td>
<td>2.86 ± 0.08</td>
<td>4</td>
</tr>
</tbody>
</table>

+ Results were calculated based on 95% confidence level by using equation:

\[ \mu = \bar{x} \pm t s \sqrt{\frac{1}{n}} \]

where \( s \) is the measured standard deviation, \( n \) is the number of experiments, \( t \) is a number of Student's \( t \), \( \bar{x} \) is the measured mean and \( \mu \) is the true mean.

The use of releasing agents in this work was necessary in order to overcome the interferents present in iron ores because standard additions alone would not work. The releasing agents used were found to be suitable in eliminating all the interference effects in this work.
Several conditions have to be met for the accurate determination of calcium in iron ores by a flow injection sample introduction system, i.e.:

i) The dispersion must be designed so that the standards and the sample experience the same effects. This can be done either by changing the volume injected or tube length, whichever is possible.

ii) The concentration of reagent or reagents added into the sample solution must be enough to overcome completely the interference effects and to give the same effects to the injected standards.

iii) In the case of interferents that give a constant depression, the amount of the interferent added to the sample solution must be such that when the standard is dispersed in the flowing stream, the interferent to calcium ratio never falls below the value required to give the constant depression.

iv) The pumping rate of the sample solution must not be less than the aspiration rate of the nebuliser of the instrument in order to get maximum peak height.

4.8. Conclusions

The flow injection standard additions method has the advantages over the conventional normal nebulization method and the normal standard additions method in a way that: it uses the same standard solutions for a number of samples, thus reduces considerably the amount of
volumetric manipulation necessary; it is an interpolative method, thus increasing the accuracy over the normal extrapolative method; it is also possible to use in the alternative configuration, i.e., sample is injected into standards used in turn as a carrier. This method also reduces the amount of reagent used by addition only to the sample rather than to sample and standards in the normal methods.

Although the flow injection standard additions method has several advantages, two problems arise from the method, i.e.; it requires preliminary experiment to determine the ratio of interferent to analyte as well as the dispersion value in the carrier stream, and although the releasing agents used overcome the interferences in the sample, the enhancing effects of the releasing agents may give higher signal than the pure analyte should produce. This may lead to the false measurements of the signals of the sample and standards.

Some aspects of this work have been published in the Analyst (19).
REFERENCES


4) David, D. J., Analyst, 84(1959)536-545.


Chapter 5

APPLICATION OF A SIMPLIFIED MODEL FOR DISPERSION - DETERMINATION OF CHROMIUM IN STEELS

5. Introduction

A procedure for the determination of chromium in low-alloy irons and steels by air-acetylene flame AAS has been described by Kinson, Hodges and Belcher (1). The procedure involved the dissolution of sample in a mixture of phosphoric-sulphuric acid before atomization. It was claimed that the method was rapid, preliminary separations were not required and the accuracy obtained was well within the permissible range for routine determinations. The interferences caused by the presence of some metals such as iron, nickel, tungsten and molybdenum were accommodated by preparing calibration standards of the same composition as the sample being analysed.

Barnes (2) in his determination of chromium in steel by flame AAS used aluminium and ammonium chloride to reduce the depressive effect of iron. The effects of flame conditions, acids and alloying elements such as molybdenum, manganese, nickel and tungsten have also been investigated. The effect of iron and the use of aluminium and ammonium chloride in overcoming it have been studied under various flame conditions. The optimum conditions for the determination of chromium have been investigated by Feldman and Purdy (3) by studying the solution matrix, flame composition and solvent extraction procedures.
The analysis of chromium in iron and steel by flame AAS has been reviewed by Scholes (4).

Maruta, Suzuki and Takeuchi (5) have examined the interferences of hydrochloric, perchloric, nitric and phosphoric acids and have tried to elucidate the mechanism of their interferences on the flame AAS of chromium. They suggested that the enhancing effect on chromium by hydrochloric acid was due to the chlorinating action in which formation of the chlorides of this element have relatively low melting and boiling points, which are also related to the rate of vaporization. The oxidation of chromium by perchloric acid enhanced the chromium signal and the production of volatile compounds such as oxychloride may also have contributed to the enhancement. The depressive effect caused by phosphoric acid was due to the formation of thermally stable compounds. The signal was also depressed in nitric acid solutions in the lower region of the flame but showed little interference effect in the upper region of the flame.

Cationic interferences have also been studied by Yanagisawa et al (6) under various flame conditions. They found that chromium was subject to serious interferences in fuel-rich flames. For example, sodium, potassium, strontium, zinc and tin showed pronounced depressive effects in the lower region of the flame. Besides the temperature of the flame, the formation of mixed oxides may also contribute to the interferences. The enhancement effect caused by barium, aluminium, magnesium and calcium was suggested due to their presence in large amount in the solution and thus, would compete with chromium in the formation of stable oxides, so that, the production
of chromium atoms was enhanced. Iron was found to depress chromium (III) but enhanced chromium (VI). The depression increased with the increasing of iron concentration and it may be derived from the formation of non-volatile compounds with chromium such as chromite, since iron and chromium have similar properties.

Thomerson and Price (7, 8) have overcome the interference of iron on chromium by using perchloric acid in the dissolution procedure and a nitrous oxide-acetylene flame. It was suggested that the use of perchloric acid as the final medium has the advantage that it causes the least interference of all other common acids used.

The mechanism of interference of iron on chromium absorption have been investigated by Roos and Price (9). They suggested that the controlling factors in the depression of chromium by iron were particle size and volatility, and the rate of evaporation of aerosol particles in flame determined the overall pattern of interference. The aspiration of chromium-iron solution would cause relatively large dried particles in the flame which, after reduction by the flame gases, would consist of chromium (boiling point 2480°C) in less volatile matrix of iron (boiling point 3000°C). Atomization of the chromium in the air-acetylene flame would therefore be considerably impaired. In the nitrous oxide-acetylene flame (7), this depression was not observed because the vaporization was complete. Incomplete vaporization of particles in the flame may result in a noticeable departure from proportionality between absorbance and concentration.
The releasing effect of ammonium chloride on chromium in the presence of iron in an air-acetylene flame has also been investigated by Roos (10). He suggested that the releasing action on chromium by ammonium chloride was due to a distillation effect rather than to changes in particle size caused by the rapid volatilization of ammonium chloride in the matrix. Partial saturation of the ammonium chloride vapour (as it distilled from the clotlets) with chromium and iron as chlorides would lead to the enhancement of both of these elements. However, since the concentration of iron in the clotlet was greater than that of chromium, a much larger proportion of the chromium actually present in the particles would accompany the ammonium chloride during the distillation. Under these conditions, enhancement of chromium would be far greater than that of iron.

A method involving the use of an air-acetylene flame and incorporating 8-hydroxyquinoline as a releasing agent to suppress metallic interferences in the determination of chromium in steel has been described by Ottaway and Pradhan (11). Iron (III) was completely removed under optimum and fuel-lean flame conditions, but not under fuel-rich flame conditions. A complete oxidation of sample is necessary to convert iron (II) to iron (III), since oxine (8-hydroxyquinoline in hydrochloric acid) does not remove iron (II). Oxine also enhances the signal from chromium. Addition of ammonium bifluoride (NH₄HF₂) and sodium sulphate to suppress the diverse effects of elements on chromium in the determination of chromium has also been recommended by Purushottam, Naidu and Lal (12). Bifluoride forms complexes with some metal ions such as
iron (III) and tin (IV) and precipitates calcium, magnesium, barium and lead, and thus, reducing the total ion content of the sample solution. Ammonium bifluoride, not only suppresses the interferences from foreign metals and metal ions but also enhances the absorption of chromium. The use of hydroxylamine hydrochloride (NH$_2$OH.HCl) under fuel-rich flame in the determination of chromium in steel has been suggested by Pandey et al (13) in order to eliminate the depressive effect of iron on chromium signal.

Fogg, Soleymanloo and Burns (14) have overcome the interference of iron by masking the iron with fluoride after extraction with 4-methyl-2-pentanone, whereas, Donaldson (15) has oxidized chromium (III) to chromium (VI) with ceric ammonium sulphate before extracting it with chloroform containing tribenzylamine.

Green (16) has observed the effect of oxidation state in the determination of chromium in perchloric acid media and he suggested that the addition of hydrogen peroxide overcomes this problem and ensures conversion of all chromium into chromium (III) state. Cresser and Margitt (17) have found that variation in pH can cause a small but significant interference when chromium (VI) was determined by an air-acetylene flame. The interference is due to 4CrO$_4^{2-} \rightleftharpoons$ CrO$_4^{2-} + H^+$ equilibrium. The signal of chromium decreases as the pH of the solution increases. Thompson (18) has studied the effect of different oxidation states on the shape of the calibration graphs of chromium under various flame conditions in the air-acetylene flame. The use of fuel-rich air-
acetylene flame, optimised for maximum chromium sensitivity in
the determination of chromium was not recommended because the
calibration graph found under these conditions exhibited regions
near zero and negative slope. The effect was much more pronounced
for chromium (III) than for chromium (VI) and it was thought to
be caused by the kinetics of the reactions resulting in the for-
mation of free chromium atoms in highly reducing air-acetylene
flame.

Aggett and O'Brien (19, 20) have studied the formation of chromium
atoms in an air-acetylene flame from several pure chromium com-
pounds and in the presence of matrix electrolytes. The major
cause of differences in the atom formation behaviour of the com-
pounds investigated was the chemical nature of the species formed
on droplet desolvation and also the mean particle size. Both of
these factors affect the rate at which subsequent thermal decom-
position takes place. In the presence of alkali metal salts the
formation of chromium atoms in an air-acetylene flame is modified
in different ways that depend on the behaviour of the salt in the
flames. Chloride and nitrate systems tend to retard atom formation
in the lower region of the flames because of the relatively low
volatility of the salts or their oxides. The chromium atom pre-
cursors are eventually released higher in the flame as much smaller
particles than those formed in the absence of matrix. This leads
to enhancement of absorbance signal along the flame axis.

Hall, Brumhead and Whitham (21) have described a scheme for the
determination of chromium in all types of steel by using an air-
acetylene flame, whereas Cobb, Foster and Harrison (22) have determined chromium in steel by using a nitrous oxide-acetylene flame. The nitrous oxide-acetylene flame eliminates the interference of iron because of its high temperature. Price (23) has discussed dissolution methods in the analysis of metals in steel. A general method for the determination of all elements of interest in steel samples has also been described (24).

5.1. Basic Model for Dispersion

This chapter describes the determination of chromium in steel in air-acetylene flame by using flow injection sample introduction system. The dispersion process in the carrier stream between the point of sample injection and the detector is controlled to compensate for the matrix interferences so that the standards and sample solutions always experience the same matrix effects when the absorbance is made.

The concentration-time relationship when a step change is made in concentration from zero to \( C_m^a \) has been given in Chapter 4, Section 4.2, page 112, and the concentration at the peak maximum, \( C_p^a \), which occurs at a time equal to \( V_i/u \) is given by

\[
C_p^a = C_m^a \left[ 1 - \exp\left(\frac{-V_i}{V}\right) \right]
\]  

(5.1)

Equation (5.1) shows that if volume \( V_i \) of an analyze solution is injected into a carrier stream containing water, the absorbance signal equivalent to concentration \( C_p^a \) will be observed on the
chart recorder of the spectrometer.

5.1.1. Reagent Additions Method

If the carrier stream contains a reagent of concentration \( c^R_m \), then, as the sample plug passes through the "mixing chamber", the reagent concentration varies according to

\[
c^R = c^R_m \exp(-ut/V) \tag{5.2}
\]

and the reagent concentration at the peak maximum is given by

\[
c^R_p = c^R_m \exp(-V_1/V). \tag{5.3}
\]

The dispersion of the reagent, \( D^R \), is given by

\[
D^R = \frac{c^R}{c^R_p} = \left[ \exp(-V_1/V) \right]^{-1} \tag{5.4}
\]

Thus, combining equations (4.4) in Section 4.2 page 112 and (5.4)

\[
D^R = D/(D - 1) \tag{5.5}
\]

From equations (5.4) and (5.5)

\[
c^R_m = c^R_p D/(D - 1) = c^R_p c^S_m/c^S_p (D - 1) \tag{5.6}
\]

If a matrix component or an interferent which gives a constant depression on the analyte (see Chapter 4, Section 4.4.1., page 115)
is used as a reagent in the carrier stream and if the depressive effect is constant at interferent to analyte mass ratio, \( R_{1/a} \), and if the most concentrated standard used in the calibration is \( C_{t}^{\text{std}} \), then equation (5.6) can be generalized to

\[
C_{m}^{R} = \frac{R_{1/a} \cdot C_{t}^{\text{std}}}{(D - 1)} \tag{5.7}
\]

Thus, the relation between dispersion and peak concentration enables the minimum interferent concentration at the peak to be calculated.

### 5.1.2. Analogue of Standard Additions Method

When a standard of concentration \( C_{t}^{\text{std}} \) is injected into the carrier stream containing the analyte element at concentration \( C_{m}^{S} \), the concentration at peak maximum is the total contributions from the concentration of standard and the concentration of carrier, i.e.,

\[
C_{p} = C_{t}^{\text{std}} \cdot [1 - \exp(-V_{1}/V)] + C_{m}^{S} \cdot \exp(-V_{1}/V) \tag{5.8}
\]

The change in concentration, \( \Delta C \), at peak maximum is equal to \( C_{p} - C_{m}^{S} \), thus equation (5.8) becomes

\[
\Delta C = C_{t}^{\text{std}} \cdot [1 - \exp(-V_{1}/V)] + C_{m}^{S} \cdot \exp(-V_{1}/V) - C_{m}^{S}
\]

\[
= (C_{t}^{\text{std}} - C_{m}^{S}) \cdot [1 - \exp(-V_{1}/V)] \tag{5.9}
\]
Thus, equation (5.9) becomes

\[ \Delta C = D^{-1}(C^{\text{std.}} - C^a). \]

A plot of \( \Delta C \) versus \( C^{\text{std.}} \) would intercept the \( C^{\text{std.}} \) axis at concentration \( C^a \). If absorbance is a linear function to concentration, then

\[ \Delta A = (k/D)(C^{\text{std.}} - C^a) \quad (5.10) \]

where \( \Delta A \) is the observed change in absorbance and \( k \) is the proportionality constant relating absorbance and concentration.

If the concentration of a standard, \( C^{\text{std.}} \), is lower than the concentration of the sample carrier stream, \( C^a \), then the change in concentration or absorbance (\( \Delta A \) or \( \Delta C \)) at peak maximum, will be negative, and if \( C^{\text{std.}} > C^a \), then \( \Delta A \) or \( \Delta C \) will be positive, and also if \( C^{\text{std.}} = C^a \), then \( \Delta A \) or \( \Delta C \) will be zero.

In order for the method to compensate for any interference effect in the sample, the dispersion must be designed so that interference effects in the sample stream operates to the same extent on the injected standard as it does the sample.

If the concentration of top standard used in the calibration is \( C_t^{\text{std.}} \), combining equations for dispersion, (5.4), (5.5) and (5.8),

\[ D = \left[ 1 - \exp(-V_1/V) \right]^{-1}. \]
Thus the concentration of interferent $C_P^R$ at the peak maximum must be

$$C_P^R = \frac{R_i}{a} \left[ \frac{(C_t^\text{std.}/D) + C_m^s(D - 1)/D}{D} \right]$$

(5.12)

and, from equations (5.5) and (5.6), the minimum concentration of interferent in the sample carrier stream can be calculated as

$$C_m^R = \frac{R_i}{a} \left[ \frac{C_t^\text{std.}/(D - 1) + C_m^s}{D} \right]$$

(5.13)

5.2. **Apparatus, Standards and Reagents**

**a) Apparatus**

An Altex type 201-25 8 port injection valve with two external sample loops (manually operated).

A Gilson minipuls 2 peristaltic pump.

Anachem PTFE 0.58 mm internal diameter tubing as the basis for flow injection manifolds.

Connectors.

A Shandon Southern AJ300 Atomic Absorption Spectrometer.

A Recorder.

**b) Standards and Reagents**

Chromium (III) standards were prepared by dilution of 1000 ppm
stock solution (BDH Chemicals Limited).
Iron (III) solution was prepared by dissolving the appropriate amount of high-purity iron granules (British Chemicals Standard 149/3) in hydrochloric and nitric acids (21).
Hydrochloric (32%), nitric (69%) and sulphuric (98%) acids from BDH Chemicals Limited.
Phosphoric (60%) and perchloric (70%) acids from Fisons Chemicals Limited.

The flame conditions and the flow injection parameters used in the determination of chromium in steel were as follows:

- Air flow rate = 8.2 l/min.
- Acetylene flow rate = 4.9 l/min.
- Lamp current = 6 mA.
- Burner height = 4.2 unit.
- Monochromator band pass = 0.18 nm.
- Wave length = 357.9 nm.
- Pumping rate = 5.95 ml/min.
- Tube length = 200 cm.
- Volume of injection = 50 µl.

For the standard additions method the carrier stream was the sample solution containing the necessary amount of iron and for the reagent additions method the carrier was an iron solution.
5.3. Procedure

In the preliminary studies, the optimum flame conditions were obtained according to the instruction manual and the pumping rate, tube length and volume injected were varied to give the appropriate dispersion (see Chapter 2).

In the studies of interferences of iron and acidity, increasing amount of iron or acids or both were added into a fixed concentration of chromium. For the study of the effect of fuel-oxidant ratio, the acetylene flow rate was varied from 4.0 l/min to 5.5 l/min in the presence and absence of iron.

Four types of mixture of acids were investigated in the dissolution of steel samples, i.e., a mixture of phosphoric-sulphuric-nitric acids (1), a mixture of hydrochloric-nitric-perchloric acids (7), a mixture of sulphuric-nitric-hydrofluoric acids (15) and a mixture of hydrochloric-nitric acids (21). The dissolution procedure by Nall et. al (21) was chosen for the determination of chromium in this work because of its simplicity and suitability for the steels under investigation.

The absorbance measurements were made by injection of a series of 50 μl of standards into the sample carrier stream containing the appropriate amount of iron (to give a constant depression on the chromium signal). In the reagent additions method, standards and sample were injected into a water carrier containing the appropriate amount of iron.
A dispersion of 4 was obtained when 50 μl of sample was injected into the carrier stream at tube length of 200 cm.

5.3.1. **Dissolution Procedure for British Chemicals Standards**

**Samples (BCS)**

Weight of sample used: BCS 220/2, 241/2 and 261/1 = 0.1 gram.

- BCS 251/1 = 0.2 gram.
- BCS 254/1 = 0.4 gram.
- BCS 255/1 = 0.5 gram.

10 ml of hydrochloric (sp. gr. 1.18) and 5 ml of nitric (sp.gr. 1.42) acids were transferred to a 250 ml PTFE beaker containing the sample. The beaker, covered with PTFE cover, was heated gently on a hot plate for 30 to 45 minutes, until about 5 ml of the solution was left. Another 10 ml of hydrochloric acid was added to the beaker and heated for another 30 to 45 minutes. The solution was cooled and was then transferred into a volumetric flask. For BCS 220/2 and BCS 241/2, the solution was transferred to a 500 ml volumetric flask containing sufficient amount of iron so that the final solution contained 500 ppm. For BCS 261/1, the solution was transferred to 100 ml volumetric flask, diluted with tridistilled water to mark, pipetted, a 25 ml aliquot was transferred to a 500 ml volumetric flask containing sufficient iron so that the final solution contained 500 ppm. For BCS 251/1, 254/1 and 255/1, the solution was transferred quantitatively into a 100 ml volumetric flask and made up to volume.
For the reagent additions method, the blank solution was prepared in the same manner as the sample containing the appropriate amount of iron in the final volume. Since there was sufficient amount of iron in the blank carrier stream to give a constant depression, all of the samples were prepared by diluting them with tridistilled water to volume without the addition of external iron solution, as in the analogue of standard additions method.

A series of standard of 3, 6, 9, 12, 15, 18 and 21 ppm of chromium solution was prepared from 1000 ppm chromic nitrate stock solution in 1M nitric acid, by diluting it with tridistilled water in 100 ml volumetric flask to the necessary concentrations.

5.3.2. Absorbance Measurements

In standard additions method, standards were injected into the sample carrier stream and the changes in absorbance between the sample and the standards were plotted against the concentration of the standards. The chromium content of the sample was obtained when the change in absorbance was zero, i.e., at the intercept on the standard concentration axis.

In the reagent additions method, a series of standards and sample were injected into the blank carrier solution containing the appropriate amount of iron. The normal calibration graph was plotted and the chromium content of the sample was obtained by interpolation.
5.3.3. Calculations of Reagent for Standard Additions and Reagent Additions Methods.

The minimum mass iron to chromium ratio which was necessary to give a constant depression, \( R_{1/a} \), was found to be 30 to 1 as shown in Figure 5.1. The top standard used in the calibration sequence was 21.0 ppm, and the concentration of the chromium in the sample was expected to be about 10 ppm.

For standard additions method, equation (5.13) applies, i.e.,

\[
C_m^R = R_{1/a} [\frac{C_{std}^t}{(D - 1)} + \frac{C_m^s}{(D - 1)}]
\]

\[
= 30 \left[ \frac{21}{(4 - 1)} + 10 \right] = 30(7 + 10) = 510 \text{ ppm}.
\]

The minimum concentration of iron needed in the sample carrier stream to give a constant depression is 510 ppm. Thus the 500 ppm of iron added to the sample carrier solution was more than enough because there was always some iron already present in the sample itself.

For the reagent additions method, equation (5.7) applies, i.e.,

\[
C_m^R = R_{1/a} \frac{C_{std}^t}{(D - 1)}
\]

\[
= 30 \times \frac{21}{(4 - 1)} = 210 \text{ ppm}.
\]

Thus, the minimum concentration of iron needed in the blank
Figure 5.1. The effects of iron on the absorbance of a 10 ppm chromium solution. (X) fuel-lean flame. (Δ) fuel-rich flame.
carrier stream to give a constant depression is 210 ppm.

5.4. Results and Discussion

By using a peristaltic pump at the appropriate pumping rate, the aspiration rate to the flame was greatly improved and could be varied very easily to give maximum chromium sensitivity. When a deuterium lamp was used for determining background correction, no sign of scattered light or molecular absorption was observed in the sample.

Figure 5.1 shows the degree of depression of iron on chromium signal increases sharply as the amount of iron was added and levels off at 30:1 mass iron to chromium ratio. The depressive effect was more significant in fuel-rich flame than in fuel-lean air-acetylene flame.

The effects of hydrochloric and nitric acids in the presence and absence of iron can be seen in Figure 5.2. In the absence of iron both acids had little effects on chromium signal. This little effect is probably due to the change in viscosity or surface tension of the solution. On the contrary, in the presence of iron, chromium signals in hydrochloric and nitric acids were severely depressed, but the extent of the depression in each medium was not the same. The releasing effect of chloride was observed in hydrochloric acid medium, whereas, a complete depression was observed in nitric acid medium. Depending on various other factors, such as in the presence or absence of diverse cations or anions, different
Figure 5.2. The effects of acids on the absorbance of a 10 ppm chromium solution in the presence and absence of iron. (X) chromium in nitric acid and (Δ) chromium in hydrochloric acid in the absence of iron, (◯) chromium in hydrochloric acid and (□) chromium in nitric acid in the presence of 1000 ppm iron.
acids give different effects on chromium signal.

Figure 5.3 shows the effects of fuel-oxidant ratio on chromium signal. The chromium signal was severely depressed under fuel-lean but not so severely under fuel-rich air-acetylene flames. The optimum fuel flow for the determination of chromium was at 4.9 l/min which was slightly fuel-rich flame. It was found that in the presence of large amount of iron, a cut-off point was observed at this fuel flow. This odd behaviour of chromium has been discussed by Thompson (18) and is thought to be caused by the kinetics of the reaction or reactions resulting in the formation of free chromium atoms in highly reducing air-acetylene flames.

In finding the most suitable method of dissolution of steel sample with less interferences from acids, the mixture of hydrochloric-nitric acids was found to be satisfactory because of its simplicity, speed and suitability for the samples under investigation. The other three mixture of acids either needed extra precautions in handling them or needed further treatment to complete the dissolution procedure. The presence of small amount of undissolved silica did not affect the result obtained. This was proved by filtering the undissolved materials and treating them with hydrofluoric acid. The results obtained suggest that this extra procedure was unnecessary (see Table 5.1).

Typical examples of signal observed and the calibration graph in the flow injection analogue of the standard additions method are
Figure 5.3. Effects of fuel-oxidant ratio. (X) 10 ppm chromium, (Δ) 10 ppm chromium and 160 ppm iron, (○) 10 ppm chromium and 660 ppm iron and (□) 10 ppm chromium and 1160 ppm iron.
shown in Figure 5.4 and Figure 5.5. The results obtained for a number of BCS Steels containing from 0.19% to 17.4% of chromium is shown in Table 5.2. Additional iron was added to the first three samples to achieve the necessary 30:1 iron:chromium mass ratio to give the maximum interference effect on all the standards in the calibration sequence. In the experiments described here the sample solutions were diluted so that the chromium concentration was about 10 ppm and sufficient iron (III) solution added so that the final solution contained an additional 500 ppm iron. This together with the iron already present in the samples was considered to provide an adequate 'safety margin'. As can be seen from Figure 5.5, satisfactory results could have been obtained if the 15 ppm standard were considered the 'top' standard and so, in fact there was a considerable safety margin. The other three samples contained a much higher ratio of iron to chromium and thus the dispersion could be decreased while still achieving the necessary maximum depressive effect. The effect of a change in top standard or sample concentrations on the concentration of interferent necessary for the successful application of the standard additions method can thus readily be calculated. Similar calculations can be performed for other interfering components of the solutions. In this study, it was necessary to ensure that the effects due to the hydrochloric and nitric acids used in the dissolution procedure (see Figure 5.2) were taken into account when the final acidity of the sample solutions was considered.
Figure 5.4. Typical chart recording for the flow injection standard additions method. The steel sample contained about 10 ppm chromium and the injected standards covered the range 0–21 ppm in 3 ppm increments.

- Tube length = 2.3 cm.
- Flow rate = 5.95 ml/min.
- Volume injected = 50 μl.
Figure 5.5. A typical calibration plot for the flow injection standard additions method (ΔA, change absorbance vs. $c_{\text{std.}}$, concentration of standard). The sample concentration, $c_s^m$, is obtained from the intercept on the $c_{\text{std.}}$ axis.
In theory the equation could be used to calculate the dispersion necessary for the method to work for given values of the other parameters. Rearrangement of equation (5.13) gives:

\[ D = C_t^{\text{std}} \left[ R_1/a \left( C_m^R - C_m^S \cdot R_1/a \right) \right] + 1 \]  

(5.14)

The value of \( D \), by definition, cannot be less than 1, so it is immediately apparent that there is a lower limit for \( C_m^R \) (equal to \( C_m^S \cdot R_1/a \)) for successful application of the method. However, as \( C_m^R \) approaches this limit the value of \( D \) required becomes very large and there are two practical difficulties associated with large values of \( D \). Firstly, the sensitivity, i.e. the slope of the calibration plot, is inversely proportional to \( D \) and thus as \( D \) increases, sensitivity decreases and the uncertainty in the interpolated value at \( \Delta A = 0 \) (see Figure 5.5) increases. Eventually, of course, at large values of \( D \), \( \Delta A \) becomes indistinguishable from the noise on the signal.

The second problem concerns the way in which dispersion is increased. If \( D \) is increased by increasing the length of tubing between injector and nebuliser then the peak is broadened and thus the time between injections must be increased to avoid carry-over, and cross contamination. If \( D \) is increased by decreasing the volume injected then precision becomes a problem as small changes in the volume injected cause large changes in the value of \( D \) (see Figure 2.11, Chapter 2, page 67). There is also a minimum volume which can be injected due to the mode of construction of the injection valve. There are thus a number of
Table 5.1. Results for analysis of British Chemical Standard Steels by treating with hydrofluoric acid for complete dissolution by the Flow Injection Standard Additions Method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chromium found, %</th>
<th>Certified value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS 220/2</td>
<td>5.12, 5.14, 5.16</td>
<td>5.12</td>
</tr>
<tr>
<td>BCS 261/1</td>
<td>17.4, 17.6, 17.5</td>
<td>17.4</td>
</tr>
</tbody>
</table>

Table 5.2. Results for analysis of British Chemical Standard Steels by the Flow Injection Standard Additions Method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chromium found, %</th>
<th>Certified value, %</th>
<th>η</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS 261/1</td>
<td>17.4 ± 0.1</td>
<td>17.4</td>
<td>5</td>
</tr>
<tr>
<td>BCS 241/2</td>
<td>5.34 ± 0.02</td>
<td>5.35</td>
<td>5</td>
</tr>
<tr>
<td>BCS 220/2</td>
<td>5.13 ± 0.02</td>
<td>5.12</td>
<td>5</td>
</tr>
<tr>
<td>BCS 251/1</td>
<td>0.51 ± 0.03</td>
<td>0.51</td>
<td>2</td>
</tr>
<tr>
<td>BCS 254/1</td>
<td>0.27 ± 0.01</td>
<td>0.27</td>
<td>2</td>
</tr>
<tr>
<td>BCS 255/1</td>
<td>0.19 ± 0.03</td>
<td>0.19</td>
<td>2</td>
</tr>
</tbody>
</table>

The terms ‘±’ indicate the 95% confidence limits about the mean for two to five replicate analyses.

Table 5.3. Results for analysis of British Chemical Standard Steels by the Flow Injection Reagent Additions Method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chromium found, %</th>
<th>Certified value, %</th>
<th>η</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS 261/1</td>
<td>17.4 ± 0.1</td>
<td>17.4</td>
<td>5</td>
</tr>
<tr>
<td>BCS 241/2</td>
<td>5.34 ± 0.07</td>
<td>5.35</td>
<td>5</td>
</tr>
<tr>
<td>BCS 220/2</td>
<td>5.12 ± 0.06</td>
<td>5.12</td>
<td>5</td>
</tr>
</tbody>
</table>
practical restrictions on an upper value of $D$ and so it appears sensible to select $D$ with due regard to sensitivity, peak width and precision and then to calculate the concentration of interferent required from equation (5.13). This may mean that interferent has to be added to samples, if the concentration is not high enough, as was done in three of the samples described here.

Table 5.3 shows the results obtained by reagent additions method for comparison with standard additions method. A dispersion of 4 was used and thus the concentration of iron added to the carrier was 210 ppm or greater.

Rearrangement of equation (5.7) gives:

$$D = \frac{C_{t}^{std} \cdot R_{1/a} / C_{m}^{R}}{D} + 1$$  (5.15)

Thus, the value of $D$ cannot be less than 1, and as $C_{m}^{R}$ approaches zero the value of $D$ becomes very large, and the same difficulties associated with large values of $D$ occur.

5.5. Conclusion

There are several advantages in using reagent additions and analogue of standard additions in flow injection-atomic absorption techniques. In both methods, the standard and sample solutions are always in the same matrix effects before the absorbance concentration measurements are made. The calculation of unknown by interpolation is an advantage over normal standard additions method which
uses a less accurate extrapolation technique. These methods also reduce the manipulation of volumetric glassware and thus, increase the speed of operation and reduce the amount of sample, standards and reagents used.

Two conditions determine the success of these methods, i.e.,

i) The correct dispersion must be used for the sample and the standards with due regard to sensitivity, peak width (or peak height) and precision.

ii) The measurement must be made on the plateau of the plot of the interfering element (see Figure 5.1), i.e., the carrier must always contain enough amount of interfering element or elements in order to give a constant depression for all the standard and the sample solutions.

That the first condition is obtained can be determined from the shape of the calibration graph; if insufficient dispersion is used, a slightly S shape calibration graph is obtained.

Several minor problems are observed in these methods, i.e., uncertainty in determining the absorbance of the chromium sample in the standard additions method because of noise levels, a blank solution with the appropriate amount of interfering element must be used as carrier stream in the reagent additions method and measurements are always made in the presence of interferences, thus, decreasing the sensitivity of the chromium signal.
Some aspects of this work have been published in the Analyst, Volume 108, 1983, pages 153 to 158, in the Analytica Chimica Acta, Volume 145, 1983, pages 159 to 168 and in the Analyst in press.
REFERENCES

16) Green, H. C., Analyst, 100(1975)640-642.
Chapter 6

METHODS OF CALIBRATION

6. Introduction

The aims of this chapter is to demonstrate several methods of constructing calibration graphs for evaluation of unknown concentrations of samples by flow injection methods and to discuss the characteristics of each of the calibration graph in atomic absorption spectrometry. The useful range of the graphs and their limitations are also discussed.

According to Beer's Law the absorbance of an absorbing species is proportional to its concentration and this can be represented by a straight line, on a calibration graph, through a series of absorbance, concentration points. Assuming the calibration is linear between the origin and the absorbance-concentration points of standards, the concentration of an unknown solution can be obtained from the calibration graph by interpolation. Often the linear relationship between the absorbance and the concentration is only true at low absorbance values and deviations from linearity towards the concentration axis are usually apparent as the absorbance increases. The reason for the non-linearity of these calibration graphs have been discussed in some textbooks (e.g., 1, 2). This graphical curvature must be accommodated either by reducing the concentration range being studied or by resorting to electronic means of compensation.
Generally, the unknown concentration of an analyte can be determined by three techniques in AAS. These are the standard working curve method, the standard additions method and the internal standards method. The last of these methods is used more frequently in emission analysis because of the difficulty of measuring more than one element at a time by absorption.

6.1. **Standard Working Curve Method**

The most common technique for quantitative analysis is to construct a standard working curve using known amounts of the desired element in a solution with a similar composition to the unknown. It is critical that the composition or the matrix of the standards be as close as possible to that of the unknown, because different solutions have different types of interferences that affect the absorbance.

If absorbance is a linear function of concentration, the general form of the equation of a straight line is represented as

\[ A = mC + b \]

where \( A \) is the absorbance at concentration \( C \), \( m \) is the slope of the calibration and \( b \) is the intercept on the absorbance axis due to the blank. Ideally, the calibration line passes through the origin and follows the equation of \( A = mC \), but because of the existence of uncertainties in the points along the calibration curve, caused by systematic and random errors and also there may be a significant blank absorbance, the first equation is more frequently used.
Asterdenbos (3) has suggested that several statistical conditions have to be fulfilled first in order to get accurate results from a calibration curve. The precision of the concentration values was much better than the precision of the measurement of the absorbance values. Values found for the absorbance for parallel determinations at the same concentration value have a Gaussian distribution. The standard deviation of the measured absorbance values must be the same for the whole range of concentration values covered by the calibration curve, i.e., the standard deviation of the absorbance should be independent of concentration. The influence of the distribution of calibration measurements over the concentration range, the location of the concentration of the sample within this range and the number of replicate sample measurements have also been discussed and considered under certain conditions (4).

The non-linearity of typical calibration plots for atomic absorption spectrometers and the possible bias involved in drawing a smooth curve through the calibration points leads to significant errors in estimation of the metal content of test solution. A simple numerical method for evaluation of data from AAS which assumes an exponential relationship between absorption reading and concentration has been described by Andrews and Jowett (5). The exponential expression for the calibration curve was given as

$$A = A^0 \left[1 - \exp(-BC)\right],$$

where $A$ is the absorption reading of a solution with metal concentration $C$, $A^0$ is a parameter having the dimension of $A$, and $B$ is a parameter having the inverse dimension of $C$. This expression can be linearized for fitting to a set of data to find the estimators.
of the parameters in the equation. Criteria for the goodness of fit between equation and the data were discussed.

Harnly and O'Haver (6) have used simultaneous multielement atomic absorption with a continuum source to extend the linearity of the calibration curves in high analyte concentrations. The advantage of a continuum source when used with a wavelength modulation technique is that absorption measurements can be made at a series of wavelengths at selected distances from the centre of the line profile. A technique for an extension of calibration curves by using the Zeeman effect has been described by Veinot and Stephens (7). By applying a magnetic field to the light source and by increasing its strength the upper limit of the calibration curve may be extended without moving the spectrometer off the optimum resonance line. This technique is of potential value in extending the working range of AAS especially for instruments employing the Zeeman effect for background correction.

Mandel and Linnig (8) have studied a basis for judging the reliability of the slope, intercept and any value derived from the calibration line by using the method of joint confidence regions. The points for slope and intercept were obtained by least squares method and the joint confidence region was constructed by using these points and some other quantities in the equation of ellipse.
Aarons (9) has suggested that the effect of the number and arrangement of the calibration standards in experiments, known as the experimental design, on the calibration graph influenced the precision with which the parameters of the calibration line can be estimated. A general theoretical approach of the experimental design has been developed and applied in a variety of situations which involved the construction of a calibration graph over a finite range of concentration. The optimised design would have the most benefit where only a limited number of standards was possible or desirable. The precision obtained by using this design would become less and less as the number of calibration standards increased.

Schwartz (10) has described that in a linear calibration curve, the unknown value of the analyte, $X_i$, can be calculated from the equation $X_i = \bar{x} + (Y_i - \bar{y})/b$, where $\bar{x}$ and $\bar{y}$ are the averages of $x_i$ and $y_i$ respectively, $b$ is the slope and $Y_i$ is the measurement response from the analyte. Methods of estimating confidence limits associated with $X_i$ in linear and non-linear calibration curves have also been described (10).

Some factors affecting the shape of analytical curves in AAS have been discussed in detailed by Rubeska and Svoboda (11). Factors related to properties of spectral lines, such as the hyperfine structure of the line, the ratio of the absorption and emission line widths and the resonance line broadening and line shift in the absorbing medium have been considered. An empirical equation expressing the analytical curves for different ratios of emission
line width to absorption line width has also been given. Some factors causing the bending of the analytical curves in atomic emission flame spectrometry such as self-absorption, ionization, compound formation, variation in solution flow rate and atomization efficiency, entrance optics and the effect of measuring spectral line multiplets, have been described by Vickers, Remington and Winefordner (12). De Galan and Samaey (13) have proved theoretically and demonstrated experimentally that the atomic absorption analytical curves would be bent towards the concentration axis if more than one spectral line emitted by the source of radiation falls within the spectral bandwidth of the instrument, unless all radiation is absorbed in the flame to exactly the same extent. Incomplete volatilization which may be due to the flame temperature may also lead to the bending of the analytical curve (13). The effect was more significant in the presence of interfering ions in the sample matrix or if the concentrated analyte solutions were employed (13). A reduction of flame path-length by rotation of the burner head in flame AAS has also affected the linear range of the analytical curve (14). Rotation of the burner may increase the linear range at the upper end of the curve and may also increase the linear range at the lower end of the analytical curve (14).

Van Dalen and De Galan (15) have proposed the formulation of analytical procedures involving flame AAS that take account of the sensitivity, precision and shape of the analytical calibration graph. It has been suggested that the analytical procedure should not be limited to the linear portion of the calibration graph, but
rather to the useful concentration range that was derived from the the acceptable precision (15). General procedures for measuring and maximizing precision in calibration graph analysis based on confidence-band statistics have been presented (16, 17, 18). In calibration graph analyses, confidence bands around both the sample signal and the calibration graph can be calculated to yield a confidence band around the predicted concentration and to estimate the precision of the sample analysis.

Butler (19) has discussed the importance of analytical range and has proposed that it be defined in terms of the best precision of measurement obtained over a concentration range multiplied by a factor acceptable to the analyst. The factor causing a decrease in precision of measurement at lower and higher concentration ranges with respect to AAS and AES using inductively coupled plasma source has also been discussed (19).

Kateman (20) recently has summarized some new developments and modifications of well-known calibration techniques in analytical chemistry. The three main lines of development in statistical methods, in mathematical treatment and in hardware for correction of interference effects.

The calibration by linear regression and by calculation of the slope and intercept from the means of replicates blanks and top standards has been discussed by Hilton et. al (21). The rectilinear range has been described as the range of concentration over which the slope and intercept of a calibration line were not significantly different
for the two methods at the required confidence level.

6.1.1. Least-squares Method (22)

If there are n pairs of data \((C_1, A_1), (C_2, A_2), \ldots \ldots (C_n, A_n)\), where \(C\) is the concentration of the analyte and \(A\) is the absorbance measurement, and there is a linear relationship between the concentration of the analyte and the absorbance measurement, the equation of the calibration line will be in the form

\[ A = mC + b \]  

where \(m\) is the slope and \(b\) is the intercept on the absorbance axis. \(m\) is calculated from the equation

\[ m = \frac{\sum(C_i - \bar{C})(A_i - \bar{A})}{\sum(C_i - \bar{C})^2} \]  

where \(\bar{C}\) and \(\bar{A}\) are the means of the \(C_i\) and \(A_i\) data respectively. Intercept \(b\), can be calculated from equation \(b = \bar{A} - m\bar{C}\), where \(m\) has been found. Substituting \(\bar{A} - m\bar{C}\) for \(b\) in equation (6.1), gives the equation of the line as

\[ A - \bar{A} = m(C - \bar{C}) \] 

Detailed discussion of the calculation of the best straight line by the method of least squares has been given by York (23), Wentworth (24, 25) and Pattengill and Sands (26).

6.2. Standard Additions Method

In the standard additions method, known amounts of the element of interest are added to the sample analyte and the increase in signal is measured. Each solution is diluted to the same total
volume and should have the same final composition (except for 
analyte concentration). If the absorbance is linearly related to 
concentration and if the concentration of unknown is \( C_x \) and the 
concentration of added standard is \( C_s \), then,

\[
\frac{C_x}{(C_x + C_s)} = \frac{A_x}{A_{x+s}}, \quad \text{and} \quad C_x = \frac{A_x C_s}{(A_{x+s} - A_x)} \tag{6.4}
\]

where \( A_x \) and \( A_{x+s} \) are the absorbances of the unknown and the 
unknown plus standard respectively. Alternatively, a series of 
standard additions can be made and a calibration graph can be 
constructed to find the concentration of unknown. The unknown 
concentration can be obtained at the intercept of the extrapolation 
line at the concentration axis, i.e., when \( A_{x+s} = 0 \), equation 
(6.4) becomes \( C_x = -C_s \).

Although the main advantage of the standard additions method is 
that the matrix remains constant for all samples and standards, 
the accuracy of an extrapolation method is never as good as an 
interpolation method because the value found may be well include 
background and scatter signals.

A statistical approach on the evaluation of the standard additions 
method in AAS using linear regression analysis has been discussed 
by Tiam, Diehl and Harbach (27, 28). Franke, de Zeeuw and Hakkert 
(29) have evaluated and optimised the standard additions method 
for AAS based on the assumption of the equality of the coefficients 
of variation rather than the equality of the variance. The optimum \textit{precision} 
can be obtained by applying a single addition of the largest 
possible concentration of standard within the linear range of the 
response concentration curve (29). An approximation to the experi-
mental error of the standard additions method based on linear least squares regression has been presented by Larsen et. al (30).

Several possible variations with linear and non-linear response instruments for the standard additions method procedure have been presented by Bader (31). Among the variations presented were continuous variation of standard at constant total volume, continuous variation of unknown at constant total volume, continuous variation of both unknown and standard at constant total volume, variation of volume of single addition of standard and variation of total volume with continuous variation of standard.

6.3. Internal Standards Method

In the internal standards method, the ratio of the absorbance for the known mixtures of the internal standard element $C_s$ and analyte, $C_x$, are measured to construct a standard curve. The concentration of the unknown can be obtained from the calibration curve when the amount of internal standard added, $C_s$, to an unknown sample is known.

\[
\frac{\text{Absorbance ratio in standard mixture}}{\text{Concentration ratio in standard mixture}} = \frac{\text{Absorbance ratio in unknown}}{\text{Concentration ratio in unknown}}
\]

or

\[
\frac{R_x/C_s}{C_x/C_s} = \frac{R_x/C_s}{R_x/C_s}
\]

or

\[
C_x = \frac{R_x/C_s \cdot C_s/R_x}{C_s}
\]

(6.5)
Internal standardization is normally used to correct for variations in nebulizer performance and flame characteristics. A detailed study on the selection of an internal standard in flame AAS has been described by Takada and Nakano (32).

6.4. Flow Injection Analogue of the Standard Additions Method

This method is used in conjunction with flow injection analysis, where standards are injected in sequence into the sample carrier stream (see Chapter 3, Section 3.6, page 101, Chapter 4, Section 4.1, page 111 and Chapter 5, Section 5.1.2). The concentration of the injected standard at the peak maximum is given by

\[ C_{p}^{\text{std.}} = C_{m}^{\text{std.}} \left[ 1 - \exp\left( -\frac{V_{i}}{V} \right) \right] \]  \hspace{1cm} (6.6)

and the concentration of the analyte in the sample carrier stream at the peak maximum is given by

\[ C_{p}^{s} = C_{m}^{s} \exp\left( -\frac{V_{i}}{V} \right) \] \hspace{1cm} (6.7)

where \( C_{m}^{\text{std.}} \) is the concentration of the standard, \( C_{m}^{s} \) is the concentration of the analyte in the sample carrier stream, \( V_{i} \) is the volume injected and \( V \) is the volume of the hypothetical mixing chamber.

Thus, the concentration at peak maximum is obtained by combining equations (6.6) and (6.7) to give
\[ c_p = c_{\text{std.}} \left[ 1 - \exp(-V/V) \right] + c_{\text{a}}^s \exp(-V/V) \]  

(6.8)

The change in concentration at peak maximum, \( \Delta C \), is given as

\[ \Delta C = c_{\text{std.}} \left[ 1 - \exp(-V/V) \right] + c_{\text{a}}^s \exp(-V/V) - c_{\text{a}}^s \]  

(6.9)

\[ D = \left[ 1 - \exp(-V/V) \right]^{-1}, \text{ thus,} \]

\[ \Delta C = D^{-1}(c_{\text{std.}} - c_{\text{a}}^s) \]  

(6.10)

If absorbance is a linear function of concentration, then, equation (6.10) becomes

\[ \Delta A = (\lambda/D)(c_{\text{std.}} - c_{\text{a}}^s) \]  

(6.11)

If \( c_{\text{std.}} > c_{\text{a}}^s \), \( \Delta C > 0 \), if \( c_{\text{std.}} < c_{\text{a}}^s \), \( \Delta C < 0 \), and if \( c_{\text{std.}} = c_{\text{a}}^s \), then \( \Delta C = 0 \). Thus, a graph of \( \Delta C \) versus \( c_{\text{std.}} \) will intercept the \( c_{\text{std.}} \) axis at \( c_{\text{a}}^s \).

Since the normal use of standard additions method in AAS is to compensate for interference effects in the sample, in the flow injection method, the dispersion must be designed so that the interference effects in the sample stream operate to the appropriate extent on the injected standards. This can be achieved by calculating the amount of reagent or interferent needed to give a constant depression to the analyte in the sample and in the standard.
(usually top standard) for a chosen dispersion. The relationship between the relevant parameters for this calculation has been given in Chapter 5, Section 5.1.2, page 149. The use of this method of calibration was applied successfully in the determination of calcium in iron ores (see Chapter 4) and in the determination of chromium in steels (see Chapter 5).

Figure 6.1 and 6.2 show typical peaks observed in the flow injection analogue of the standard additions method and its associated calibration graph. The concentration of the analyte of the sample is found from the intercept on the concentration axis.

The advantages of this method have been discussed in Chapter 4 and Chapter 5.

6.5. Variable Dispersion Calibration Method

As previously mentioned, dispersion is defined as the ratio of the concentration of injected analyte to the concentration of the peak maximum, and, the dispersion may be varied either by changing the tube dimensions (i.e., tube length and tube diameter) or by changing the volume injected. Although dispersion may also be varied by changing the pumping rate of the carrier, this is not recommended for AAS because the performance of the atomic absorption instrument depends on the aspiration rate of the nebuliser. Increasing the dispersion by changing the tube dimensions may reduce the speed of analysis as it requires alteration of the length or the diameter of the tube in the flow injection system. Changing
Tube length = 200 cm.
Flow rate = 5.95 ml/min.
Volume injected = 50 μl.

Figure 6.1. Typical chart recording for the flow injection standard additions method. The sample contained about 10 ppm chromium and the injected standards covered the range 0-18 ppm in 3 ppm increments.
Figure 6.2. A typical calibration plot for the flow injection standard additions method ($\Delta A$, change absorbance vs. $C_{\text{std.}}$, concentration of standard). The sample concentration, $C_s^a$, is obtained from the intercept on the $C_{\text{std.}}$ axis.
the volume injected is a simple way of varying the dispersion and allows the speed of analysis to be maintained in the flow injection techniques. Another advantage of changing the volume injected is that it can be used to construct a calibration curve from a single concentration of standard. This idea of injecting variable volume at constant concentration has been used by Serbeck et. al (33) and Chuang et. al (34) to construct the analytical curve in flame spectrometry. Although the FIA method was not yet introduced by then, both of them have used a technique which was very similar to FIA. They obtained a linear analytical curve when a series of volumes of standard was injected into a flowing carrier stream.

When a concentration of standard is injected into a large dispersion system, which can be achieved either by increasing the tube dimensions or by using a real mixing chamber, a gradient of concentration is produced. This concentration gradient, which is at the rising part of the peak, can be used to construct a calibration curve. The use of small volume injection to obtain a large dispersion is not recommended because of its limited rising part of the peak it produces.

6.5.1. Injection of Variable Volumes at Constant Concentration

From previous equation (see Chapter 3, Section 3.1.1., page 84) the concentration-time profiles is given by

\[ C = C_m \left[ 1 - \exp\left(-\frac{ut}{V}\right) \right] \]  

(6.12)
Provided that the absorbance has a linear relationship with concentration (i.e., this has to be determined experimentally), equation (6.12) can be converted to the form of

\[ A = A_m^s \left[ 1 - \exp(-ut/V) \right] \]

If a series of volumes of a single concentration is injected into a carrier at a particular tube dimensions and flow rate, at peak maximum, \( t = V_1/u \), and equation (6.12) would become

\[ C_p^s = C_m^s \left[ 1 - \exp(-V_1/V) \right] \]

or \( A_p^s = A_m^s \left[ 1 - \exp(-V_1/V) \right] \)

The volume of mixing chamber, \( V \), can be calculated from the slope of the linear curve of \( V_1 \) against \( \ln \frac{A_m^s}{A_m^s - A_p^s} \) as shown in Figures 6.3a and 6.4a. Once the volume of mixing chamber, \( V \), is known, the concentration at peak maximum, \( C_p^s \), for various volumes injected can be calculated from equation (6.13) and the absorbance-concentration relationship at peak maximum can be constructed as shown in Table 6.1 and Table 6.2 and their respective calibration curves in Figure 6.3b and Figure 6.4b.

If an analyte is injected with known volume and at the same conditions as above, the concentration at peak maximum of an analyte can be obtained from its absorbance by interpolation from the calibration graph of \( A_p^s \) versus \( C_p^s \). The initial concentration of the analyte can be calculated by substituting all the...
Figure 6.3a. A plot of volume injected, $V_1$, vs. $\ln A_m^S/(A_m^S-A_p^S)$, at tube length 2.6 cm and flow rate 115.8 µl/sec.

Figure 6.3b. A plot of absorbance at peak maximum, $A_p^S$, vs. concentration at peak maximum, $C_p^S$.
Figure 6.4a. A plot of volume injected, $V_1$, vs. $\ln A^S_m/(A^S_m-A^S_p)$, at tube length 200 cm and flow rate 115.8 $\mu$l/sec.

Figure 6.4b. A plot of absorbance at peak maximum, $A^S_p$, vs. concentration at peak maximum, $C^S_p$. 

Absorbance, $A^S_p$
Table 6.1. The absorbance-concentration relationship at the peak maximum for various volumes injected, at tube length of 2.6 cm and flow rate of 115.8 µl/sec. for 0.5 ppm Mg.

<table>
<thead>
<tr>
<th>Volume injected, $V_i$ µl</th>
<th>Absorbance, $A^s_p$</th>
<th>Concentration, $C^s_p$, ppm</th>
<th>Dispersion, D</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.3</td>
<td>0.099</td>
<td>0.066</td>
<td>7.57</td>
</tr>
<tr>
<td>24.8</td>
<td>0.138</td>
<td>0.115</td>
<td>4.35</td>
</tr>
<tr>
<td>49.9</td>
<td>0.249</td>
<td>0.205</td>
<td>2.44</td>
</tr>
<tr>
<td>101.3</td>
<td>0.361</td>
<td>0.328</td>
<td>1.52</td>
</tr>
<tr>
<td>149.5</td>
<td>0.406</td>
<td>0.397</td>
<td>1.26</td>
</tr>
<tr>
<td>205.5</td>
<td>0.448</td>
<td>0.443</td>
<td>1.13</td>
</tr>
<tr>
<td>298.4</td>
<td>0.461</td>
<td>0.478</td>
<td>1.05</td>
</tr>
<tr>
<td>501.5</td>
<td>0.489</td>
<td>0.497</td>
<td>1.01</td>
</tr>
<tr>
<td>1001.3</td>
<td>0.501 = $A^s_m$</td>
<td>0.500</td>
<td>1.00</td>
</tr>
</tbody>
</table>

values of $C^s_p$, $V_i$ and $V$ in equation (6.13). An example of the results obtained is shown in Table 6.1 for various volumes and concentrations of standard magnesium.

As shown in Figures 6.3a and 6.4a, the curves of $V_i$ against $\ln \frac{A^s_m}{(A^s_m - A^s_p)}$ do not give straight line at high $V_i$. This is especially true at low dispersion with large volume of injection. The reason is probably because the diffusion rate of the analyte and the carrier is not the same for different volume of injection. Since the rate of diffusion of the solution is inversely proportional to the square root of its density (which is directly related to concentration), a larger volume of injection would
represent higher concentration in the stream, thus, slows down the diffusion rates, and would produce higher signal than expected.

Table 6.2. The absorbance-concentration relationship at the peak maximum for various volumes injected, at tube length of 200 cm and flow rate of 115.8 µl/sec. for 6.5 ppm magnesium solution.

<table>
<thead>
<tr>
<th>Volume injected, (V_1, \mu l)</th>
<th>Absorbance, (A_p^s)</th>
<th>Concentration, (C_p^s, ppm)</th>
<th>Dispersion, (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.3</td>
<td>0.035</td>
<td>0.032</td>
<td>16.34</td>
</tr>
<tr>
<td>24.8</td>
<td>0.054</td>
<td>0.057</td>
<td>9.17</td>
</tr>
<tr>
<td>49.9</td>
<td>0.106</td>
<td>0.108</td>
<td>4.84</td>
</tr>
<tr>
<td>101.3</td>
<td>0.193</td>
<td>0.196</td>
<td>2.67</td>
</tr>
<tr>
<td>149.5</td>
<td>0.260</td>
<td>0.260</td>
<td>2.01</td>
</tr>
<tr>
<td>205.5</td>
<td>0.326</td>
<td>0.317</td>
<td>1.65</td>
</tr>
<tr>
<td>298.4</td>
<td>0.403</td>
<td>0.384</td>
<td>1.36</td>
</tr>
<tr>
<td>501.5</td>
<td>0.499</td>
<td>0.457</td>
<td>1.14</td>
</tr>
<tr>
<td>1001.3</td>
<td>0.523</td>
<td>0.496</td>
<td>1.05</td>
</tr>
</tbody>
</table>

But because the residence time of a large volume is longer than a small volume (large volume of standard fills a longer tube than a smaller volume of standard) in the flow injection system before reaching the detector, the end boundary or end part of the standard would experience more dispersion and diffusion than the front part of the standard, and thus, reduces the concentration...
and the signal. This is illustrated in Figure 6.5.

Table 6.3. An example of the results obtained for various volumes and concentrations of standard magnesium from the calibration graph of $A_p^s$ versus $C_p^s$.

<table>
<thead>
<tr>
<th>Volume injected, $V_i, \mu l$</th>
<th>Absorbance, $A_p^s$</th>
<th>$C_p^s$, found from curve, ppm</th>
<th>$C_m^s$, found, ppm</th>
<th>$C_m^s$, injected, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>49.9</td>
<td>0.018</td>
<td>0.02</td>
<td>0.11</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>0.062</td>
<td>0.06</td>
<td>0.30</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>0.143</td>
<td>0.14</td>
<td>0.64</td>
<td>0.7</td>
</tr>
<tr>
<td>101.3</td>
<td>0.038</td>
<td>0.04</td>
<td>0.11</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>0.119</td>
<td>0.12</td>
<td>0.31</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>0.261</td>
<td>0.25</td>
<td>0.65</td>
<td>0.7</td>
</tr>
<tr>
<td>205.5</td>
<td>0.070</td>
<td>0.07</td>
<td>0.11</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>0.204</td>
<td>0.20</td>
<td>0.31</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>0.433</td>
<td>0.42</td>
<td>0.66</td>
<td>0.7</td>
</tr>
</tbody>
</table>

As a result of this effect, the peaks obtained at large volume of injection flatten at their tops as shown in Figure 6.6.

If a longer tube length is used, the difference of residence time between part (a) and part (c) (see Figure 6.5) is negligible compared to the time it travels from the injection point to the detector and this effect is eliminated, as shown in Figure 6.7.
Figure 6.5. Effect of dispersion on large and small volumes of injection. The total dispersion and diffusion is much higher at the end of part (c) than at the front of part (b) due to the longer residence time of part (c) than part (b).

6.5.2. Injection of Constant Volume at Constant Concentration

If distilled water is used as a carrier stream when injecting an analyte at known dispersion (i.e., $D = c_m^S/c_p^S$ or $A_m^S/A_p^S$), the initial concentration of the analyte can also be calculated by multiplying the dispersion value with the concentration at peak maximum obtained from interpolation of the curve $A_p^S$ versus $c_p^S$. From this principle, it is possible to construct a calibration curve based on the variation of the dispersion, either by changing
Figure 6.6. Effect of sample volume at short tube length (i.e. 2.6 cm), on the shapes of the peak. Volume injected, 13, 25, 50, 100, 150, 200, 300, 500 and 1000 µl.
Figure 6.7. Effect of sample volume at long tube length (i.e. 200 cm), on the shapes of the peak. Volume injected, 13, 25, 50, 100, 150, 200, 300, 500 and 1000 µl.
the volume of injection as above, or by changing the tube dimensions or by changing the combination of both. An example of a calibration curve constructed from the variation of the dispersion (i.e., by varying the volume and the tube length) is shown in Table 6.4 and its calibration graph is shown in Figure 6.8. A calibration graph

Table 6.4. An example of a calibration graph for 0.5 ppm magnesium standard constructed from the variation of dispersion, based on changing the tube length and volume injected.

<table>
<thead>
<tr>
<th>Volume injected, $V_i$</th>
<th>Tube length, cm.</th>
<th>Dispersion, $D$</th>
<th>Absorbance, $A_p$</th>
<th>Concentration, $C_p$, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.8</td>
<td>200</td>
<td>9.68</td>
<td>0.054</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.20</td>
<td>0.116</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.31</td>
<td>0.177</td>
<td>0.15</td>
</tr>
<tr>
<td>49.9</td>
<td>100</td>
<td>2.91</td>
<td>0.207</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>2.52</td>
<td>0.240</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>2.22</td>
<td>0.272</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.99</td>
<td>0.294</td>
<td>0.25</td>
</tr>
<tr>
<td>101.3</td>
<td>100</td>
<td>1.73</td>
<td>0.349</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.39</td>
<td>0.434</td>
<td>0.35</td>
</tr>
<tr>
<td>205.5</td>
<td>100</td>
<td>1.21</td>
<td>0.498</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>1.10</td>
<td>0.548</td>
<td>0.45</td>
</tr>
<tr>
<td>298.4</td>
<td>60</td>
<td>1.05</td>
<td>0.573</td>
<td>0.48</td>
</tr>
</tbody>
</table>

based on normal nebulization is also shown for comparison.

A calibration method based on the variation of dispersion is
Figure 6.8. A calibration curve constructed from the variation of the dispersion i.e., by varying the volume injected and the tube length. For comparison, a calibration curve obtained by normal nebulization is also shown.
and fast, i.e., only one standard concentration is needed to construct a range of calibration line within the standard selected, by varying the volume injected or tube dimensions, or the combination of both. It also gives better precision in terms of signals obtained and more accurate, i.e., by reducing errors in the preparation of standard, than normal method.

6.5.3. Concentration Gradient Formation

If a suitably chosen standard is injected into a water flowing stream with a real mixing chamber located between the injection point and the detector, the standard solution is subjected to a large dispersion and a concentration gradient is formed as a function of time along the rising part and the falling part of the peak according to

\[ C = C_{\text{std.}} \left[ 1 - \exp(-ut/V_m) \right] \text{for } t < t_m \]  

(6.14)

for the rising part, where \( t \) is less than \( t \) at the peak maximum (i.e., \( t_m \)), and,

\[ C_p = C_{\text{std.}} \left[ 1 - \exp(-V_i/V_m) \right] \text{for } t = t_m \]  

(6.15)

and,

\[ C = C_p \exp[u(t - t_m)/V_m] \text{for } t > t_m \]  

(6.16)

where \( V_m \) is the volume of mixing chamber, \( u \) is the flow rate and
If absorbance is directly proportional to concentration, the absorbance-time profile can be converted to the concentration-time profile. This profile can be used to construct a calibration curve.

If the various absorbances on the rising part of the peak of the standard are plotted against time, and the unknown signal is obtained at the same conditions as the standard, either by direct nebulization or by injecting a discrete volume, the concentration of the unknown can be calculated as follows:

i) The time (minus t error) for the unknown is obtained by interpolation from the absorbance versus time curve.

ii) For normal nebulization, the unknown concentration can be calculated from equation (6.14), where $C_{\text{std.}}$, $u$ and $t$ being known. $V_m$ is obtained by analysing the absorbance-time profile of the rising part of the peak to the steady state signal according to equation

$$t = \frac{V_m \ln[A_{\text{std.}}/(A_{\text{std.}} - A)]}{u}$$  \hspace{1cm} (6.17)

A plot of $t$ against $\ln[A_{\text{std.}}/(A_{\text{std.}} - A)]$ in equation (6.17) would give a slope of $V_m/u$.

iii) For unknown obtained by injection through flow injection
system, the signal corresponding to the initial concentration of the unknown must first be calculated by using equation

\[ A_s^m = A_p^s \left[ 1 - \exp(-ut/V_m) \right]^{-1}, \text{ or } A_m^s = A_p^sD. \]

Once \( A_m^s \) is known, its time (minus \( t_{\text{error}} \)) can be obtained by interpolation from the same curve and the initial concentration of the unknown can be calculated by using equation (6.14).

The methods above are illustrated in Figure 6.9. The unknown signal obtained by injection would probably be under exactly the same conditions as the standard in flow injection system and would represent the better way than obtaining the unknown signal by normal nebulization. This is because of the difficulty in obtaining the unknown signal by normal nebulization without disturbing the FIA-AAS system and at the same time maintaining its settings, especially the aspiration rate to the instrument.

Although the method of constructing a calibration graph by using a gradient forming device has not been tested extensively yet, some positive results have been obtained (35, 36).
Figure 6.9. Methods of determining the concentration of unknown based on a calibration curve of absorbance vs. time, by volume injected and normal nebulization.
REFERENCES


35) Tyson, J. F., Appleton, J. M. H., and Idris, A. B., Analyst,
FLOW INJECTION - SOLVENT EXTRACTION TECHNIQUE

7. A Brief Introduction

The use of solvent extraction in AAS as a concentration technique and to remove the analyte element from potential concomitant chemical, physical and other matrix interferences has been described by Cresser (1). Solvent extraction methods for use with flame AAS has also been described by Kinrade and Van Loon(2). Several characteristics, such as the choice of buffer and the choice of chelating agent have also been described (2).

Allan (3) has discussed the effect of organic solvent on the concentration of the atom and the temperature of the flame. The extent of increase in sensitivity depends on the types of solvent used and the atomizer. Ammonium pyrrolidinedithiocarbamate (APDC) reagent has been used to complex copper prior to extraction into an organic solvent (4). Many heavy metals can be chelated with APDC and extracted into an organic solvent. Methyl isobutyl ketone (MIBK) or 4-methyl-2-pentanone was found to be a satisfactory solvent for this procedure used with AAS. By using a small volume of MIBK, it would be possible to concentrate the element being determined and increase the sensitivity.

Factors such as acidity and extraction ratio which influence the extraction efficiency must be equalized between samples and
standards to eliminate errors due to the solvent extraction procedure (5). The solubility of MIBK in water, for example, is influenced by the acidity.

Subramanian and Meranger (6) have determined several trace metals in drinking water by solvent extraction with APDC-MIBK and graphite furnace AAS. Parameters such as the effect of pH of the aqueous phase prior to extraction, extraction efficiency, the optimum APDC concentration, the length of time needed for complete extraction and the time-stability of the chelate in the organic phase have been investigated in detailed (6). The decomposition of APDC in aqueous medium has been shown as

$$\text{R}_1\text{N}^-\text{C}^\text{S}_2\text{S} \xrightleftharpoons{H^+}\text{R}_1\text{N}^+\text{C}^\text{S}_2\text{S} \rightarrow \text{R}_1\text{N}^+\text{G}^\text{S}_2 \rightarrow \text{NH}_2 + \text{CS}_2$$

and pH dependent. The complexation efficiency of APDC is considerably influenced by its hydrolytic stability. The stability of the APDC complex for several metals in the MIBK phase after extraction from aqueous solution at various pH values has also been presented (7). The use of APDC for the extraction of copper from a wide range of aqueous solutions into organic solvent suitable for direct AAS determination has been recommended by the Analytical Methods Committee (8). Copper-APDC complex is readily extracted from aqueous solution within the range of strongly acidic to pH 10 into polar organic solvents (8).
The construction and performance of extraction system in flow injection analysis have been described by Karlberg and Thelander (9). The effects of sample dispersion, the length of mixing coil (as short as possible to prevent dispersion of the injected sample), flow rates and the ratio of organic to aqueous phase on the sensitivity of the method have also been discussed (9). This system has been applied successfully in the determinations of codeine as the picrate ion-pair in acetylsalicylic acid tablets (10), vitamin B₁ (Thiamine) by thiochrome method (11) and extraction constants (12).

Nord and Karlberg (13) have designed several membrane phase separators and have tested these for use in flow injection extraction manifold. Three main groups of phase separators have been described, namely, the chamber separator which takes advantage of the density difference only; the two-material separator, which utilizes the difference in properties of two different materials and the respective affinity to the two phases; and the membrane separator, which consists of a lipophilic membrane that expels the aqueous phase but lets through the organic phase. Another phase separator with a polyethylene-backed porous PTFE membrane has also been described (14).

Kina et. al (15) have described the extraction of potassium ions into 1,2-dichloroethane by use of macrocyclic compounds in a simple flow injection-solvent extraction system with fluorimetry. The aqueous sample was injected into the flowing organic stream, transported through the mixing coil, mixed with the carrier stream and the potassium complex was extracted into the organic phase with anilinonaphthalene sulphonate (ANS) as counter-ion.
Bergamin et. al (16) have determined molybdenum in plant material by a thiocyanate method by using a flow injection-solvent extraction system with the use of a phase separation chamber. The geometry of the phase separation chamber, pumping rates, coil lengths, reagent concentration, salting-out effect and sample volumes have been investigated and discussed (16). Lead and cadmium have been determined by Klinghoffer, Ruzicka and Hansen (17) by solvent extraction with dithizone by using the same principle described by Karlberg and Thelander (9). Optimum conditions such as pH, type of masking agents and reagent concentrations for the determination have been investigated by using a flow injection scanning technique. In this technique, the pH of the aqueous phase was continuously altered and a pH profile to indicate maximum extraction and the influence of the metal ion hydrolysis may be obtained by repeated injection of a sample of the same composition.

Imasaka, Harada and Ishibashi (18) have studied the sensitivity and the selectivity of the flow injection determination of gallium with lumogallion by using solvent extraction-fluorimetry. The sample aliquot was injected into lumogallion reagent, transported into the mixing coil, mixed with a stream of isooamyl alcohol through the extraction coil, and the aqueous and organic segments were separated by a membrane device before fluorimetric detection. The effect of pH, reaction and extraction coil lengths, diverse ions and the transient phenomena in extraction process have also been examined (18). Careful adjustment of the residence time was necessary in order to get complete extraction with less
dispersion of the extract and high injection rate.

A multiple solvent extraction system with FIA has been described by Shelly et. al (19, 20). The system consisted of three extraction sections connected one another and when the sample was injected into the system and by resampling the proper extraction phase, three steps were linked together with reagents and solvents added in confluence at the appropriate points before it was analysed. The system was optimised by studying the three basic performance characteristics, i.e., increased sample recovery, increased reproducibility of recovered sample and decreased analysis time. The parameters which affected these characteristics have also been discussed (20).

Fossey and Cantwell (21) have reported the design of an extraction system with FIA employing a membrane separator. Equations relating peak area of the extracted sample to flow rates have been derived and verified (21). The characteristics of the extraction system were studied by using caffeine as a sample component. The variations of sample peak area, peak width and peak height were investigated as a function of extraction coil length, sample injection volume and flow rates.

The purpose of this preliminary study is to investigate the possibility of using a solvent extraction-flow injection system with AAS. The system design is based on home-made extracting and separating chambers and consists of the following components (19):
a) A solvent segmenter - a device that produces alternating segments of two immiscible liquids.

b) An extraction coil - a length of small diameter tubing which carries the segmented solvents and promotes transfer of the extractant from one phase to the other.

c) A phase separator - an apparatus which allows the phases to separate in such a manner that one or both phases may be recovered.

The system is modified from Nord and Karlberg (22) extraction system for flame AAS.

Several parameters of the system were studied using a copper standard solution and the APDC-MIBK method.

7.1. Description of the System

Figure 7.1 shows the flow injection manifold used with solvent extraction and AAS. The system consists of peristaltic pump, injection valve, mixing coil, home-made displacement bottle for organic solvent, extracting chamber, extraction coil, separating chamber, AAS and waste system. The extracting and separating chambers were made from glass. The extracting chamber of model A has a volume of 0.50 ml whereas model B has 0.10 ml volume. The use of extracting chamber model A would produce globules of
Figure 7.1. Flow injection manifold used with solvent extraction and atomic absorption spectrometry.
organic phase flowing in a discontinuous manner, into the extracting coil, while the use of extracting chamber of model B would produce organic phase flowing continuously into the extracting coil. The separating chamber would separate the organic phase from the aqueous phase.

If a volume of sample containing copper is injected into the carrier stream of APDC, a copper complex is form along the mixing coil. The complex formed is extracted by the MIBK from the displacement bottle through the extracting chamber and extracting coil. The organic phase containing copper complex is separated from the aqueous phase by the separating chamber before continuously detected by AAS.

7.2. Apparatus, Standards and Reagents

a) Apparatus

A peristaltic pump.

PTFE tubing, 0.53 mm i.d., for mixing and extracting coils.

A home-made glass extracting and separating chambers (see Figure 7.1).

A displacement bottle for organic solvent (MIBK).

An injection valve model 201-25 and a plastic syringe.

Atomic Absorption Spectrometer.

b) Standards and Reagents

A copper stock solution containing 1000 ppm in 1M nitric acid
(BDH Chemical Limited).

Ammonium Pyrrolidinedithocarbamate (APDC), (Fisons SLR grade), prepared by dissolving the appropriate amount with tridistilled water to make 1% w/v (pH about 5).

4-methyl-2-pentanone (methylisobutylketone), (Fisons).

7.3. Procedure

A series of standard copper (2, 4, 6, 8, 10 ppm) was extracted in a conventional manner into the MIBK with 1% w/v APDC complexing reagent as follows:

a) 1, 2, 3, 4, 5 ml of 50 ppm copper solution were transferred into 5, 50 ml extraction funnels.

b) 1 ml of 1% APDC solution was added into each of the funnels - a brownish precipitate complex was formed.

c) The copper complex was extracted by shaking the funnels for a few minutes with 10 ml of MIBK.

d) The aqueous phase was collected and the organic phase was transferred quantitatively into 25 ml volumetric flask and diluted to mark with MIBK.

For comparison, a series of 2, 4, 6, 8 and 10 ppm of aqueous copper was also prepared.

The aqueous phase, the organic phase and the aqueous copper solution were aspirated into the flame by conventional nebulization by following the optimum conditions for copper according to the
instructor's manual of the manufacturer as follows:

- **Wave length** = 324.7 nm.
- **Acetylene** = 2.00 l/min.
- **Air flow rate** = 8.0 l/min.
- **Burner height** = 4.0 unit.
- **Band pass (slit width)** = 2 nm.
- **Lamp current** = 3.0 mA.

The flow injection with solvent extraction system in Figure 7.1 was tested by injecting the aqueous copper standard and the extracted copper in the organic phase with the following conditions:

- **Mixing coil** = 25 cm.
- **Extracting coil** = 500 cm.
- **Extracting chamber** = model B.
- **Length of tube from separating chamber to nebulizer** = 30 cm.
- **APDC flow rate** = 3.3 ml/min.
- **MIBK flow rate** = 1.8 ml/min.
- **Volume of injection** = 50 or 1000 µl.

The efficiency of the mixing and the separating coils were investigated by varying their length in the system and by collecting the aqueous and organic phases and aspirated them into the flame in the conventional manner.

The influence of volume injected for flow injection-solvent extraction system was investigated by injecting a series of volumes of 10 ppm copper solution into the system by using extracting chamber model B.
with mixing coil length 25 cm and extracting coil length 500 cm.

The shape of calibration graph was also investigated by using the same parameters above by injecting 50 μl of a series of 5, 10, 15, 20, 25 and 30 ppm copper solution into the system. The graph obtained was compared with the graph obtained when no separation between aqueous and organic phases was made, (both of the phases were nebulized into the flame).

7.4. Results and Discussion

Figure 7.2 shows the sensitivity of copper in MIBK by conventional extraction and by normal nebulization compared with the sensitivity of the aqueous copper. The sensitivity is increased by a factor of almost two. No absorbance was detected in the aqueous phase.

Figure 7.3 shows the shape of the curves when 50 μl and 1000 μl of the copper in the organic phase after extraction were injected into the flow injection-solvent extraction system. For comparison, a series of concentration of copper was also injected into the solvent extraction system. As shown in Table 7.1, the extraction efficiency was found about 75.3% relative to conventional extraction method. Table 7.2 compares the results obtained between the extracted copper in MIBK and the aqueous copper when injected into the system.
Figure 7.2. Sensitivity of copper in MIBK compared to aqueous copper, by normal nebulization.
Figure 7.3. The shape of the calibration curves of copper when injected into flow injection-solvent extraction system.
It would take about 6.5 minutes to complete the whole procedure in conventional solvent extraction and less than 10 minutes to complete the extraction procedure and to obtain the results by using flow injection system.

Figure 7.4 compares the shapes of calibration graph by flow injection-solvent extraction when the aqueous and organic phases were separated and not separated before they were nebulized into the flame. It shows that when the organic and aqueous phases were not separated, the absorbance of copper decreases significantly with high noise level (RSD 3.9% compared with 1.5% when separation was made). The presence of the aqueous phase has somehow reduced the efficiency of the nebulizer. The increase in flow rate when the waste system was closed was probably the reason why the reduction of the signal occurred.

Table 7.1. Comparison between conventional extraction and flow injection-solvent extraction. \( V_1 = 50 \mu l \).

<table>
<thead>
<tr>
<th>Concentration, ppm.</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional extraction, Absorbance.</td>
<td>0.178</td>
<td>0.397</td>
<td>0.549</td>
<td>0.753</td>
<td>0.987</td>
</tr>
<tr>
<td>Flow injection-solvent extraction, Absorbance</td>
<td>0.160</td>
<td>0.271</td>
<td>0.433</td>
<td>0.567</td>
<td>0.643</td>
</tr>
<tr>
<td>Extraction Efficiency, %</td>
<td>89.9</td>
<td>68.3</td>
<td>78.9</td>
<td>75.3</td>
<td>65.3</td>
</tr>
</tbody>
</table>

Average extraction efficiency = 75.5 %. 
Figure 7.4. The shapes of calibration graph for copper obtained by flow injection-solvent extraction when the aqueous and organic phases were separated and when they were not separated before nebulized into the flame.
Table 7.2. Comparison between the extracted copper in MIBK and the aqueous copper when injected into the FI-solvent extraction system. \( V_1 = 50 \mu l. \)

<table>
<thead>
<tr>
<th>Concentration, ppm.</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracted copper</td>
<td>0.064</td>
<td>0.115</td>
<td>0.163</td>
<td>0.206</td>
<td>0.235</td>
</tr>
<tr>
<td>Aqueous copper</td>
<td>0.034</td>
<td>0.060</td>
<td>0.098</td>
<td>0.144</td>
<td>0.199</td>
</tr>
<tr>
<td>Extraction efficiency</td>
<td>53.1</td>
<td>52.2</td>
<td>60.1</td>
<td>69.9</td>
<td>84.7</td>
</tr>
</tbody>
</table>

Average extraction efficiency = 64.0%.

Figure 7.5 shows the effect of volume injected on the signal of 10 ppm copper. From the graph shown it is suggested that the volume of injection needed to reach the steady state signal would be very large.

1) Extracting chamber - The use of extracting chamber model B gives several advantages over model A, i.e., in terms of speed of analysis, the extracting chamber model B would produce a complete peak between 12 to 15 seconds for the injection of 50 \( \mu l \) of 10 ppm aqueous copper, whereas model A would take 24 to 30 seconds to produce the same complete peak; model B would give higher sensitivity than model A for the same concentration of copper, and results obtained by using model B shows better precision than results obtained by using model A. This is because model A would
Figure 7.5. Effect of sample volume on the signal of copper in flow injection-solvent extraction system.
give longer residence time for the copper complex formed in the mixing chamber than model B due to its large volume, and thus, increase the dispersion.

ii) Mixing and Extracting coils – As shown in Table 7.3, the efficiency of the system depends on the length of the extraction coil, thus, the extracting coil must be chosen to give a reasonable sensitivity and speed of analysis. The length of mixing coil is not very critical because the reaction between copper and APDC is instantaneous. In flow injection-solvent extraction, the reproducibility of the results from the system and the speed of analysis are considered more important than the efficiency of the system as long as the signal obtained lies within reasonable limits.

Table 7.3. Efficiency of the flow injection-solvent extraction system based on the length of the extracting coil.

<table>
<thead>
<tr>
<th>Method</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous layer</td>
</tr>
<tr>
<td>Conventional solvent extraction</td>
<td>0.006</td>
</tr>
<tr>
<td>Flow injection-solvent extraction (200 cm).</td>
<td>0.025</td>
</tr>
<tr>
<td>Flow injection-solvent extraction (500 cm).</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Mixing coil length = 200 cm., copper concentration = 4 ppm.
Several advantages of this system over conventional solvent extraction are: high speed of analysis, economical use of reagents and solvents, simple and reduced manipulation of extraction glassware.
REFERENCES


CONCLUSIONS

The two main features of flow injection analysis compared with other types of continuous flow analysis, namely, the absence of air bubbles between samples and direct injection of sample into the flowing stream have made this method very versatile with a high degree of flexibility. It can be coupled to almost any detection system and any analytical technique such as, solvent extraction, ion exchange, etc. The manifolds are normally robust and are easily assembled, exchanged or modified for a particular determination. With a readout time between 3 to 40 seconds and with low carry-over, a high sample throughput can be attained. The absence of air bubbles reduces the pulsation effect caused by the compressibility of the air. The unsegmented flow in FIA provides the possibility of adapting the flow pattern according to the requirements of a particular analysis, by selecting a small, medium or large dispersion of the sample zone.

The combination of flow injection methods with atomic absorption spectrometry provides a very simple way of introducing samples and standards into the nebuliser, which gives the same advantages as in discrete nebulization techniques with some additional possibilities based on using the sample as a carrier stream.

The effects of high solid content and viscosity of the sample can be easily reduced. Discrete volumes of sample or reagent can be injected into the carrier stream and dilution of the sample or reagent can be easily controlled by controlling the dispersion of
the system.

The proposed single well-stirred tank (simplified exponential) model to account for the absorbance-time behaviour and the peak shape produced by FIA-AAS techniques, leads to the development of methods of calibration, such as the analogue of standard additions and reagent additions methods. The applications of the proposed model to the development of the analogue of the standard additions and reagent additions methods for the determination of calcium in iron ores and of chromium in steels led to the development of other calibration methods for AAS, such as the continuous dilution gradient concentration method and variable dispersion method. Both methods are based on the use of a single concentrated standard. Results obtained by these methods show that the proposed model is viable for the development of future calibration methods in flow injection systems.

The measurement of the volume of hypothetical mixing chamber based on the proposed model provides the basis for calculating of several experimental parameters for prediction purposes. A particular determination can be easily performed according to its requirements once these parameters are established for the system.

A system designed for flow injection-solvent extraction with AAS gives a simple and rapid way of analysing samples that need separation by solvent extraction without performing conventional solvent extraction procedures. The system can be constructed easily and cheaply based on home-made extracting and separating chambers.
SUGGESTIONS FOR FUTURE WORK

The investigations of the various factors affecting the optimum performance of atomic absorption spectrometer with flow injection techniques provide the basis for developing coupled FIA-AAS instrumentation and for automation of the system in the future. The development of an automated system for FIA-AAS requires more flexible manifolds for variable dispersion from limited to large to achieve the requirement for a particular analysis with the possibility of computer control.

The proposed model may have applicability to other FIA systems in which the detector volume is not so dominant and some consideration should be given to investigating peak shapes for colorimetric, fluorimetric methods, etc.

Further investigation on the proposed model for large dispersion system in FIA-AAS techniques is needed as shown by the restricted value of the model for predicting several experimental parameters at medium and large dispersion. The possibility of combining the Gaussian model with the proposed model to explain the peak shape produced at a large dispersion which shows a tendency towards a Gaussian or slightly skewed shape should be investigated. It may be also interesting to investigate whether the hypothetical volume of mixing chamber in the proposed exponential model consists of one volume or two separate volumes, namely, the volume of the mixing chamber of the atomic absorption instrument and the volume of the mixing chamber produced from the flow injection manifold.
as was suggested in equation (3.15), Chapter 3, page 90.

Another possible application for calibration and prediction purposes is that by using another proposed exponential model as was given in equation (6.16), Chapter 6, page 199, which makes use of the falling part of the peak.

A method of constructing a calibration graph by using a gradient forming device should be investigated further and this could be modified so as to form the basis of an automatic calibration method.

The technique of flow injection-solvent extraction system with atomic absorption instrument may be developed further. The system may be modified or redesigned to suit the specific application on solvent extraction. Several modifications to extracting and separating chambers can be made for use in the system to increase the extraction efficiency. Various other factors affecting the sensitivity of the techniques require further study, and the use of these techniques for separation methods and for direct analysis of samples may be examined by analysing some real samples.

Other sample pretreatment and clean up procedures such as ion-exchange chromatography, electrodeposition, etc., could be adapted to a FIA configuration for use in conjunction with AAS to the analysis.