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DEVELOPMENT AND CHARACTERIZATIN OF A FLOW SYSTEM FOR VOLTAMMETRIC ANALYSIS

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Development and characterization of a flow system for voltammetric analysis

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by

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Development and characterization of flow systems for voltammetric analysis

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SYNOPSIS

Flow-through systems are progressively becoming more important in all branches of analytical chemistry. The increased demand for analysis of more complex environmental samples has resulted in development of reliable, versatile and sensitive techniques. A combination of voltammetric analysis such as differential pulse anodic stripping voltammetry (DPASV) with flow systems satisfy all the above requirements. Although this combination has great potential, practically it has not been realised yet. This may be partly due to the use of traditional mercury electrodes which has many complications.

In order to improve the use of this technique, a glassy carbon electrode (GC) which is more suitable to flow systems compared to mercury-based electrodes was studied. This study describes the electrochemical behaviour of chemicals on the GC electrode in flow systems. The use of a GC with flow systems as a means of detection proved to be very effective and can make electrochemistry more accessible to unskilled scientists.

Having characterised and optimised all instrumental parameters of the system, practical evaluation of the technique as a trace metal analyser was explored. Future improvements necessary for optimum development of the technique are also mentioned.

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SAMEVATTING

Deurvloei sisteme word toenemend belangrik in alle vertakkings van analitiese chemie. Die toenemende aanvraag vir analises van meer komplekse omgewingsmonsters het gelei tot die ontwikkeling van betroubare, veelsydige en sensitiewe tegnieke. Al bogenoemde vereistes kan bevredig word deur'n kombinasie van voltammetriese analise soos differensiële puls anodiese stroping (DPASV) met vloeisisteme. Alhoewel daar'n potensiaal vir hierdie kombinasie is, het dit nog nie prakties gerealiseer nie. Dit kan gedeeltelik daaraan toegeskryf word dat die gebruik van die tradisionele kwikelektrode gekompliseerd is.

Om die gebruik van hierdie tegniek te bevorder, is die glasagtige koolstofelektrode (GC), wat meer geskik vir vloeisisteme is, bestudeer. Hierdie studie beskryf die elektrochemiese gedrag van chemikalieë op die GC electrode in vloeisisteme. Daar is bewys dat die gebruik van die GC as detektor met vloeisisteme baie effektief is en dat dit elektrochmie meer toeganklik maak vir onopgeleide wetenskaplikes.

Nadat alle instrumentele parameters van die sisteem gekarateriseer en geoptimiseer is, is die tegniek prakties geëvalueer. Verdere verbeterings vir optimum ontwikkeling van die tegnieke word ook gegee.

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ABBREVIATIONS USED

Methods

AAS	Atomic Absorption Spectrometry
adSV	adsorptive Stripping Voltammetry
adV	adsorptive Voltammetry
ASV	Anodic Stripping Voltammetry
CFA	Continuous Flow Analysis
CSV	Cathodic Scanning Voltammetry
CV	Cyclic Voltammetry
DPASV	Differential Pulse Anodic Stripping Voltammetry
DPV	Differential Pulse Voltammetry
FIA	Flow Injection Analysis
HPLC	High Performance Liquid Chromatography
ICP	Inductively Coupled Plasma
LC-EC	Liquid Chromatographic Electrochemical Techniques
PSA	Potentiometric Stripping Analysis
TLC	Thin Layer Chromatography
XRFS	X-ray Florescence Spectrometry

Electrodes

AE	Auxiliary Electrode
CME	Chemically Modified Electrode
DME	Dropping Mercury Electrode
TFME	Thin Film Mercury Electrode
GC	Glassy Carbon Electrode
HME	Hanging Mercury Electrode
RE	Reference Electrode
WE	Working Electrode
WJE	Wall Jet Electrode

Chemicals

FA	Fulvic Acid
HA	Humic Acid
SDS	Sodium Dodecyl Sulphate

Apparatus

DCD	Direct Cell Dearation
DC	Deaeration Chamber
TL	Thin Layer

WJ	Wall Jet

Nomenclature

D_T	Deposition Time
Ep	Maximum Current Potential
HD	Hydrodynamic Voltammetry
LOD	Limit Of Detection
RSD	Relative Standard Deviation

1. INTRODUCTION

1.1 TRACE METAL ANALYSES

Determination of heavy metals in environmental and biological samples is of interest in studies of pollution, toxicology, bio-availability and biogeochemical cycles. The fact that heavy metals are non-biodegradable in nature and are massively introduced in the environment has focused attention on their analysis and characterization. Another important factor is that only a few metals are completely nontoxic at any level and some of the so-called essential nutrients also have toxic action depending on their concentration levels. These factors make metal analysis a number one priority in toxicology. The narrow concentration window that exists between toxic and essential levels demand reliable and accurate knowledge of the metal contents in various matrices. It is also important to determine the actual form present for understanding of the reactivity, availability and toxicology of the metal. Therefore, speciation studies are a necessity, not a choice for trace metal analysis in a variety of samples.

Reliable and versatile trace metal analysers are needed for many practical applications. Among these are the establishment of a meaningful tolerance limit for trace metals pollution, elucidation of the roles of trace metals in biological functions, characterization of ultrapure materials for technological advances, speciation of trace metals in industrial processes as well as forensic science investigations. Techniques commonly applied for the job are methods such as AAS (especially ETA-AAS), ICP-OES, XRF, stripping analysis or neutron activation. Unfortunately some of these methods have high detection limits. Therefore, they cannot be used without lengthy preconcentration steps. In addition their usage is limited by instrumentation costs, analysis time, sample preparation or selectivity, especially when constant monitoring is required.

It is worth noting at this point that flow based stripping analysis belongs to a powerful group of trace metal analysers especially for constant monitoring. Its instrumentation is cost effective and most of its components can easily be made in-house. They are capable of doing trace,

speciation and simultaneous analysis within a short period of time.

Stripping analysis started around 1952 out of Baker's idea[1] of the possibility of preconcentrating analytes on the electrode in order to lower their detection limits. Over the years we have witnessed the development of the technique, introduction of sophisticated commercial instruments and its coupling with other techniques. Several studies on environmental, biological and clinical problems have demonstrated the essential advantages of this technique over the others. These advantages are:

- The possibility of determining a considerable number of chemical elements (more than 40) and many organic substances;
- Low detection limits (attaining 10^{-9} -10^{-10} mol/ℓ for some elements);
- High selectivity, good accuracy and reproducibility;
- The possibility of determining correlation of the composition properties (speciation studies);
- Ease of computerisation and automation of analytical procedures;
- Relative simplicity and low price of the instruments used.

1.2 FLOW-THROUGH BASED STRIPPING ANALYSIS

The research needed to provide an understanding of the electrochemical response requires significant investment of time and resources for preparation of solutions and performance of necessary experiments. Both expense and repetitive nature of this type of work usually limit the choice and range of parameters to be varied, often to the serious detriment of the science. Automation of sample handling and experimental execution allows the electrochemical response to be characterized more efficiently and thus more thoroughly.

Flow-through configuration methods are progressively becoming more important in all branches of analytical chemistry, including environmental analysis. These involve continuous monitoring of substances and laboratory measurement of discrete samples using continuous flow analysis (CFA), flow injection analysis (FIA) and high performance liquid chromatography (HPLC). Of the three general types of continuous non-segmented techniques,

FIA offers better features for the development of stripping methods, namely, greater flexibility and versatility due to the different FIA modes, lower sample and reagent consumption and higher sampling frequency.[2,3]

An intrinsic combination of flow-through configuration and electroanalytical techniques may broadly be defined by the term hydrodynamic voltammetry (HD). This coupling offers several advantages over batch measurements, namely minimum contamination as a result of minimum sample handling, avoidance of concentration depletion by continuous replenishment of the sample and effective, reproducible mass transport is provided by the flowing solution. The technique of medium exchange (deposition occurs while the sample is flowing through the detector and stripping done after the sample solution has been replaced with another secondary electrolyte) enhances the selectivity offering multi-element determination capabilities.[4,5] In addition they are highly selective, sensitive and easily automated.[6] As a result there are regarded as one of the most efficient techniques in trace metal analysis.

Anodic stripping voltammetry (ASV) is an electroanalytical technique that can easily be adapted for flow measurements. Due to the two steps usually required (deposition and stripping), a continuous signal cannot be obtained. However, data can be obtained in rapid succession, making the method suitable for a highly sensitive flow-through system. Various flow measurements for automated ASV analysis are reported;[7-12] most of which are based on FIA,[7-11] CFA [12] and a few on HPLC principles. These techniques are suitable for an environmental surveillance, industrial quality control and clinical laboratories where a high sample throughput is needed. Usually, the cathodic deposition is followed by anodic stripping (ASV). The reverse procedure of anodic deposition followed by cathodic stripping has been reported by some researchers.[13,14]

As a trace analysis technique ASV is very efficient and has been applied to (or is potentially applicable to) about 30 elements as well as a handful of organic compounds. Therefore it is not as universal as spectrometric methods, but for certain important groups of substances and

in speciation studies it exhibits unrivalled sensitivity. Its application is still not popular mainly because knowledge of the principles of electrochemistry and a certain amount of experience is required which often compares unfavourably with other techniques. Despite these limitations several researchers have used flow systems based on ASV for on-line determination of Cu, Pb, Cd, Tl, Zn and Cr. Investigations done were on simultaneous,[15] kinetic and speciation [16-18]. Reviews discussing different parameters of on-line ASV systems have been published.[19,20] Samples such as natural waters,[21] drugs,[22] food [23,24] and industrial processes were monitored.

1.3 PRINCIPLE

The usual practice of ASV involves the deposition (concentration) of metal ions of interest onto a surface or in the body of the working electrode (WE), under convective diffusion conditions. Deposition is carried out employing a controlled-potential electrolysis for a definite time under reproducible hydrodynamic conditions in the solution. This process can be divided into two main groups: redox chemical reaction (electro deposition) and on adsorptive step (adsorption deposition). Preconcentration procedures in various electrode materials are considered in detail in the literature. [25-27]

After deposition most researchers brought the solution to rest and stripped the deposit purely under diffusion control. Here the electrolysed or adsorbed species are redissolved in the solution during a potential scan. The stripping involves scanning the potential anodically (ASV) linearly or with another potential-time waveform. On rare occasions cathodic scanning (CSV) was used. When the potential reaches the standard potential of a metal-metal ion couple, that particular metal is reoxidized back into solution and a current is recorded. However some workers have stripped the deposit in a flowing carrier steam instead of quiescent state.

A number of articles and books discussing stripping voltammetry theory in detail are published.[26,29] Unfortunately these equations which are used to solve rigorously for batch and flow

systems with steady-state deposition currents discussed in the majority of these articles are not applicable to stripping voltammetry in continuous systems because the dispersions in these systems are different. The theory applicable in other types of flow systems including continuous flow has been examined in detail before.[25,34] The limiting current equation described in terms of the hydrodynamic parameters of the flowing solution was given :

$$I_l = KnFCD^{2/3}\nu^{-5/12}a^{-1/2}R^{3/4}V^{3/4} \qquad (1)$$

Where

I_l = limiting current,

k = constant (1.38),

n = number of electrons involved in charge transfer process,

F = Faraday's constant,

C = concentration of electroactive species in the bulk of the solution (mol/mℓ),

D = Diffusion coefficient $cm^2\ sec^{-1}$,

ν = kinematic viscosity,

a = inlet diameter,

R = electrode radius,

V = volume flow rate.

The equation clearly defines the current in terms of volume flow rate, diameter of the nozzle and the radius of the electrode. Notably, the effect of separation between the nozzle and electrode is not included. This is because it is commonly assumed that the jet of the liquid issuing from the nozzle does not break up before it impinges on the electrode surface. However several workers had observed significant dependence of current on the nozzle-electrode separation.[34]

The stripping peak is subject to the type of electrode used as well as the stripping procedure followed. Gunasingham, Ang and Ngo [25] did the theory and practical verification of the different stripping procedures using TFME. They assumed that the stripping step is essentially a thin layer

process wherein rapid depletion of metal on the surface (adsorbed or reduced) occurs.

In discribing a theory for ASV at GCWJE(Glassy carbon wall jet electrode), Gunasingham et. al.'s [25] reasoning was adopted which involved the correlations derived by Devries and Van Salem. For a general case of a solid electrode (e.g. Thin film mercury electrode) coupled with a solution of infinite thickness, the equation for stripping peak current is given by:

$$i_p = 1.1157 \times 10^6 n^2 A c \ell v \qquad (2)$$

Where i_p = stripping peak current

A = area of the electrode,

c = concentration of metal deposited on the electrode surface,

ℓ= electrode thickness,

v = scan rate,

n is the same as in (1)

This equation assumes that the deposition step is carried out under conditions of convective diffusion. During the stripping process, however, the solution is brought to rest and the flux is assumed to reduce to zero. Nevertheless, the equation is still applicable in a flowing and quiescent solution on the basis that the stripping step is essentially a thin-layer process wherein rapid depletion of the metal adsorbed or deposited on the electrode occurs. In this regard the stripping peak current is assumed to be governed by the metal concentration on the electrode which can be determined using Faraday's law:

$$c = I_l t_d / n F B \qquad (3)$$

where I_l , F are the same as in (1)

c, n are the same as in (2)

t_d = deposition time

B = volume of the electrode

$B = A\ell$

A, ℓ are the same as in (2)

By substituting equations (1) and (3) in equation (2) The equation for the stripping peak current was obtained. This equation was derived and veryfied by Gunasingham, Ang and Ngo[25] on a mercury-film wall jet electrode.

$$i_p = 1.1157 \times 10^6 kn^2C\, D^{2/3} \nu^{-5/12} a^{-1/2} R^{3/4} V^{3/4}\, vt_d \qquad (4)$$

where

i_p = stripping peak current,

v = scan rate,

t_d = deposition time,

k,n,C,D,ν,a,R and V are as indicated in (1)

In this regard the stripping peak current is assumed to be governed by the amount of metal deposited at the electrode which is a function of the following: concentration of the metal in the solution, pre-electrolysis potential and time, flow rate, electrode active area, solution temperature, composition and the number of electrons involved in the transfer (n). Factors such as rate of polarization, product transport and solubility (in case of a chemical reaction), and character of the reaction products also affect the peak height.

1.4 INSTRUMENTATION

A continuous-flow voltammetric flow-through trace analyser system requires a series of valves, cells, electrodes and potential or an intensity generator. In addition, changes in a chosen variable must be monitored. Valves used in this configuration are injection (valves which insert the sample or reagents into the continuous system (FIA)) or selecting valves (those which select different electrolytes). Six or eight way valves are adequate for this job.[30,31]

The cell is the important unit of the detector, and its construction must be suitable to the hydrodynamic conditions used, to permit sensitive and reproducible measurements. This means that the cell must have the smallest possible effective volume, minimum IR drop and a uniform polarization of the working electrode.

Various cell designs and approaches to on-line monitoring have been investigated by several authors.[32,33] Optimum choice of the detector configuration and design requires certain conditions to be fulfilled; these include the following:

- Ease of disassembly and reassembly for cleaning the electrode;
- No leakages;
- Low noise and potential drop across the electrodes.

Two designs are found to satisfy the above conditions; wall-jet (WJ): a cell which has the solution impinging perpendicular to the working electrode (WE) surface and then spreading radially over the surface of the WE[33-38] and thin-layer (TL): the solution is passed over the WE. Different versions of these designs have been used. The WJ has been found to be most satisfactory for a versatile electrochemical detector.[39,40]

The WJ is more frequently used than the TL mainly due to its high sensitivity and freedom of the WE from surface adsorption as a result of the mechanical washing effect of the impinging liquid. A major drawback of this design is that liquid electrodes cannot be used because of difficulties with the collection of the used liquid. Position of reference and auxiliary electrodes has no significant influence on the behaviour of the cell provided the distance between them and the WE does not increase the potential drop.[30]

1.4.1. ELECTRODES

In general the use of a three electrode system with a suitable working electrode (WE), reference electrode (RE) and an auxiliary (counter) electrode (AE) is required in differential

pulse systems since in these systems resistance effects as well as other artifacts are minimized or eliminated.

1.4.1.1. WORKING ELECTRODE (WE)

The WE is a key to the cell. Its nature and shape is a function of the stripping mode used and of the analyte features. The WE position in relation to flow direction depends on the type of cell design used. It can be parallel (flow-by) in TL, perpendicular (flow-on) in WJ, or the solution can pass through the electrode (flow-through) when porous or tubular electrodes are engaged.[41]

Selection and construction of the electrode material must be in such a way that the accessible potential range is suitable for the given purpose. It is important that the residual current and noise are sufficiently low and constant. The surface activity of the WE must be constant at least during the time of a single complete analysis and kinetic parameters of the analyte electrode reaction are favourable (ideally, the electrode reaction should be very rapid, simple and free of interferences from adsorption effects and side reactions).

Automation of measurements favours application of flow-through cells, those with a tube or porous electrode.Their larger inner surface area ensures high efficiency of the analyte electrolysis. Irrespective of this advantage, porous or tube electrodes are not often used. Materials used for them are similar to those used in conventional electrodes.[42,43]

A number of materials including mercury, carbon, platinum and gold have been used. Despite difficulties associated with mercury electrodes in the flow-through configuration (see above section) mercury electrodes namely the hanging mercury (HME)[44] and thin-film mercury electrode (TFME) [35,45] had so far been the most commonly used. Reasons for the frequent use being HME's simplicity, economy and reproducibility. However, mercury's toxicity, low surface area to volume ratio, and use of only low stirring rates seriously limit the use of

HME. The extremely high ratio of surface area to volume of TFME gives high resolution. In addition mercury's wider potential range makes these electrodes very attractive.

TFME's are prepared by pre-electroplating/electrodepositing or simultaneously depositing mercury and a sample on a suitable support (noble metals or carbon). Metal substrates are not favoured, because most of them dissolve in mercury forming an amalgam, resulting in intermetallic reactions with metals of interest. Although these electrodes have been successfully used, their usage is seriously hampered by the inability to reproduce a film surface on replating and the fact that the films are easily mechanically damaged at high liquid flow rates.[46,47]

Various forms of carbons (impregnated graphite, pyrrolytic graphite, reticulated vitreous carbon, glassy carbon, carbon paste and composite electrode) have proved to be useful as TFME substrate and in their own right.[8] They have been used as discs, foil, rods and grids. Literature on these electrodes clearly indicates that pastes are superior in having lower residual current to noise ratio and are cheaper. Performance of other carbon electrodes strongly depends on the quality of polishing of the electrode surface.When well polished, they yield reproducible signal values over a prolonged time. Other solid electrodes are not commonly used and in few occassions when they are used were found to have similar problems encountered with carbons, in addition they have limited potential range and are difficult to polish.[48]

The successful application of carbon electrodes results from high chemical and electrochemical stability of the material, a relatively high hydrogen and oxygen overvoltage, the broad working potential range and the simplicity of the mechanical renewal of the electrode surface. The inertness of the carbon and practically gastight, extreme low porosity of the GC is contributing to its wide use. [26,27]

A promising new trend is the use of modified electrodes, referred to as chemically modified

electrodes (CME).[39-43] Modification is usually done by coating the electrode surface with chemicals which alter its characteristics. The coating is achieved by electrodepositing of chemicals or by directly binding of chemicals with the oxide groups on the electrode surfaces. Modifiers such as nafion, various polymers, lichens and cellulose acetates have successfully been applied. CME's can be classified into three different groups according to the type of modifiers used. Organic substances like lichens and cellulose films have been used and inorganic complex ions and metal-ad-atoms have been studied. Although some success has been achieved with these electrodes, they have no practical use due to their poor stability and irreproducible surface.

1.4.1.2. REFERENCE ELECTRODE (RE) AND AUXILIARY ELECTRODE (AE)

The RE provides a stable potential insensitive to the composition of the solution under study and with which the potential of the WE is compared. Commercially available liquid junction electrodes such as Ag/AgCl and saturated calomel electrodes are popular choices. Unfortunately they are prone to leakages and pressure changes, a cause for concern where the presence of chloride ions may affect the stripping peak. This set back is solved by isolation of the RE. Occasional checks for salt clogging are important to resistance.

In a flow-through configuration the use of a solid RE is convenient. Solid electrodes such as carbon rods, paste of mercury, calomel, KCl and graphite fixed in a PVC tubing with Teflon glass membrane had been employed. Pellets containing KCl, alumina and Teflon pressed around an Ag/AgCl wire as well as Pt rod had been used successfully. [9,31]

Counter or auxiliary electrodes are usually made of a chemically inert conducting material with reasonable surface area. Pt, carbon (GC, graphite) and stainless steels are used. They are usually incorporated into the cell as stainless steel tubing or screws of the cell outlets. Rods, wires and discs are also used.

1.4.2 MAINTENANCE OF ELECTRODE SURFACE

The surface of solid electrodes changes with time due to adsorption of species from the solution or chemical changes to the surface itself. These changes often result in variations in sensitivity or reversibility and in extreme cases lead to complete inhibition of charge transfer. As a result the performance of these electrodes depends on the ability to maintain a reproducible surface, which is a major problem. Electrode regeneration is always a necessity when solid electrodes are used because maintaining their surface unchanged is an impossible task.

Electrode cleaning and activation involve mechanical, chemical or electrochemical treatment of the electrode surface. As a rule mechanical treatment used are cutting off a thin layer or polishing with abrasives. Commonly used abrasives are metallographic paper (2/0 or 5/0), emery paper and alumina powder (0.3 and 0.5 μm). The nature of these treatments renders them difficult to apply directly in the solution of interest and they are not repeatable on a short time scale. Despite this disadvantage they are commonly used.

Electrochemical and chemical regeneration methods are more adaptable for flow-through configurations, the former being more popular. In all cases treatment include the application of a relatively large positive potential and on few occasions sufficiently negative potentials are used. There is no standard procedure followed, therefore widely varying surface cleanliness is achieved resulting in varying effects on charge transfer rates.

GC electrochemical treatment used by a number of workers is applying +2.0 V for 3 minutes or -1.0 V for 1 minute.These procedures although used, indicates considerable surface roughness. If the treatment is conducted under square wave conditions at a frequency of 1 Hz and an amplitude of +2.0/-1.0 V for 10 min no observable change on the surface occurs. Other methods which are used are vacuum heat treatment, ion etching and laser radiation. The latter had been used successfully *in situ* cleaning of GC and Pt electrodes.[48] Chemical treatments are unpopular mainly due to a considerable amount of time required to identify the appropriate chemicals and

availability of inert pure chemicals. Common chemical treatments are acid cleaning with 3 mol/ℓ HNO_3 between samples or measuments and wiping the GC electrode with a filter paper soaked in methanol to remove adsorbed organic substances.

1.5 LIMITATIONS OF DPASV

DPASV limitations in flowing liquids concerns the carrier liquid, sample matrix and the working electrode. Problems related to the electrode were discussed above. An appropriate carrier liquid must have sufficient electrical conductivity to yield a small and constant signal. Hence non-polar solvents cannot be used, therefore carrier liquids must be water, polar solvents, electrolyte solutions and a mixture of organic and aqueous solutions.

One of the main concerns in a sample matrix is the presence of dissolved oxygen and organic compounds. Oxygen reduction current in these instances can be up to 1000 time higher than the oxidation currents of the elements of interest. Minimization or elimination of dissolved oxygen is usually done by inert gas purging of the sample before analysis. Detailed procedures on deoxygenation in DPSAV studies is dealt with in chapter 3.

Adsorption of organic or surface active substances hinder metal reduction during the plating step or change the reversibility of the metal oxidation during the stripping. This results in serious distortions of the peak (changes in current, potential and shape). In the presence of these substances, DPASV analysis of most metals, especially copper (its potential is closer to the potentials which are usually affected, see chapter 2 for details) is complex and unexpected observations have been made in analysis of interstitial and natural water samples. A number of studies have indicated occurance of the additional peaks or shoulders and in extreme cases the copper peak is not observable at all.[50-52] This phenomenon was generally minimised by modification of experimental conditions such as dilution, changing the deposition potential or applying a different electrochemical technique. At the moment comprehensive studies on the effects of these compounds on the plating and stripping response have been done on mercury

electrodes.[52]

Other minimization and elimination of these matrix effects have been achieved in several procedures for sample treatment. The most common being ultraviolet irradiation, which unfortunately is lengthy and complicated to preform in flow-through configuration systems. Electrochemical or chemical oxidation of the organic substances has been reported. Chemicals like gelatin, triton and several complexing agents have been used to either selectively complex the interfering ions or to shift the potential of the analyte away from that of the interfering compound. However the most promising method is the use of fast voltammetric techniques such as linear ASV with ring collection and sampled dc versus pulse voltammetry and modified electrode (see section 1.4.1). Problems concerning interferences raises some concern when using the DPASV techniques and they have been tackled by several researchers but are far from being solved.[26,27,30] Therefore when developing a new method it is always advisable to check for possible interferences.

1.6 THE SCOPE OF THIS STUDY

Recent increased usage of flow-based stripping analysis made it necessary to consider the technique and its applications in various samples. Apart from the obvious advantages of the flow based systems stated above, the call for the determination of not more than one species as well as their chemical forms dictated the increased usage of ASV. A lot of literature deals with development, theory and various aspects of the technique,[27,30] but very little has been done on solid electrodes. At present mercury electrodes have a wider application despite a number of difficulties associated with them not, to mention mercury hazards.

The goals of this study were to expand the practical possibilities for elucidation of the electrochemical behaviour of chemical systems and to exploit the potential of GC electrodes in flowing systems. A flow system for voltammetric analysis, especially ASV, was developed and characterized. The system was designed so that sample manipulation and electrochemical

experiments can be performed under automated control. Once the system has been characterized systematically to establish optimum conditions, voltammograms were used for absolute determination of trace metals.

The applicability of this system was illustrated by analysis of reference materials , real samples and comparison with adopted or commonly used standard methods. In accord with the task, this study presents examples on trace analysis, speciation and simultaneous analysis of metals. The work was done on natural waters and beverages. Detailed experimental problems that have been barriers for the widespread usage of solid electrodes were investigated. They include interferences, regeneration, deoxygenation as well as instrumental parameters. A GC electrode was chosen because it posses isotropic properties and do not require a particular orientation in the cell. Besides, the use of GC is simple, relatively inexpensive, environmentally friendly and require little reactivation.

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2. TRACE ANALYSIS OF COPPER

2.1 INTRODUCTION

Copper is widely distributed in nature and is a major component of a wide range of minerals. Its ores occur naturally in two forms; native and oxidised. The metal together with its alloys are commonly used in various industries in production of utensils. It is also used medicinally to combat plant diseases, pests as well as an antiseptic.[1] The importance of copper in living things is well documented.[2] Human beings can tolerate relatively large amounts if orally ingested (1 g/ℓ) and smaller amounts if intravenously administered (1 mg/ℓ). This tolerance discrepancy is explained mainly due to the fact that under optimum conditions less than one third dietary copper is assimilated and as it is assimilated is transported by serum albumin to the liver for ceruloplasmin synthesis.[3]

One of the foremost contaminants of the environment, especially in water, is copper, the content of which should not exceed 1 mg/ℓ.[4] A major problem in the determination of trace concentrations of copper in natural water is the ease with which sample contamination can occur during analysis. Techniques which require sample handling and multiple reagent additions are more prone to errors, which can be satisfactorily eliminated only by the use of *in situ* preconcentration or monitoring methods. An example is the filtration and enrichment procedures of Martinotti et. al.[5]

Various methods including gravimetry, electrogravimetry,[6] potentiometry,[7] spectrophotometry,[8] atomic absorption spectrometry (AAS), inductively coupled plasma (ICP) [9] and voltammetry [10,11] have been used successfully for copper analysis. Unfortunately these methods are frequently difficult, time consuming and sometimes can not be used for trace analysis. Automated or flow based methods of analysis in combination with reliable, highly sensitive instruments have a potential to solve the problems of monitoring the conditions of ecologically important mater-ials such as water. Several researchers have demonstrated that FIA or CFA in conjunction with photometric,[12,13] potentiometric[14,15] and electrochemical methods[16-20] for continuous monitoring of trace levels of copper are very advantageous.

Of all the techniques employed, it is not surprising to find that electrochemical techniques are the most commonly used in water analysis. Apart from being highly sensitive, they allow simultaneous determinations of several elements, they require very little or no sample pretreatment. These are the features which make them the darlings of *in situ* analysis.

2.1.1 VOLTAMMETRY OF COPPER

DPASV has proved to be suitable for the determination of low concentrations of Cu ($< 10\ \mu g/\ell$) in a variety of samples, with sensitivities comparable to those of graphite furnace atomic absorption spectrometry.[11] However it is not readily adaptable to *in situ* measurements. Despite the obvious problems of removal of oxygen with nitrogen before measurements, maintaining the working electrode surface and removal of used mercury (when mercury electrodes are used) this technique has been used successfully.

The ability of copper to bind strongly with both organic and inorganic ligands (see section 2.3), its ability to exist in more than one oxidation state as well as the closeness of the copper peak potential to the first oxygen reduction peak renders copper analysis with DPASV as the most difficult. Studies have revealed the complexity of these processes associated with electrodeposition in estuarine waters, chloride media, organic substances and in presence of a surfactant when mercury electrodes are used.[21] Although they were predominantly used, the mercury electrodes are inconvenient for flow analysis detection methods. In addition, the low solubility of copper in mercury, the occurrence of copper stripping peak at a potential where many organic species are adsorbed on the mercury renders use of this electrodes in copper analysis very difficult and riddled with erroneous results.

In flow analysis the tendency is to use thin film mercury electrodes (TFME) more frequently than hanging mercury electrodes (HME) because the utility of HME was found to be limited due to the mechanical instability in flowing liquid. However the small volume of the TFME leads to very high concentrations of amalgams resulting in serious problems of intermetallic compound formation.

A number of supporting electrolytes such as HNO_3,[22,23] H_2SO_4, $NaNO_3$, $HClO_4$;[24] acetate

buffer pH 5, ammonium bromide, ethylenediamine[25] and ethylene diaminepentaacetic acid [26] have been employed.Anderson et. al.[27] extensively studied the pH dependence of Cu(II) reduction in acidic aqueous nitrate and perchlorate solutions using cyclic voltammetry. Reviews on electrochemistry and typical kinetic parameters for Cu(II) reduction have been presented. Studies reported were on speciation,[28,29] complexation capabilities, simultaneous analysis mostly with Pb, Cd and Zn and trace analysis. [30]

Trace determination of copper is not restricted to natural water samples, some important applications have been reported. Which include analysis of soils, sediments, sludges and clinical samples such as blood, hair and urine.[31] Recently analysis of industrial effluents [24] and food samples has increased. This led to several companies publishing developments in instruments based on voltammetric techniques; Chemtronic (Australia) have reported a portable instrument for detection of a number of heavy metals in industrial effluents and Eutech Cybernetics (Singapore) a hand held instrument for monitoring of Cu and Pb.

To our knowledge there is no report about the determination of copper using a GC electrode. This electrode is the most commonly used electrode in flow-through configurations usually as a substrate for mercury film. The use of this electrode is advantageous because, the electrode is easily adaptable to flow based analysis, it has a relatively high hydrogen and oxygen overvoltage and has a broad working potential. As stated earlier (chapter 1 section 1.4) this electrode is one of the most frequently used solid electrodes.

In this study a DPSAV method for the analysis of copper using a GC electrode in a flow- through configuration was developed. Various parameters were optimised and possible interferences studied. Applicability of the developed method was tested on potable (fresh) water samples. Accuracy, precision as well as repeatability were checked and verification of the method was done using a standard material.

2.1.2 FACTORS THAT AFFECT COPPER ANALYSIS

As mentioned above, the copper DPASV analysis using HME or TMFE has some difficulties, mainly due to the adsorption of organic compounds onto the mercury electrodes.

Comprehensive studies of the copper anodic peak in the presence of natural organic matter such as fulvic acid (FA), humic acid (HA) and extracellular polymeric substances or model organic compounds have been made. Peak distortions, appearance of subsidiary peaks and shifts of the baseline represents the alteration of the ASV response and have been attributed to the adsorption of these organics.[32-34]

A considerable amount of these organic substances are found in environmental samples such as lakes, rivers, oceans and to a lesser extent tap water. Clinical samples also suffer from the same problem. Therefore analysis of copper in these samples require some elaborate sample preparation or some minimisation methods. In addition to measures discussed in chapter 1 section 1.5, for copper analysis the following have been used:

- Addition of gelatin or sodium dodecyl sulphate (SDS) in FA or HA rich samples respectively.[24]
- Use of linear ASV with ring collection, sampled dc versus pulse voltammetry and a potential step procedure.
- Recently modified electrodes and microelectrodes especially *in situ* monitoring are commonly used.[35,36]
- Using an internal standard like indium.[37]
- Application of medium exchange.[38]

Another important problem in DPASV copper analysis is the intermetallic compound formation between copper and other metals. Intermetallic copper compounds known are Ag-Cu, Cu-Cd, Cu-Ga, Cu-In, Cu-Mn, Cu-Ni, Cu-Sb, Cu-Sn, Cu-Tl and Cu-Zn. These intermetallic compounds are a problem when determining low concentrations of copper in the presence of high concentrations of these metals. Their presence may result in an increase or decrease of the copper anodic peak as well as baseline changes and broadening of the peaks.[32]

It is obvious from the considerable number of papers on the Zn-Cu intermetallic compound that the determination of trace levels of Cu in solutions bearing high concentrations of Zn is an important problem.[23,40,41] From the literature a range of elimination techniques are employed. The following devices have been used to reduce this form of interference;

I) Careful selection of the deposition potential to exclude the interfering element, e.g application of a deposition potential more anodic than -0.9 V have been used to minimise the interference of Zn, Ni and Co.

ii) Intentional addition of a metal that forms a more stable compound with the interfering metal,e.g addition of Ga or Ge which preferentially combines with Cu are used in solutions that contain high concentrations of Zn.

iii) Removal of the interfering element prior to introduction of the sample into the voltammetric cell, like using twin electrode thin layer cells (two parallel working electrodes sandwiching the thin solution layer) with selective plating, measuring Cu at one electrode and Zn and Cd at the second electrode.

Commonly used interference eliminating techniques for trace analysis are standard addition, dilution of the sample, use of medium exchange and to a lesser extent mathematical calculations. In most instances a suitable choice of the working electrode and supporting electrolyte are very important factors.

2.2 EXPERIMENTAL

2.2.1 APPARATUS

The continuous flow voltammetric flow-through trace analyser system (see figure 2. 1) used in this work was constructed from the following components: a Watson-Marlow peristaltic pump; a 647 VA-Stand (Metrohm, Herisau, Switzerland) with a home made flow-through cell and a 646 VA-processor (Metrohm, Herisau, Switzerland). Tygon and Teflon tubing was used throughout. The 646 VA-Processor was used to execute all the voltammetric instructions and to evaluate the voltammograms. The sample flow was manually controlled.

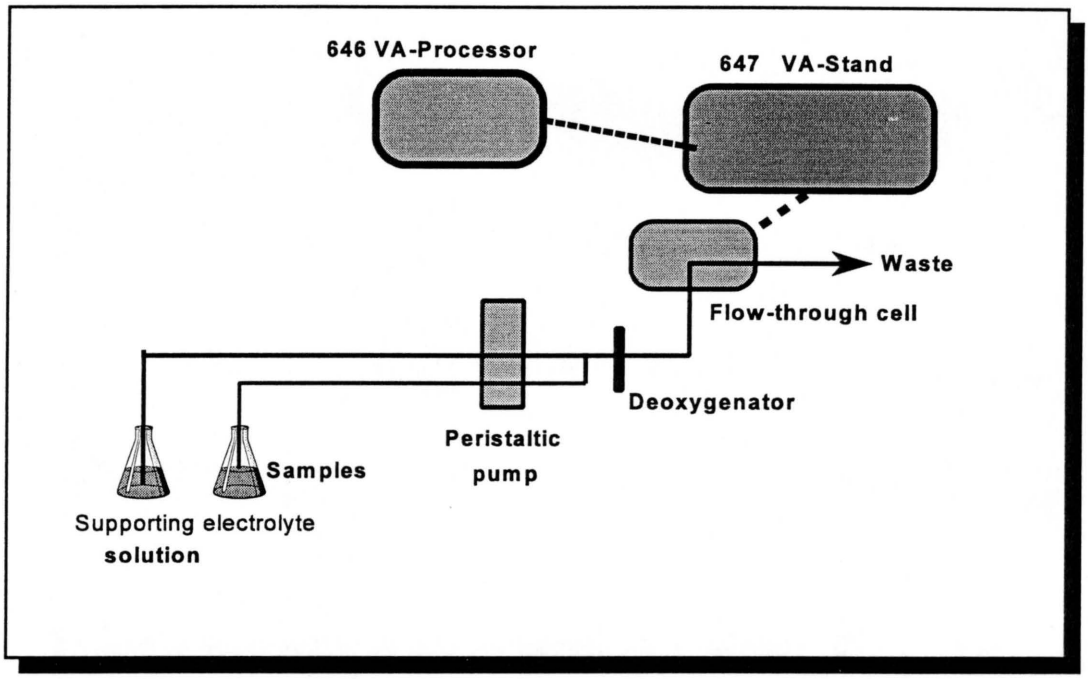

Figure 2.1: Systematic flow-through trace analyser

2.2.2 DETECTOR CELL

The wall-jet flow-through cell was made of Perspex, with a geometric cell volume of 5 mℓ. A schematic diagram of the cell is outlined in Figure 2.2. Samples entered the wall-jet flow-through cell via a jet-nozzle (i.d. 0.3 mm) shown as the inlet in the lower block and left via the outlet in the upper block to waste. The three electrodes were positioned into the flow-through cell from the upper block.

A 3 mm diameter glassy carbon disc (WE in Fig. 2.2) was used as working electrode, glassy carbon rod (AE in Fig. 2.2) as an auxiliary electrode and a silver/silver chloride as reference electrode (Ag/AgCl/KCl with [KCl] = 3 mol/ℓ) illustrated as in Fig. 2.2. The blocks were pressed together by screws and spacing material. The surface of the WE was hand polished every morning to a mirror like finish with a slurry of 0.3 μm alumina powder and washed with 1 mol/ℓ NaOH solution between solutions.

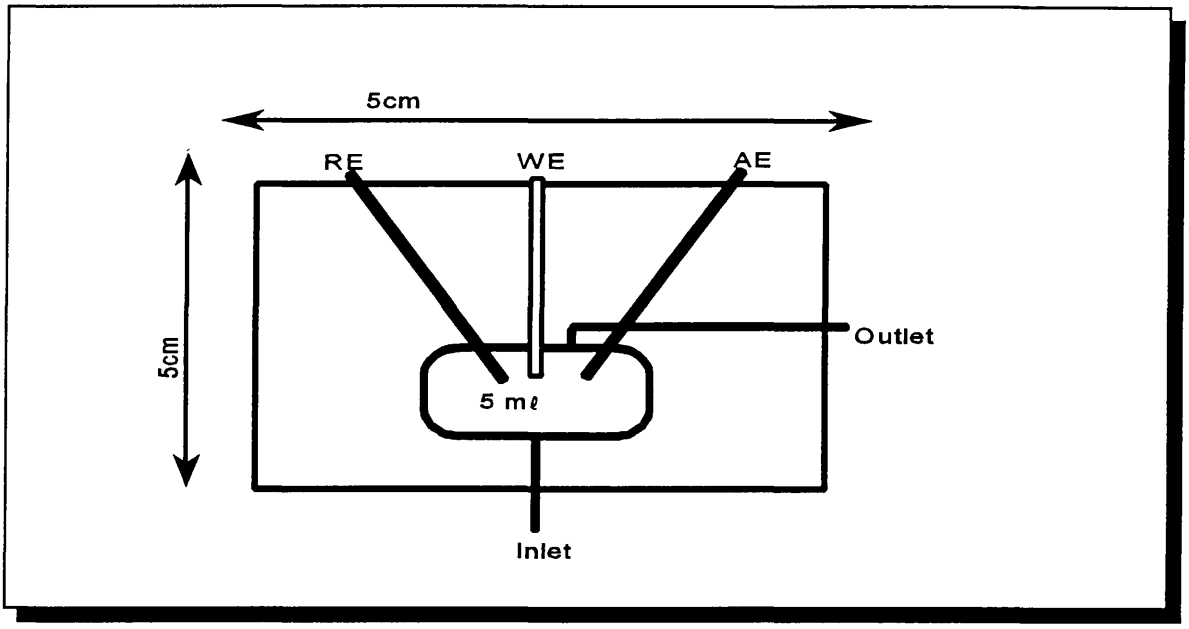

Figure 2.2: ***Schematic diagram of the homemade flow-through cell. WE = working electrode, RE = reference electrode, and AE = auxiliary electrode. Cell volume = 5 mℓ; inlet jet-nozzle i.d. = 0.3 mm: WE = 3 mm diameter GC. Other dimensions are given and discussed in text.***

2.2.3 PROCEDURE

The following conditions were used unless otherwise stated: supporting electrolyte: 1 mol/ℓ HNO_3, deposition potential time: 5 min , deposition potential: -400 mV, stripping range : -400 mV to +400 mV, pulse amplitude: 150 mV, resting time : 5 s, flow rate: 11 mℓ/min (during deposition and otherwise none), scan rate: 10 mV/s, pump tubing bore: 2.79 mm, deaeration time: immediately prior to analyses for about 10 minutes or during analyses. The flow was generated by pumping the test solution through the flow-through cell using the peristaltic pump.

2.2.4. REAGENTS AND SAMPLES

All reagents were prepared from suprapure analytical-reagent grade purity. All aqueous solutions were prepared from specially treated doubly distilled deionised water. A stock copper solution

was prepared by dissolving accurately weighed $Cu(NO_3)_2 \cdot 3H_2O$ to a known volume with pure water([Cu] = 500 $\mu g/\ell$). Solutions and dilutions were all made using ultrapure water. Nitrogen used was of high purity grade. Samples were collected in polythene bottles from three different sources in the University of Pretoria campus (SA) and analysed within a day of sampling. Tap water was sampled after running the tap for 20 minutes, rain water was collected from the roof drains and ground water was obtained from bore holes.

2.2.5 SAMPLE PREPARATION

All vessels (beakers, flasks, polythene bottles) were cleaned by keeping them for at least three days in 3 mol/ℓ HNO_3, rinsing them thoroughly with purified water before use. Standard working solutions and samples were prepared by adding enough concentrated nitric acid into appropriate volumes of stock solution or water samples to give a solution containing a final concentration of 1 mol/ℓ HNO_3. These solutions were then diluted to the mark with ultrapure water and mixed thoroughly.

2.3 RESULTS AND DISCUSSION

2.3.1. OPTIMISATION OF THE SUPPORTING ELECTROLYTE

The appropriate electrolyte was determined by comparing mean peak heights of different supporting electrolytes (Table 2. 1). The copper stripping peak potential in these electrolytes was around 0.00 V. Nitric acid had the highest peak current especially at a concentration of 0.32 mol/ℓ, but this concentration unfortunately did not give reproducible results when real samples were analysed. A 1.0 mol/ℓ nitric acid solution was therefore chosen as the supporting electrolyte.

To obtain a better response performance from the DPASV, pulse amplitude, the distance between the inlet and the working electrode (inlet-WE separation) and deposition potential were optimised. The results are shown in Table 2.2. In each case, one parameter was varied, while the others were kept constant. A higher pulse amplitude resulted in an increase in sensitivity, but also an increase in background. A pulse amplitude of 150 mV was mostly adopted as the best compromise.

TABLE 2.1. ***Dependence of peak current on supporting electrolyte at a constant flow rate of 11 mℓ/min ([Cu] = 10 μg/ℓ; 5 min deposition time at - 400 mV). The numbers in brackets represent the relative standard deviations for four measurements.***

SUPPORTING ELECTROLYTE (mol/ℓ)	MEAN PEAK CURRENT (μA)
1.0 KNO_3	10.92 (1.84)
1.0 $NaNO_3$	10.80 (2.01)
1.0 HNO_3	12.94 (0.96)
0.32 HNO_3	20.55 (1.03)
0.06 HNO_3	5.13 (0.96)

Preliminary results revealed that the deposition potential must be more negative than 0.00 V for accurate measurements. The peak height of Cu measured at different deposition potential between −200 mV and −400 mV (Table 2.2) indicated a decrease in sensitivity with an increase in deposition potential. The decrease may be due to insufficient copper deposition as the deposition potential becomes more anodic and closer to the stripping peak. A −400 mV deposition potential was the best choice because it gave the highest peak current and is closer to the copper stripping peak thus eliminating some interferences, which might result from reducible substance at voltages below -400 mV.

TABLE 2.2 ***Effects of different instrumental parameters on the peak current. ([Cu] = 20 μg/ℓ; conditions as in experimental except for the variables studied). The numbers in brackets represent the relative standard deviations for four measurements.***

PARAMETERS	MEAN PEAK CURRENT (μA)
PULSE AMPLITUDE (mV):	
20	0.557
50	3.233
150	9.855
200	12.71
INLET-WE SEPARATION (mm):	
3	18.21 (0.33)
5	19.31 (0.35)
8	10.45 (1.42)
DEPOSITION POTENTIAL (mV):	
-400	25.46 (2.58)
-300	22.15 (0.79)
-200	17.46 (1.42)

2.3.2 CHARACTERIZATION OF THE FLOW-THROUGH CELL

To acertain that the flow-through cell build has wall jet properties, the stripping peak current equation (eq. 4, chapter 1) was experimentally verified. This equation describes the current in terms of the hydrodynamic parameters of the flowing solution. It defines the performance in terms of the volume flow rate, diameter of the nozzle and radius of the electrode. In this studies diameter of the nozzle and the radius of the electrode were constant, therefore only the relationship between the flow rate and peak current was used to verify the performance of the cell. From the equation, a wall jet electrode's (WJE) response should increase almost linearly with three quaters of the power of the solution flow rate. As expected a plot of peak current versus flow rate to the power of three quaters resulted in a linear graph with a slope of 1.65 μ**A** /min$^{3/4}$,

a correlation coefficient of 0.989 and an intercept of 0.411 μ**A**. This confirmed that the cell has WJE properties.

2.3.3 LINEARITY OF DPASV RESPONSE AND OXYGEN EFFECT ON THE RESPONSE

The dependence of the DPASV Cu peak on the deposition time exhibits a good linear relationship with a slope of 2.998 μ**A** /min, a correlation coefficient of 0.991 and an intercept of 5.51 μ**A** up to a deposition time of 10 minutes. Although the highest deposition times produce highest peak currents, low deposition times are advantageous because they shortened the analysis time and reduced interferences. Due to the consideration of time and to maintain the electrode surface for a longer period between washing, a lower deposition time of 5 minutes was adopted unless stated. The effect of deaeration on the response (Fig.2.3) clearly indicated the need to deaerate solutions for at least 10 minutes to increase sensitivity, but to be more accurate a 20 minutes deaeration time was adopted. Deaeration reduced the first oxygen peak by decreasing the amount of oxygen in the solution thus enabling an easy measurement of the copper peak.

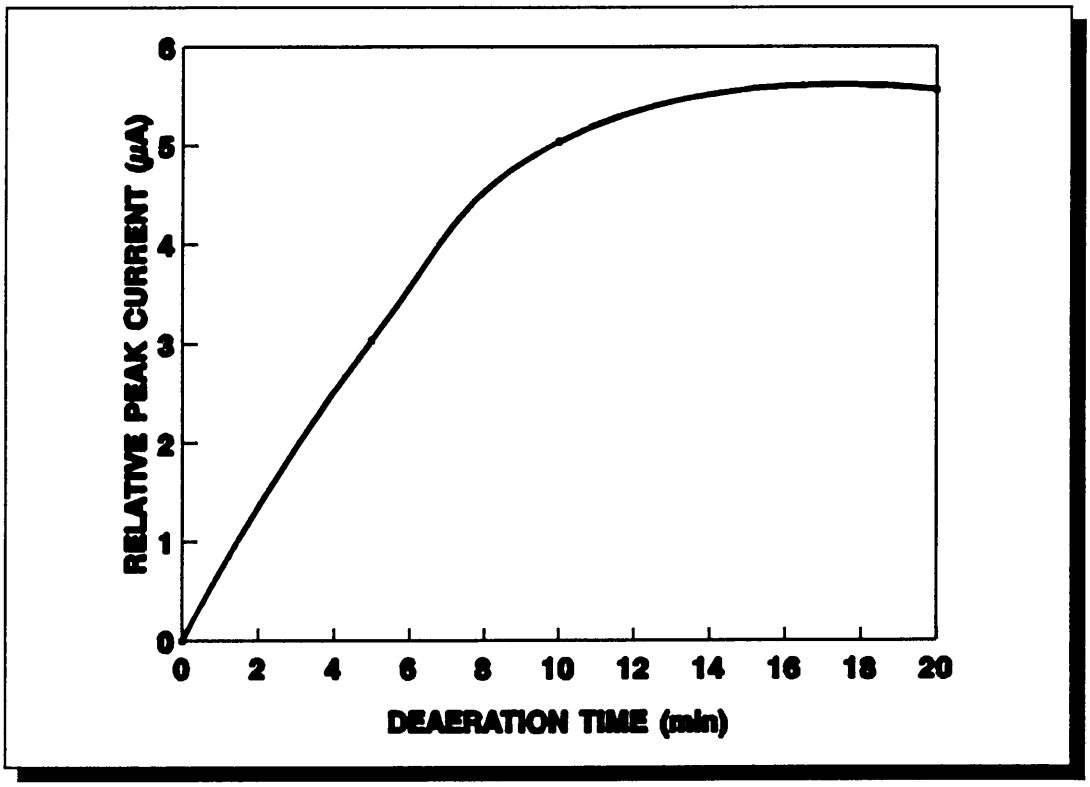

Figure 2.3: Effect of deaeration time on the peak current.

The influence of flow-rate on peak current was also investigated. A good linear relationship with a slope of 1.01 μA/mℓ.min $^{-1}$, a correlation coefficient of 0.969 and an intercept of 1.07 μA was achieved up to a flow rate of 12 mℓ/min.The highest linear flow-rate combined with a low deposition time gave reasonable sensitivity within the shortest analysis time was used.

Quantitative evaluation is based on the linear correlation between peak currents and concentrations. A comparison of the calibration graphs for different regeneration methods (with the proposed procedure described in 2.2.3) after successive measurements of copper solutions of increasing concentration (1.0 - 20.0 μg/ℓ) showed a linear relationship for all regeneration methods. A plot done without regeneration had a slope of 1.84 μA/μmolℓ^{-1}, a correlation coefficient of 0.997 and an intercept of -1.54 μA. A similar experiment done with electro-chemical regeneration yielded a calibration graph with a slope of 2.76 μA/μmol.ℓ^{-1}, a correlation coefficient of 0.998 and an intercept of 5.66 μA. The best results were obtained by chemical regeneration where the calibration graph yielded a slope of 2.70 μA/μmol.ℓ^{-1}, a correlation coefficient of 0.999 and an intercept of 0.85 μA. The results clearly indicated a need for periodical cleaning of the electrode.

2.3.4 REGENERATION METHOD FOR THE ELECTRODE

Table 2.3 shows the percentage recovery of standard copper solutions using three regeneration methods (no cleaning), electrochemical and chemical (cleaning with a 1 mol\$\ell$ NaOH solution between analyses). Four replicates of each sample were done and the mean results are reported. Agreement between the results and input values was very satisfactory especially with chemical cleaning. Without washing, both the accuracy and the precision were poor. Chemical cleaning with a 1 mol\$\ell$ NaOH solution gave the most accurate and reproducible results with the lowest detection limit and this regeneration method was used throughout the rest of the analysis.

The limit of detection was calculated from 3 x the standard deviation of the lowest standard in the calibration graph after thirteen successive measurements were made. [42] From the results it is clear how important the pretreatment of the GC electrode is, because it determines the reproducibility and accuracy of the results. Since the nature and extent of the pretreatment

procedures depends on the electrode material as well as the supporting electrode it is necessary to establish the best pretreatment procedure for our method (see table 2.3).

TABLE 2. 3: Comparison between regeneration method conditions using the proposed procedure described in 2.2.3.

Regeneration of the electrode	**Amount of copper added (μg/ℓ)**	**Amount of copper found (μg/ℓ)**	**% copper recovered**	**Detection limit (μg/ℓ)**
NONE	4.00	2.97	74.2	1.49
	8.00	5.81	72.6	
CHEMICAL	5.00	4.56	91.2	0.56
	10.00	9.50	95.2	
ELECTROCHEMICAL	5.00	4.44	88.9	1.31
	12.00	11.00	91.7	

The results can be explained by the fact that during deposition and plating steps a number of chemicals are adsorbed, deposited and formed a film on the electrode. Some of these chemicals are not stripped off the electrode and as a result they accumulated resulting in decreased currents and inaccurate results as indicated in table 2.3. During electrochemical cleaning which involved stripping from -1.5 V to 1.5 V four times without plating, oxidizable compounds stuck on the electrode are removed, thus cleaning the electrode of contaminants or film introduced during deposition.

On the other hand chemical cleaning simply removes any chemicals which are soluble in NaOH solution. Another possible explanation for the better performance of the GC after chemical cleaning might be due to the addition of the hydroxide ion to the electrode.[43]

2.3.5 INTERFERENCES

An important problem in analytical voltammetry has been reproducibility of measurements on

the solid electrodes due to adsorption and passivation processes. The problem can be minimised partly by regeneration of the electrodes. However, for real sample analysis, a thorough investigation into diverse ion and organic effects in copper analysis was necessary. Inorganic species like Zn^{2+}, Fe^{3+}, Al^{3+} and organic species like acetate, phenol and humic acid in fresh water samples may interfere in the copper determination. Appearance of the peak current in the presence and absence of these species (Zn^{2+}, Fe^{3+}, Al^{3+} , acetate, phenol and humic acid) was therefore studied. The results obtained, when adding 2 mg/ℓ of each of the species in 16 μg/ℓ copper in 1 mol/ℓ HNO_3 solution, are given in table 2.4.

Studies made by adding varying concentrations of the species from 0 to 2 mg/ℓ in 16 μg/ℓ copper solutions, show no apparent change in the peak current up to a concentration of 300 μg/ℓ with all species except humic acid. Humic acid showed a decrease in peak current at a concentration of 40 μg/ℓ, while Zn^{2+}, Fe^{3+}, phenol and acetate showed a decrease above 300 μg/ℓ. Al^{3+} showed no apparent change in peak current up to 2 mg/ℓ. Interferences of Zn^{2+} and humic acid in copper analysis using mercury or mercury film electrodes are well documented.[7,38-41,44] These interferences appear at concentrations which are higher than those normally obtained in fresh water samples and it was therefore safe to assume that they will not interfere in our real samples.[44]

2.3.6 ACCURACY AND PRECISION

Precision of the method was estimated from fifteen replicates measurements of a 16 μg/ℓ standard Cu solution. The mean peak current found was 27.52 μA with a range of 23 - 31 μA and a relative standard deviation of 7.52 %. Accuracy was tested by determining the Cu content in a British certified reference material (No. 251) of low alloy steel containing 0.090 ± 0.004 % Cu. The mean result of six measurements was 0.090 ± 0.005 % Cu with a range of 0.084 - 0.091μg/ℓ.

Table 2. 4: ***Effect of the addition of other elements/ compounds in 16 μg/ℓ copper in 1 mol/ℓ HNO_3 solution***

ELEMENT	PEAK CURRENT (μA)	% Decrease [a]
Cu alone	16.47(0.57)	-
Al^{3+}	16.80 (1.44)	0
Acetate	9.02 (0.43)	45
Fe^{3+}	11.35 (1.01)	31
Zn^{2+}	9.45 (1.21)	43
Humic acid[b]	4.79 (0.32)	71
Phenols	6.32 (0.79)	62

[a] % decrease at 2 mg/ℓ =[{(peak current of pure copper) - (peak current of interfering element)}/peak current of pure copper] x 100,

[b] Humic acid at 40 μg/ℓ had a % decrease of 15.

2.3.7 APPLICABILITY

The suitability of the glassy-carbon electrode for real sample analysis was demonstrated by determining copper in fresh water samples (Table 2.5). After collection, samples were acidified with nitric acid to obtain a final concentration of 1 mol/ℓ. Quantification was experimentally done using standard addition for two deposition times and two standard additions. The concentration of the metal in the sample was determined from the intercept of the two graphs. This standard addition method [40] was reported to be useful in compensating for the effects of intermetallic

interference and for variations in instrumental parameters between runs. The calibration method was also employed for quantification purposes, and gave similar results to those provided by standard addition. In these samples we can safely assume that there was no interferences since the concentrations of the interfering elements are below the interfering concentrations.

TABLE 2. 5: ***Results of fresh water samples***

Water type	Ground	Rain		Tap
		Arcadia	Campus	
pH	4.32	6.96	6.54	7.24
Cu ($\mu g\backslash \ell$)	1.57	3.36	5.80	13.11
RSD (%)	1.40	2.36	2.16	1.50

A successful flow-through system was developed for trace analysis of copper using DPASV with a glassy-carbon electrode. Periodical regeneration of the electrode with a 1 mol/ℓ NaOH solution increased sensitivity and precision. The method was shown to be applicable with a detection limit of 0.56 $\mu g/\ell$, with a determination time less than 7 minutes per measurement (omitting deaeration time). The draw back of the system is the deaeration period. This DPASV limitation is being investigated in the next chapter.

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3. DEOXYGENATION IN A FLOW-THROUGH ANODIC STRIPPING VOLTAMMETRY

3.1. AN OVERVIEW ON DEOXYGENATION

Dissolved oxygen is a major problem in anodic stripping voltammetry (ASV) as mentioned in earlier chapters. The presence of oxygen in the solution results in a large background current, baseline noise or sloping baseline which obscures the metal peaks. In every aqueous solution open to the atmosphere the concentration of dissolved oxygen is about 0.001 mol/ℓ. It has been established that it is necessary to reduce the amount of dissolved oxygen in view to reduce background current and to obtain lower detection limits.[1]

Oxygen is reduced polarographically giving two waves of approximately equal heights that overlap with metal waves occurring over a wide potential range.[2] These prevent utilisation of higher galvanometer sensitivities. In addition to this problem, presence of oxygen may also affect the response due to the chemical oxidation of the metal or precipitation of metal hydroxides by the hydroxyl ion (a product formed on the electrode surface in the presence of oxygen).[3,4]

The removal of oxygen can be achieved either by chemical reduction or physically by deaeration with indifferent gases (inert gases). In the first technique sulphite had been used depending on the composition of the solution. In alkaline solutions, sulphites of alkali metals can be used, because at a pH greater than eight they do not give polarographic waves. The products of this reaction with oxygen are sulphates which are also polarographically inactive. In ammonia solutions some strong reducing agents (e.g. salts of divalent manganese or iron) react faster with oxygen than sulphite.[5]

The application of chemical reduction is limited to those instances in which sulphite does not react with other solution components. Furthermore successful use of chemical reduction required addition of the sulphite crystals or 0.2 mℓ of freshly prepared saturated sodium sulphite solution for few minutes before the solution comes into contact with mercury. In organic and ammonia solutions at least 10 minutes is require before starting the analysis. This results in increased

analysis time. Deoxygenation may be prevented in the presence of traces of kerosene, alkaloids and some aromatics. Although this technique might be more adaptable to flow-through systems, it is severely limited by availability of inactive and pure chemicals.[5] Oxygen removal can also be done by the use of enzyme-catalysed reactions.[6] The enzyme reactor inserted before the injector, deoxygenated the carrier solutions by reacting with glucose added to the carrier. The reaction was catalysed by glucose oxidase and catalase co-immobilized in an enzyme reactor. The porous glass reactor was effective for several months.

Generally, the physical removal of oxygen predominates over chemical reduction. In order to remove oxygen effectively from the flow-through system, several methods have been used, including electrical scrubbers,[7] semi-permeable membranes[8,9] and discriminating methods like subtractive stripping voltammetry.[10] These background correction methods are often incomplete because of changes in the analytical and background scans. All require specialised equipment to be fitted to the flow-system, making the system cumbersome and difficult to adapt for field work. Comparatively, electrical scrubbers seem efficient, but suffer as a result of their limited applicability, they cannot be used when copper determination is desired.[7] Hydrogen peroxide, which is formed in the reaction, interferes seriously in many cases. A detailed discussion on these methods has been reviewed by Wallace.[11]

Deoxygenators that have the widest applicability, highest efficiency and fewest drawbacks are semi-permeable membranes. Unfortunately their efficiency is limited only to flow systems that have a tubing length of at least 250 cm, thickness of less than 0.024 cm and a flow rate less than 3 mℓ/min. This accounted for their frequent use in HPLC detection and predominant use of gas bubbling in other systems. A promising method employs chromatomembranes of interphase exchange[12] which is based upon capillary effects that occur within a porous hydrophobic matrix. The performance of this method is much higher when higher flow rates are used. It produces water of oxygen content below 20 μg/ℓ. It results in a better efficiency than boiling, which produces water of oxygen content of 900 μg/ℓ and inert gas bubbling with 200 μg/ℓ oxygen content. In general, these methods are quite complicated to implement.

Some of the interesting techniques that have recently been used are the dual coulometric voltammetry cells.[13] This approach utilises an upstream coulometric cell with a mercury-coated

vitreous carbon working electrode and a downstream wall-jet detector with a mercury coated GC disc working electrode. The strategy is to selectively and exhaustively electrolyse the interfering constituents at the upstream cell prior to the ASV detection at the downstream cell. Nitrogen activated glass nebulizer employing a Teflon capillary for aspirating the sample solution, has been used to spray the sample into a nitrogen atmosphere; oxygen removal is practically instantaneously.[14] The limiting stage for this technique is the time required for sufficient sample collection in the cell which is about 1 minute.

3.1.1 INERT GAS DEAERATION

Physical removal by deaeration with an indifferent gas is the most commonly used technique, [15-17] in spite of several drawbacks which arise from bubbling of indifferent gases (removal of volatile compounds, lengthy time and inconvenience in flow-through configurations). Introduction of the inert gas into the sample results in a decrease in the partial pressure of the oxygen in the gaseous phase above the solution. This decrease of the partial pressure of the oxygen causes a decrease in the dissolved oxygen concentration. Efficient deoxygenation in flow-through systems has been realised by introducing nitrogen into the stream to form a two-phase flow combined with a membrane gas-liquid separator.[18] There is a report on removal of oxygen by permeation through the walls of a silicone tubing into a surrounding nitrogen flow.[19]

A number of deaeration devices or chambers have been described[20] and direct cell deaeration has also been reported recently.[21] Gases such as pure nitrogen (oxygen free), hydrogen, helium, argon and to a lesser extent other inert gases, carbon dioxide and even fuel gas such as methane, provided that they are polarographically indifferent in the solution under tests, have been employed for degassing solutions. In order to reduce the deaeration time as well as developing a simple and effective method of oxygen removal for a continuous flow-through anodic stripping voltammetric system, two simple yet efficient methods of deoxygenation are compared. Analysis potential of direct cell deaeration (DCD) was compared with that of a deaeration chamber (DC) (deaeration in a chamber prior to detection). The two methods are optimised, their limitations as well as their effectiveness in oxygen removal are evaluated.

3.2 EXPERIMENTAL

3.2.1 APPARATUS

The continuous flow-through trace analyser system used and the voltammeter were described in chapter 2 section 2.2. The modified version of the wall-jet described earlier (see chapter 2 section 2.2) was used. Modifications include an addition of an inlet for nitrogen purging in the lower block of the cell, as was done by Tay.[20] The inlet can be closed with a perspex stopper when direct cell deaeration is not desired. Electrode setup described in chapter 2 was used.

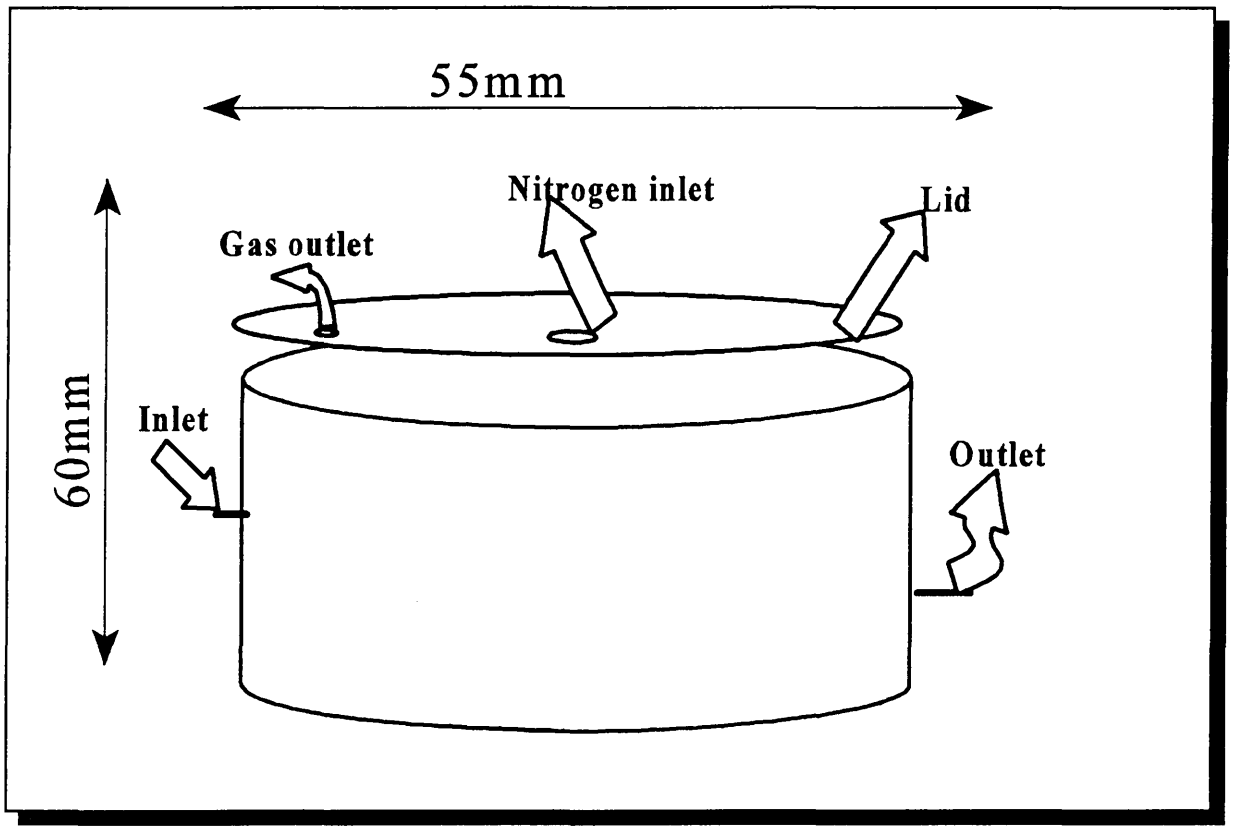


Figure 3.1: Schematic diagram of a continuous flow deaeration chamber

The deaeration chamber (75 mℓ bottle, 4 cm in diameter and 6 cm high, see figure 3.1) was placed between the pump and the flow cell with a tygon tubing connection to each. Two solution

openings, an inlet and an outlet, were placed in opposite sides of the bottle wall. The lid had two openings that allowed the nitrogen in and the oxygen to escape. High purity grade nitrogen was used. The sample flow was continuously maintained through the chamber and cell except during the stripping.

3.2.2 CHEMICALS

All chemicals were of analytical-reagent grade and were used without further purification. A stock solution of copper was prepared by dissolving accurately weighed $Cu(NO_3)_2$. $3H_2O$ in distilled water to give a copper concentration of 500 $\mu g/\ell$. Dilution of the stock solution was performed to acquire the desired working concentration. The final solutions were acidified with nitric acid to have a final concentration of 1 mol/ℓ HNO_3. Dilutions and preparations of solutions were done with deionised water from a Modulab system (Continental water systems)

3.2.3 PROCEDURE

The deaeration chamber (DC) measurements were performed in the following way: 10 mℓ of the sample was placed into the chamber, purged with nitrogen gas for five minutes and passed through the flow cell at a rate of 9 mℓ/min for a deposition time (D_T) of either 3 or 5 minutes at -400 mV. The solution flow stopped just before stripping began. Metal stripping was done by applying an anodic potential scan up to 400 mV or 300 mV and a voltammogram was recorded simultaneously at a scan rate of 20 mV/sec.

Flow-cell purging in direct cell deaeration (DCD) was performed as follows: the sample was passed through the cell and purged with nitrogen at the same time; purging was continued even during the stripping period; the deposition potential, deposition time and the flow rates used were exactly the same as those used for DC measurements.

3.3 RESULTS AND DISCUSSION

3.3.1 OPTIMISATION OF THE DEAREATION CHAMBER

Preliminary investigation and observation made in chapter 2 suggests that effective deoxygenation depends on sample volume. Therefore the study of the effect of volume on deoxygenation was undertaken. The investigations were done using different sample volumes containing 10 $\mu g/\ell$ of Cu. Copper was the element of choice in this study due to the closeness of copper peak to the second reduction peak of oxygen. The efficiency of the oxygen removal could thus be evaluated by measuring the copper peak current. In an undeaerated solution the oxygen reduction peak predominated, which resulted in an inability to detect the copper. The ability to measure the copper peak current increased gradually as the oxygen was removed.

Table 3.1: Effect of sample volume on peak current. The solution was deaerated for 5 minutes before analysis ([Cu] =10 $\mu g/\ell$)

SAMPLE VOLUME (mℓ)	PEAK CURRENT (μA)	
	D_T = 3 min	D_T = 5 min
10	8.52	10.33
15	6.99	9.51
20	6.56	8.61
30	6.43	8.75
35	5.99	8.14

It is evident from Table 3.1 that the peak intensity decreased with an increase in sample volume up to 20 mℓ where the intensity remained almost constant throughout the investigated volumes (10 to 35 mℓ).The results clearly show that five minutes deaeration was not sufficient for removal of oxygen in the sample volumes above 15 mℓ. These might have been due to the fact that 5

minutes deaeration only removed a fixed amount of oxygen and this amount was less than the amount of oxygen present in samples of 15 mℓ or more. An increase in sample volume is directly proportional to an increase in dissolved oxygen, therefore complete deaeration in larger volumes will require more time. A smaller volume results in a reduced analysis time, as a result a 10 mℓ volume was preferred

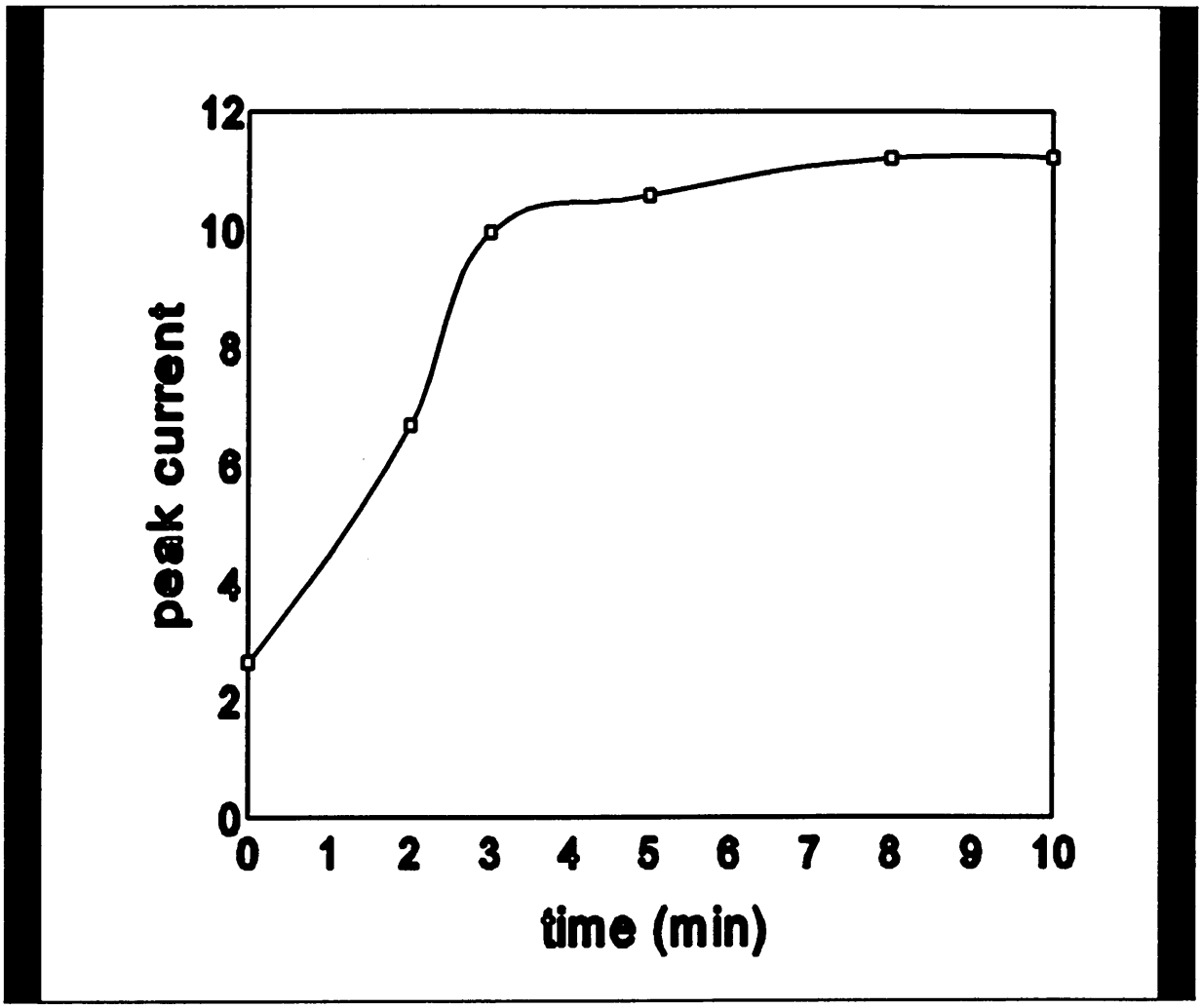


Figure 3.2: Effect of deaeration time in the chamber on the peak current ([Cu] =20 μg/ℓ)

The effects of deaeration time on peak current is shown in figure 3.2. It is clear from the graph that a deaeration time of at least 3 minutes is necessary to remove oxygen sufficiently. This time

was an improvement from the conventional 20 minutes usually adopted and used in chapter 2.

3.3.2 OPTIMISATION OF THE DIRECT CELL DEAERATION (DCD)

To determine the effective method that can be adopted, comparison between continuous purging during stripping and stopped purging was done (see table 3.2). A higher sensitivity was observed with continuous purging. When purging, the solution lost oxygen together with some volatile compounds, resulting in a change in solution composition especially if it is done for a long time. The change in composition may have medium exchange effects. In addition the nitrogen prevents oxygen diffusion from the electrodes.

Table 3.2: Effect of continuous purging during stripping on the peak current intensity. Flow rate = 9 mℓ/min, D_T = 5 minutes

[Cu] (μg/ℓ)	PEAK CURRENT (μA)		
	PURGING	NO PURGING	DIFFERENCE
8	4.29	2.24	2.05
16	6.31	5.71	0.60
32	11.57	9.36	2.21
40	14.22	12.88	1.34
sensitivity (slope μA/μgℓ$^{-1}$)	0.312	0.310	0.002

3.3.3 COMPARISON BETWEEN DCD AND DC

To evaluate the applicability of the DCD and DC deaeration methods, as well as to demonstrate their advantages compared with each other, several investigations were carried out. Both methods show an expected linear dependence of the peak current vs preconcentration time (eq.

4 chapter 1). However, the current sensitivity in DCD is almost double that in DC, a clear indication that DCD is more sensitive than DC.

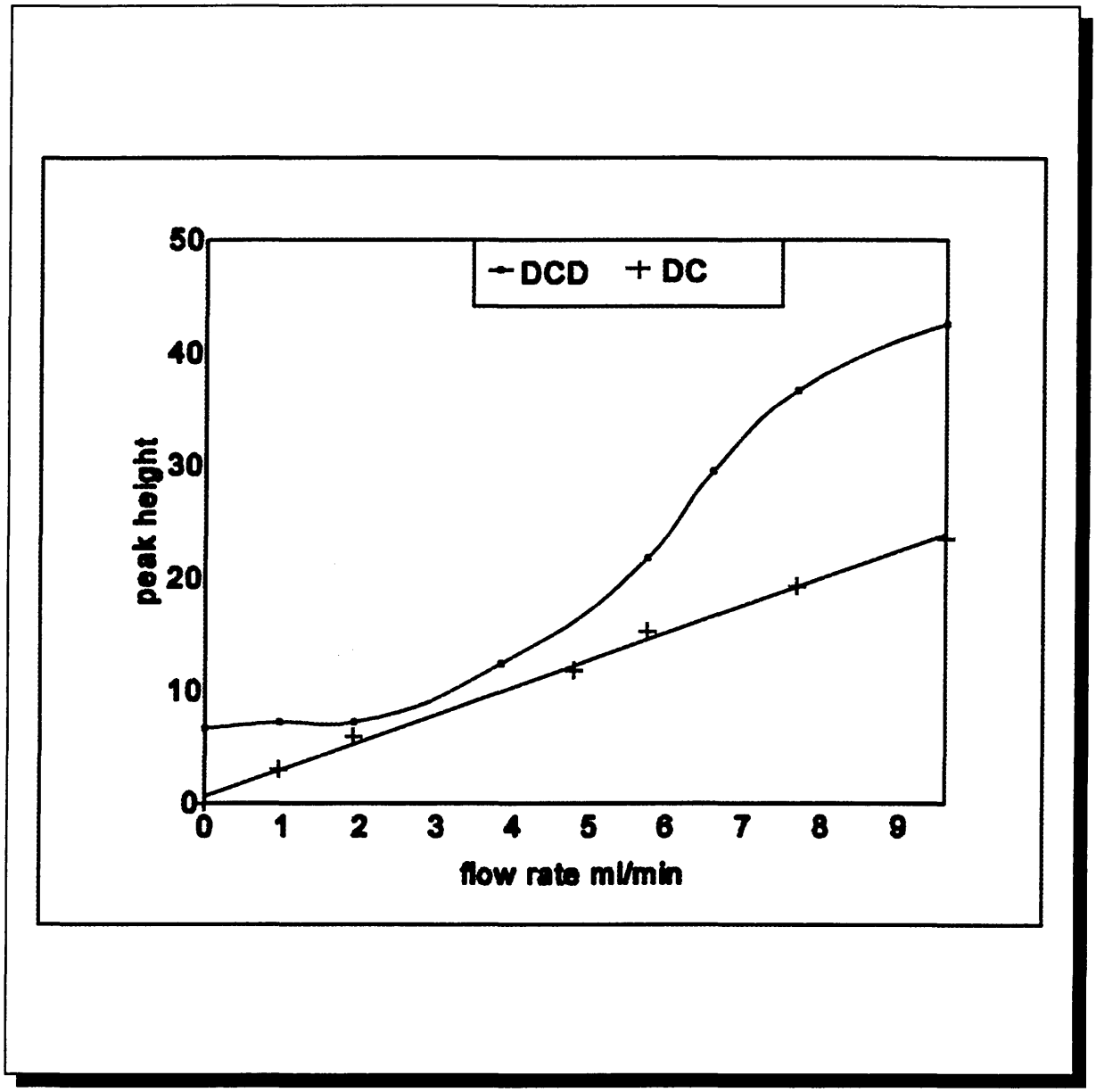


Figure 3.3: Effect of flow rate on peak current. ([Cu] = 16 μg/ ℓ, D_T= 5 minutes)

Figure 3.3 shows the relationship between flow-rate and peak current. A linear increase of peak current in DC and an S-shaped curve in DCD is observed. A higher flow-rate resulted in higher signal, due to a thinner diffusion layer. In DCD the curve tended to form a plateau at a flow-rate

above 8 mℓ/min, probably because of too little time available for detection, as the contact time is too short between the electrode and the solution, or it may be due to insufficient oxygen removal. This behaviour may be explained further by the fact that for a wall jet electrode (WJE), the response should increase almost linearly with three quarters of the power of the solution flow rate and eventually level off when the equilibrium concentration is achieved.[12]

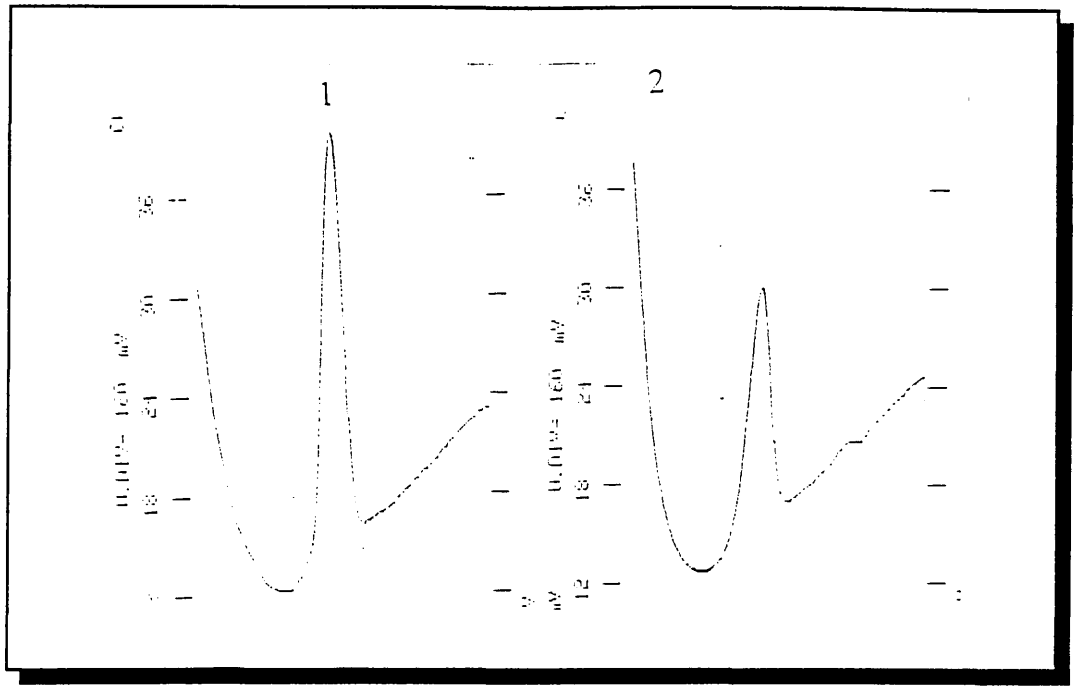
3.3.4 OXYGEN REMOVAL STUDIES

The DPASV scans obtained by purging the cell contents with nitrogen (DCD method) and purging the solution in the chamber (DC method) at optimum conditions are shown in figure 3.5 a and b respectively. As can be seen, the DCD method of oxygen removal was more effective in eliminating background current due to excess dissolved oxygen. Therefore the DCD method will be more suitable for trace analysis than DC methods. With the DC method oxygen might diffuse back into the flow system through the tubes or connections and the electrode grooves, resulting in a highly sloping base line that obscures the metal stripping peak. The problem is more severe when short deposition times or low concentrations are used, because in these conditions peak currents are small. It is evident that DCD method is more suitable than DC for trace analysis, where low concentrations of the orders of 10^{-9} in natural waters are determined.

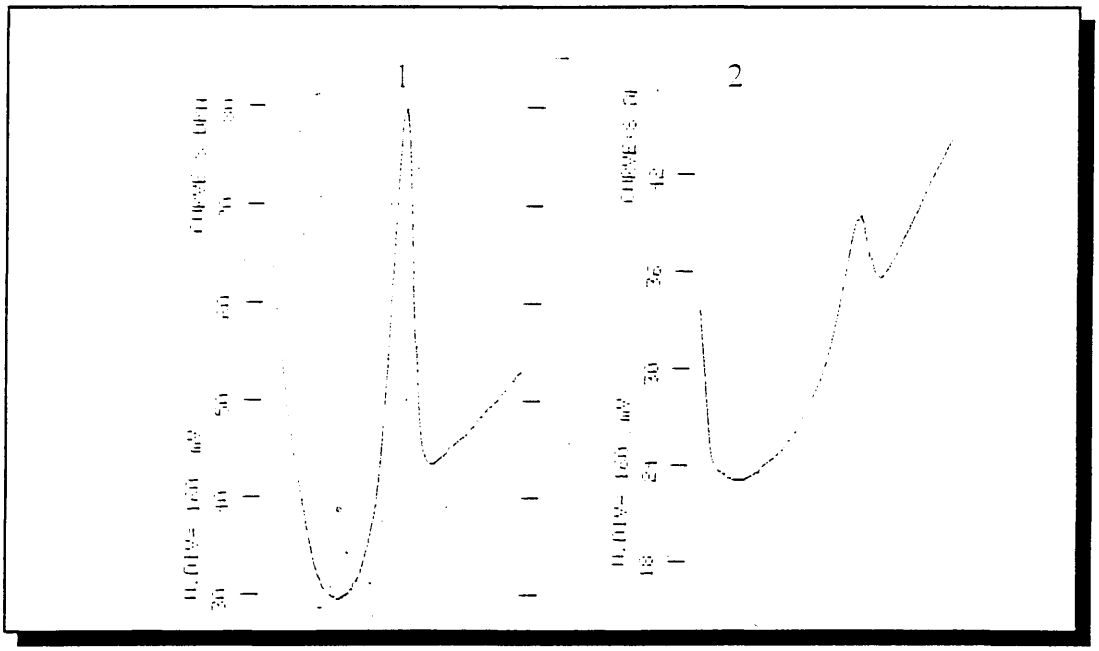
3.3.5 SAMPLE THROUGHPUT, LIMITS OF DETECTION AND PRECISION

The sample throughput was 10 and 13 measurements per hour for DC and DCD respectively, using a sample containing 16 μg/ℓ copper at D_T = 3 min, a flow rate of 9 mℓ/min and a 3 min deaeration time for a 10 mℓ solution with DC. They is no significant difference in sample throughput. This maybe not be the case if different samples were analysed instead of using the same sample,because more time is required for cleaning the cell between runs in DC than in DCD.

The detection limits for copper were calculated as three standard deviations of the lowest concentration (1.6 μg/ℓ). Detection limits of 0.91 and 0.17μg/ℓ at 3 and 5 minutes D_T respectively were obtained using DCD. When DC was used, there was no detection at 3 minutes D_T,



(A)



(B)

Figure 3.4: ***Effect of oxygen on polarograms: A) DCD method where1 and 2 were obtained with a deposition time of 5 and 3 minutes respectively. B) the same as A but using DC method.***

while a detection limit of 0.15μg/ℓ at 5 minutes D_T was obtained. This indicates that detection limits are dependent on the preconcentration time and the deaeration method. These detection limits differences are a direct result of the differences in background. The method with the smallest background has the lowest detection limit. From the studies it is clear that detection limits can be lowered further by increasing deposition times.

Thirteen consecutive measurements of a 16 μg/ℓ copper solution at a flow rate of 9 mℓ/min gave a relative standard deviation (RSD) less than 7 % at a deposition time of 3 minutes and less than 5 % at a deposition time of 5 minutes for DC. Similar experiments with DCD resulted in a RSD of less than 5 % at a deposition time of 3 minutes and less than 2 % at a deposition time of 5 minutes. These RSDs' are similar to those usually obtained in FI-ASV systems

3.3.6 CONCLUSION

Comparison of these well known simple, cheap deaeration methods clearly illustrates the need to be able to effectively remove oxygen without compromising on the analysis time. It is obvious that DCD and DC methods are not equivalent. DCD methods tend to be faster than DC methods. This is mainly due to the fact that in DCD, removal of oxygen can be achieved at the same time when depositing the analyte. This feature results in considerable gains in convinience, time saving, low detection limits and higher sensitivity. Furthermore, sample throughput is higher with a reproducibility less than 5%. The system is not cumbersome and can easily be used for trace analysis. DC on the other hand is not entirely useless. Its main drawback is that it is time consuming especially when different samples are analysed, a lot of time will be spent cleaning the apparatus between analysis. An additional gadget makes it cumbersome and easily contaminated. Sensitivity of both methods can be improved by increasing flow rate, but the increase is limited to between 3 to 8 mℓ/ min for DCD. This flow rate limit may be due to the fact that higher flow rates lead to higher pressure in the system, thus increasing sensitivity to small air-bubbles and cosequently higher noise levels.

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4. SPECIATION OF TRACE AMOUNTS OF IRON(II) AND IRON (III)

4.1 INTRODUCTION

One of the toughest problems facing analytical chemists on the verge of the 21st century is that of speciation. Speciation may be defined as the different physico-chemical forms of an element which together comprises its total concentration in a given sample. It has become especially clear that it is not the total concentration of a certain element that produces a negative or positive effect, but rather the specific compound form that decisively influences the toxic effect of an element. For example, inorganic mercury species are generally unable to cross biological membranes and thus have low toxicity, but alkyl-mercury species are lipid soluble and hence extremely toxic.

It is very important therefore to do the determination of the actual form of an element in environmental compartments for accurate assessment of its biological activity (bioaccumulation, bioconcentration, bioavailability and toxicity).[1,2] This entails quantification of the different forms as well as oxidation states of an element in a sample. The oxidation state of an element and its presence in the dissolved complexes are important considerations related to its availability and toxicity. A complex scheme of operations designed to simplify the analytical procedures involved in the acquisition of accurate data on individual forms of elements is used. Several approaches that have been followed include utilisation of analytical methods based on selective or sequential extraction, like ion-exchange chromatography, coupled instruments e.g. FI- spectrophotometry and electrochemical methods (anodic stripping voltammetry, adsorptive stripping voltammetry and ion selective electrodes).

Electrochemical techniques especially anodic stripping voltammetry (ASV) is undoubtedly the most widely used technique in the speciation of trace metals. The application of this technique to metal speciation is based on the fact that it allows differentiation between the labile (i.e weakly complexed) and total metal value. In addition different electroactive species undergo reduction

(or oxidation) at different electrode potentials; this enables differentiation of element oxidation states as well as their complexes. Some researchers have coupled ASV with flow injection analysis (FIA) as well as with other detectors used effectively in speciation.

The choice of the technique for an investigation of trace metal speciation depends on a number of factors. A prime requirement of any speciation method is to involve little sample manipulation and to offer the minimum sample contamination. This is sufficiently satisfied by the flow-through configuration. Other considerations include the concentration of the metal in the sample, the complexity of the system (especially interferences) and the level of information required. Most methods that were mentioned above provide only a crude fractionation profile; the more sophisticated techniques such as HPLC-ICP-MS may provide greater sensitivity as well as adequate information regarding the nature of the species present but they are very expensive to set up and require a greater degree of analytical expertise.

A number of these analytical methods have been discussed for the speciation of elements such as aluminium, arsenic, cadmium, chromium, copper, mercury, platinum, lead, iron, selenium, tellurium, tin, vanadium and zinc.[8-15] Chromium, arsenic and mercury are among the most frequently analysed due to their toxicity problems. Several review articles[16,17] have been published on this topic, and it is still clear that some work on simultaneous speciation analysis is lacking, hence these studies were taken on this subject with iron as an element of choice.

4.1.2 IRON DETERMINATION

Iron is widely distributed in nature and is one of the most important elements in biological systems. Its biological effectiveness is influenced by its chemical properties such as valency, solubility and degree of chelation or complex formation. In general iron can exist in two oxidation states i.e iron(II) and iron(III). The possible iron species in aqueous solutions are hexaaquairon(II) and hexaquairon(III) ions, various negative and positively charged iron(II) and iron(III) complexes and uncharged organic compounds of iron(II). The predominant iron species depends on the oxidation-reduction conditions and on the concentration and stability constants of the anions present in the solution.

The content of iron in natural waters can vary over several orders of magnitude from tens of $\mu g/\ell$ (sea water) to tens of mg/ℓ (ground water and mineral waters). In wine it is present in concentrations ranging between 0.5-25 mg/ℓ. Although the iron concentration is not very important, it influences the organoleptic properties of the solution, mainly colour, taste and turbidity. In addition, irons' bioavailability and metabolism are strongly dependent on the species involved. Particularly the iron uptake is influenced by its oxidation state and complexation through inorganic and organic ligands, thus making iron discriminating methods' essential.

Methods used to determine iron in environmental and geological samples include spectro-photometry, voltammetry, and amperometry.[18-20] These methods have also been applied in differentiation of the two oxidation states. The determination of both iron valences in geological and biological materials is a problem that has not yet been solved. Recently a publication on the use of flow injection system (FIA) in conjunction with amperometric detection with a glassy carbon electrode in which the two oxidation states were differentiated by an appropriate choice of indicator potential has been released.[21] Amperometry combined with spectrophotometry[22] and atomic absorption detection as well as a combination of HPLC (electrochemical detection) with flame atomic absorption detection[23] have made a useful contribution to iron speciation studies. The potential of FI spectrophotometry followed by flame atomic absorption spectro-photometry was also shown in determination of iron(II) and total iron.

Typically, one form together with total iron is determined allowing for the calculation of the second oxidation state by the difference between measured values. Methods based on 1,10-phenanthroline as the complexing agent allow the determination of Fe(II) and total iron after reduction of Fe(III) by spectrophotometry. Alternatively, iron(III) may be determined directly using a chelating agent with a strong affinity for Fe(II) like thiocyanate or hydroxamine acids. In such systems, the carrier stream is split into two parallel streams, one of which is used for the direct measurement of a particular species, whereas in the other a redox step is carried out before the measurement, which subsequently gives the total concentration of the element.

The use of a valve to switch between streams, with or without a redox reagent, for the sequential determination of oxidation states of elements has been the most common method employed for

speciation. This approach has been applied in the spectrophotometric determination of both iron oxidation states. The method usually utilises one of the following approaches: a method not requiring a redox reagent and which uses selective indicator reactions for each oxidation state, with a valve to switch between the streams of buffered 1,10-phenanthroline and acetohydroxamic acid has recently been reported. Similarly both iron forms have been determined spectrophotometrically by synchronized sample injection into two parallel flow streams in which Fe^{2+} and Fe^{3+} were determined with 1,10-phenanthroline and thiocyanate respectively. The use of FIA for speciation have been reviewed by Luque de Castro.[24]

An alternative approach applied to the quantification of two oxidation states of a metal is a multi-detection. A combination of spectrophotometry and atomic absorption spectrometry has been employed for sequential determination of Fe^{2+} and total iron. Almost all the schemes described, are not simultaneous analysis, but splitted streams, two injections or multi-detection systems.[25-29] Although these methods can differentiate between the two iron forms they are tedious, time consuming and mostly inaccurate. Their applicability is usually limited when the ratio of the oxidation states are almost the same.

Simultaneous analysis of both oxidation states has been done based on FIA and kinetic spectroscopy. The system used a silver reductor and two flow cells aligned in the same optical path to yield two peaks.[30] Amperometric detection coupled with FIA and differential pulse polarography were also used in analysis of rocks.[30] In some cases the assays were performed by simultaneous extraction into chloroform of tris (8-quinolinato)-iron(III) and tris (4,7-dipheny-1,10,-phenanthroline) iron(II) prior to their electrochemical reduction in propylene carbonate.[31] An electrochemical detector in series with a flame atomic absorption spectrophotometer have also been applied for simultaneous speciation of different iron oxidation states. These methods, although they worked very well, are labourious.

Flow-through systems are highly automated enclosed systems of relatively small capacity that virtually eliminates the use of volumetric glassware and reduce the risk of contamination and dependence on the manual skills of the analyst. These features make flow-through configuration a powerful tool for developing speciation analysis. Furthermore, flow configuration can help

speciation, mainly, by manipulating the propulsion unit, the injection valve, the transport systems and /or the detector. However, their application to the simultaneous determination of more than one oxidation state in a single sample, has been limited, and those which have been reported have favoured the split-stream approach. These study has focused on the detector and the transport system. The transport system used (described in Fig.4.1), utilized the deaeration chamber to effectively remove dissolved oxygen in the carrier steam before adding the sample. These enabled maintenance of the iron oxidation states in solution as well as developing a discriminating method that determines both iron forms simultaneously with one stream and one measurement.

The method is based on the powerful advantages of a flow-through configuration combined with a versatile detector that is able to determine both Fe(II) and Fe(III). Differential pulse anodic stripping voltammetry (DPASV) has been applied in analysis of different oxidation states of an element. The DPASV in flow-through configuration using a glassy carbon electrode (GC) was employed to accomplish discrimination of the two common iron oxidation states. Differentiation between Fe(II) and Fe(III) was achieved by an appropriate choice of the supporting electrolyte. Optimum conditions, possible interferences and the electrode reactions were studied.

4.2 EXPERIMENTAL

4.2.1 APPARATUS

The continuous flow- through trace analyser system, cell design and three electrodes arrangement used were described in detail previously (see chapter 2 experimental section). An exception was the use of an μautolab system (Ecochemie, Utrecht, The Netherlands) connected to a model 663 VA stand (Metrohm, Herisau, Switzerland) for voltammetric measurements replacing the 647 VA stand and 646 microprocessor (Metrohm) previously used. The μautolab controls the experimental parameters such as initial and final potential scan increments and provides peak location, peak height, peak area readings as well as blank corrections. The electrode used was hand polished using 0.3 μm alumina every morning and electrochemically cleaned with 3 mol/ℓ HNO_3 between samples.

4.2.2 REAGENTS AND SAMPLES

All chemicals used were of analytical grade. Ammonium iron(II)sulphate and iron(III) nitrate were used for preparation of the standard solutions of iron(II) and iron(III) respectively. A 200 mg/ℓ stock solution was prepared in deaerated 2% (v/v) HNO_3 (it was stable for a week) and working standards were prepared daily by suitable dilution of this stock solution in the matrix required. High grade nitrogen was used for deaeration of samples, solvents and during analysis. All reagents and samples were prepared in distilled water (ultra pure water).

4.2.3 PROCEDURE

A study of the relevant literature and some optimization experiments (see below) led to the following recommended procedure.

A supporting electrolyte containing 0.1 mol/ℓ $Na_4P_2O_7$ and 1 mol/ℓ NH_4Cl buffer at pH 9.0 were separately pumped into the deaeration chamber (described before in chapter 3 experimental section) simultaneously using the peristaltic pump as illustrated in fig. 4.1. The mixture was deaerated with nitrogen and passed through the flow-through cell at 4.7 mℓ/min. Samples were introduced into the reagent mixture at 2 mℓ/min just after deaeration in the chamber. The solutions were allowed to merge, react and flow through the cell. Subsequently the accumulation potential or deposition potential (Dp) of -1.2 V was applied for a selected time (D_t). After the deposition period the flow was stopped, a rest period of 5 seconds was observed and a positive going differential pulse scan (25 mV/s) was commanded. The scan was stopped at 0.1 V, and the system was immediately ready for the next run. Each set of experiments was repeated three times and averages calculated.

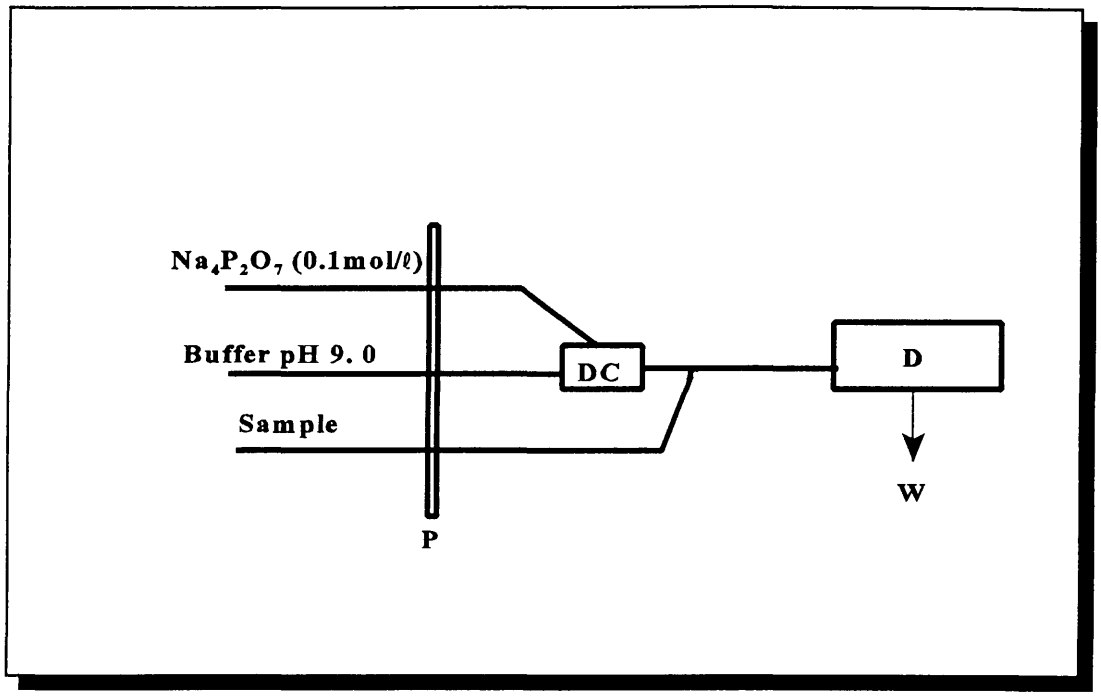

Figure 4.1: The flow-through manifold used, D = detector, DC = Deaeration chamber, P = Pump, W = waste

4.2.4 CYCLIC VOLTAMMETRIC STUDIES

Cyclic voltammetric studies were performed using a concentration of $1x10^{-4}$ mol/ℓ for both oxidation states. The solutions were pumped through the cell at a rate of 4.7 mℓ/min for two minutes. Subsequently the elements were deposited on the electrode at a selected potential (usually the starting potential). After two minutes the flow was stopped, and a resting period of 5 seconds was observed before scanning. Each set of experiments were repeated three times.

4.3 RESULTS AND DISCUSSION

4.3.1 SELECTION OF THE SUPPORTING ELECTROLYTE

A supporting electrolyte was required which formed well defined, sufficiently separated peaks with the two different oxidation states. Ideally there should be no interaction of the given valencies on each other's wave. If present, the interaction should be small and easily correctable. Several solutions were investigated in order to choose the more efficient supporting electrolyte.

Substances tried included tartrate, citrate, thiocyanate, borate, ethylenediaminetetraacetic acid (EDTA), cyclohexane diamino-tetra-acetic acid (CDTA), ethylene glycol-bis(β-amono ethyl ether)N,N'tetra acetate (EGTA), cathechol, thioglycollic acid and o-phenanthroline. It was found that almost all of them formed at least a peak with one form of iron. EDTA and its derivatives formed peaks with both iron forms. It was however impossible to use any of these reagents due to unresolved stripping peaks of both iron forms even after varying the pH of the solutions.

Preliminary examination of pyrophosphate suggested that this might be a satisfactory supporting electrolyte. Its application appeared to be uncomplicated and furthermore, detailed studies of copper and iron couple pyrophosphate systems had been reported. [32,33] In addition Brennan and Svehla had used pyrophosphate in speciation of iron using a mercury electrode.[34] Their work highlighted difficulties encountred with differential pulse, therefore it was important to study in detail the behaviour of the iron couple in alkaline pyrophosphate solution. It was found during these investigations that the peak potentials of iron(II) and iron(III) were affected by pH as shown in table 4.1. The best resolution of the peaks was obtained at pH 9.0.

During this investigation it was found that the method works better if the supporting electrolyte was degassed effectively prior to addition of the sample. This might be due to the fact that the Fe^{2+} pyrophosphate complex ($Fe_2P_2O_7$) is very unstable. On exposure to air the Fe^{2+} complex oxidises to Fe^{3+} by the oxygen resulting in determination of total metal. Therefore to prevent this happening the solution has to be degassed first, thus maintaining the solutions oxidation states. A similar situation was observed when using a pH 4.0 buffer solution instead of a pH 9.0. At pH 4.0 hydrogens evolution on the electrode might result in oxidation of the Fe^{2+} complex. Although pH 9.0 buffer was ideal for this method, its use was complicated by frequent precipitation of Fe(III). This problem was solved by adding nitric acid into the sample.The final pH of the solution ranged from 7.5 to 8.5 depending on the sample solution.

Table 4. 1: ***Comparison of peak potentials of Fe^{3+} and Fe^{2+} in different pH mediums using pyrophosphate.***

Sample	PEAK POTENTIAL (mV)		
	$Na_4P_2O_7$ alone	$Na_4P_2O_7$ buffered at pH = 4.0	$Na_4P_2O_7$ buffered at pH = 9.0
Fe^{2+}	-349	-597	-597
Fe^{3+}	-445	-603	-758

4.3.2 VOLTAMMOGRAMS OF THE IRON COUPLES IN ALKALINE PYROPHOSPHATE

A condition for the appearance of an anodic stripping voltammetry (ASV) signal is the accumulation of a compound (adsorbed or reduced) of interest on the electrode surface with a potential shift in the anodic direction. The accumulated compound is stripped off the electrode and the ASV peak appears. To determine if such accumulation takes place as well as its nature cyclic voltammetric (CV) polarograms and DPASV scans were run. The different oxidation states of iron in pyrophosphate buffered at pH 9.0 formed two distinct stripping peaks shown in Fig. 4.2a. Under these conditions Fe^{3+} forms a very stable complex with the pyrophosphates, the stripping of which occurs at a more negative potential than that of Fe^{2+} as shown by Fig.4.2b.

Fig.4.3 and Fig.4.4 clearly show the differences of Fe^{3+} and Fe^{2+} in this medium. A Fe^{3+} CV scan (Fig. 4.3) at the starting potential of -1.5 V exhibits two anodic peaks; an almost symmetric large peak at -0.609 V (1) and a small wave at -0.086 V (2). The latter disappears as the resting potential becomes more positive than -1.2 V. Subsequent cathodic scans were characterised by a small wave at -0.975 V (3) not visible as a result of its small current. This cathodic scan shows that Fe^{3+} is effectively masked by the strong complex formed with the pyrophosphate. Areas and peak heights of these peaks increased as the resting potential becomes more negative. There is thus a strong indication that the deposition reaction of Fe^{3+} and therefore accumulation in ASV, is more adsorption controlled as opposed to reduction controlled, due to the very stable complex formed [35] (fig 4.2).

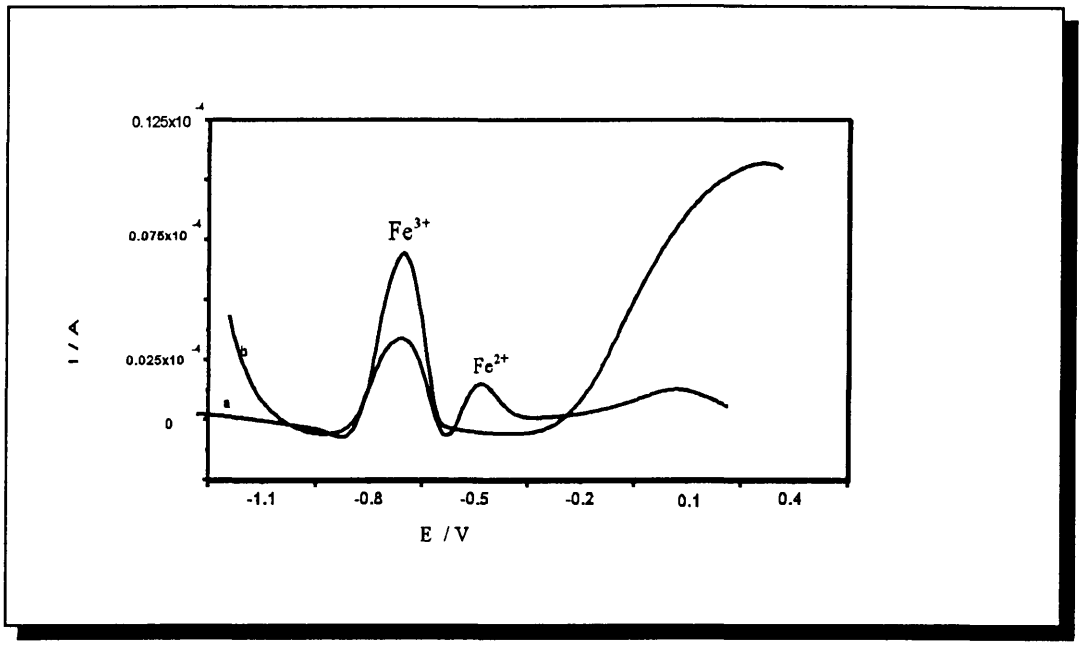

***Figure* 4.2:** *Differential pulse anodic stripping voltammograms of the iron oxidation states in pyrophosphate at pH = 9.0; a) both Fe^{2+} and Fe^{3+} b) Fe^{3+} alone*

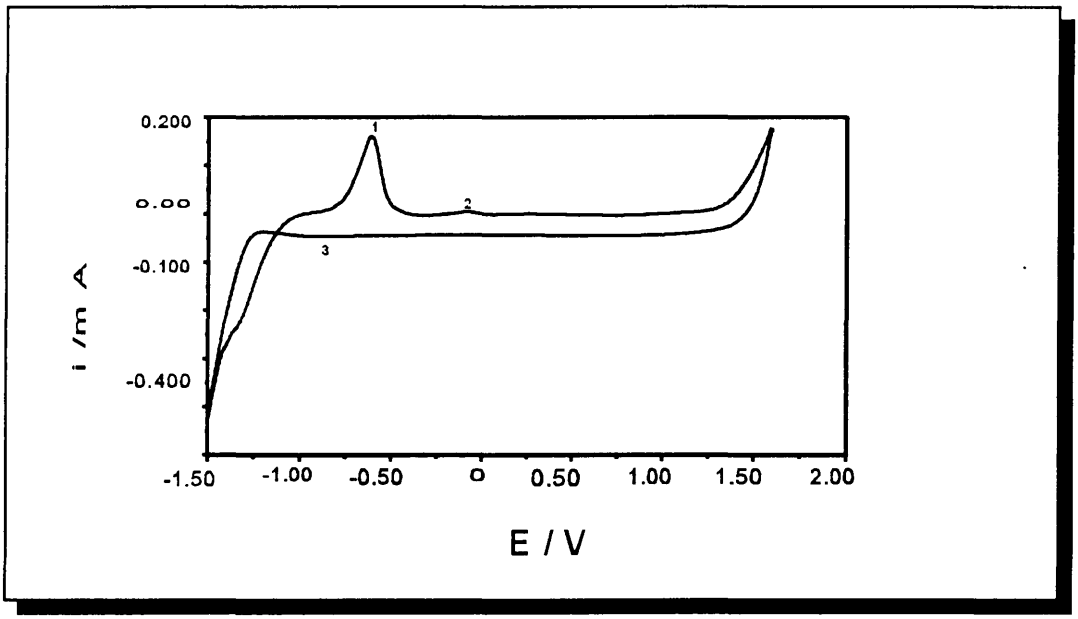

***Figure* 4. 3:** *Cyclic voltammogam of Fe^{3+} in pyrophosphate at pH= 9.0*

The Fe^{2+} CV scan at the same starting potential (Fig. 4.4), is characterised by two cathodic peaks (- 0.909 V (c) and -0.489 V (d)) and two anodic (-0.760 V (a) and -0.101 V (b)). Correlation between the area and peak heights of the anodic peaks b and the cathodic peaks c strongly suggest that we have a chemically reversible system. The two peaks may be assigned to oxidation of Fe^{2+} to Fe^{3+} and reduction of Fe^{3+} to Fe^{3+} respectively. Peak a can be attributed to Fe contamination present in Fe^{2+} solutions, that can also explains its disappearance as the starting potential changes since it is of low concentration.It was further found that as the starting potential becomes more anodic, only one anodic and one cathodic waves at -0.514 V and - 0.438 V appear. These waves increased in size and shifts to a more anodic potential of -0.304 V and -0.362 V as the starting potential becomes more anodic (-0.9 V). The system becomes more reversible as the starting potential becomes more anodic.

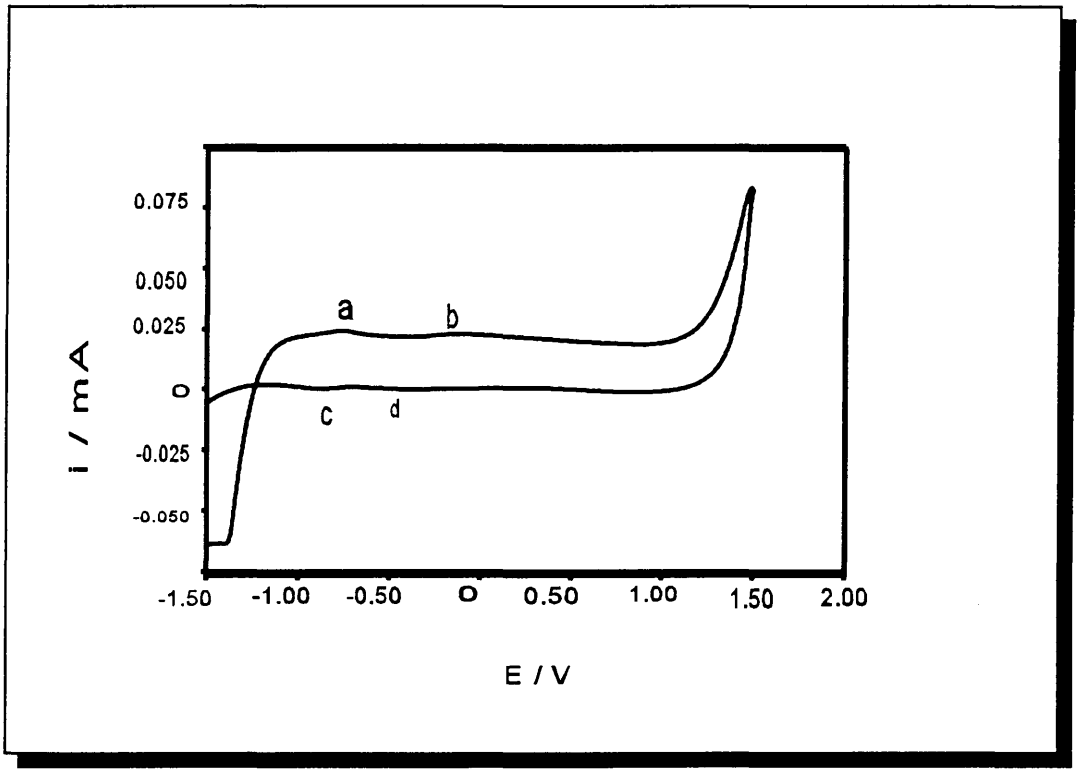

Figure 4. 4: *Cyclic voltammogram of Fe^{2+} in pyrophosphate at pH = 9.0*

Based on these we can conclude that the Fe^{2+} accumulation in ASV is based on a simple electron transfer reaction not on an adsorption process. It is clear from the studies that the positions of these peaks depend on the resting potential, they become more negative as the starting potential becomes more cathodic. At the starting potential of -1.2 V two sets appear. It therefore seems that the pyrophosphate bufferefed medium at pH 9.0 supplied the necessary bit of irreversibility for the determination of both Fe^{2+} and Fe^{3+} which confirmed the work of Parry and Anderson.[32]

4.3.3 OPTIMISATION OF PARAMETERS

To obtain a better response performance from the system (high sample through put, adequate sensitivity, working range and minimum wastage of the carrier solution) optimisation of the instrumental parameters was a necessity which was done by varying the flow rates, deposition time and deposition potential. Fe^{3+} and Fe^{2+} yields well defined stripping peaks at -0.8 and -0.5 V respectively as shown in Fig.4.2. The peak intensity increases with increasing deposition time,increasing flow rate and as the deposition potential becomes more cathodic. This indicates enhancement of deposition on the electrode surface. However the peak increase was accompanied by appearance of multiple peaks with an increase in D_t and more cathodic Dp, which resulted in irreproducible stripping peaks. An accumulation potential of -1.2 V and a D_t of 3 min were recommended. A flow rate of 4.7 mℓ/min, which was the highest studied, was used.

4.3.4 INTERFERENCES

Two kinds of interferences are expected in this system, namely presence of strong complexing agents in the sample which are capable of removing both iron(II) and iron(III) from pyrophosphate complex and the presence of metal ions in the sample which can be deposited or absorbed on the GC electrode resulting in unresolved stripping peaks from those of the iron forms. There are reports that state that the basic Fe^{2+} pyrophosphate complex reduce solutions of silver, gold and mercury salts.[35] Therefore the presence of these elements in the sample may affect the iron couple system. Fortunately these elements are not common in most samples and as a result they were not studied.

Metals that are deposited on a GC electrode and have potentials closer to that of the two oxidation states are Cu, Pb, Cd and Zn. Cu interference had been dealt with in detail in the literature. The correction method proposed were tried successfully. [20] The effect of the other metals were studied by observing the appearance of the iron peaks in the presence and absence of these metals. It was found that when 1 x 10^{-3} mol/ℓ of each of these species (Pb, Cd, Zn) are added into 2.5 x 10^{-4} mol/ℓ of Fe^{2+} or Fe^{3+}, Zn showed no possible interference. It formed a completely resolved stripping peak from both of iron's stripping peaks and their peak heights were unchanged. On the other hand both Pb and Cd produced overlapping peaks with Fe^{2+} and Fe^{3+} respectively. The effect in both cases were additive. An additive interference can easily be corrected if the amount of the interferent is known. The amounts of Pb and Cd can easily be determined in the presence of iron.

Another unexpected interference was the presence of iron (III) in iron (II) standards, resulting in a small stripping peak, the intensity of which increased with increasing concentration. Two methods can be applied to overcome this particular interferent. First the amount of iron (III) in iron (II) can be determined and the appropriate calculation can be done. Secondly the standard addition method can be used without any corrections, because the concentrations of the standards used are very small.

During analysis of real samples there was no interference correction made, because the standard addition method was used. Cd and Pb interference was not present in wine samples, tap water had Pb. In this sample the Pb and Fe^{2+} stripping peaks were resolved probably because their concentration levels were small. Investigation of organic interference was not done based on the assumption that their concentrations are small due to the dilution of the samples.

4.3.5 ANALYTICAL APPLICATION

Under the experimental conditions selected, analytical results in terms of the linear range of concentration vs peak current signal, limit of detection (LOD) and precision expressed as percent relative standard deviation (%RSD) are reported in Table 4.2. The limit of detection was determined by a signal to noise ratio of 3 from the lowest concentration.

TABLE 4.2. ***Quantitative parameters for both iron(II) and iron(III). Flow rate 4.7 mℓ/min, deposition potential -1.2 V.***

PARAMETERS	**Fe^{3+}**	**Fe^{2+}**
% RSD(n = 14)	3.7	2.3
LOD (x 10^{-8} mol/ℓ) (n=14)	2.4	2.7
Linear range (mol/ℓ)	10^{-6} - 10^{-4}	10^{-6} - 10^{-4}
Regression coefficient	0.987	0.998

To test the accuracy and to determine the analytical application of the method, some wine and tap water samples were analysed. The samples were diluted 1:5 with 2% (v/v) HNO_3 prior to analysis. The determinations of the different iron oxidation states were done using the standard addition method. These results were compared with those obtained by standard spectrophotometric methods based on the use of KSCN and 1, 10-phenanthroline, at the wavelengths of 508 nm and 475 nm for Fe^{3+} and Fe^{2+} respectively. Relevant results are summarised in Table 4.3. As can be seen in table 4.3 they is a good agreement between the two methods with tap water and white wine results. Agreement of the red wine results improved with the values obtained using blanks containing uncomplexed samples (spectrophotometry). These blanks corrected the colour intereference observed when the conversional blanks (without the sample) were used.

This proposed method is promising for the applicability of the simultaneous speciation determination of iron(II) and iron(III) in complex matrices. Its main advantage being its ability to analyse both oxidation states in one run. In addition unlike spectrophotometry it is not affected by the colour of the solution. The analysis time was 5 to 7 minutes indicating a possibility of at least 10 runs per hour. This is an improvement compared to other reports of at least 10 minutes

analysis time. The analysis time can be improved with an upgrading of the deaeration technique.

TABLE 4. 3: **Mean** ***results (n =4) obtained for simultaneous determination of iron (II) and iron (III) in real samples with the proposed method in comparison with spectrophotometric analysis.***

Sample	**Proposed method (DPASV)** [c]		**Spectrophotometric method**	
	Fe^{3+} (mmol/ℓ)	**Fe^{2+} (mmol/ℓ)**	**Fe^{3+} (mmol/ℓ) (508 nm)**	**Fe^{2+} (mmol/ℓ) (475 nm)**
White Wine (Graca)	0.031 ± 0.004	0.263 ± 0.005	0.025 ± 0.009	0.258 ± 0.045
Red wine	0.226 ± 0.007	0.338 ± 0.006	0.219[a] ± 0.093 (0.806) [b]	0.330[a] ± 0.067 (0.815)[b]
Tap water	0.492 ± 0.004	0.153 ± 0.0245	0.481± 0.042	0.149 ± 0.035

[a] ***The colour of red wine interfered in the spectrophotometric determinations of both Fe^{2+} and Fe^{3+}. These results were obtained by correcting for colour interference by running the samples without any reagent as blank***

[b] ***Results obtained without any correction for colour interference for the spectrophotometric methods.***

[c] ***± values are the standard deviations of the four results***

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5. SIMULTANEOUS ANALYSIS OF COPPER, LEAD, CADMIUM AND ZINC.

5.1 INTRODUCTION

The necessity to measure several analytes rapidly in the same sample in areas such as clinical chemistry, environmental and industrial has resulted in great developments in automated methods. Determination of several elements in one sample with a single measurement and/or injection is commonly referred to as simultaneous analysis. Although it is possible to carry out simultaneous analysis with automated methods, unfortunately a very small number of them have been described.

Typical procedures for simultaneous analysis have usually been based on chromatography technique.[1,2] Often these techniques are coupled with spectrometric methods, anodic stripping voltammetry and potentiometric stripping voltammetry. Although these couplings have been successfully, they are unsuitable for analysis of large sets of samples because they are tedious and time consuming especially their separation step. However, they still dominate in simultaneous analysis. Alternative multielement analysis of several analytes is also possible using techniques such as energy dispersive XRFS,[3] AAS, ICP[4] and electrochemical techniques. These techniques present advantages over chromatography as they are faster, have reduced sample treatment time and the removal of interferences is not strictly required in many instances.

Recently, with the development of flow systems (especially flow injection analysis (FIA)), the alternative techniques' efficiency and potential have improved. FIA offers an opportunity to avoid contamination by working with closed systems, thus solving the main problem of simultaneous analysis. In addition it enhances sample frequency as well as saving sample and reagents when miniaturised flow systems are used. An examination of the literature reveals that application of FIA to simultaneous analysis have focused on the following:[5]

- Multi-detection in series or parallel involving more than one detector;
- Sequential injection;
- Splitting up of the stream with two or more cells aligned in the same optical path;[6]
- Use of two or more synchronized or special injectors for samples and /or reagents when

a single detector is used.[7]

Multi-element detection is frequently done in rain water samples. Various methods are available for this task. Other samples analysed are foods, wines, waters and zinc electrolytes.

5.2 ELECTROCHEMICAL METHODS

Due to their capacity to perform simultaneous multi-element analysis, and to their ability to display the information on all elements at the same time, electrochemical techniques could offer a veritable alternative for simultaneous analysis of inorganic elements. In the literature descriptions of the use of anodic stripping voltammetry (ASV) procedures for simultaneous analysis of Cu, Cd, Pb and Zn have been reported.[8-10] These elements have also been determined by potentiometric stripping analysis (PSA),[11,12] adsorptive voltammetry (adV),[13] differential pulse voltammetry (DPV) [14,15] and adsorptive stripping voltammetry (adSV).[16] An automatic system for the *in situ* determination of these five elements in fresh waters composed of a filtration unit, a separation and enrichment unit and an electrochemical unit for DPASV was reported by Martinolli et al.[17]

A carbon paste electrode modified with amberlite IRC 718 chelating resins has been used for simultaneous analysis of Zn, Cd, Pb, Cu and Hg.[18] Use of other chelating resins with different functional groups as electrode modifiers have been reported.[19] Some electrodes such as mercury (hanging (HME), dropping (DME) and thin film (TFME) and a platinum disc are commonly employed.

In most cases by using a suitable supporting electrolytes combination or addition of complexing agents, the identification and determination of the whole range of elctroactive species present in the sample is possible by a few steps without any previous separation stage. A number of solutions have been used as supporting electrolytes including NH_4Br, $(NH_4)_3C_6H_5O_7$, EDTA, $NaNO_3$, ammonium tartrate, 2-quinoline thiol at suitable pH values and acetate buffer at pH 4.0. Usage of the different types of acids was also reported, but their application is complicated with zinc determination, especially in strongly acidic solutions due to hydrogen evolution at a voltage

closer to the stripping peak voltage of Zn.[22]

The capacity of ASV in trace analysis of heavy metals has been demostrated in the previous chapters and in the literature.[23] Its application limitations as a result of electrode acivity lost has been found to be a problem. Furthermore, difficulties associated with actual electrochemical multi-component sample analysis, as a result of the complicated polarograms, sevirely limits the application of this technique; the information for each particular sample is rarely tabulated or described. The other problem involves the handling of the data compilation in order to design a suitable analytical method for each specific problem.

The choice of a suitable combination of supporting electrolytes has been employed predominately in determination of multi-elements analysis. Unfortunately this requires handling of a lot of data concerned with the electrochemical behaviours of the metals in several media and also to take into account the following:

- Chemical interferences between an individual element and each sample constituents;
- Mutual interferences between the present ions and overlapping or peak potential's displacements;
- Minimal analysis time and effort.

These clearly indicate that the research needed to provide an understanding of the electrochemical response requires a significant investment of time and resources for the preparation of solutions and performance of the necessary experiment. Therefore, it is not surprising that all papers have only been applied to groups of a few metals under specific conditions. Most papers report analysis of two elements, occasionally five elements, but rarely more than five elements have been determined simultaneously. Automation of sample handling and experimental execution allows the electrochemical response to be characterised more efficiently and thus more through. It is hoped that with flow configuration the number of elements that can be analysed simultaneously can be increased.

Alternatively the electrochemical techniques are coupled to various column liquid chromatographic techniques (LC-EC). Chromatographic techniques such as ion-exchange,[22]

HPLC and TLC[23] have been used successfully in LC-EC couples. A square wave anodic stripping voltammetric method for low parts per million determinations of heavy metals separated by TLC on carboxymethyl cellulose plates has been used for simultaneous analysis of Pb, Cd, Cu and Zn.

5.2 EXPERIMENTAL

5.2.1 CHEMICALS AND REAGENTS

Ultrapure water obtained with a milli-Q-milli-R.O system (millipore) was used throughout. All reagents were of analytical reagent grades. Cadmium, copper and iron stock solutions were prepared by dissolving the nitrate salts and zinc stock solution by dissolving the sulphate salt in 2% nitric acid. A 0.100 mol/ℓ lead stock solution was obtained from Merck. All prepared stock solutions were stored for a month, after which new stock solutions were made.

5.2.2 PROCEDURE

Instruments and apparatus used, have been described in detail in chapter 4. Section 4.2.1 and the manifold illustrated in figure 4.1. From the preliminary studies the following procedure was adopted.

A supporting electrolyte mixture of 0.1 mol/ℓ $Na_4P_2O_7$ and 0.25 mol/ℓ acetate buffer solution at pH 3.5 was prepared by separately sucking the solutions into the deaeration chamber at a flow rate of 4.70 mℓ/ min and 1.12 mℓ/ min respectively. The solutions were mixed and deaerated with nitrogen and passed through the flow though cell at a rate of 4.5 mℓ/ min. Samples were introduced into the supporting electrolyte mixture at a rate of 2.73 mℓ/ min just after deaeration. The solution merged, reacted and flows through the cell. Subsequently the deposition potential (D_p) of -1.5 V was applied for three minutes. After deposition the flow was stopped and a rest period of 5 seconds was observed before a positive differential pulse scan of 30 mV/s was commanded. Before starting the next run, the electrode was conditioned at 1.0 V for 1 minute. After about 2 hours of continuous use the electrode was cleaned by rubbing it over a filter paper soaked in ethanol, then on a dry filter paper followed by electrochemically cleaning with at least

three scans. Furthermore, the electrode was polished on a daily basis with alumina powder.

5.2.3 ANALYSIS OF SAMPLES AND RECOVERY EXPERIMENTS

In a 15 mℓ water sample, 2.0 mℓ of concentrated HNO_3 and a suitable amount of standard mixture were added. After dilution to the mark (50 mℓ) with ultra pure water, analysis was performed as described above in the procedure. Three standard additions were made for each sample.

For recovery experiments a standard mixture containing all elements was added into a 50 mℓ volumetric flask containing a water sample. The mixture was then treated and analysed as described above.

5.3 RESULTS AND DISCUSSIONS.

5.3.1 PRELIMINARY STUDIES

The main difficulty associated with the analysis of several elements simultaneously in a single polarogram is the determination of a suitable electrolyte. The properties of the supporting electrolyte used as the carrier liquid significantly influence the stripping reaction through redox reactions, formation of complex compounds and poorly soluble compounds readily adsorbing on the electrode surface and frits used in the cells. The stripping peak potentials of the supporting electrolytes tested are given in table 5.1.

An appropriate electrolyte is selected by a trial that indicates a distinct peak potential (Ep) values for the elements studied. To enable adequate separation for simultaneous measurement an Ep difference of at least 100 mV is desired. Resolution between Cu and Pb signals in a KSCN carrier electrolyte was insufficient owing to the vicinity of their stripping peak's potentials. A similar problem was observed between Fe and Pb in acetate buffer pH 4.0. Lowering the scan rate may solve the problem but significantly long analysis times will be required. From table 5.1 $(NH_4)_3C_6H_5O_7$, EDTA, and $Na_4P_2O_7$ have the desired Ep difference among all five elements in

their decreasing sequence.Therefore, best resolutions are expected with the use of $(NH_4)_3C_6H_5O_7$. Its utilization is made more attractive because it has been used successfully with HME and it had higher peak intensities than the other electrolytes.

Table 5.1 ***Peak potential of the elements in different electrolytes (V). The element's concentration*** **was $4x10^{-5}$ mol/ℓ**

Electrolyte	STRIPPING PEAK POTENTIAL *(V)*				
	Cu	**Pb**	**Fe**	**Cd**	**Zn**
0.1mol/ℓ EDTA (pH 4.0)	0.043	-0.461	-0.306	-0.682	-1.129
0.25 mol/ℓ KSCN	-0.364	-0.389	-0.789	-0.581	-0.829
0.1mol/ℓ $Na_4P_2O_7$	0.054	-0.492	-0.594	-0.804	-1.050
0.25mol/ℓ Acetate buffer pH 4.0	0.109	-0.223	-0.296	-0.546	-0.998
0.25mol/ℓ $(NH_4)_3C_6H_5O_7$	0.172	-0.412	-0.065	-0.879	-1.017

The polarogram of a mixture of all elements in $(NH_4)_3C_6H_5O_7$ is shown in figure 5.1. As expected good separation was obtained. However, the polarograms were not reproducible. Trials with various pH 's and buffers were not able to correct the problem. The loss of the electrode activity was not due to adsorption, because the second scan produced no peaks, indicating that all elements are stripped completely in the first scan. The only report in the literature on the use of this electrolyte is sketchy and HME was used.

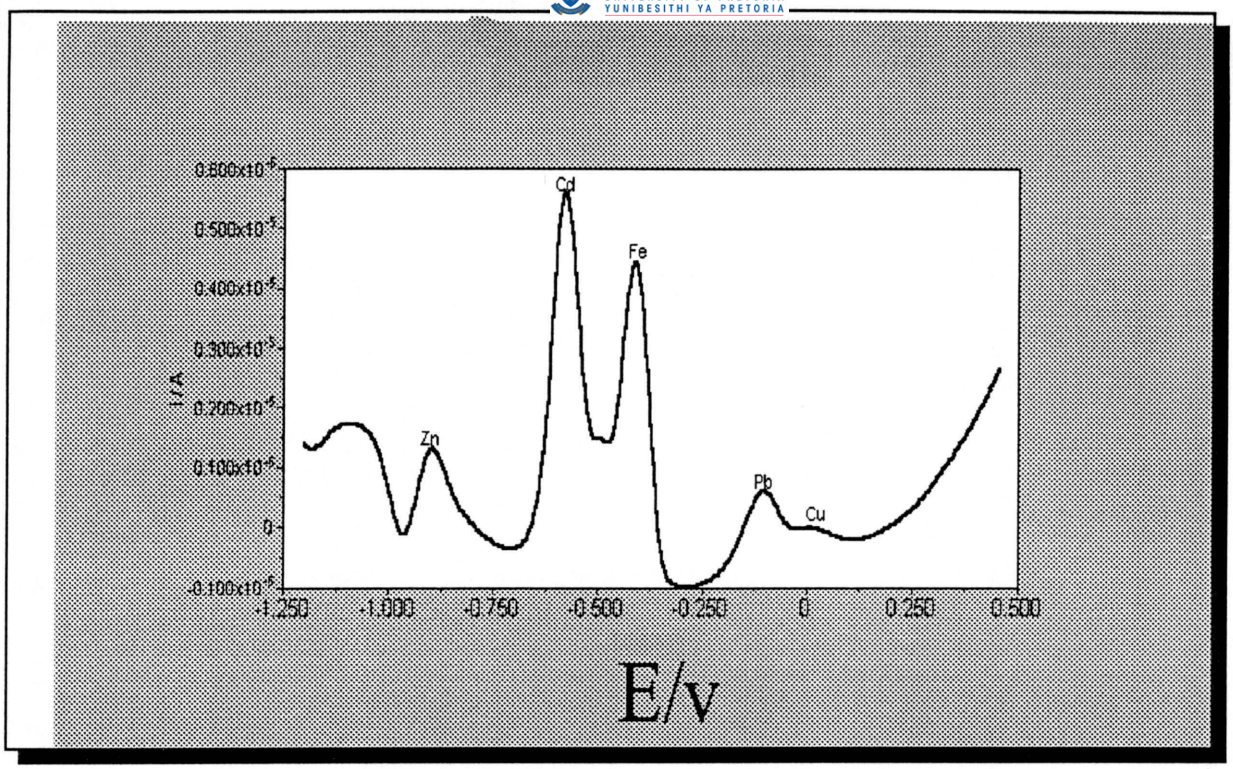

Figure* 5.1:** ***Polarogram of Cu, Cd, Fe, Pb and Zn in ammonium citrate pH 4.0

Failure to use $(NH_4)_3C_6H_5O_7$ led to EDTA studies because it was the second best electrolyte. Since most EDTA complexes are pH dependent it was important to establish if Ep values of the elements change with pH. These pH effect studies on the five elements in EDTA are shown in table 5.2. The Ep values of all elements were found to be pH dependent. Due to the evolution of hydrogen on the glassy carbon electrode in acidic medium during deposition, which results in increased hydrogen content in the solution as well as decreased pH level, thefore a higher pH was desired. A pH above 4.0 seemed ideal for the system, since at this pH their was no precipitation of the EDTA.

Although, good separations were observed, use of EDTA was complicated due to the following:

- At the optimum pH (above 4) to use to minimize the hydrogen evolution effect on Zn determination, Pb and Cd don't give a stripping peaks. Also, Zn peak is not reproducible;
- When lower pH values are used, EDTA precipitates;

- The negative cutoff cathodic potential of a glassy carbon electrode (-800mV) shifts with a change in pH becoming more negative with an increase in pH. As a result, determination of Zn at lower pH's was a problem.

Table 5. 2 ***Ep values of the elements in EDTA solutions at different pH values (V)*****,(peak currents in (μA) are shown in brackets** ***all elements' concentrations were 150 $\mu g/\ell$)***

pH	Pb	Cu	Cd	Fe	Zn
5	no peak	-0.336(1.274)	no peak	-0.356(0.949)	-0.998 [a]
4	-0.474(1.332)	-0.043(3.543)	-0.731(2.952)	-0.306(0.328)	-1.063(1.116)
3	-0.461(1.642)	0.043(0.798)	-0.682(1.893)	-0.247(0.897)	-1.129(0.725)

[a] **The peak was not reproducible**

On the basis of the previous studies in chapter 4, application of pyrophosphate in acetate buffer at pH 4.0 as a supporting electrolyte was studied. A polarogram of a mixture of all elements in this medium is shown in figure 5.2(a). An adequate separation was obtained with almost all elements except that Pb and Fe were too close. Addition of 0.005% gelatin into the supporting electrolyte was tested (figure 5.2(b)). In the presence of gelatine Pb and Fe separate but the intensity of Cd and Zn, peaks decreased. It was better to use the solution without gelatin, because in addition to loss of sensitivity of the Cd and Zn, the electrode required cleaning more frequently than without the gelatin.

The selection of the deposition potential depends on the metal that has the most negative peak potential which happens to be zinc at -1.050 V. The zinc signal increased as the deposition potential becomes more negative; unfortunately hydrogen evolution occurs at negative potentials. A reduction potential of -1.25 V was chosen as the best compromise for zinc signal and hydrogen evolution. Deposition time was controlled by the zinc and copper signals because they had low sensitivity. A deposition time of 3 minutes, and pulse amplitude of 50mV were preferred because

good sensitivity and separation of the peaks for all metals were achieved with these settings.

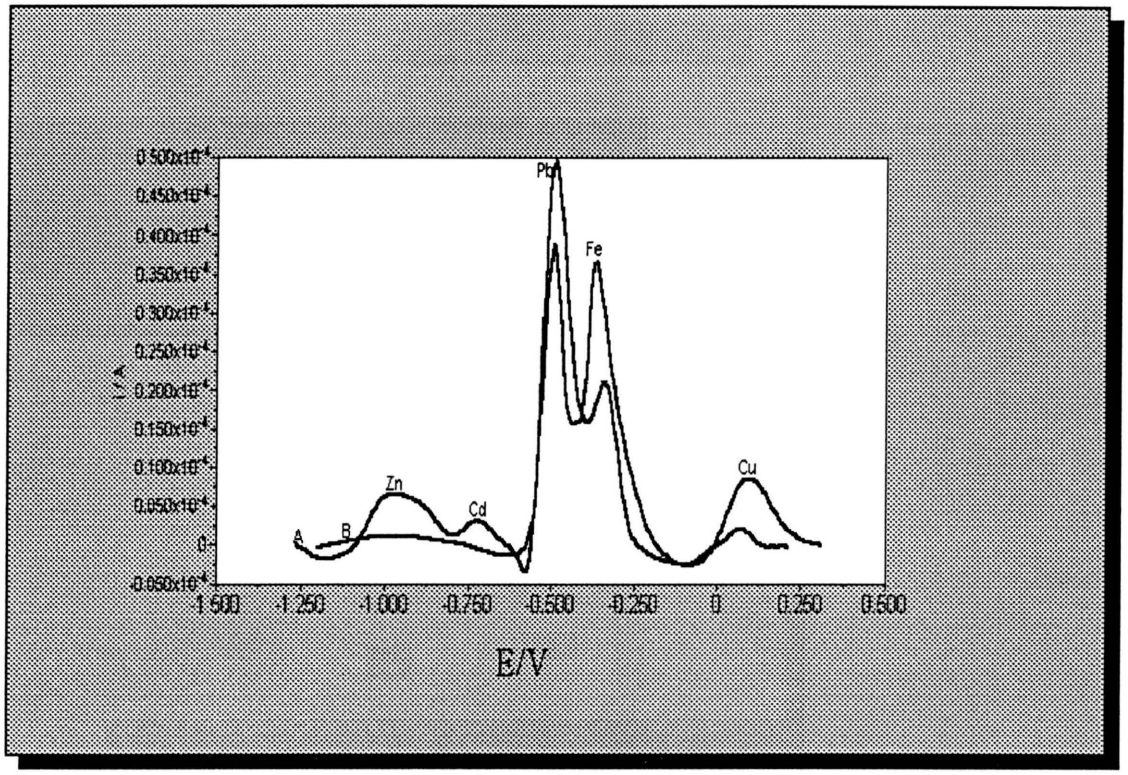

Figure 5.2: ***Polarogam of Cu, Cd, Pb and Zn in pyrophosphate buffered at pH = 4.0 with acetate; A= without gelatin, B = 0.005% gelatin***

5.3.2 INTERMETALLIC EFFECTS

To determine if they are any intermetallic effect between elements, simultaneous deposition of all four elements from 3 minute to 13 minutes was studied. Slope values are tabulated in table 5.3. The slope values obtained in this study are representative of the sensitivity of this technique for each metal. From these values, it could be concluded that the sensitivity of cadmium is better than that of copper (mCd/mCu = 2.576) for the same concentration level. The same can be said about

lead and zinc (mPb/mZn =2.8). The slope of copper alone is smaller than that of copper in the presence of the others; the opposite is observed for zinc. These differences are more pronounced in higher concentrations, this can be attributed to the formation of an alloy between zinc and copper, which has a stripping peak potential closer to that of copper. The final effect is an increase in the copper signal and a decrease in the zinc signal. This intermetallic effect has been reported previously in the determination of zinc and copper on the mercury electrodes.

Table 5. 3 ***Slope values for the peak heights with deposition time for the simultaneous determination of 100 μg/ℓ of Pb and Zn and 50 μg/ℓ Cd and Cu in comparison with Slopes obtained for the same concentrations of Zn and Cu determined alone***

METAL	CADMIUM	COPPER	COPPER (ALONE)	LEAD	ZINC	ZINC (ALONE)
slope (μA/ min)	0.268	0.121	0.104	0.285	0.108	0.134

It was observed that the simultaneous determination of four metal ions requires careful attention to the concentration ratio at which the metal ions are present especial for cadmium and lead. A large excess of one metal ion may affect the response of the other due to a change in the coverage of the electrode surface and the increase in peak areas since the vicinity of their stripping peaks is closed. The peak potentials shift, and the peak shapes change in extreme cases. Presence of iron affects Cu, Cd and Pb peaks. The Iron effect on copper can be a result of the formation of copper ferric pyrophosphate ($Cu(I)_3Fe(III)_3(P_2O_7)_3.12H_2O$) complex. This complex is known to form when solutions of ferric pyrophosphates in sodium pyrophoshates are reacted with copper.[24] In the presence of iron the copper peaks broadens because the stripping peaks of the three different spieces (Cu, $(Cu(I)_3Fe(III)_3(P_2O_7)_3.12H_2O)$, Fe complex) are not resolved. Although, iron has a distinct stripping peak from both Cd and Pb simultaneous determination of these three elements is not easy. The effect on the Cd and Pb can be attributed to the vicinity of their stripping peaks especially the Pb, but it may also be due to the change in the electode surface. In chapter 4

it was observed that Fe^{3+} accumulation is due to adsorption, therefore it is possible that these occur thus changing the electrode surface and its properties. Only a very narrow ratio range can be separated. The ratio range might change with concerntration. Despite these problems the presence of low concermtrations of iron do not interfere due to its low sensitivity.

5.3.3 APPLICATIONS

To evaluate the method, some analytical parameters such as linear range and detection limits were also determined. The detection limits, taken as the concentration that gave a signal to three times the standard deviation of the blank signal were calculated from the calibration slopes, for simultaneous determination. Results are tabulated in table 5.4 .The detection limits and linearity can be increased by increasing deposition time and pulse amplitude.

Table 5.4 **Parameters obtained for *deposition time of 3 minutes, pulse amplitude of 50 mV, flow rate of 4.75mℓ/ min and a scan rate of 30 mV/ sec.***

METAL	LINEAR RANGE ($\mu g/\ell$)	SLOPE ($\mu A/\ \mu g\ell^{-1}$)	DETECTION LIMITS ($\mu g/\ell$)	% STANDARD DEVIATION
Cu	50-500	0.494	39.00	14.00
Cd	50-500	0.704	9.60	5.55
Pb	50-500	0.030	16.65	13.20
Zn	100-400	0.009	14.69	13.56

The method was tested by simultaneous analysis of Cu, Cd, Pb and Zn in two water samples, results of which are shown in table 5.5. The sensitivity and accuracy was determined by recovery experiments. The results for most elements are good except for copper and zinc in water 2 which contained high amounts of zinc. In this sample the % recovery is too high for Cu and low for Zn.This is due to intermetallic effect of copper and zinc. Other elements show good recoveries of about 95% and relative standard deviations values < 14%. One run takes about 5 minutes,

which when the wash time is included approximately 8 to 12 runs per hour can be achieved, this is very fast for monitoring heavy metals in water samples, especially those low in iron content and organic compounds.

Table 5. 5 Simultaneous determination of Cu, Cd, Pb and Zn in water samples.

METAL	WATER 1	WATER 2	ADDED in ($\mu g/\ell$)		FOUND in ($\mu g/\ell$)		%RECOVERY	
			water 1	water 2	water 1	water 2	water 1	water 2
Cu	9.52	2.38	50	75	54.45	83.29	91	108
Cd	16.67	-	50	75	67.75	76.53	102	98
Pb	9.54	-	100	150	103.89	160.62	95	107
Zn	14.29	114.0	100	150	105.23	178.67	92	83

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6. CONCLUSION

A flow system for differential pulse anodic stripping voltammetry (DPASV) was described and characterized. This coupling of voltammetric analysis to flow systems was proved to be very effective in trace metal analysis. Analytical parameters such as detection limits, linear range, precision and accuracy were improved due to convective mass transport, protection of reagents and samples from atmospheric gases and contamination.

The utilization of traditional mercury electrodes in flow systems is complicated, due to their sensitivity to mass transport. Because of this problem it was important to evaluate the applicability of other electrodes. Unlike liquid electrodes (mercury electrode) solid electrodes are not subject to mass transport. Therefore, they are suitable for flow systems. Glassy carbon electrodes (GC) are solid electrodes and they are commonly used as substrates (in thin film mercury electrodes) or on their own. These electrodes are suitable for flow systems, but do not enjoy their full potential because they are not always clearly understood. In addition, there is a strong long-term drive to provide robust low maintenance techniques that can give critical information on chemical composition without distracting from the demand of accuracy and precision. These observations prompted these studies.

The main aim of this work was to design and characterize a flow system for voltammetric detection so as to improve the efficiency of electrochemical studies. A secondary objective was to characterize the flow cell detector, optimise the solution conditions and analyse complex real samples. Guidelines for the development of the voltammetric detection in flow systems and principles were discussed in detail in chapter 1. These objectives were realised through use of the flow system with a home made flow-through cell and computer controlled voltammeter. Experimental parameters' adjustments, blank subtractions and on-line analysis of the voltammograms can be done, and has been used to perform intensive investigations of electrochemical systems without time investment required using manual operations.

In the present investigation the technique was characterized by optimising instrumental and

analytical parameters. The evaluation of accuracy and reproducibility of this more productive, robust and less tedious voltammetric technique showed that they are dominated by variations in flow rate, deposition potential, deposition time, the distance between the inlet and the working electrode(WE). The sensitivity was higher at high flow rates, longer deposition times and smaller inlet WE gap. Stopped flow detection provided ideal conditions for both analytical and physical chemical investigations of chemical species adsorbed or otherwise deposited on the electrode. The results confirmed eq. 4 in chapter 1.

Furthermore, studies clearly showed that suitable deaeration methodologies and management of electrode surface maintenance are crucial for the efficiency of the system. Evaluation of these factors revealed that this two are the major hindrances in the development and wide spread acceptance of the technique. Accuracy, precision and the analysis time of the measurements are the factors which are greatly influenced (see chapters 2 and 3). The methods used to overcome these problems in this study were found to be time consuming, tedious and difficult to perform *in situ*. Therefore the results obtained can still be improved with more effective deaeration and regeneration of the surface electrode.

Having characterized the system, the technique was extensively used in exploring its potential, especially in:

- Analysis of trace metals
- Performing speciation of trace metals
- Simultaneous analysis

After optimising the following: flow rate, distance between the electrode and the inlet hole, deposition time, deaeration time as well as establishing the best electrode surface cleaning method, the applicability of GC in the three tasks outlined above was investigated. On the basis of the studies discussed in detail in chapters 2,4, and 5 it can be concluded that the GC electrode can be employed successfully to perform all three tasks. Recoveries of about 95% on average as well as relative standard deviations less than 14% were achieved with simultaneous analysis, and better results were obtained with speciation as well as trace analysis. Although the technique is definitely

effective for trace analysis with relatively fewer problems; it can be used for simultaneous analysis as well. It was obvious that the applicability of the technique is influenced by the regeneration of the electrode surface and the deaeration method. These factors are more important in simultaneous and speciation studies where complex samples are analysed.

6.1 FUTURE WORK

It is clear that there is much room for innovative research in development of the deaeration and regeneration devices. There is a need to improve the existing deoxygenation and electrode surface cleaning methods where solid electrodes are used, to adapt them to continuous monitoring. To achieve this, development of miniaturised deaeration, cell and electrode devices is desired. Those that have been used so far, were more like research curiosities than devices ready for commercialisation. To date, the electrode surface cleaning methods are tedious, time consuming and not suitable for flow systems. There is a strong need for the development of an efficient electrode surface cleaning method that can be performed within the system in the shortest possible time.

There are many unanswered questions about the flow systems with the voltammetric determination concerning the instrument and sample monitoring. This questions can be answered easily with use of proper instruments. It is hoped that with computer controlled sample and experimental adjustments there will be some valuable information and improvement on the existing systems. There is a strong need to make the technique accessible for routine analysis so that analysis can be done overnight without any human supervision. Commercially upgraded polarometers which are computer controlled are a step towards that. It is necessary to complete these developments with a computer controlled stopped flow system.