Determination of aminopolycarboxylate complexes of transition metals (Mn^{2+}, Cu^{2+} and Fe^{3+}) in pulp by Capillary Zone Electrophoresis (CZE-DAD)

By

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Abstract

The determination of the concentrations of the transition metals Mn^{2+}, Cu^{2+} and Fe^{3+} in various types of pulps was carried out using a combination of chelation with Ethylenediaminetetraacetic acid (EDTA) and ethylenediaminedisuccinic acid (S,S-EDDS) and capillary zone electrophoresis (CZE) coupled with diode array uv-detection. The metals were found to be inhomogeneously distributed in the pulps, and therefore a relatively large (20 gms) pulp samples were taken for analysis. Heating the pulp samples at 45 °C with stirring for three hours was used to leach the metals into an aqueous phase for complexation with the ligands. Leaching with EDTA enabled the quantitative determination of 0.0091,0.0048 and 0.0014 mM Cu^{2+} and 0.0088, 0.0012 and 0.0016 mM Mn^{2+} in the three types of pulps analyzed respectively while detection of Fe^{3+} was not possible using EDTA. The use of [S,S-EDDS] as the leaching agent on the other hand enabled the quantitative determination of 0.0041, 0.0036 and 0.0031 mM Cu^{2+} and 0.0024, 0.0018 and 0.0047 mM Fe^{3+} in the three types of pulps analyzed respectively while determination of Mn^{2+} was not possible using [S,S-EDDS] as the leaching agent. [S,S-EDDS] was generally better in complexing iron and copper than EDTA. The leaching procedure employed and the detectors response to specific analytes formed were found to be the most important factors in the analysis of the metals in pulp.

Opsomming

Die konsentrasies van die oorgangsmetale Mn^{2+}, Cu^{2+} and Fe^{3+} in verskeie tipes pulp is bepaal met behulp van 'n kombinasie van kelasie met etileendiamientetra-asynsuur (EDTA) en etileendiamiendisuksiensuur (S,S-EDDS) en kapillêre elektroforese (CE) gekoppel met UV-deteksie. Daar is bevind dat die metale oneweredig versprei is in die pulp monsters, en gevolglik is relatiewe groot (20 g) monsters gebruik vir die analiese. Die metale is ge-ekstraeer deur kompleksasie met die kelasie-agente in die water-fase, deur die pulp monsters vir drie uur te roer by 45 °C. Ekstraksie met EDTA is gebruik om 0.0091, 0.0048 en 0.0014 mM Cu^{2+} en 0.0088, 0.0012 and 0.0016 mM Mn^{2+} in drie tipes pulp te bepaal, terwyl Fe^{3+} nie waargeneem is met behulp van EDTA nie. Deur [S,S-EDDS] te gebruik as ekstraksie-agent, is 0.0041, 0.0036 en 0.0031 mM Cu^{2+} en 0.0024, 0.0018 en 0.0047 mM Fe^{3+} in die drie tipes pulp waargeneem. Die analise van Mn^{2+} was nie moontlik met die metode nie. Daar is gevind dat die keuse van ekstraksie metode en die detektor-sensitiwiteit vir spesifieke metale die mees bepalende faktore in die analise van metale in pulp monsters is. EDDS was 'n beter kelaat vir Fe^{3+} en Cu^{2+} as EDTA.

Dedication

TO MY BELOVED PARENTS

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List of abbreviations

APCAs	Aminopolycarboxylic acids
AEAA	Aminoethyl aspartic acid
BAS	Batch activated sludge
BAC	Biologically activated carbon
CE	Capillary electrophoresis
CGE	Capillary gel electrophoresis
CTAB	cetyltrimethylammonium bromide
CZE	Capillary Zone electrophoresis
COD	Chemical oxygen demand
DOC	Dissolved organic carbon
DTPA	diethylenetriamminepentaacetate
ECF	Elemental chlorine free
EDTA	Ethylendiaminetetraacetic acid
ED3A	Ethylenediaminetriacetic acid
EDDS	Ethylenediaminedisuccinic acid
EOF	Electroosmotic flow
ETAAS	Electrothermal atomic absorption spectroscopy
HPCE	High performance capillary electrophoresis
ICP-MS	Inductively coupled plasma mass spectrometry
IEEC	Ion exchange electrokinetic chromatography
cIEF	Capillary isoelectric focussing
cITP	capillary Isotachophoresis

ISO	International standards organization
IUPAC	International union of pure and applied chemistry
JESS	Joint expert speciation system
MECC	Miceler electrokinetic capillary chromatography
NPE	Non-process elements
NTA	Nitrilotriacetic acid
OECD	Organization for economic cooperation and development
TCF	Totally chlorine free
UV	ultraviolet

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Chapter 1

1.Introduction

In the pulp and paper industry, bleaching of the pulp is regarded as a requirement because it is responsible for the bright, white characteristics of paper, which is considered as a marker of its quality by both the industry and the consumer. Chlorine and its compounds have traditionally been used for bleaching of pulp. Because of the environmental risk associated with organochlorine compounds, hydrogen peroxide is now the most important bleaching chemical in the production of totally chlorine free (TCF) pulp replacing chlorine and its compounds. However, certain transition metals such as manganese, copper and iron which are either naturally found in wood itself or coming from process waters can have a profound negative effect on the bleaching performance of hydrogen peroxide. The effective metal-ion removal is thus pivotal to the efficiency and success of totally chlorine free bleaching of pulp using hydrogen peroxide.

Aminopolycarbxylic acids such as EDTA and DTPA have been used for the purpose of chelating the metals and keeping them from interacting with the bleaching agent by forming stable and water-soluble complexes. However the release of such aminopolycarboxylic acids and their metal complexes into the environment is also another threat to the environment because it affects the distribution of metals within acquatic ecosystems and remobilisation of heavy metals from sediments. Furthermore, EDTA which is the most commonly used chelating ligand in the pulp and paper industry for metal sequestration because of its ability to form stable and

water soluble complexes with most of the metals is none biodegradable and entails a constant threat to the environment. Researchers have therefore been focusing not only to finding reliable and effective analytical methods for the determination of the levels of free EDTA and its metal complexes in waste streams but also on the search for a suitable ligand to substitute EDTA in its applications.

[S,S]-Ethylenediaminedisuccinic acid [S,S-EDDS] has been recently synthesized and shown to be more readily biodegradable agent than EDTA and is believed to be considered for use as an alternative ligand for EDTA in the pulp and paper industry.For determination of metals present in pulp, prior complexation of the metals with an appropriate ligand and subsequent gas chromatographic or ICP-MS analysis have been practiced although these techniques have proved to be either too expensive or time consuming.

The work in this project is aimed at reaching the following main objectives :

1. Determination of the transition metals of main concern in the pulp and paper industry namely manganese, copper and iron in pulp using EDTA as a complexing agent and subsequent capillary zone electropherotic (CZE) analysis to quantify the amount of each metal being sequestered.

2. Determination of the metals manganese, copper and iron in pulp using [S,S-EDDS] as a complexing agent and subsequent CZE analysis to quantify the respective species.

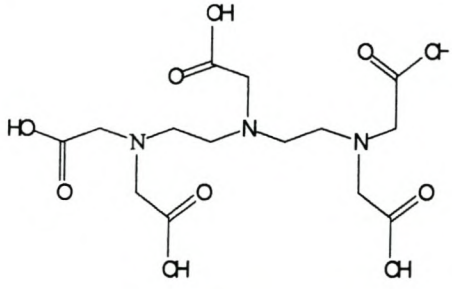
3. By way of doing so, evaluate the potential of [S,S-EDDS] to be used as an alternative ligand for the removal of pivotal metal ions in the pulp and paper industry.

Capillary Zone Electrophoresis coupled with diode-array uv-detection is chosen here as

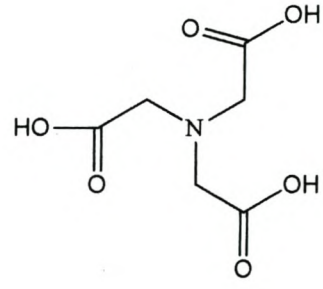
a technique because it is cheap, versatile, simple and offers short analysis times.

1.1 Aminopolycarboxylic Acids

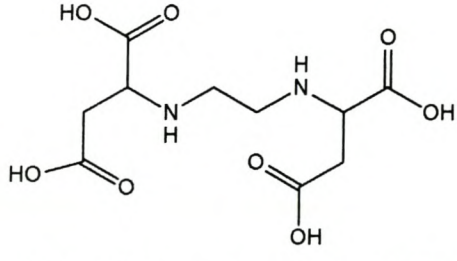
Aminopolycarboxylic acids (APCAs) are compounds that contain several carboxylate groups linked to one or more nitrogen atoms in their structure (fig.1) and are capable of reacting with polyvalent metal ions to form stable hetroatomic rings around them [1].



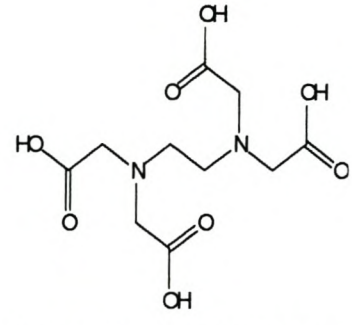
Diethylenetriaminepentaacetic acid



Nitrilotriacetic acid (NTA)



Ethylenediamine disuccinic acid (EDDS)



Ethylenediaminetetraacetic acid (EDTA)

Fig.1.1.Common aminopolycarboxylic acids

1.2 Fields of application of Aminopolycarboxylic acids

In an aqueous environment, metal ions are capable of producing a catalytic effect leading to unwanted chemical reactions. Among others, this includes the formation of insoluble precipitates of Ca and Mg salts during washing, the decay of bleaching agents such as hydrogen peroxide (H_2O_2) in the pulp and paper production processes or in detergents and the rancidity of fats and oils **[1]**.

Because of this fact, especially di- and tri-valent transition metal ions can cause various unwanted effects in a wide range of processes involving chemical reactions. It is often necessary to control their availability and accessibility in processes to reduce their effect. Aminopolycarboxylic acids are capable of forming stable and water-soluble ring-complexes with polyvalent metal ions, a phenomenon known as the chelate effect. They have the effect of restricting these metal ions from performing their normal chemical activity. As a result, they are used in a wide range of applications mainly to prevent the formation of precipitates, reduce the effect of metal catalysed reactions and the removal of metal ions or sometimes to increase their availability **[1].** A wide variety of compounds do exhibit chelating properties. The Aminopolycarboxylic acids and salts such as NTA, EDTA, DTPA and EDDS are the ones, which have found a dominant use worldwide for chelation purposes. The total consumption of aminopolycarboxylic chelating agents excluding NTA in the United States, Western Europe and Japan during 1998 was approximately 340 million pounds (155 thousand metric tons). These chelating agents are used as components or process chemicals in their applications as cleaning compounds,metal scavengers in the pulp and paper manufacturing process, water treatment, photography and

agriculture accounting for about 80% of worldwide consumption of the chelating agents, the overall market growth being heavily dependent on a country's economy and environmental shifts **[1,2]**.

1.2.1 Use of Aminopolycarboxylic acids to prevent the formation of precipitate

In addition to being active washing ingredients, detergents do contain considerable amounts of metal-complexing agents in order to prevent the formation of insoluble calcium and magnesium salts there by preventing the deposition of scale in both washing machines and textile fibres. The first detergents employed with this regard were di-and triphosphates **[3].**It was however found in a later time that phosphates have the effect of promoting the eutrophication of lakes and rivers. In the search for substitutes, Nitrilotriacetic acid, NTA, was proposed and has been used as a detergent builder for household utilities even though it has been facing various oppositions due to its carcinogenic effect **[4].** In industrial cleaning, NTA, Ethylenediamine tetraaceticacid, EDTA, and other ligands belonging to the aminopolycarboxylic acids have been used to prevent precipitation of calcium, magnesium and other heavy metal salts **[3].** Ethylenediaminetetraaceticacid (EDTA) is widely used in the textile and photographic industry and in electroplating processes instead of cyanide to serve the same purpose.

1.2.2 Use of Aminopolycarboxylic acids in preventing catalysis mediated by metal ions

Aminopolycarboxylic acids are used as additives in many detergent products to increase the stability of bleaching agents. Ethylenediaminetetraacetic acid (EDTA) is used to prevent the bleaching agent per borate by inhibiting metal ions from their

catalytic activities that can otherwise cause the decomposition of the compound **[3]**. Hydrogen peroxide is increasingly used instead of chlorine compounds in the bleaching process of the pulp and paper industry. The addition of the aminopolycarboxylic acids EDTA or DTPA is known to avoid the catalytic decomposition of the bleaching agent, H_2O_2, caused by metal ions mainly of iron , copper and manganese **[3].** Other applications in this area include the use of APCAs as additives in pharmaceuticals, cosmetics and food to prevent constituent transformations or rancidity that can happen because of metal-catalysed chemical reactions **[5]**.

1.2.3 Application of aminopolycarboxylic acids in the removal of metal ions

Aminopolycarboxylic acids are also widely used in the nuclear industry for decontamination purposes due to their ability to form water-soluble complexes with many radionuclides,in the remediation of metal contaminated soils or sediments to support electro kinetic processes, in phytoremediation strategies by increasing metal desorption from soils which facilitates metal uptake by plants and in medical treatments for heavy metal intoxication.Ca-EDTA is for example used for the intoxication of heavy metals such as lead where the EDTA complexes the toxic metal enhancing its excretion. APCAs are also used to increase metal availability to supply plants nutrient trace metals such as iron, cupper, zinc and manganese.EDTA has been used as an additive to fertilizers to serve similar applications **[6].**

The application of aminopolycarboxylic acids is usually seen from the chelate's

power to form a stable complex with the metal ions of interest and its biodegradability once released to the environment.

1.3 Biodegradability of aminopolycarboxylic acids and their metal complexes

1.3.1 Biodegradation

Biodegradation represents to the natural way of recycling of waste materials or the breakdown of organic matter into substances that can be used by other organisms.Biodegradation, as can be understood from the term itself, is carried out by organisms that can eat dead substances and recycle them into new forms. Among others these include bacteria, fungi, insects and worms. The biodegradability of chelating agents is evaluated on the basis of certain criteria set by international organisations to assess ready biodegradability of chemicals such as the Organisation for Economic Cooperation and Development (OECD) which includes features such as 70% of dissolved organic carbon (DOC) being lost to biodegradation within 28 days measured within 10 days of the biodegradation exceeding 10% **[7]** and the International standards organisation (ISO) **[8].**

The main concern of biodegradation to the chemist in general and to the environmental chemist in particular is to make sure that substances of environmental concern be easily broken down into other naturally existing substances like CO_2 and H_2O that can be used by organisms. The biodegradation of synthetic aminopolycarboxylic acids such as NTA, EDTA and DTPA is one such area attracting researchers. This awareness originates from the fact that this group of

compounds are enormously used in a wide range of applications ranging from household washing to reagents in big industrial processes. EDTA, Ethylenediaminetetraacetic acid, is the most widely used chelating agent in many of the above-mentioned areas regardless of its poor biodegradability. Here we present some discussion on the biodegradability of EDTA and EDDS, Ethylenediaminedisuccinic acid- A chelating agent coming into application only recently and with a potential of replacing EDTA because of its ready biodegradability. The discussion is concentrated on the comparison of the biodegradability of these two chelating agents because this project considers these two ligands attempting to evaluate the capability of EDDS to quantitatively sequester toxic metals, mainly copper, iron and manganese from pulp so that it can be used instead of the poorly biodegradable chelating agent, EDTA, for the sequestration of metals before the hydrogen peroxide bleaching of pulp in the pulp and paper industry.

1.3.2 Biodegradation of EDTA.

EDTA, Ethylenediaminetetraacetic acid, is known for its poor biodegradability despite its numerous uses. It has been shown in many of the works regarding the biodegradation of EDTA that this chelating agent does not meet the criteria for ready biodegradability as set by OECD **[7].** Several attempts have so far been made towards the enhancement of the degradation of EDTA. The recalcitrance of EDTA towards biodegradation in wastewater treatment plants or the environment has directed much attention to be given to other mechanisms of elimination of the ligand.

Direct photodegradation, oxidation by metal hydroxides and, to a smaller extent, sorption of EDTA to particles and subsequent sedimentation of these EDTA loaded particles are some of the important processes that seem to lead towards the partial elimination of EDTA from aquatic systems **[1]**

The process which is considered the most important elimination method for EDTA from surface waters is direct photolysis, resulting at wavelengths below 400 nm. Only Fe(III)-EDTA is however susceptible to photodegradation while other environmentally relevant EDTA species including Cu^{2+} , Zn^{2+} , Ca^{2+} and Mg^{2+} complexes of EDTA are not sensitive to photodegradation **[9].**

K.Mochidzuki & Y. Takeuchi have worked on the improvement of the biodegradability of EDTA in biologically activated carbon by chemical oxidation. Enhanced biodegradation of EDTA was shown using chemical oxidation by Fenton's reagent or Ozone followed by semi-continuous biologically activated carbon (BAC) treatment resulting in an increased biodegradation of EDTA owing to the chemical oxidation treatment. It was shown that the overall removal rate of EDTA increased when the preozonation step was introduced, and more than 80 % of EDTA was found to be removed by the ozone plus BAC treatment confirmed by the increased ratio of BOD/COD (biological oxygen demand to chemical oxygen demand) as a result of the oxidation process **[10].**

Biodegradation of EDTA by activated sludge in the pulp and paper effluents promoted the degradation of the ligand via the formation of Ethylenediamine triacetic acid (ED3A). The sludge was capable of splitting the ED3A into readily biodegradable Iminodiacetic acid and Iminoacetaldehyde acetate. Caltivated

activated sludge degraded Fe-EDTA under neutral conditions but the degradation was only partial or at low rate. In addition to the lack of complete degradation of EDTA species, some of the most common degradation products of EDTA like acetic acid are known to be biocidal **[8].**

It is therefore implied that considering the poor biodegradability behaviour of EDTA and the fact that only Fe-EDTA is sensitive to biodegradation, a considerable amount of EDTA species are being left in the environment where they can negatively affect various ecosystems and the bioavailability of essential metals via remobilization. It is hence very important that EDTA be replaced by ligands of ready biodegradability behaviour.

1.3.3 Biodegradation of EDDS.

EDDS is a member of the aminopolycarboxylate group of compounds and is a structural isomer to EDTA. It differs from EDTA in that it has two chiral centres in its structure enabling it to exist in three stereoisomeric forms namely, the [S,S]-EDDS , [R,R]-EDDS and the [R,S/S,R]-EDDS isomers.

EDDS was originally synthesized by condensation of maleic acid and 1,2-diaminoethane which was later replaced by the 1,2-dibromoethane and 1-Aspartic acid route, which lead to a substantial reduction in the cost of its production. It was first patented as an important detergent agent in 1987 though its patent history for other purposes dates back to 1964**[7]**

Biodegradation studies on EDDS have shown that this ligand is readily biodegradable under all test conditions. An in depth biodegradation test analysis results of the various stereoisomers of EDDS in a ^{14}C-labelled EDDS isomer mixture in a Batch Activated Sludge (BAS) revealed that the S,S-isomer is rapidly and completely mineralized in all test systems. In contrast the R,R-isomer remained undegraded in a Sturn test (OECD 301B) but very slowly biotransformed into the recalcitrant metabolite aminoethyl aspartic acid (AEAA) in a BAS test while the S,R/R,S racemic form had undergone biotransformation into AEAA in both high and low biomass systems **[11].**

The photodegradation of EDDS within a natural UV-radiation range (315-400 nm) was shown to be very fast and in fact proven that all the three stereoisomers of EDDS are by far more easily biodegradable than EDTA.The analysis was carried out by taking samples from humic lake water and distilled water by exposing them to sunlight and using a lamp emitting light in the UV-range for experiments done in the laboratory. Degradation was studied using the Fe(III)-complexes and sodium salts of the chelates **[12].**

1.3.4. Metal ligand interactions

The interaction of complexing agents with metals is usually studied using speciation analysis.

According to the International Union of Pure and Applied Chemistry (IUPAC), the term speciation refers to the chemical form or compound in which an element occurs

in a living system or the environment. It may also correspond to the quantitative distribution of an element. It is also supported by the views of the OECD that modelling the chemical speciation prevailing is very important to understand complex systems and phenomena in order for appropriate measures to be taken on information obtained. For the production of fine chemicals in the chemical industry, it must be pointed out that not only the purity but also the quantity of the product is dependent on the chemical species within the chemical vessel rather than the total amount of reactants considered. The efficacy of using such chemicals can be improved by the knowledge of speciation that will increase the cost effectiveness of processes and ultimately bring about a decrease in the emission of wastes. The use of EDTA and its structural isomer EDDS in the removal of Cu (II) from a solution containing Copper , Iron and Nickel at alkaline pH where the use of EDDS is more preferable since it strongly binds to Copper at the specified pH than EDTA decreases the amount of ligand that can be disposed to the environment. It clearly shows how optimized speciation conditions like pH adjustment may influence the efficiency of a process **[13].**

The environment contains a large number of ligands and metal complexes that originate from both endogenous as well as exogenous sources. Reports on experimental determinations of chemical speciation in the environment have contributed to the growth of literature as methods are adapted to examining separate fractions and even individual species rather than total amounts of components, the areas of major interest being the environment and the Industry mainly associated with waste disposals **[13,14].**

Chemical speciation simulation programs such as the Joint Expert Speciation System

(JESS) are commonly used speciation methods in the assessment for ready biodegradable replacement ligands to the poorly biodegradable traditional ligands like EDTA in the industrial cleansing of contaminant radionuclides. The chelation of radionuclides by [S,S]-EDDS have been compared with traditional decontamination agents like EDTA and has been indicated in several reports that in many respects [S,S]-EDDS favourably compares with EDTA, in most cases achieving equal performances **[14].**

The speciation of EDTA has been modelled and simulated in process, waste and river waters to the mode of its occurrence in the pulp and paper mill effluents and in subsequent receiving waters. It has been shown according to the specified analysis that the main species of EDTA are:

I. Mn and Ca complexes of EDTA in pulp mill process waters

II. Fe (III) and Mn complexes of EDTA in waste waters and

III. Fe (III) and Zn complexes of EDTA in receiving waters

The effects of increasing the complexing agent's concentration on the speciation of EDTA were seen to favour alkaline earth metals rather than transition metal ions.In a solution of known metal and ligand concentrations, the metal ligand complexation can be shown as curves (Fig.1.2) representing the distribution among complex species of ligands as a function of pH **[15].**

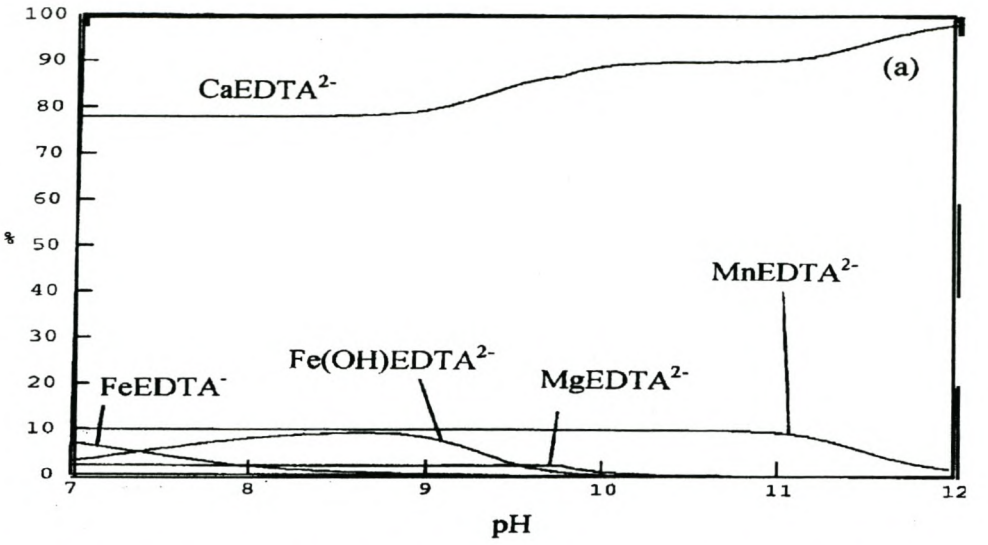


Fig.1.2 percentage of metal-chelate concentration distribution of EDTA among other ligands as a function of pH in process waters. Taken from ref. 15.

In waste and receiving waters where the presence of natural organic ligands, humic and fulvic acids and their influence on equilibrium is evident, the analysis may not be as accurate as analysis done within controlled laboratory conditions where the amount of all components is known. The difference however is expected to be minimal when the overall speciation process is considered **[15].**

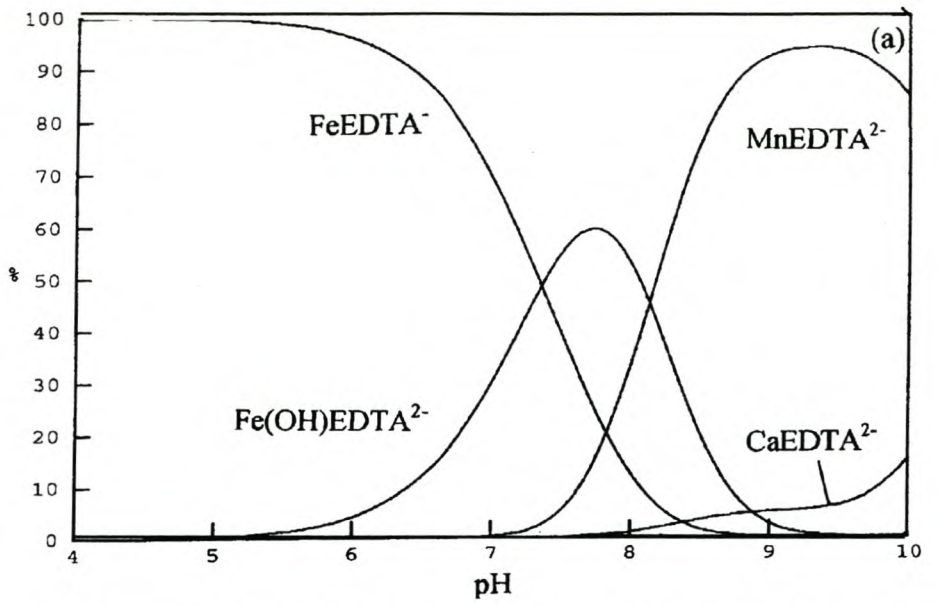


Fig.1.3 A speciation plot of the relevant chemical species of EDTA in waste water effluents of Finish pulp and paper mill at a typical metal ion concentration as a function of pH. Ref.[15]

It can be seen from fig. 1.3 that at acidic pH, the dominant species is Fe (III)-EDTA while at more alkaline pH Mn-EDTA becomes the more dominant species of EDTA. In conjunction with the increasing pressure to replace EDTA by more readily biodegradable ligands, computer simulation of chemical speciation analysis has been used to assess EDDS for its technical compatibility as a substitute for EDTA.Two important points can be considered in this case:

I. The reaction kinetics of the chelants with metal ions.

II. Chemical speciation prevailing in industrial processes so as to get maximum efficiency out of readily biodegradable ligands.

It has been shown that in aqueous systems containing transition metal ions and aminopolycarboxylates, the reaction is completed within the time of mixing. The

question regarding chemical speciation is best answered using computer simulation modelling which requires accurate values of formation and protonation constants of ligands and metal ion ligand complexes formed **[7].**

In a recent report by Paul. W. et.al [14], the two new readily biodegradable aminopolycarboxylate ligands, EDDS and IDS, Iminodisuccinic acid, were assessed against EDTA using a simulation method called JESS in terms of fulfilling the desired criteria to be used instead of EDTA in the pulp and paper industry.

The main roles expected of chelating agents in the pulp and paper processing are:

I. Transition metal ions sequestration (mainly Mn, Fe and Cu).

II. Retention of Magnesium in pulp.

III. Avoidance of ligand destruction by Calcium.

The investigation was performed at different pulp processing stages namely Cooking, washing and O_2-delignification stages (a discussion on pulp and paper is given later in this chapter) paying attention to the metal ion being complexed by each ligand in an unmodified pH of each pulp processing stage.

The results of the simulation demonstrated that at the unmodified alkaline pH of the pulp, [S,S]-EDDS was shown to be the best sequestering agent for transition metal ions in three of the four pulp processing stages. It has also been shown that the pH at which the chelating agent is added is more important than the processing stage itself. Table 1.1. shows the pH range in which the transition metal ions are completely complexed by the ligands according to the analysis **[16].**

Metal ion	[S,S-EDDS]	EDTA	NTA	IDS
Mn	6.5-10	4.5-9.5	6.5-9.0	8.5-9.5
Cu	4-12	4-7.5	4-10.5	4-9.5
Fe	4-12	4-10.5	4-7.5	4

Table 1.1 pH regions in which 100 % of the transition metal-ion is fully sequestered by the chelating agent. [Taken from reference 16].

The concept of speciation is discussed here to imply that knowing the most stable form of a chemical species at a given condition is helpful in the quantitative determination of the species considered.

1.4. Analytical techniques

Because of the ultimate dependence of the toxic characteristics of heavy metals on their chemical occurrences, it has been found to be very important to deal with the qualitative and quantitative determinations of specific metal species. The significance of determining different species of trace elements in the environment and in biological systems has become a growing interest of analytical chemists since the toxic effects of trace elements greatly depends on their chemical forms and concentrations.

Originally, analysts were only concerned (or allowed by analytical methods) upon the total content of an element in a sample. It was soon identified, however, that this analytical information was not sufficient as biochemical and toxicological studies revealed that not only the quantity but also the chemical form and oxidation state in which an element is introduced into a living system or environment is highly important. In order to obtain realistic information on the activity of a specific element in the environment therefore, it is important to determine not only the total

content but also have an understanding of the chemical forms of an element **[17].**

The attainment of a successful determination of species is dependent on two basic factors namely selectivity in order to achieve the aim of identifying the proper species present and sensitivity in order to match the analyte's level in the sample.

The use of either chromatography or capillary electrophoresis coupled with an appropriate detector system such as atomic absorption, emission spectrometry, inductively coupled plasma mass spectrometry and UV-detectors has been a breakthrough in terms of the above-mentioned principal objectives [**17**].

Knowing to the insufficiency of just recognizing or determination of total amounts of metals and their complexes to completely understand their bioavailability, toxicity and hence their environmental impact, various analytical techniques have come into existence during the last decade or so that improved quantitative analysis.

Nowadays, several such techniques are used in the determination of trace and micro trace components of species in a wide array of samples. The most commonly used analytical techniques at the present include Gas chromatography, Liquid chromatography (partition, ion exchange, gel or affinity chromatography) and Capillary electrophoresis.

Gas chromatography is the universally practiced method for the determination of volatile, thermally stable species. While liquid chromatography is used for non-volatile species analysis, there are mostly no limits in the use of capillary electrophoresis, especially capillary zone electrophoresis [**17,18,19**].

1.5 Introduction to capillary electrophoresis

Several methods have been used in the determination of aminopolycarbxylic acids and their metal complexes. In most of the methods however, a sample loss can be expected since a pre-analysis extraction and derivatization is involved in most of the techniques. Capillary electrophoresis is growing to be a method of choice because of its most direct ways of analysis.

Developed in the early 1990s, capillary electrophoresis is now a commonly used technique in several areas of analytical chemistry. In clinics and hospitals, CE is widely used in the analysis of proteins and disease markers. It is also gaining an increased demand in DNA profiling analysis for criminal investigations.

1.5.1. Principles of Capillary electrophoresis

1.5.1.1. Introduction

Electrophoresis is defined as the differential migration of charged species in solution resulting from an applied electric field. Since the introduction of narrow bore tubes (internal diameter less than 100 nm) by Jorgenson and Lukacs **[20]** in 1981, Capillary electrophoresis (CE) has attained rapid development both in theory and practice **[21-24].** CE was mainly applied in the field of analysing biological macromolecules such as proteins, peptides and nucleic acids. Nowadays, the application of CE has already infiltrated into the analysis of small molecules and ions **[25,26].** CE has now become an applicable technique in the assaying of inorganic cations and has drawn great attention.

Although the separation of inorganic cations by CE has been tried long ago **[27]**

when Hjerten pioneered the first separation of bismuth and copper applying capillary zone electrophoresis mode using a relatively large diameter capillary, it was not inspiring because of mainly selectivity leading to lack of discrimination of cations having identical ionic size and lack of sensitivity as most metal ions are transparent in the UV-Vis region. It was the introduction of an indirect uv-detection **[28]** that broke the dilemma. Recently, the introduction of CE separation of metal ions as their complexes have enhanced CE analysis due to the advantages of combination of high performance separation techniques and high sensitivity derivatization agents. Thus, since the early 1980's the theory on CE was fully developed and the potential of high performance capillary electrophoresis (HPCE) as an analytical technique was demonstrated.

Separation of analytes in CE is due to the differences in their migration velocities (v) resulting from the presence of an electric field given by:

$$V = \mu_e E \qquad (1)$$

Where μ_e is the electrophoretic mobility of the solute and E is the applied electric field. While the mobility of a given ion in a given medium is a constant characteristic to each ion, the electric field is a function of the applied voltage and the capillary length. The ion mobility is determined by the electric force that it experiences which is balanced by its frictional drag through the medium. Taking the two forces into account, the mobility of a spherical ion can be calculated using the following equation.

$$\mu_e = q/6\pi\eta r \qquad (2)$$

Where q is the ion charge, η the viscosity of the solution and r is the ion radius.

1.5.1.2. Electroosmotic flow

It is one of the principal constituents in HPCE.The electroosmotic flow (EOF) is the result of the charge on the surface of the capillary wall and the effect of the applied voltage on the solution double layer at the capillary wall. Under aqueous conditions, an excess of negative charge is created on the surface of the fused silica capillary because of the deprotonation of silanol groups, which becomes more significant as the pH increases. Counter ions build up a double layer near the charged surfaces to maintain charge balance creating the zeta potential. The cations forming this double layer are attracted towards the cathode as a voltage is applied giving rise to EOF which its magnitude can be given as

$$V_{eof} = (\varepsilon\zeta/\eta) E \text{ or} \quad (3)$$

$$\mu_{eof} = (\varepsilon\zeta/\eta) \quad (4)$$

Where ζ is the zeta potential and ε the dielectric constant. The magnitude of the EOF can be varied by changing the pH as the surface charge of the wall determines the zeta potential, which is in turn determined by the pH.

1.5.2. Modes of operations in CE

Using the same CE instrument hardware, CE separations can be accomplished with various modes including the most popularly used capillary zone electrophoresis

(CZE) **[29]** micellar electrokinetic capillary chromatography (MECC) **[30],** capillary isotachophoresis (cITP) **[31],** capillary gel electrophoresis (CGE)**[32],** ion exchange electrokinetic chromatography (IEEC) **[33]** and capillary isoelectric focusing (cIEF) **[34].**

In the application of CE for the analysis of metal complexes, CZE is the predominant separation mode used. The following discussion will focus on CZE and its principles, as CZE is the CE mode applied in this project.

1.5.2.1. Capillary Zone Electrophoresis (CZE)

CZE based separation is the most widely used operation mode in CE. Separation in CZE is based on the differences in the electrophoretic mobilities of analytes. Analytes with differing electrophoretic mobilities migrate in separated zones within the capillary and analytes with the same mobility will co-migrate as the same zone in the capillary under an applied electric field. In 1991, Swaile and Sepaniak proposed the first demonstration of metal complex separation with CZE **[35]**. The migration behaviour of metal complexes under CZE conditions is determined by the sum of the analyte's intrinsic electrophoretic mobility (μ_{ep}) and electroosmotic mobility (μ_{eo}), due to the action of the electroosmotic flow (EOF).

$$\mu_{obs} = \mu_{ep} + \mu_{eo} \qquad (5)$$

Where μ_{obs} stands for the observed electrophoretic mobilities of the complexes. Based on whether the electrophoretic mobility is opposite or in the same direction as

the direction of the electroosmotic mobility, CZE can be classified into two namely counter electroosmotic CZE where the direction of the EOF is consistent with the migration direction of cations as long as no modification has been made in the capillary. Since, in most cases, metal complexes are anionic species, the direction of electrophoretic mobility of the complexes is in opposite direction to EOF in general. The second CZE mode is co-electroosmotic CZE where CZE separation is accomplished by effectively reversing the EOF.Many approaches can be taken to reverse the EOF such as derivatization of the capillary wall **[36]** and addition of long chain cationic surfactants **[37].** Addition of cationic surfactants such as cetyltrimethylamonium bromide (CTAB) is a very convenient and mostly used way to reverse the EOF. This kind of CZE mode is used in this project but also applying a negative voltage to change the polarity of the electrodes where fast separation of anionic species is achievable.

The choice of the running buffer is of a primary importance to achieve successful CZE separation. It is always worth considering the following points while choosing a buffer:

- Good buffering capacity in the pH range chosen
- Low absorbance at the wavelength of detection
- Low mobility
- Matching conductivity to the sample solvent to reduce peak shape distortion.

1.5.3. Instrumental aspects of capillary electrophoresis

The basic instrumental aspects of capillary electrophoresis are briefly discussed and is essentially the same for all CE modes of operations. In short, a CE instrument

consists of a separation capillary (10-100 μm I.D, 20-100 cm length), an injection system, a high voltage source (capable of delivering up to 30 kv and current up to 300 μA), electrodes and detectors.

The capillary is the compartment where separations in CE take place. The capillary should be chemically as well as physically resistant, transparent to UV-radiation, able to dissipate joule heating through good thermal conductivity and possibly inexpensive. Fused silica capillaries meet almost all the requirements and are the widely used ones in CE analysis. The capillary inlet and outlet ends are placed in buffer vials during CE analysis. During injection, the inlet buffer vial is replaced by a sample vial, which will later be replaced by the inlet buffer vial before the application of the separation voltage. The inlet vial can also be replaced by cleaning solutions like NaOH and water for flushing and conditioning the capillary.

Injection in CE can be done in either of two ways namely: hydrodynamic injection and electrokinetic injection. Hydrodynamic injection is based on the pressure difference between the two ends of the capillary, which can be produced using gravity, overpressure or applying a vacuum.

UV-absorption detection is the universally adopted detection method in CE.Optical detection is directly accomplished in column through a detection window obtained by burning off the external polyimide coating of the capillary. The high transparency of the fused silica capillary wall enables the use of low wavelengths down to 190 nm.

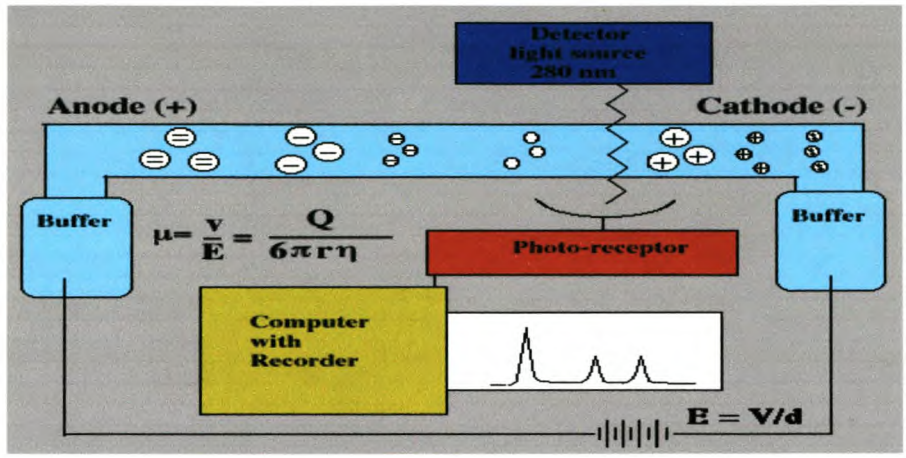


Fig 1.4. An overview of the main events happening during a CE analysis

Capillary Zone Electrophoresis (CZE) is the most commonly used technique in CE analysis now offering significant advantages including high efficiency and resolution, speed and reduced consumption of reagents.

The separation of metal ions by CZE can be accomplished in either of two main approaches. A weak complexing agent can be added to the electrophoretic buffer to attain a larger difference in mobilities. Ions that strongly complex to the reagent will tend to move slowly while those that complex only partially will move faster along the capillary. UV-Absorbance is widely used to monitor metal ions in CZE, but most metal ions do not show significant absorbance in this range. The use of indirect UV-detection where analytes substitute a UV-absorbing organic cation (electrolyte coion) has been used to detect metal ions as negative peaks. Poor sensitivity has however limited the use of this approach to resolve many analysis problems. A more sensitive approach constitutes the use of complete conversion of ions into stable, negatively charged complexes that have high molar absorbtivities and mobilities **[19].**

1.5.4. Quantitative aspects

The output of a CE analysis is displayed as an electropherogram in an absorbance versus migration time plot. The peak area for an electropherogram can be calculated either by manual integration or given automatically depending on the instrument software available. The peak area obtained is proportional to the concentration of a specific analyte present and hence is used to determine the unknown concentration of a sample present in a given matrix. Calibration curves for quantitative determination can be prepared using either the three methods of preparation of a calibration curve namely: external calibration, internal standard or the standard addition methods. The standard addition method is easier to use for determination of relatively lower concentrations and is used in this project.

1.6 Pulp and paper

The pulp and papermaking process is a huge industry operating in the manufacture of a wide variety of paper products. Papermaking is the formation of a cohesive sheet as the result of the rebonding of separated fibres. The pulp and paper industry produces a variety of paper and board products for writing, copying and newsprint-papers, wrapping and packaging products as well as specialist products such as filtration and photography papers and building materials [**38**,**39**]. The primary raw material for the paper and board industry is obtained from wood. Wood is composed of cellulose fibres that are bound together by a resinous substance called lignin. In order to get the suitable form of pulp for the manufacture of paper, the wood or plant material must be pre-treated to separate the fibres by removing the lignin and other

impurities. This pre-treatment process is referred to as pulping and the resulting material called pulp (fig 1.5).

The global consumption of paper and board products has increased from 237.11 million tonnes to 323.38 million tonnes in the last decade **[41].** Continued growth in paper consumption is leading to increased demand in fibre, creating an additional pressure on the world's diminishing forest resources. Meanwhile, the paper industry is also facing a mounting resistance from conservatory and environmental groups.

Furthermore, negative environmental impacts associated with the use of chlorine based bleaching agents required the development and implementation of pulp bleaching technologies that eliminate the use of elemental chlorine (ECF) or any chlorine based (TCF) compounds. Though commercial implementation of such pulp bleaching technologies has occurred during the last decade, understanding of the impact of effluents from those new bleaching technologies on the environment and existing biological treatment processes still needs to be researched **[42,43].**

1.6.1 Bleaching

Depending on the type of wood species used for papermaking, method of pulping adopted and presence of extraneous components, pulps do considerably differ in colour after pulping. For a number of paper types, particularly printing types, bleaching is required. Bleaching is used to whiten the pulp or help change other pulp properties without affecting the strength of the fibres that compose the pulp **[39].**

Chlorine has traditionally been used as a bleaching agent in the pulp and paper industry. The discharge of organochlorine compounds to the environment however is known to be contamination to the environment and these compounds are toxic to humans and fish **[40]**. This has rendered the replacement of bleaching using chlorine

by elemental chlorine free (ECF) chlorine dioxide (ClO_2) bleaching and later by totally chlorine free (TCF) bleaching using Hydrogen peroxide H_2O_2.

Hydrogen peroxide, now in worldwide use for pulp bleaching, is however believed to be highly sensitive to metal catalysed reactions leading to its decomposition **[44-47]**.

The H_2O_2 bleaching stage in the pulp and paper industry is preceded by the addition of chelating agents to the raw pulp in order to sequester transition metal ions (mainly Fe (III), Mn(II) and Cu(II)), which can otherwise cause unwanted chemical reactions leading to the above mentioned problems and thereby the degradation of the bleaching agent.

The hydrogen peroxide bleaching is more importantly done in an alkaline environment. This is mainly because of the formation of hydrogen peroxide anion (HOO^-), which undergoes nucleophilic reactions with chromophores of lignin. The alkalinity itself plays an important role because many degradation compounds produced in previous acidic stages of pulp processing are dissolved in alkaline solutions. The removal of transition metals mainly Fe, Mn and Cu from pulp prior to hydrogen peroxide bleaching is essential because they catalyse the degradation of hydrogen peroxide, diminishing the concentration of HOO-ion, and thus bleaching efficiency. Furthermore, these metals cause the darkening of the final product. In addition to the alkaline H_2O_2 bleaching stage, transition metals can also disturb the O_2-delignification stages where Magnesium sulphate is added to stabilize the effect. Magnesium is beneficial in the H_2O_2 bleaching stage. Sodium, as the main component of the non process elements (NPE) load and Calcium, though a distracter of targeted chelation, are important process elements to protect corrosion of titanium

construction in the alkaline bleaching process. Complexing agents like EDTA can form stable water-soluble complexes with those metals and keep them in solution without interfering with the bleaching process. Addition of such chelating agents to pulp before the alkaline H_2O_2 bleaching stage increases the quality of products. Thus, the pH of the pulp can be adjusted to a value where the metals of concern can be removed by forming stable complexes with the ligands. It can then be readjusted to an appropriate value for alkaline bleaching. The removal of the metals is basically dependent on the pH at which the chelating agents are added to the pulp rather than the processing stage as such. The determination of the transition metals Mn, Cu and Fe has been done using chelating agents like EDTA and DTPA or acid digestion as leaching procedures **[44, 59, 60].** Detection methods such as ICP-MS and ETAAS were used to quantify the metals. These detection methods are expensive compared to uv-detection despite the good sensitivity that they offer.

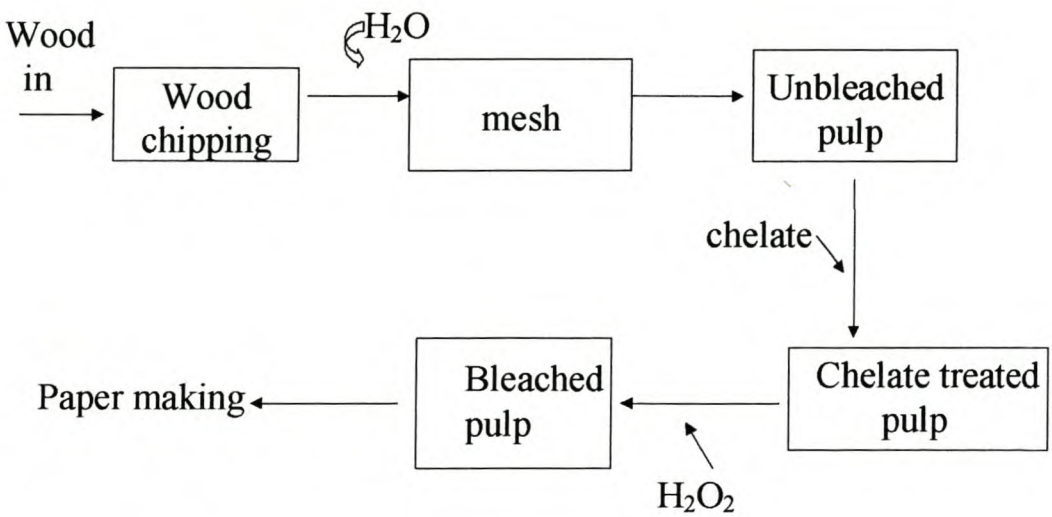


Fig.1.5 a flow diagram describing the pulp and paper making process

Chapter 2

2. Experimental

2.1 CZE Instrumentation

Capillary Zone electrophoretic (CZE) separations were performed on a high performance CE system ($HP^{3D}CE$, Fig.2.1) equipped with a built-in UV-diode array detection with wavelength filters of 200-300 nm from hewltt-packard (Waldbronn, Germany).Data analysis was carried out with a ChemStation software (Rev. A.06.01), which is also from Hewlett-packard.

All CZE separations were carried out using fused silica capillaries of 65 cm of total length, 56.5 cm effective length (length of capillary from inlet end to detection window or to the detector) and 50 μm internal diameter. The window for on-column detection was made by burning off a small section of the polyimide coating of the fused silica capillary.

Sample introduction (injection) was carried out by applying a hydrodynamic pressure of 50 millibars (mbs) throughout the experiments. The temperature of the capillary was kept at 25 ^{0}C throughout the experiments and detection was carried out using direct uv-detection. The instrument is capable of generating high voltage between 15-30 kv.

Fig. 2.1. A high performance capillary electrophoresis ($HP^{3D}CE$) system.

2.2. Reagents and chemicals

All chemicals and reagents used were of analytical-reagent grade. All sample solutions, electrolytes and standard solutions were prepared using deionised water obtained using a milli-Q laboratory water purification system. Standard solutions containing the metals Cu (II), Fe (III) and Mn (II) were purchased from Fluka as $Fe(NO_3)_3$, $Cu(NO_3)_2$ and $Mn(NO_3)_2$. Ethylenediaminetetraacetic acid disodium salt (Na_2-EDTA) and Ethylenediaminedisuccinic acid disodium salt (Na_2-S,S-EDDS) were both obtained from Fluka.

Stock solutions of EDTA and [S,S-EDDS] were prepared by dissolving appropriate mass/volume (8 mM each) of the disodium salts of the ligands in deionised water to

form a 0.3 % solution of each acid, as prescribed by the industries from which the pulp samples were collected. Stock solutions of the metal complexes of the metals Fe (III), Cu (II) and Mn (II) with the ligands were prepared by dilution of appropriate volumes of the metal standard solutions purchased from fluka with appropriate volumes of the 8 mM solutions of each ligand prepared.

2.3. CZE conditions

Several carrier electrolytes including tetraborate, acetate and phosphate buffers were examined in order to establish a working CE method that would enable the successful determination of the metal ligand complexes. While a 30 mM phosphate buffer at pH 6.5 was found to be appropriate for the CZE analysis of EDTA and its metal complexes, a 25 mM borate buffer at pH 7 was used for the analysis of [S,S]-EDDS and its metal complexes.Cethyltetradecylammonium bromide (CTAB) was used as a surfactant to reverse the EOF. 0.1 Molar solutions each of NaOH and HNO_3 were used for the desired pH adjustments.

2.4. Procedure

The capillary was rinsed for 5-10 min with each of 0.1 M NaOH and deionsed water and then reequilibrated with the running electrolyte for 5- 10 min at the beginning of each day to keep the capillary properly conditioned before analysis is started (flushing stage). The capillary was rinsed with carrier electrolyte for 3 min in between all electrophoretic separations. All sample and electrolyte solutions were degassed by ultrasonication and filtered through a 0.45 μm membrane filter to remove suspended particles before each CZE analysis.

2.5. Pulp

Three South African unbleached wood pulps (I,II and III) were considered in this analysis. Each pulp sample was treated with each complexing ligand in a separate procedure before the CZE analysis could be carried out.

2.5.1. Treating pulp with EDTA and [S,S-EDDS]

In the pulp pre-treatment procedure with the ligands, 20 g of each pulp sample (I, II and III) was weighed and transferred into three separate 250 ml round bottom flasks. To each round bottom flask containing the pulp sample, 0.8 mM of either of the two ligands EDTA or [S,S-EDDS] was added. The content in the flasks, ligand plus pulp sample, was made to dissolve in 100 ml of deionised water. The resulting solution in the flask, which now contains the ligand and the pulp sample dissolved in 100 ml of deionised water, was heated in an oil-bath system with stirring and temperature controlling system at 45 ^{0}C for three hours to disintegrate the pulp matrix. After cooking and cooling, the resulting solution was filtered using a 0.45 μm membrane filter.

Chapter III

3. Determination of EDTA and its metal complexes in pulp.

3.1. Method Development

Separations of anions and cations using capillary electrophoresis have attained large attention in the previous few years. High resolution, short analysis times and low background electrolyte and reagents consumption has made capillary electrophoresis a more advantageous technique in the separation and determination of anions and cations compared to ion chromatography **[48].** Several research groups have dealt with CE and in many of their works showed the potential of CE in the analysis of anionic **[49-50]** and cationic **[51-53]** species. The separation and determination of cations and anions has often been done using separate CE systems using different background electrolytes. It has, however, been shown recently that capillary electrophoresis is a potentially suitable technique for the simultaneous determination of anionic and cationic species. The analysis is normally carried out in an electrolyte system where one group of analytes migrate in the same direction as the electroosmotic flow (EOF) while the other group of analytes migrate in the opposite direction to the electroosmotic flow. This situation holds true as long as the migration velocity of the later group of compounds does not exceed the velocity of the electroosmotic flow **[54]**

Among the various modes of capillary electrophoresis, Capillary Zone electrophoresis

has been shown to be a highly efficient technique that comprises all types of electrophoresis in fused silica capillaries of less than 100 μ meters internal diameter. Separations in capillary zone electrophoresis are based on the differential mobilities of charged compounds along a capillary under the effect of an applied voltage. The migration behaviour of species in capillary electrophoresis is significantly affected by several factors including pH, conductivity and ionic strength of background electrolyte **[55].**

3.2. Analysis of metal ions and their complexes

The determination of metal ions in medicines, soil samples, drinking water and industrial waste-effluents has demanded the use of rapid and reliable analytical techniques. Several analytical techniques including ion-chromatography, atomic spectrophotometric and electrochemical methods have been used for this purpose despite many of these techniques being tedious, time consuming, involve preliminary separation steps based on solvent extraction, coprecipitation or ion-exchange and the needed of expensive detection systems.

In contrast, capillary electrophoresis, specifically capillary zone electrophoresis coupled with UV-detection, has become a routine technique for the determination of metals and their complexes due to its simplicity, separation speed and high efficiency **[56,57].**

One of the major problems in the CE analysis of metal ions is that in some metal ion groups, individual cations have almost the same mobilities due to their similar size

and identical charges which arises because of the effect of hydration on the size of the metal ions upon hydration. Divalent transition metal ions are examples that typically exhibit this effect. Varied degree of hydration causes the majority of these metals to attain a similar size and hence identical mobilities **[56].**

In capillary electrophoresis enhanced separations can be achieved by making use of complexing agents that selectively bind to the metals. There are two main approaches that can be considered here:-

I. Addition of a weak complexing agent that will complex to the metals to a varying degree to the electrophoretic buffer to promote a larger difference in effective mobilities of the metals resulting from the accompanying degree of complexation of metal to ligand. Metals that complex to a higher degree to the complexing agent migrate slower than those that complex only partially. This happens because complexation basically has the effect of reducing the net charge on the resulting compound.

II. The complete conversion of metal ions into stable negatively charged complexes of high molar absorbitivities and different mobilities. This approach has shown to be more preferable because a more sensitive spectrophotometric detection of metal ion complexes can be performed. Aminopolycarboxylic acids such as ethylenediamintetraacetic acid, EDTA, are commonly used complexing agents for this purpose because of their ability to form very stable complexes with the metals in a 1:1 molar ratio **[56].**

The use of aminopolycarboxylic acids in the analysis of metal ions by capillary electrophoresis has been reported by several authors. The use of EDTA for the simultaneous determination of Cr (III), Fe (III), Cu (II) and Pb (II) as UV-absorbing EDTA complexes by capillary electrophoresis has been shown **[55,56].**

Apart from this, the development of trace analytical methods is required for the study of the environmental fate of aminopolycarboxylic acids. These acids are used in a wide range of industrial processes to serve for various purposes ranging from detergent reagents to their use for the removal of transition metal ions to prevent unwanted chemical reactions. The environmental fate of APCAs therefore needs to be properly addressed to help control their release to the environment where they can negatively affect various ecosystems **[57].**

EDTA has so far been used in the pulp and paper industry for the removal of transition metal ions, mainly Cu, Mn and Fe, from pulp before the hydrogen peroxide bleaching stage. These metals severely affect the bleaching process by basically destroying the bleaching agent if not removed which normally results in the production of a poor quality paper. The addition of a complexing agent to the pulp before bleaching keeps these metals from interacting with the bleaching agent by the formation of stable water-soluble complexes.

Despite its important use EDTA is not a ligand of choice because of its poor biodegradability behaviour, which does not comply with the OECD criteria for ready biodegradability. Being poorly biodegradable and having a strong affinity to complex

with the majority of metals, its release to the environment can severely affect metal bioavailability and be toxic to certain organisms. Gas chromatographic determinations of EDTA together with other aminopolycarboxylic acids in sewage treatment plant (STP) and paper mill effluent by Hing-Biu Lee et.al **[17]** has already shown the presence of up to 11micrograms of EDTA in the sample from a paper mill effluent.

[S,S]-Ethylenediaminedisuccinic acid [S,S]-EDDS is a newly synthesized alternative complexing agent believed to serve as a potential substitute for EDTA. It has been shown that it is readily biodegradable and compares well with EDTA in most of its applications.

3.3. Electrophoretic behaviour

The development of a CZE procedure for the separation and quantification of metals using complexing agents requires the formation of stable complexes of the metals with the complexing agent as unstable complexes tend to dissociate within the capillary making CZE separations difficult. In addition, as electrophoretic separations are based on differences in mobilities, separations will only be possible if the complexed forms of the metals have differences in charge or size. Aminopolycarboxylic acids are known for their ability to form stable negatively charged complexes with many metals stable at a wide range of pH and show good absorbance at wavelengths between 200-300 nm. Under the normal electrophoretic conditions using a fused silica capillary (injection at anode and detection at cathode), the electroosmotic flow (EOF) is towards the cathode. With this type of configuration, positively charged species elute first while negatively charged species elute late or

may not elute depending on their size to charge ratio. Only anions with mobilities less than that of the EOF are detected with this type of configuration. The relatively small charge to size ratio of metal aminopolycarboxylic acid complexes provide a possibility of separating these analytes using the configuration with positive injection side, i.e under counter electroosmotic conditions, but with longer analysis times. A rapid separation of anionic species like metal complexes with aminopolycarboxylic acids can be achieved more easily by adding surfactants like CTAB to reverse the electroosmotic flow, i.e. under coelectroosmotic conditions. In this case, reversal of EOF renders anions to elute first and be detected at very short analysis times.

Depending on the charge of the metal ion, the metals interact with the ligand according to the reaction scheme below:-

$M^{n+} + H_2Y^{2-} = MY^{n-4} + 2H^+$, Where, M represents the metals Cu, Mn and Fe

Y = EDTA

In order to determine the best separation conditions (peak efficiency, analysis time) selection of appropriate background electrolyte is essential in CZE analysis. Buffer solutions of borate, acetate and phosphate were investigated and 30 mM phosphate buffer (pH 6.5) with a 0.5 mM CTAB as a surfactant to reverse the EOF was found to give the best results.

Under the pH conditions considered, the following EDTA species were present:[Cu-EDTA]$^{2-}$,[Mn-EDTA]$^{2-}$ and [Fe-EDTA]$^{1-}$ **[34,56].**

3.4. Separation of standard solutions

Several carrier electrolyes were studied to find out the most convenient experimental conditions for the separation and detection of the metal complexes of Fe (III), Cu (II)

and Mn (II) with EDTA.While a borate buffer seems good for Cu and Mn complexes of EDTA, since their complexes are very stable even at higher pH values. Iron tends to be destabilized and form hydroxy compounds at alkaline pH. Using Phosphate buffer, pH 6.5, 245 nm wavelength, a satisfying condition for the separation and detection of the standard mixtures of $[Cu\text{-}EDTA]^{2-}$,$[Fe\text{-}EDTA]^{1-}$ and $[Mn\text{-}EDTA]^{2-}$ was seen and resolution and peak shapes were good.Thiourea,a neutral compound, was used as an electroosmotic marker since it carries no charge and hence migrates with the electroosmotic flow. According to previous work and the theoretical background underlying capillary zone electrophoresis (coelectroosmotic mode), the elution order of the analytes was expected to be $EDTA^{4-}$> $[Cu\text{-}EDTA]^{2-}$ > $[Mn\text{-}EDTA]^{2-}$> $[Fe\text{-}EDTA]^{1-}$ and the results obtained were in line with this trend. It was found to be, as it is usually the case, important to first qualitatively identify the species by separately injecting the analytes to enable one to know which analytes elute and at what migration time. Each species including the free ligand, EDTA, thiourea, marker compound, and the metal-EDTA complexes of each of Cu (II), Mn (II) and Fe (III) was studied. By injecting different concentrations of each species, their identification was possible.

3.4.1. $[Cu\text{-}EDTA]^{2-}$

Copper (II) ion is known for its ability to form a very stable complex with EDTA in a 1:1 Molar ratio under a wide range (5-10) of pH [**19,37,57**]. The resulting electropherogram that was obtained from the standard CZE analysis of [Cu-EDTA] **(fig.3.1)** also demonstrates that this metal ion forms a stable complex with EDTA.The presence of a sharp and well-shaped electropherogram in the CZE analysis is also an

indication of the stability of the analyte.

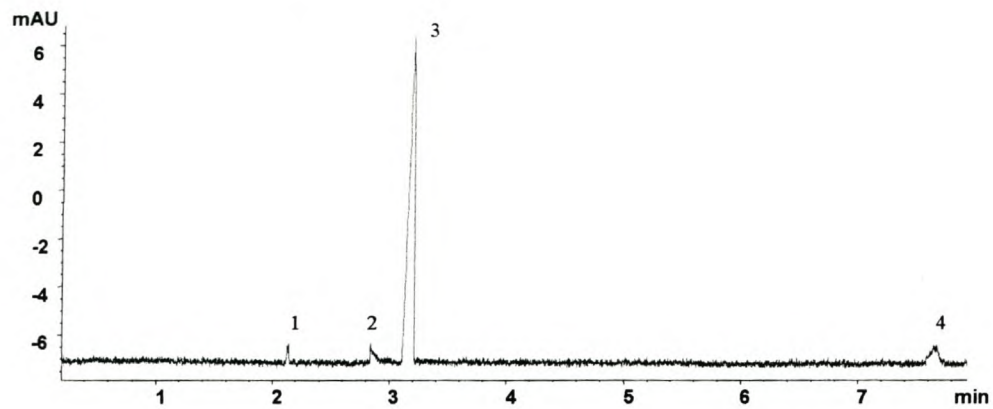


Fig.3.1. Electropherogram for the analysis of $[Cu\text{-}EDTA]^{2-}$, 0.15 mM Cu(II), Phosphate buffer pH 6.5 ,0.5 mM CTAB as a surfactant, 8 mM EDTA , hydrodynamic injection for 2 sec, applied voltage -25 kv.direct detection at 245 nm.

The reproducibility of the analysis results was shown by considering 5 CZE runs and the reproducibility in peak areas and migration times together with their relative standard deviations is given in the table below.

Analysis number	Concentration Cu (II), mM	Peak area	Migration times
1	0.15	18.26	3.146
2	>>	18.63	3.146
3	>>	18.11	3.149
4	>>	18.81	3.154
5	>>	18.74	3.151
% RSD		1.6	0.1

Table.3.1. Data of peak areas and migration times for the standard CZE analysis of $[Cu\text{-}EDTA]^{2-}$ together with their relative standard deviations.

3.4.2 $[Fe\text{-}EDTA]^{1-}$

Equilibrium reactions of iron with EDTA show that this metal forms a stable $[Fe\text{-}EDTA]^{-}$ in the pH range between 5 and 7. This makes the CZE analysis of iron most appropriate at a pH range 5 to 7 because instability of the complex formed at pH

greater than 7 makes CZE analysis difficult, as there is a shift in equilibrium along the capillary. This effect has been shown by analysing the complex at pH greater than 7 where broad and tailed electropherograms were obtained. This happens due to the formation of the hydroxy complex of iron with EDTA at alkaline pH. At the analysis pH of 6.5, a sharp and well-shaped electropherogram **(fig.3.2)** was obtained. The analysis results were quite reproducible as demonstrated by considering 5 consecutive CZE runs of the sample. Table 3.2 shows the data used to check the reproducibility of peak area and migration times together with their relative standard deviations.

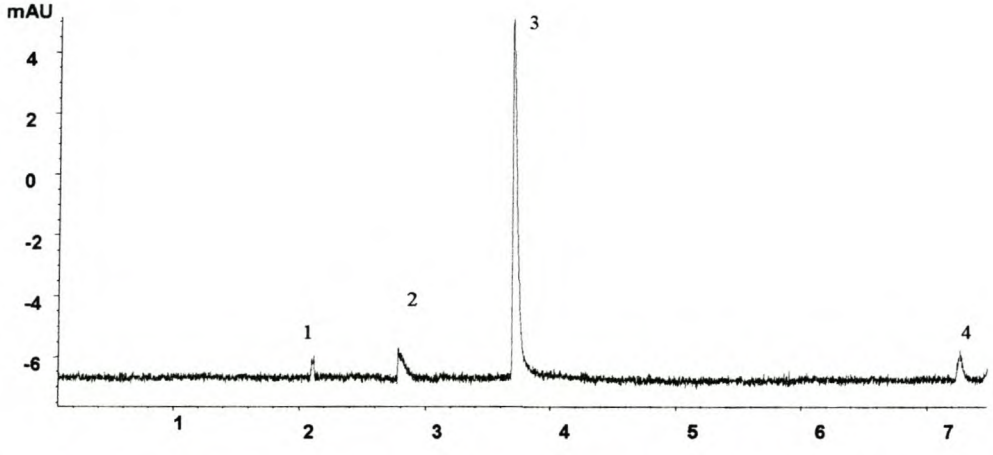


Fig.3.2. electropherogram for $[Fe\text{-}EDTA]^{1-}$,10 ppm Fe(III), 8mm EDTA, 30 mM phosphate buffer, pH 6.5, 0.5 mM CTAB surfactant, hydrodynamic injection for 2 sec, applied voltage -25 kv. Direct detection at 245 nm.

Analysis number	Concentration of iron, mM	Peak area	Migration time
1	0.17	30.83	3.709
2	>>	30.75	3.696
3	>>	30.19	3.695
4	>>	30.11	3.688
5	>>	30.77	3.726
% RSD	>>	1.1	0.4

Table.3.2. Data of peak areas and migration times for the standard CZE analysis of $[Fe\text{-}EDTA]^{1-}$ together with their relative standard deviations.

3.4.3. $[Mn\text{-}EDTA]^{2-}$

Manganese –EDTA complexes as well are stable at the pH (6.5) of analysis but the complexes generally have lower absorbance at wavelengths considered (200-245 nm) compared to the EDTA complexes of copper and iron. It was however still possible to analyse the complex at those wavelengths. The formation of $[Mn\text{-}EDTA]^{2-}$ was always evident at pH greater than 6 by the appearance of an electropherogram (fig.3.3) after a CZE analysis of its EDTA complex and despite the suppressing effect of other peaks resulting from species that strongly absorb , it could be analysed.

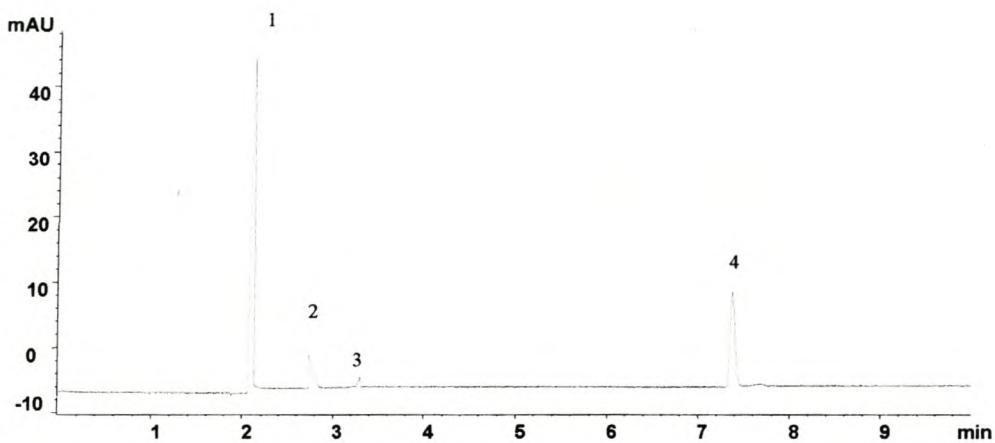


Fig.3.3 Electropherogram for $[Mn\text{-}EDTA]^{2-}$, 0.9 mM Mn, 8 mM EDTA 30 mM phosphate buffer, pH 6.5, 0.5 mM , hydrodynamic injection for 2 sec,applied voltage -25 kv, direct detection at 245 nm.

The following table shows how the reproducibility of the results of the analysis was checked together with the relative standard deviations of peak areas and migration times.

Analysis number	Concentration of Mn, mM	Peak area	Migration times, min
1	0.9	2.82	3.30
2	>>	2.87	3.27
3	>>	2.84	3.26
4	>>	2.80	3.26
5	>>	2.80	3.26
% RSD	>>	1	0.5

Table.3.3. Data of peak areas and migration times for the standard CZE analysis of $[Mn\text{-}EDTA]^{2-}$ together with their relative standard deviations

From the electropherograms above the conditions chosen demonstrate a good CZE separation of the species considered.

Once the species identification and migration time of each species under the chosen experimental condition was studied, a mixture of all metal ligand complexes and marker compound was injected. From the figure below, under the conditions used, the results demonstrate clear resolution between peaks.

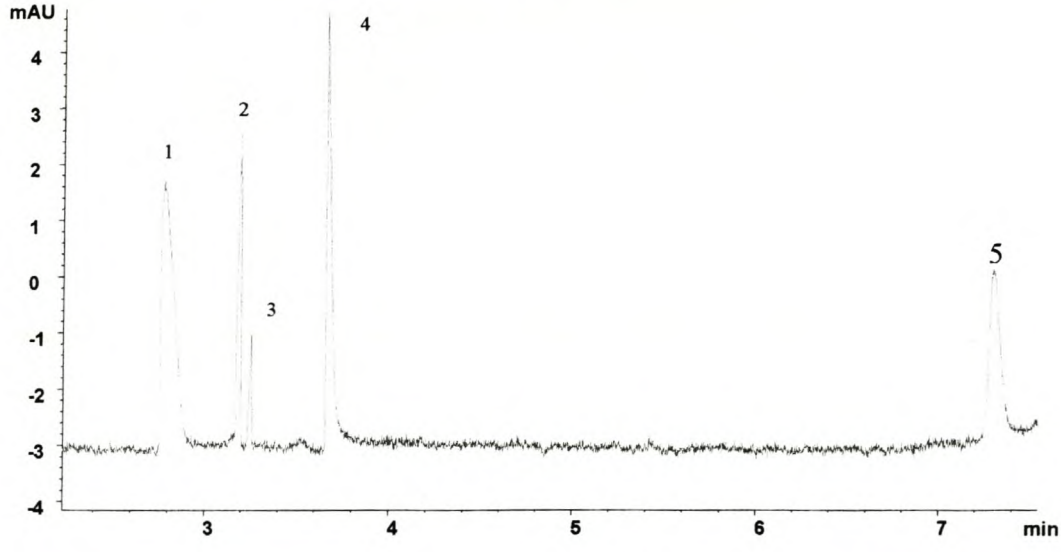


Fig.3.4 Eelectropherogram of a mixture of standard solutions of Cu-EDTA, 0.17 mM Cu, Mn-EDTA, 0.9 mM Mn, and Fe-EDTA, 0.15 mM Fe. 30 mM phosphate buffer, pH 6.5 and 0.5 mM CTAB as a surfactant.

Detection at 245 nm. Peak identification; 1.EDTA^{4-} 2. [Cu-EDTA]$^{2-}$ 3. [Mn-EDTA]$^{2-}$ 4. [Fe-EDTA]$^{1-}$ 5. Thiourea, marker compound.

3.5. Quantification

Several parameters, which are important for quantitative analysis such as linearity, reproducibility and minimum detectable concentrations were examined under the above optimised experimental conditions.

In order to envisage quantitative applications, the reproducibility of the results obtained was studied by making several consecutive injections of each species. The results demonstrated good reproducibility and detection was possible up to the lower ppm range for the three metal complexes considered (fig 3.4). The results obtained clearly show that this method can be used for the determination of metals in pulp.

3.6. Analysis of the filtrate

Because of its varied composition, though the components may not be present to a detectable amount, pulp treated with EDTA and adjusted to the experimental conditions was analysed. It can be seen from the electropherogram below **(fig.3.5)** that the presence of the metals Copper and Manganese could be observed but Iron was not detected. Accordingly, based on the results obtained in developing the CZE method, identification of the species was carried out.

3.7. Results of the CZE determination of the metals Cu^{2+}, Fe^{3+} and Mn^{2+} in pulp I after complexation with EDTA

The CZE method developed using the standard solutions of the metal complexes were applied for the determination of the complexes of the metals in pulp. The initial CZE analysis of pulp I is depicted in figure 3.5

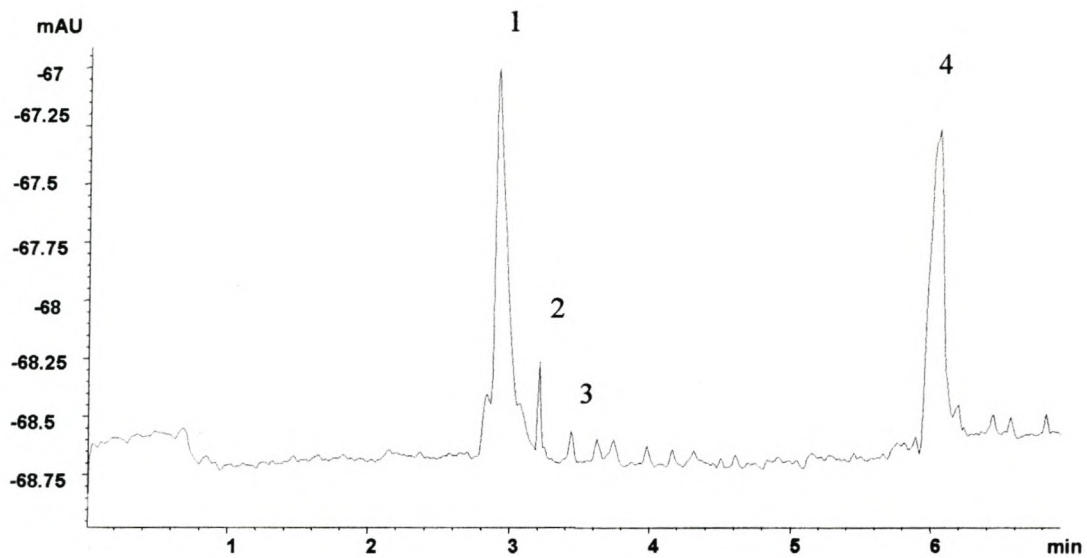


Fig.3.5 Electropherogram for the CZE analysis of 20 % pulp-I, Peak identification: 1. EDTA 2. [Cu-EDTA]$^{2-}$ 3.[Mn-EDTA]$^{2-}$ 4. marker. CZE conditions: 30 mM phosphate buffer, pH 6.5, 8 mM EDTA, 0.5 mM CTAB, hydrodynamic injection for 10 sec, applied voltage –25 kv, direct uv-detction at 245 nm

Using the peaks of the metal complexes that were obtained from the initial analysis of the pulp sample, calibration curves were constructed for Cu-EDTA and Mn-EDTA. A four-point calibration graph was constructed for each metal complex using the standard addition method. The following electroferogram (fig. 3.6) shows a pulp sample (20 %) analysed after spiking the pulp sample with 1 ppm Cu^{2+} and 1 ppm Mn^{2+} ions. It could easily be seen that the peak height and the area of the peaks that was obtained from the unspiked pulp sample increases as the concentration of the metals increases.

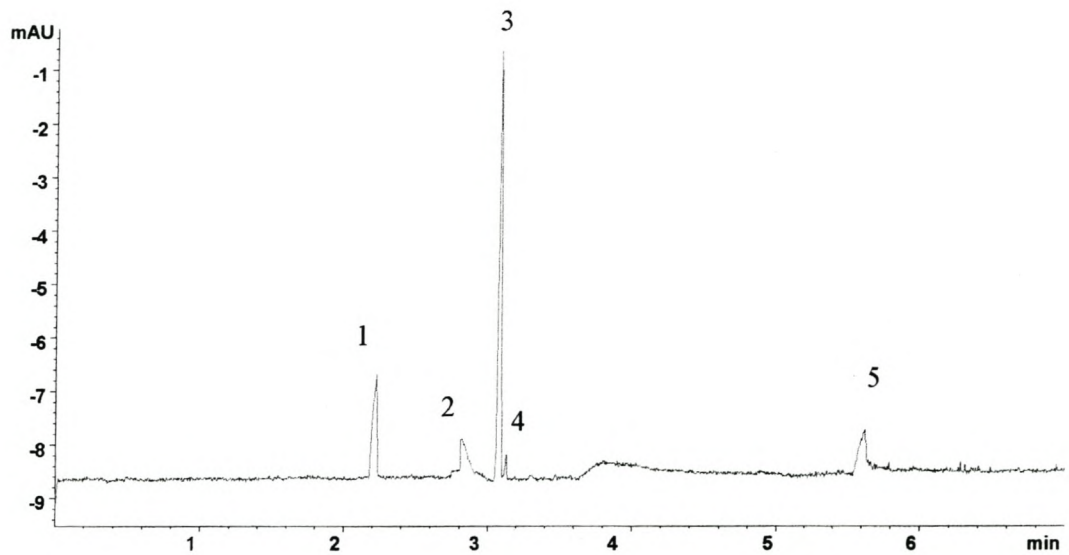
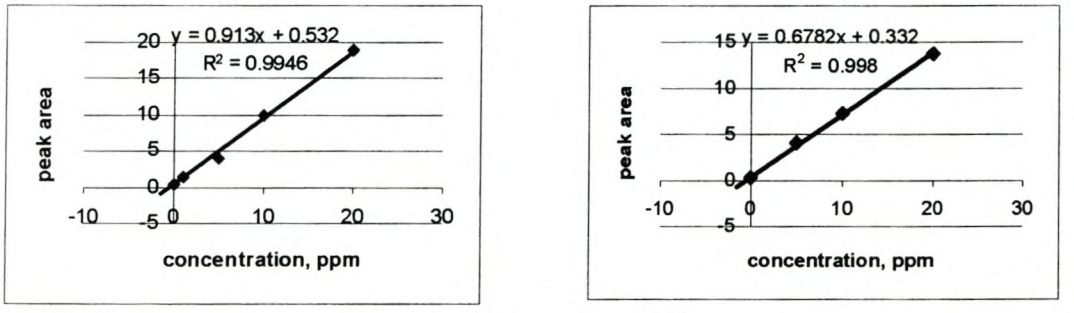


Fig.3.6. Electropherogram for a 20% pulp spiked with 1 ppm Cu^{2+} and 5 ppm Mn^{2+} ions. Peak identification, 1. NO_3^- 2. EDTA 3. $[Cu\text{-}EDTA]^{2-}$ 4. $[Mn\text{-}EDTA]^{2-}$ 5. Thiourea

Copper (II)		Manganse (II)	
Peak area	migration time (min)	peak area	migration time (min)
0.532	3.071	0.332	3.31
0.49	3.081	0.36	3.28
0.51	3.11	0.36	3.36
0.54	3.12	0.320	3.39
0.52	3.15	0.30	3.41
%RSD = 3.7 %	1 %	6 %	1.6 %

Table 3.4. Data showing the reproducibility of the results of the initial CZE analysis of pulp-I. For the determination of the metals copper and manganese.

A linear relationship between the peak areas and concentrations were obtained in the 0.015-0.047 mM range for Cu (II) and in the 0.018-0.055 mM range for Mn (II) The linear correlation coefficients calculated from the calibration curves obtained with 4 **(fig. 3.7,a , b)** different concentrations of Cu (II) and Mn (II)) were 0.9945 and 0.9980 respectively. The concentrations of each species taken and their corresponding peak areas and migration times are given in **table 3.5 a, b**.



(a) (b)

Figure 3.7. Calibration curves for metal-EDTA species of Cu^{2+} (a) and Mn^{2+} (b) for the determination of the corresponding species in pulp-I.

From the calibration curves constructed, it was then possible to determine the concentration of the metals in the pulp sample. The concentrations of Cu^{2+} and Mn^{2+} found in the pulp were 0.58 ppm and 0.48 ppm respectively.

Sample number	concentration of Cu (II) (ppm)	peak area	migration time (min)
1	0	0.532	3.071
2	1	1.45	3.15
3	5	4.05	3.14
4	10	10.06	3.088
5	20	18.855	3.14
% RSD			1.5 %

(a)

Sample number	concentration of Mn (II), ppm	peak area	migration times (min)
1	0	0.332	3.31
2	5	4.08	3.16
3	10	7.29	3.18
4	20	13.72	3.16
% RSD			2.2 %

(b)

Table.3.5. Data used for construction of calibration curves for $[Cu\text{-}EDTA]^{2-}$ (a) and $[Mn\text{-}EDTA]^{2-}$ (b) for the determination of the respective metals in pulp-I

3.8. Results of the determination of the metals Cu^{2+} and Mn^{2+} in pulp-II after complexation with EDTA

3.8.1. Effect of mixing

In dealing with such applications aimed at removing undesirable transition metals from a pulp matrix, one can easily understand that mixing plays an important role because it enhances the mass transfer into the aqueous phase by basically disintegrating the pulp matrix. Mass transfer into the aqueous phase in part facilitates the removal of metals by making them more accessible to the complexing agent. Nitrogen bubbling or magnetic stirring are the common ways by which mixing can be done. The effect of the mixing type on the recovery of metals have in fact been shown **[28].**

The aim of the analysis, as explained before, is to remove certain transition metals, especially Manganese, Iron and Copper, from a pulp matrix collected from different mills before the hydrogen peroxide bleaching stage of the pulp and paper industry. These metals are known for their negative role of catalytically decomposing hydrogen peroxide under the alkaline conditions of peroxide bleaching. Chelation and acid digestion are the most common industrially used techniques for metal removal from pulp [29].Chelation is however more preferable because of the selective complexation that the ligand entertains with the metals. Acid treatment on the other hand is known to remove almost all metals including the metals of important qualities to the

product.[29].Magnesium Sulphate and free Calcium(II) ions are important auxiliary chemicals to the process. Magnesium sulphate because of its ability to stabilize hydrogen peroxide and Ca^{2+} ions because of their function as good corrosion inhibitors of titanium constructions though excess of Ca may strongly disturb the functionality of the chelating agent [28].

EDTA, in addition to being under scrutiny because of environmental impacts is known to be easily distructed by Ca decreasing its efficiency. [S'S-EDDS] on the other hand is more environmentally friendly and is known for its low selectivity towards calcium. The following electropherogram shows the results of the analysis of a softwood pulp (pine) treated with EDTA.

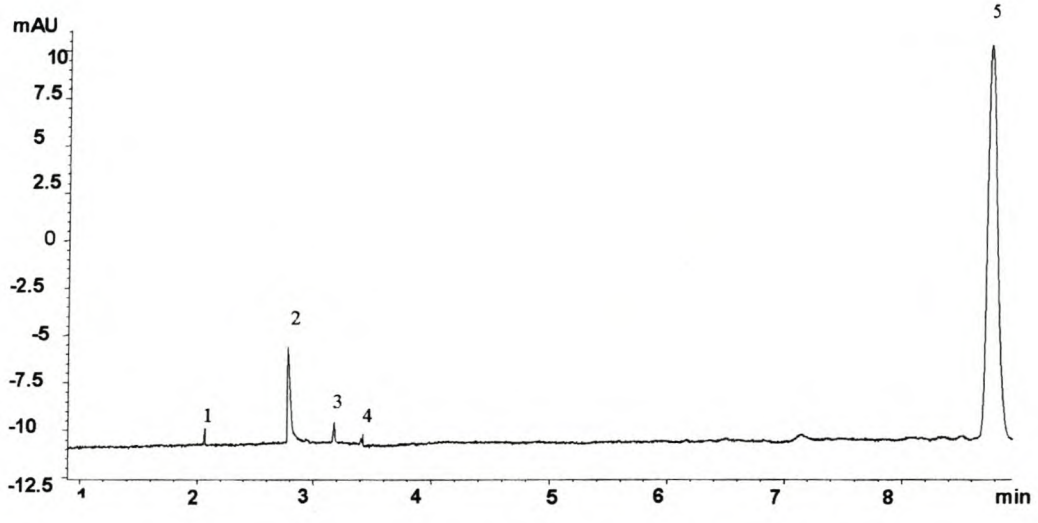


Fig. 3.8. Electropherogram for soft-wood pine pulp treated with 8 mM EDTA,0.5 mM CTAB .phosphate buffer,pH 6.5,hydrodynamic injection 10 sec, applied voltage -25 kv, direct uv-detection at 245 nm.

It can be seen from the electropherogram that there are at least four peaks. In order to identify to which compound the peaks correspond, the pulp sample was spiked with standard solutions of the metals. The result electropherogram is presented below.

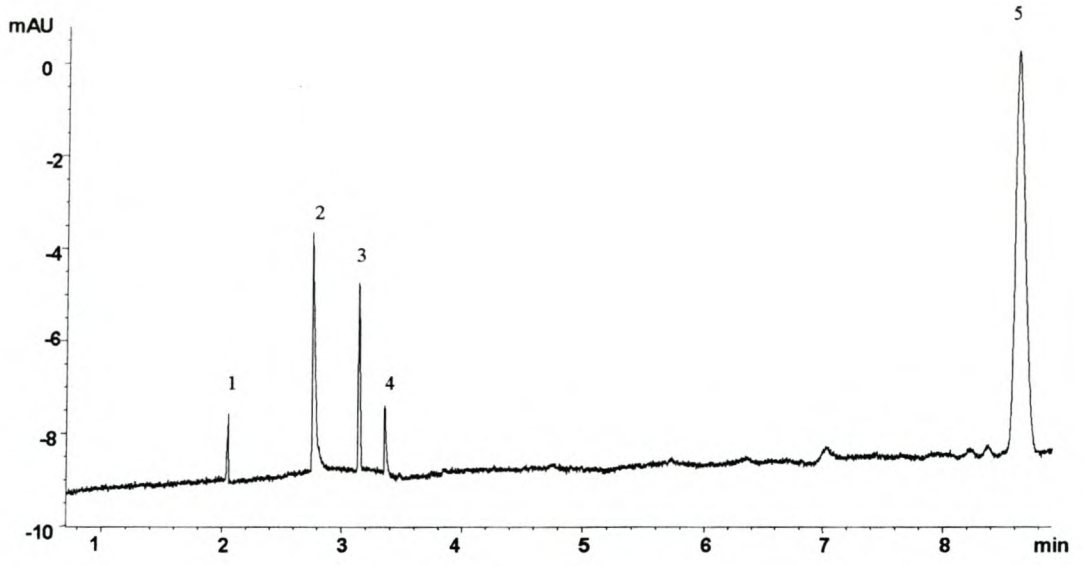


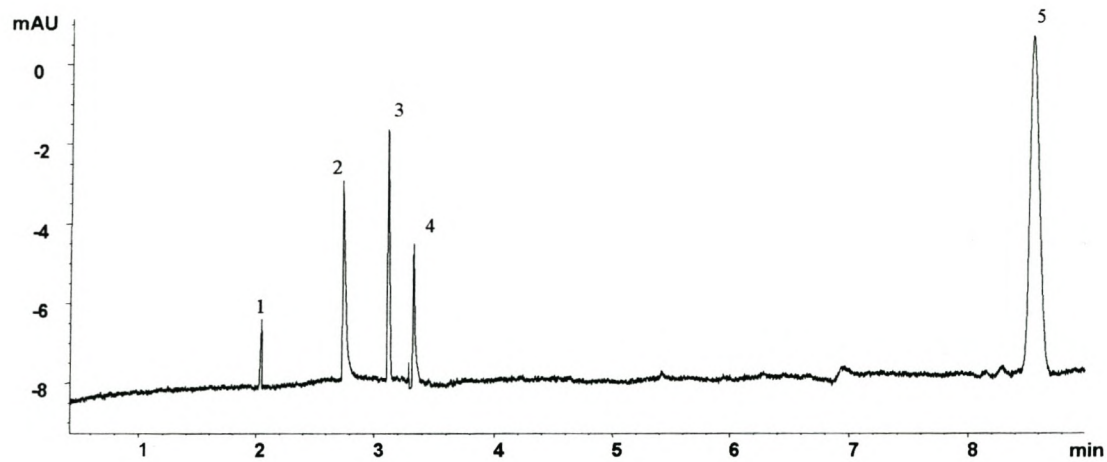
Fig.3.9. Electropherogram for soft-wood pine pulp spiked with 1 ppm of each of Cu^{2+} and Mn^{2+}, Phosphate buffer, PH 6.5,8 mm EDTA, 0.5 mm CTAB, hydrodynamic injection time 10sec, applied voltage -25 kv direct uv-detection at 245 nm.peak identification: 1. NO_3^- 2. $EDTA^{4-}$ 3. $[Cu\text{-}EDTA]^{2-}$ 4. $[Mn\text{-}EDTA]^{4-}$ 5. Marker

The peak area was seen to dramatically increase when the sample was analysed after spiking it with the standard solutions of the metals to identify the peaks representing metal-EDTA complexes found from the pulp. From the results obtained, it can be seen that a peak that represents Fe-EDTA didn't appear. Depending on studies previously made using other techniques, it was shown that no or less recovery of Fe-can be observed because of its strong binding nature to the pulp on one hand and its existence in the form of geotite, hydroxide or oxide in the pulp which are very hard to be removed from the pulp **[28,29].** The concentration of Iron in the aqueous phase might as well be below the detection limits.

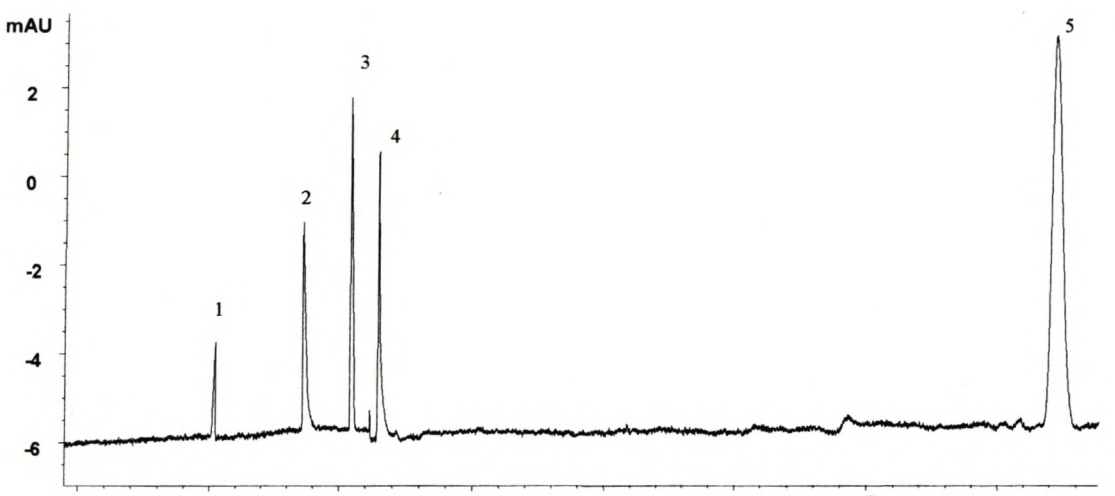
In order to determine the amount of Mnganese and Copper present in the pulp, a successive spiking of the sample was made and analysed to get electropherograms

(fig.3.10) for construction of calibration curve using the standard addition method.

A linear relationship was obtained between concentration and peak area in the lower parts per million ranges.



(a)



(b)

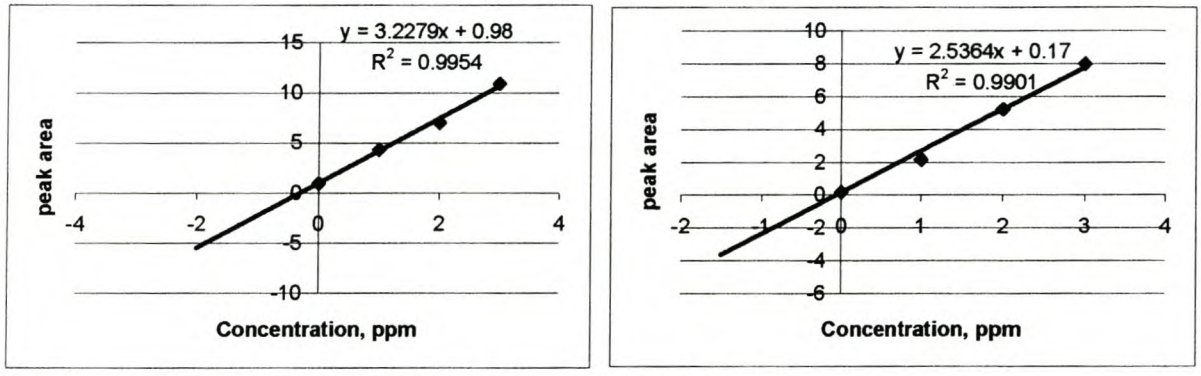
Fig.3.10. Electropherograms of pulp-II after spiking with 2 ppm standard solutions (a) and 3 ppm standard solutions (b).

The data used for the construction of the calibration curves for copper and manganese

in soft wood pin pulp; peak areas and concentrations, migration times and their relative standard deviations are given in the tables below.

Copper (II)			Manganese (II)		
concentration (ppm)	peak area	migration time(min)	concentration (ppm)	peak area	migration times (min)
0	0.98	3.17	0	0.17	3.4
1	4.33	3.13	1	2.15	3.35
2	7.02	3.11	2	5.22	3.32
3	10.90	3.15	3	7.91	3.30
% RSD		0.8			1.3

Table.3.6 Data of peak areas and concentrations used for construction of calibration graphs for the determination of the metals copper and manganese n pulp-II after complexation with EDTA.



(a) (b)

Fig.3.11 Calibration curves for $[Cu\text{-}EDTA]^{2-}$ (a) and $[Mn\text{-}EDTA]^{2-}$ (b) for the analysis of the metals in pulp- II.

3.9. Results of the CZE Analysis of the metal complexes of manganese and copper with EDTA in pulp –III

In a similar way, this soft wood pulp was treated with EDTA and analysed for its metal content using the same CZE technique. The following electropherogram shows

the results of the initial CZE analysis of the pulp sample before spiking.

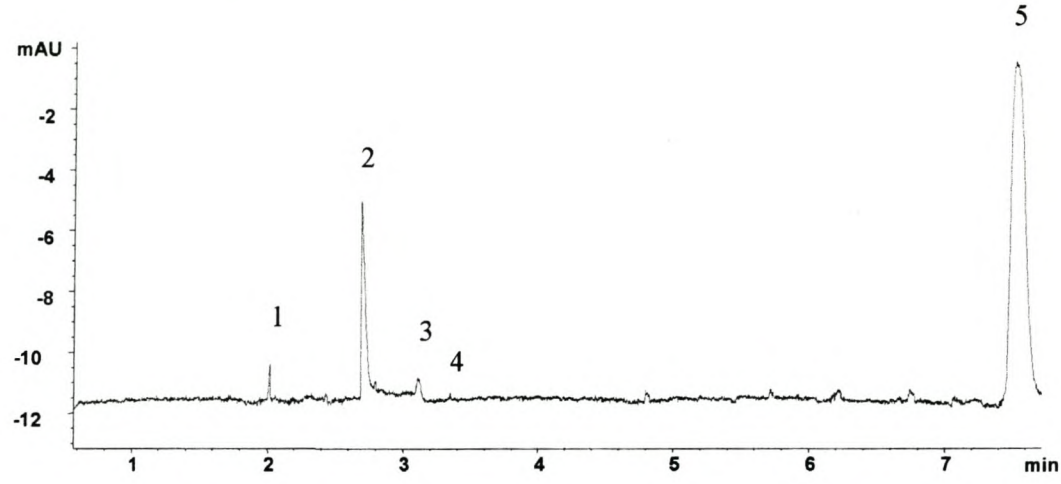


Fig.3.12 Electropherogram for soft-wood pulp-III, phosphate buffer PH 6.5, 0.5 mM CTAB, 8 mM EDTA, hydrodynamic injection for 10 sec, applied voltage -25 kv, direct UV-detection at 245 nm.

Analysis of spiked sample was done and showed the presence of Cu-EDTA and Mn-EDTA species manifested by the increase in peak area of some of the peaks as the result of spiking. Fe-EDTA was not detected which could be due to similar reasons mentioned above for the soft wood pine pulp. The following electropherogram shows the results obtained after spiking of the sample with 1 ppm of each of Cu^{2+} and Mn^{2+} standard solutions.

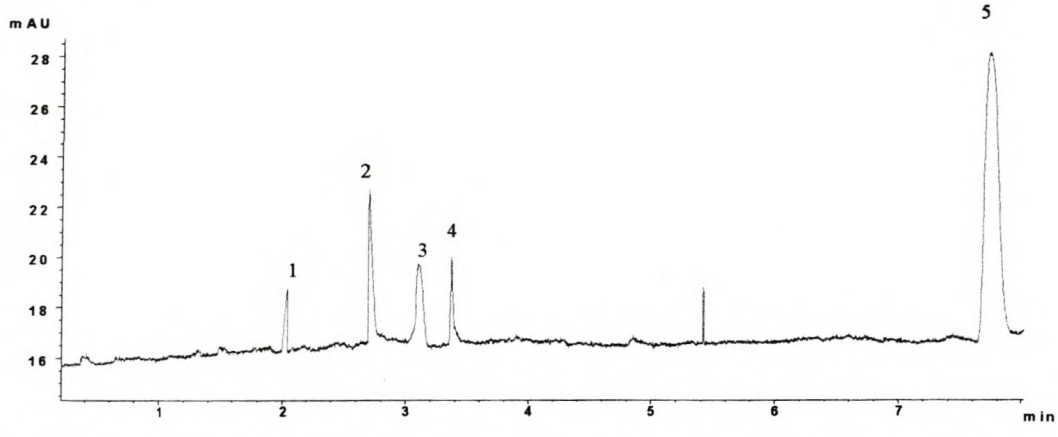


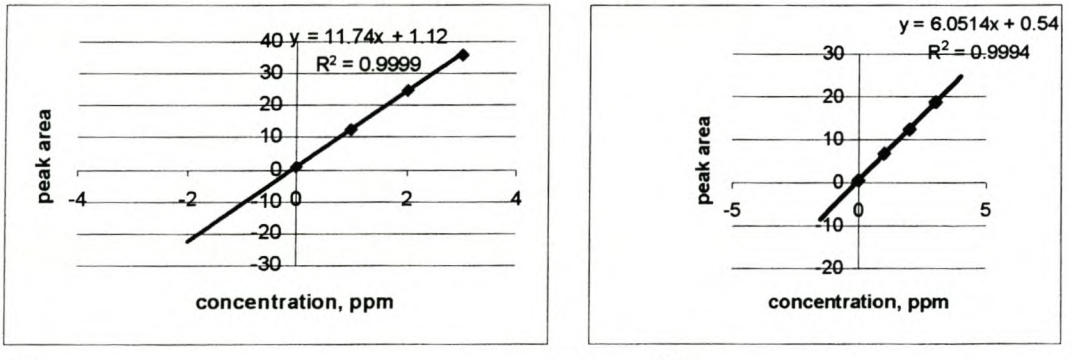
Fig.3.13 Eletropherogram for soft wood pulp III after spiking the sample with 1 ppm of each of

Cu (II) and Mn (II) ions.

Successive spiking of the sample was done in order to construct a calibration curve using the standard addition method to enable us the quantitative determinations of each species present in the pulp. The data used for construction of calibration curve and the calibration curves together with the relative standard deviation is given below.

$[Cu\text{-}EDTA]^{2-}$			$[Mn\text{-}EDTA]^{2-}$		
Concentration in ppm	peak area	migration time, min	Concentration, ppm	peak area	Migration time, min
0	1.12	3.13	0	0.54	3.39
1	12.8	3.104	1	6.8	3.37
2	24.87	3.074	2	12.41	3.34
3	36.18	3.068	3	18.78	3.33
% RSD		1.2			0.8

Table.3.7. data used for construction of calibration curves for the analysis of the metals in pulp-III



(a) (b)

Fig.3.14 Calibration curves for Cu-EDTA (a) and Mn-EDTA (b) for the determination of copper and manganese metals in pulp-III after complexation with EDTA

Using the calibration curves prepared using the standard addition method of

calibration for every species, the amount of each metal found in the pulp sample was calculated. A summary of the amount of each metal, copper and manganese, obtained in the three pulp samples taken from different paper mills after complexation with EDTA is given below in the table.

Pulp type	$[Cu\text{-}EDTA]^{2-}$	$[Mn\text{-}EDTA]^{2-}$	Correlation factor	
			$[Cu\text{-}EDTA]^{2-}$	$[Mn\text{-}EDTA]^{2-}$
Pulp-I	**9.1 ± 3.7 %**	**8.8 ± 6 %**	**0.9946**	**0.9980**
Pulp-II	**4.8 ± 0.5 %**	**1.2 ± 1 %**	**0.9954**	**0.9901**
Pulp-III	**1.4 ± 1.1 %**	**1.6 ± 2.3 %**	**0.9999**	**0.9994**

Table.3.8. Concentrations (µg/l) of $[Cu\text{-}EDTA]^{2-}$ and $[Mn\text{-}EDTA]^{2-}$ ± relative standard deviation based on four replicate determinations in the three types of pulps analysed.

It can be seen from the analysis of the three pulps taken from different sites for the determination of the metals in pulp that it was possible to determine copper and manganese after complexation with EDTA and subsequent quantification using a CZE technique coupled with a diode array uv-detection. Previous analysis using the chelating agents EDTA or DTPA and a subsequent ICP-AES or ET-AAS **[59, 60]** revealed the quantitative determinations of all the three metals. It wasn't possible to quantify iron using this technique. This may be attributed to the difference in sensitivity that can be attained with the different techniques. Iron is also known to strongly bind to or can exist in the form of hydroxides and geotites in the pulp that the amount of iron leached by the complexing agent might be below the detection limit. It

was observed that the amount of the metals determined generally decreased from pulp-I to pulp-III. Higher amount of the metals was determined especially in pulp-I compared to the other two. This could be attributed to the higher water content of pulps-II and III compared to pulp-I where in the case of pulp-II and III, the amount of the metals might have been already washed away when the sample was taken. While in pulp-I, a solid pulp sample was taken where the metal contents lose due to water could be avoided..

Chapter 4

4. Analysis of the metals, Cu (II), Mn (II) and Fe (III) in pulps using [S,S-EDDS] as the complexing agent.

The synthesis and characterisation of new complexing agents is part of the search to find more environmentally friendly chemicals. A property that distinguishes [S,S]-EDDS from other strong transition metal chelators is its ready biodegradability **[7,15].** Previous speciation analysis of [S,S]-EDDS based on computer simulation modelling **[16,7]** have shown the potential of this ligand in metal chelation. It is one of the stereoisomers of ethylene diamine disuccinic acid,[R,R],[R,S]/[S,R] and [S,S]. Only the [S,S] stereoisomer is readily biodegradable while the other isomers are biotransformed to recalcitrant metabolites[15,11].The potential of [S,S]-EDDS as a substitute for EDTA in the transition metal sequestration in the pulp and paper industry is being assessed here using capillary zone electrophoresis.

4.1 CZE analysis of standard solutions

In order to demonstrate an effective CZE separation, a separate CZE analysis of each metal complexed with EDDS was carried out first. Stock solutions of the metals Mn(II), Fe(III) and Cu(II) in their nitrates, purchased from Merck, were used to prepare dilute metal-chelate solutions. Both borate and phosphate buffers were evaluated and the best results were obtained using a 25 mM borate buffer at pH 7. 0.5 mM CTAB was added to the buffer in order to reverse the electroosmotic flow. Purified water, obtained using a Millipore Milli-Q water purification system was used to prepare all solutions. Thiourea was used as an electroosmotic marker. All analysis was done under coelectroosmotic conditions and detection was performed using a

direct UV-detection.

4.1.1. Analysis of Copper-EDDS

According to reports on speciation analysis based on computer modelling [7,16], copper ion strongly complexes with EDDS (logK = 18.45). It has been reported [16] that a complete complexation of copper ion by [S,S]-EDDS is evident in the pH region between 4-12. This characteristic formation of stable complex has been shown in this CZE analysis by the appearance of a sharp and well shaped electropherogram **(fig.4.1).** The results of the analysis were quite reproducible as demonstrated by doing several CZE runs. Table below shows data showing the reproducibility of peak areas and migration times (n =5) together with their relative standard deviations.

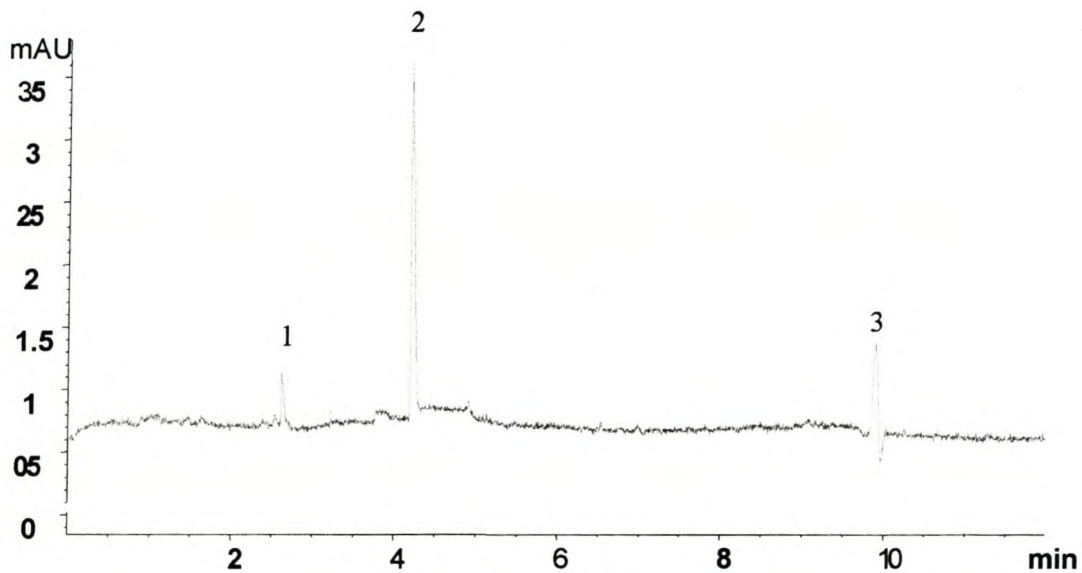


Fig.4.1. Electropherogram for the standard analysis of Cu-EDDS, 0.07 mM Cu, 25 mM borate buffer, ph 7, 0.5 mM CTAB hydrodynamic injection for 2 sec, applied voltage –25 kv. Direct uv-detection at 245 nm. Peak identification: 1. NO_3^- 2. $[Cu\text{-}EDDS]^{2-}$ 3. marker.

Analysis number	Concentration of copper, mM	Peak areas	Migration times. Min
1	0.07	9.40	4.19
2		9.35	4.20
3		9.20	4.214
4		9.24	4.216
5		9.30	4.206
%RSD		0.8	0.25

Table.4.1. Results for peak area, migration times and corresponding relative standard deviations showing the reproducibility of the analysis for $[Cu\text{-}EDDS]^{2-}$, n = 5, Standard solution.

4.1.2. Analysis of $[Fe\text{-}EDDS]^{1-}$

This metal ion also demonstrates a good complexing properties with [S,S-EDDS].In fact, as opposed to EDTA, which forms stable complex with Fe (III) ion only at lower pH, (5-6.5), this ligand, [S,S-EDDS] forms a very stable complex of Fe (III) ion at a wide range of pH's (5-9).

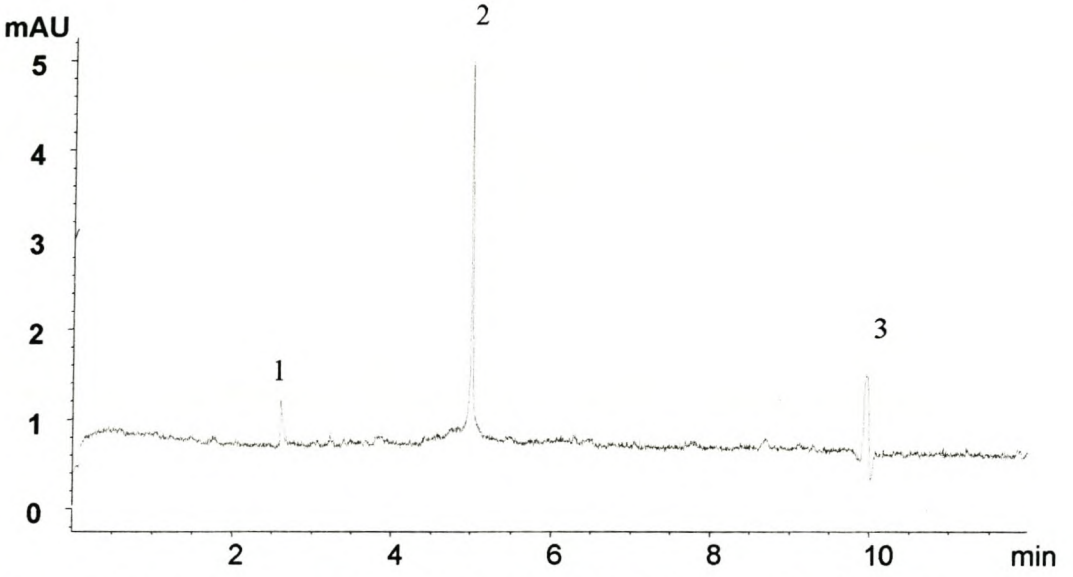


Fig.4.2. Electropherogram for $[Fe\text{-}EDDS]^{1-}$, 0.017 mM Fe^{3+}, 8 mM $EDDS^{4-}$, 245 nm. Other conditions as in figure 3.14 above. Peak identification: 1. NO_3^- 2.$[Fe\text{-}EDDS]^{1-}$ 3.marker

Consequently, the stability of the complexes formed could be clearly demonstrated by the formation of a sharp and well shaped electropherogram obtained during the CZE analysis of the [Fe-EDDS] complex (fig.4.2). The results have good reproducibility as demonstrated by considering 5 CZE runs as depicted in the table below.

Analysis number	Concentration of Fe^{3+},mM	Peak area	Migration times , min
1	0.017	8.78	5.12
2		8.72	5.057
3		8.67	5.030
4		8.80	5.07
5		8.74	5.05
% RSD		0.58	0.66

Table.4.2 Results for peak area, migration times and corresponding RSDs showing the reproducibility of the results of the analysis of $[Fe\text{-}EDDS]^{1-}$.

4.1.3. Analysis of standard mixtures of the metals with EDDS

To demonstrate the simultaneous CZE separation of the metal-EDDS species considered above, a mixture of the EDDS complexes of the metals were injected together and analysed. As depicted in the figure below, all the species considered eluted well resolved at different migration times. Based on the principles of CZE under coelectroosmotic conditions, the more negatively charged species elute faster. Consistently, the results demonstrate the elution of $[Cu\text{-}EDDS]^{2-}$ before $[Fe\text{-}EDDS]^{1-}$.

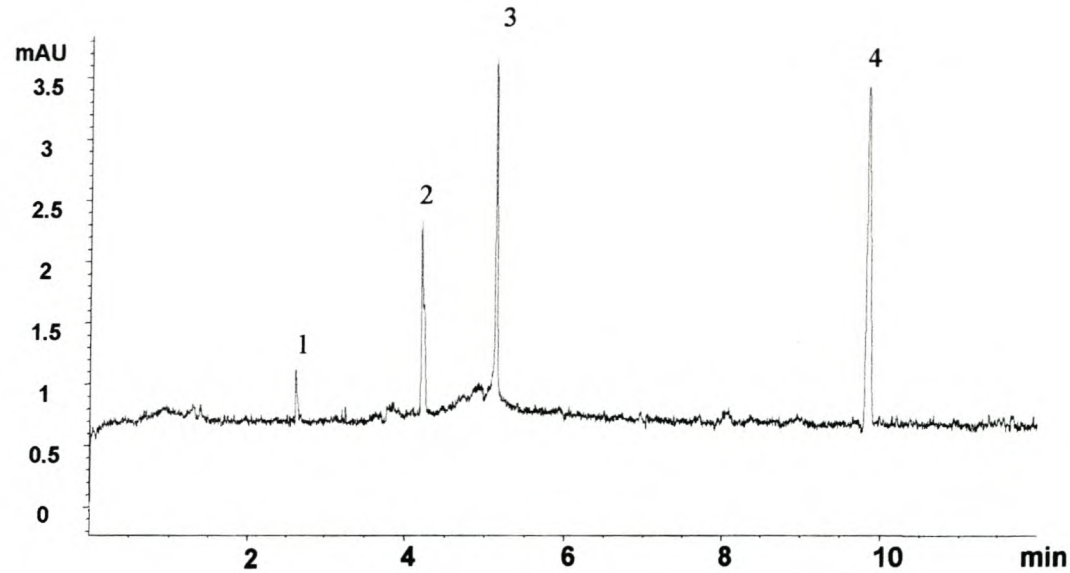


Fig.4.3 Electropherogram for the standard mixtures of $[Cu\text{-}EDDS]^{2-}$, 0.07 mM Cu (II) and $[Fe\text{-}EDDS]^{1-}$, 0.017 mM Fe (III). 25 mM boratete buffer ph 7,0.5 mM CTAB, hydrodynamic injection for 3 sec, applied voltage –25 kV. Direct uv-detection at 245 nm. Peak identification: 1. NO_3^- 2. $[Cu\text{-}EDDS]^{2-}$ 3. $[Fe\text{-}EDDS]^{1-}$ 4. marker.

It can be seen from the analysis of the standard mixtures of the complexes of copper and iron with [S,S-EDDS] (fig.4.3) that the species present in the sample elute well resolved and at different migration times which enables one to qualitatively identify and quantitatively measure the species considered in any matrix.The results obtained from the analysis of the standard solutions of the complexes of S,S-EDDS with the metals was in good reproducibility as depicted in tables above and detection limits up to the lower parts per million range was possible for both complexes.It can therefore be concluded that this method can be used to determine the metals copper and iron from a pulp matrix using the complexing agent S,S-EDDS and subsequent CZE analysis.

4.2 Analysis of metals in pulp with EDDS

4.2.1. Pulp sample pre-treatment

Three South African soft wood pulps, one from the Durban pulp and paper industry and two from industries from the Northern Province, were considered in this project. The pulp sample was treated with the chelating agent in a separate system before the subsequent CZE analysis can be carried out. This is done in order to promote a mass transfer of the metals in the pulp, which normaly are bound to the pulp, into an aqueous phase where they can be easily accessible for complexation by the chelating agent.

In the pulp pre-treatment procedure, 20 g of each pulp sample was weighed and transferred into three separate 250 ml round bottom flasks each containing 100 ml of deionised water.8 mM (570 µl) of the chelating agent, now S,S-EDDS, was added to the contents in each flask. Each mixture was then heated in three separate oil bath systems each containing a stirrer and temperature controlling systems for three hours at 45 ^{0}C (cooking) . Heating with stirring at 45 ^{0}C for a couple of hours causes the pulp to disintegrate and hence the release of the metals into the aqueous phase. The resulting solution was filtered through a Milli-Q filter to remove solid and suspended particles.

4.2.2. CZE analysis of pulp samples

Capillary zone electrophoretic analyses were carried out in accordance with the method developed. The filtrate from the pulp sample pre-treatment was taken and its pH adjusted to the working pH (7) of the method developed. 1M solution of each of NaOH and HNO_3 were used to adjust the pH.

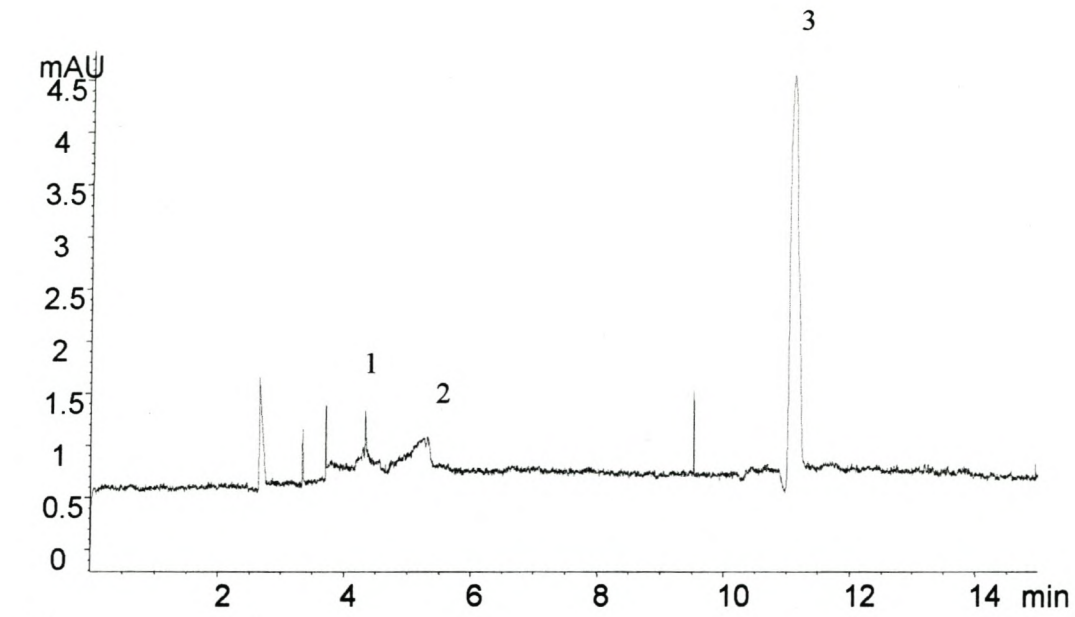
4.3. Results and discussion

The underlying objective of this project is the quantitative sequestration of the transition metals namely Manganese, iron and copper by chelation with S,S-EDDS and subsequent CZE analysis to quantify the amount of metal being sequestered by the ligand.The initial CZE analysis of the filtrate from the pre-treated pulp is aimed at getting electropherograms corresponding to the uv-absorbing complexes of the metals present as a result of treating the pulp with the complexing agent. It has already been demonstrated that the complexes of the metals with S,S-EDDS absorb well in the range between 200-254 nm in the standard CZE analysis done in this project. Their presence can therefore easily be detected by the CZE method. Detection was done at 245 nm for all the analytes.

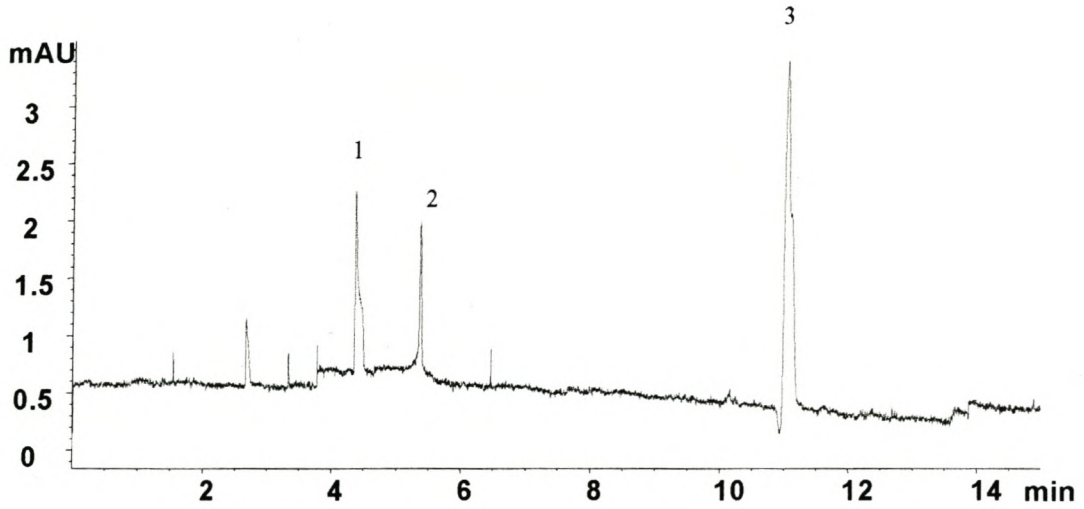
4.3.1. Results of the CZE Analysis of the metal complexes of copper and iron with [S,S-EDDS] in pulp I

After taking the filtrate and adjusting to the working CZE conditions, the pulp sample was analysed for the metal complexes. An unspiked pulp sample was first analysed and then spiked for a second analysis to identify the species present. Since pulp is a natural substance, it is obvious that it is composed of a wide range of cationic, anionic and neutral species that may give rise to a CZE peak. It is only after spiking that one can identify the species under consideration as the migration times of the same species remains the same under CZE conditions. The figure below shows the resulting electropherograms of the CZE analysis of unspiked pulp sample (a) and a pulp sample spiked with 1 ppm of each metal (b).The results obtained after spiking the pulp sample demonstrated the presence of $[Fe\text{-}EDDS]^{1-}$ and $[Cu\text{-}EDDS]^{2-}$ showed by the increase in peak area of the original peaks. Manganese complex of [S,S-EDDS] was

not found which could be because of its low absorbance even at higher wavelengths or can be present in amounts below the detection limit.



(a)



(b)

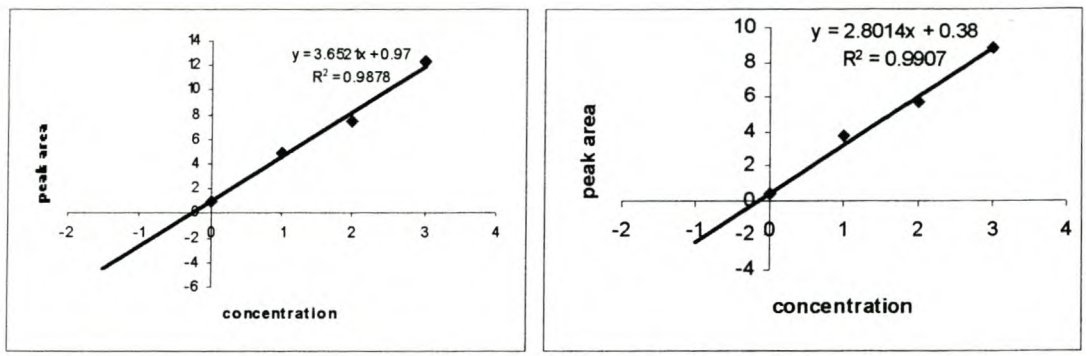
Fig.4.4. electropherograms for unspiked pulp sample (a) and after spiking the sample with 1 ppm of each of Cu and Fe(b) experimental conditions:25 mM borate buffer,pH 7, 0.5 mM CTAB surfactant,hydrodynmic injection for 7 sec,applied voltage – 25 kv,direct UV-detection at 245 nm.peak identification: 1.$[Cu\text{-}EDDS]^{2-}$ 2.$[Fe\text{-}EDDS]^{1-}$ 3.marker

For the purpose of constructing a calibration graph in order to determine the unknown amount of the metals in the pulp, the sample was continuously spiked and analysed. The results were linear between 0.015-0.047 for $[Cu\text{-}EDDS]^{2-}$ and between 0.017-0.05 for $[Fe\text{-}EDDS]^{1-}$ complexes with correlation factors of 0.9878 for $[Cu\text{-}EDDS]^{2-}$ and 0.9907 for $[Fe\text{-}EDDS]^{1-}$. The data used for the construction of calibration graphs together with their relative standard deviations of the migration times obtained are given in the table below.

$[Cu\text{-}EDDS]^{2-}$			$[Fe\text{-}EDDS]^{1-}$		
Concentration(ppm)	Peak area	Migration times(min)	Concentration (ppm)	Peak area	Migration times (ppm)
0	0.97	4.33	0	0.38	5.33
1	4.95	4.36	1	3.70	5.35
2	7.52	4.36	2	5.70	5.37
3	12.33	4.37	3	8.8	5.41
%RSD		0.3			0.6

Table.4.3. Data of concentrations and peak areas used for the construction of calibration graphs for $[Cu\text{-}EDDS]^{2-}$ and $[Fe\text{-}EDDS]^{1-}$ together with the species migration times and their relative standard deviations.

The corresponding calibration curves for the complexes are given below and from the equation of the straight line connecting the points, the unknown amount of the metals in the pulp could easily be calculated.



(a) (b)

Fig.4.5. Calibration graphs for $[Cu\text{-}EDDS]^{2-}$(a) and $[Fe\text{-}EDDS]^{1-}$ (b) used for the determination of the unknown quantities of the respective complexes in pulp –I.

4.3.2. Results of the CZE analysis of the metal complexes of copper and iron with [S,S-EDDS] in pulp –II and III

In a similar way to the previous one, a CZE analysis of these pulps was carried out under the same CZE conditions described before. The electropherograms obtained as a result of the analysis of an unspiked pulp and after spiking the pulp with 1 ppm each of the metals showed similar correlations. Accordingly, it could be observed that $[Fe\text{-}EDDS]^{1-}$ and $[Cu\text{-}EDDS]^{2-}$ are found in a quantifiable amount in the pulps.

The calibration curves so constructed showed good linearity for both $[Cu\text{-}EDDS]^{2-}$ and $[Fe\text{-}EDDS]^{1-}$ and the correlation factors obtained were 0.9974 and 0.9899 and 0.9902 and 0.9828 in pulps II and III respectively. The unknown amount of the metals found in the pulp was calculated using the equation of the straight line forming the calibration points. The amount of the metals copper and iron found in the three types of pulp samples using [S,S-EDDS] as the complexing agent is summarised in the table below.

Pulp type	$[Cu\text{-}EDDS]^{2-}$	$[Fe\text{-}EDDS]^{1-}$	Correlation factor	
			$[Cu\text{-}EDDS]^{2-}$	$[Fe\text{-}EDDS]^{1-}$
Pulp-I	4.1 ± 3.8	2.4 ± 3.5	0.9878	0.9907
Pulp-II	3.6 ± 3	1.8 ± 4.6	0.9974	0.9899
Pulp-III	3.1 ± 2.3	4.7 ± 1.9	0.9828	0.9902

Table 4.4. Concentrations of $[Cu\text{-}EDDS]^{2-}$ and $[Fe\text{-}EDDS]^{1-}$ in µg ± relative standard deviations based on four replicate determinations obtained in the three types of pulps.

In the analysis of these metals in pulp using EDTA as the leaching agent, copper and manganese could be quantified. When [S,S-EDDS] was used as the leaching agent however, quantification of copper and iron was possible. It has been shown [16] that [S,S-EDDS] and EDTA show a complete complexation with iron in the pH range 4-12. The EDTA complex of iron is known to be destabilized as the pH increases by the formation of hydroxides **[60].** On the other hand, it has been shown **[58, 59]** that the determination of the metals manganese, copper and iron in pulp was possible using other techniques like ICP-AES and ETAAS. This shows that not only the sensitivity offered by the technique but also the stability of the complex formed between each metal and the ligands clearly accounts for the differences on the metal species that could be determined as unstable complexes make CZE analysis difficult. When comparing EDTA and EDDS with regard to the metal ion concentrations determined, it was observed that [S,S-EDDS] is more suitable for some of the transition metals like iron as it was seen that it was not possible to determine it using EDTA. This is also supported by the fact that the formation constant is higher for Fe-EDDS (log K =22) **[7]** than Fe-EDTA (log K =14) **[61]** at the pH range of analysis (6.5-8).For the

determination of copper in pulp, there were observable variations in the amount of metal determined using EDTA in the different pulp types while the results look more consistent for [S,S-EDDS] that the difference between the determined amounts of metal in the three pulps is low that it can be accounted by the effect of water content of the pulps. It can be said that even for copper, the complexes formed were not stable enough that it caused a considerable differences in the amount of metal determined.

Chapter 5

5. Conclusions and Recommendations

The aim of this project was to determine transition metals (manganese, copper and iron) in various pulp samples (I,II and III) using Capillary Zone electrophoresis coupled with diode array uv-detection after complexation with EDTA and [S,S-EDDS].The main purpose of doing this was to evaluate the readily biodegradable ligand, [S,S-EDDS] of its potential to quantitatively sequester the above mentioned metals from pulp so that it can be used as a suitable substitute for the non-readily biodegradable ligand, EDTA, for applications in the pulp and paper industry. The following conclusions can be drawn out of the results obtained during this study.

In the analysis of the metals in pulp using EDTA as the leaching agent, the quantification of copper and manganese was possible while iron couldn't be determined. In the analysis of the metals in pulp using [S,S-EDDS] as the leaching agent on the other hand, the quantitative determination of copper and iron was possible while manganese couldn't be determined. In the standard CZE analysis of the metal ligand complexes of the metals prior to the analysis of pulp samples, it was observed that the iron complex of EDTA showed instability demonstrated by the appearance of recurring tailed electropherograms while the [S,S-EDDS] complex of iron is stable under a wide range of pHs. While the manganese complex of both ligands was observed to be of low sensitivity, it was not possible to determine its complex with [S,S-EDDS] using the detection method used. Determination of these metals from a pulp matrix using other more sensitive methods like ICP-MS and AAS demonstrated the quantification of all the three metals in pulp **[58,59]** It was

concluded therefore that the differences mainly occurred due to the sensitivity of the specific species formed. It was observed generally that [S,S-EDDS] forms more stable complexes with iron and copper compared to EDTA. Quantification of the metals was possible up to the lower parts per million using both complexing agents. It can therefore be recommended that regardless of the insensitivity of some of the species which can be improved by the use of more sensitive detection methods, the sequestering ability of the readily biodegradable ligand [S,S-EDDS] compares well with that of EDTA for the metals of concern in the pulp and paper industry and hence can be used instead of EDTA in the prebleaching treatment of pulp. It was observed that [S,S-EDDS] is better in complexing iron than EDTA and compares well with it in complexing copper (comparing tables 3.8 and 4.4).This could be seen from the consistency of the amounts of the metals determined in the three pulps using [S,S-EDDS] for copper and from the fact that it was not possible to determine iron using EDTA at all. This work was specifically aimed at the performance of [S'S-EDDS] to sequester transition metals of environmental concern as compared to EDTA.The results have shown differing performances of the ligands with regard to the three metals (Mn, Cu and Fe) considered and it was observed that one ligand is better than the other in sequestering one metal ion and viseversa.EDTA was ,for example, more efficient in sequestering copper than [S'S-EDDS] while the reverse is true for iron. Considering the above mentioned advantages obtained from the analysis made and combined with its ready biodegradability therefore, it should be recommended that [S'S-EDDS] can be used for this same application in the pulp and paper industry instead of EDTA.

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