INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®

SIMULTANEOUS PRECONCENTRATION OF TRACE METALS BY CLOUD POINT EXTRACTION WITH 1-(2-PYRIDYLAZO)-2-NAPHTHOL AND DETERMINATION BY NEUTRON ACTIVATION ANALYSIS

AURORA PÉREZ GRAMATGES

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
at
Dalhousie University
Halifax, Nova Scotia
1999 July

0-612-49263-X

DALHOUSIE UNIVERSITY

FACULTY OF GRADUATE STUDIES

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled "Simultaneous Preconcentration of Trace Metals by Cloud Point Extraction with 1-(2-Pyridylazo)-2-Naphthol and Determination by Neutron Activation Analysis"

by Aurora Pérez Gramatges

in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dated: August 17, 1999

[redacted]

External Examiner

Research Supervisor

Examining Committee

Dalhousie University

Author: **Aurora Pérez Gramatges**

Title: **Simultaneous Preconcentration of Trace Metals by Cloud Point Extraction with 1-(2-Pyridylazo)-2-naphthol and Determination by Neutron Activation Analysis**

Department: Chemistry
Degree: Ph.D.
Convocation: 1999 Fall

Permission is herewith granted to Dalhousie University to circulate and have copied for non-commercial purposes, at its discretion, the above title upon the request of individuals and institutions.

[redacted]

Signature of Author

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

The author attests that permission has been obtained for the use of any copyrighted material appearing in this thesis (other than brief excerpts requiring only proper acknowledgment in scholarly writing) and that such use is clearly acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Several methods for the simultaneous preconcentration of selected transition metals and lanthanides by cloud point extraction (CPE) followed by their determination using neutron activation analysis (NAA) have been developed. In general, the methods involve the use of a nonionic surfactant, namely PONPE-7.5, and a chelating agent 1-(2-pyridylazo)-2-naphthol (PAN).

A phase diagram of the surfactant has been constructed and the effects of different additives on the cloud point have been investigated. Other parameters that characterize the CPE, such as the ratio between the surfactant-rich and the aqueous phases and the percent of water in the surfactant-rich phase have been determined. High partition coefficients of PAN ($log K_d$ of 2.38-3.73) between the two phases have been obtained.

Different factors that can influence the extraction efficiency of metals have been optimized. These factors included solution pH, ionic strength, temperature, and PAN and surfactant concentrations. The most critical parameters in the CPE have been found to be the solution pH and the concentration of the chelating agent. The extraction recoveries for most of the metal chelates are >95% under optimal conditions. A preconcentration factor of 60 has been achieved leading to low detection limits of 0.03-25 ppb. The groups of metal studied are: (i) Cd, Co, Cu, Mn, Ni and Zn; and (ii) Dy, Er, Eu, Gd, Ho, La, Lu, Pr, Sm, Tb, Tm, and Yb.

Some preliminary studies have been done regarding the use of a mixture of chelating agents (TAN and PAN) in CPE. A quantitative recovery has been obtained for Hg which is not chelated efficiently by PAN only. The method has been extended to the simultaneous determination of Cd, Hg and Zn.

The precision and accuracy of measurements have been evaluated using elemental comparator standards and certified reference materials, respectively. The methods have been applied to the determination of the transition metals in samples of tap and sea water.

LIST OF ABBREVIATIONS AND SYMBOLS

Φ	activating flux
θ	relative natural isotopic abundance
ε	detector efficiency for the measured radiation energy
λ	decay constant of the nuclide
σ	absolute standard deviation, activation cross-section
3,5-diClDMPAP	2-(3,5-dichloro-2-pyridylazo-5-dimethylaminophenol)
5-Br-PADAP	2-(5-bromo)-2-pyridylazo)-5-diethylaminophenol
A	radioactivity
AAS	atomic absorption spectroscopy
APCI	atmospheric pressure chemical ionization
APDC	ammonium pyrrolidinedithiocarbamate
BL 4.2	poly (oxyethylene) (4.2) dodecanol
Brij 35	poly (oxyethylene) (23) dodecyl ether
C	concentration
C_{10}-APSO$_4$	3-(decyldimethylammonium) propylsulfate
CMC	critical micelle concentration
Copro-III	coprophorphyrin III
CPE	cloud point extraction
CRM	certified reference material
CS	chromatographic separation

Cupferron	ammonium salt of N-nitroso-phenylhydroxylamine
C_xE_y	n-alkyl-polyoxyethylene ethers
D	distribution coefficient
DDW	deionized distilled water
E	extraction efficiency
EDTA	ethylendiaminetetraacetic acid
EO	ethylene oxide
EQA	external quality assessment
FAA	flame atomic absorption
FIA	flow injection analysis
H_2Dz	dithizone
HPLC	high performance liquid chromatography
IAEA	International Atomic Energy Agency
ICP	inductive coupled plasma
INAA	instrumental neutron activation analysis
IQA	internal quality assessment
IR	infrared
K_d	partition coefficient
keV	kiloelectronvolt
KP	Krafft point
L_c	critical level (detection limit)
Ln, REE	lanthanides, rare earth elements

m	mass of the element
M	atomic mass
MCA	multichannel analyzer
MEUF	micellar enhanced ultra filtration
MS	mass spectrometry
N	aggregation number
N_A	Avogadro constant
NAA	neutron activation analysis
Neocuproine	2,9-dimethyl-1,10-phenantroline
NIST	National Institute of Standards and Technology
P_γ	probability of emission of a photon with energy E
PAN	1-(2-pyridylazo)-2-naphthol
PAP	2-(2-pyridylazo) phenol
PAPM	2-(2-pyridylazo)-5-methylphenol
PAS-C_n	4-alkylamido-2-hydroxybenzoic acids
PIPES	piperazine, N,N'-bis-(2-ethanesulfonic) acid
PNAA	preconcentration neutron activation analysis
PO	propylene oxide
PONPE-10	polyoxyethylene nonylphenyl ether (EO = 10)
PONPE-2	polyoxyethylene nonylphenyl ether (EO=2)
PONPE-20	polyoxyethylene nonylphenyl ether (EO = 20)
PONPE-7.5	polyoxyethylene nonylphenyl ether (EO = 7-8)

ppb	parts per billion
ppm	parts per million ($\mu g\ g^{-1}$)
ppt	parts per trillion ($ng\ g^{-1}$)
RNAA	radiochemical neutron activation analysis
RTP	room temperature phosphorescence
SDS	sodium dodecyl sulfate
SIM	single ion monitoring
SPA	spectrophotometric analysis
SRM	standard reference material
TAC	2-(2-thiazolylazo)-4-methylphenol
TAEP	2-(2- thiazolylazo)-4-ethoxyphenol
TAMP	2-(2-thiazolylazo)-4-methoxyphenol
TAN	1-(2-thiazolylazo)-2-naphthol
TAPP	2-(2-thiazolylazo)-4-phenylphenol
t_c	counting time
T_{cp}	cloud point temperature
t_d	decay time
t_I	irradiation time
TLC	thin layer chromatography
TRIS, Trizma	tris-(hydroxymethyl)-aminomethane
Triton X-100	polyoxyethylene *p-tert*-octylphenyl ether (EO = 9-10)
Triton X-114	polyoxyethylene *p-tert*-octylphenyl ether (EO = 7-8)

Tween 40	poly (oxyethylene) sorbitan esters of fatty acids (40: sorbitol monopalmitate)
UV	ultraviolet

ACKNOWLEDGMENTS

I would like to thank my supervisor Prof. Amares Chatt for giving me the opportunity to do this research project and for his assistance throughout my studies. His experience, vision and encouragement were crucial in the successful completion of this work.

Many thanks to Dr. Jiri Holzbecher of the Dalhousie University SLOWPOKE-2 Reactor Facility for his valuable help in my experimental work and his useful suggestions. I will always be grateful for his infinite patience and sense of humor.

It has been a pleasure for me to work with many enthusiastic people as part of Prof. Chatt's group. Many thanks to present and former members, specially to Dr. Joe Kiceniuk, Blaine Zwicker, Dr. Wayne Goodwin, Dr. Weihua Zhang, Dr. Eric Sullivan, Youqing Shi and, more recently, Tien Quach, Monica Chuang and Mark Renault. I would like to express my special gratitude to Christina Bottaro for her great support and friendship during this period; my best and sincere wishes in all your future endeavors.

I would also like to thank Prof. J.C.T. Kwak and former members of his research group, in particular Brent Jewett and Dr. Andrew Rodenheiser, for their efforts in helping me understand the complicated world of surfactants.

Special thanks to Dr. Patricia Lane, from the Biology Department, Dalhousie University, who played a very important role in my success pursuing this Ph.D. degree.

The financial assistance from the Natural Sciences and Engineering Research Council (NSERC) of Canada, Faculty of Graduate Studies of Dalhousie University, and Instituto Superior de Ciencias y Tecnología Nucleares (ISCTN), Havana, Cuba, is

gratefully acknowledged. A special thanks to the Department of Chemistry, Dalhousie University, for the D.E. Ryan Award.

This thesis would not have been accomplished without the unconditional love and support of my family and friends, specially my mother and my husband. This thesis is dedicated to them.

1. INTRODUCTION

Increasing industrialization has led to considerable pollution of the environment by toxic elemental and organic species. The levels of some of these species in aquatic environment, especially in lakes, rivers, and wells are of particular concern because of the use of these reservoirs for drinking water. The potential industrial sources of many elements include metal plating, circuit-board manufacturing, photographic and photo-processing industries, synthetic fuel plants, oil refineries, and mine tailings and leachates [1].

From a general point of view, almost every element encountered in aquatic systems can be considered as toxic, depending upon its concentration, species, and bioaccumulation. Thus, the monitoring of toxic elements and their species in water systems has become very important. The most commonly determined elements in fresh waters are Al, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn; the levels of Ag, As, Co, Se, Sn, Th, V and U are occasionally reported [2, 3, 4].

Since the concentrations of many elements in natural waters are generally below the detection limits of most instrumental techniques, preconcentration steps are necessary for reliable analysis. Several preconcentration methods have been employed for this purpose depending on the availability of equipment and the specific characteristics of the technique. The methods include evaporation, electrodeposition, surface adsorption, coprecipitation, ion-exchange, and liquid-liquid extraction [2, 3, 5].

As a preconcentration technique, liquid-liquid extraction has been widely used because of the ease of steps involved, the broad range of analytes that can be extracted, and the relatively low costs of reagents and equipment. Nevertheless, there are several problems associated with the conventional liquid-liquid extraction procedures. For example, a low extraction efficiency of an analyte in a single step necessitates the use multiple extractions. Then the experimental procedure becomes time-consuming, uses more reagents, and affects the preconcentration factor. Additionally, extensive sample manipulations during the extraction/enrichment steps can lead to problems in ensuring adequate and reproducible recovery of the analyte.

The signal produced by the reagents used in the extraction procedure can interfere with the analyte signal. The use of large amounts of organic solvents and chelating agents during an extraction can significantly increase this blank. In addition, due to the toxicity and carcinogenic risks associated with many of the organic solvents, their usage in substantial volumes can constitute a health hazard.

Therefore, the search for more effective preconcentration methods of extraction has become necessary for analytical chemists working in the environmental field. Several modifications to the standard procedures and the development of new methodologies have become evident over the last ten years or so. Still, many of them suffer from limitations to different degrees in terms of recovery, efficiency, reproducibility, volume of the extraction solvent required, processing time, and/or ease of use.

1.1 Surfactant-Mediated Processes as Preconcentration Techniques

Surfactants have mainly been used in analytical chemistry for different purposes such as the solubilization of organic compounds in water, enhancement of analytical signals, control of reaction paths, micellar catalysis, and alteration of spectral features of reaction products [6, 7, 8, 9, 10, 11]. Surfactants have also been used for compartmentalization of ionic and neutral solutes into very small volumes [12, 13]. This property of surfactants can be exploited for developing simple and efficient preconcentration methods where analytes can possibly be concentrated into a small-volume extracting phase.

Different types of surfactant have been employed in multiple extraction schemes. Among them, the nonionic type offers the best alternative to conventional extractive methods. A particular advantage of using nonionic surfactants arises from their ability to achieve the compartmentalization of solutes by a simple and reversible process. This process depends upon a distinctive physical property of nonionic surfactants, namely, the existence of a critical point in the phase separation phenomenon which is induced by changes in the temperature, ionic strength, pressure, *etc.* of the micellar solution. Above this critical point (*a.k.a.* cloud point), two isotropic phases are well defined: a dilute aqueous phase containing low concentration of surfactant and a surfactant-rich phase. Any species that associates and binds to the micellar entity in solution can be subsequently extracted into the surfactant-rich phase.

The possibility of preconcentration of trace elements in the surfactant-rich phase offers exciting opportunities for the modification and development of analytical methods. These methods can be used to overcome solubility problems, to speed up reaction rates, and to improve selectivity and efficiency in solvent extraction and chromatographic methods.

Cloud point extraction procedures have been reported for extraction and preconcentration of metal chelates and organic compounds in freshwater, seawater, leachates, and other aquatic media of environmental concern [9]. In the particular case of the analysis for metals, the method offers good selectivity when specific ligands for metals and optimum conditions for separation of phases are used. One of the pioneers in the application of this micellar-based technique to the analysis of metals in aqueous samples is Watanabe, who reported the determination of Ni(II) by cloud point extraction of its 1-(2-thiazolylazo)-2-naphthol) (TAN) chelate in a micellar solution of Triton X-100 [14]. Since this initial work, several extractions of other metal ions using similar heterocyclic azo ligands and different surfactants have been reported.

The cloud point methodology has been applied successfully to the preconcentration of metal chelates for the spectrophotometric [15, 16, 17], flow injection [18] or electrochemical analysis [19] of trace elements in a variety of different samples (tap water, coastal water, soils, alloys, *etc.*). In general, cloud point extractions provide sample purification as well as a simple and effective means for improvement of the analytical method by enhancement of both sensitivity and selectivity. Typically, the procedure is developed for only one element. A small number of elements (up to 3 or 4) has been determined in a few cases.

1.2 1-(2-pyridylazo)-2-naphthol (PAN)

Organic reagents with one anionic group (*e.g.* -OH, -SH, *etc.*) and one uncharged basic group (*e.g.* =N-, =O, *etc.*) can easily replace coordinated water molecules from many aquated metal ions forming neutral, essentially covalent chelates. Chelates in which the metal has become part of the organic structure and is more or less buried inside the molecule, are only slightly soluble in water but dissolve readily in organic solvents.

Various types of non-specific ligands have been used in metal extractions. In particular, ligands containing an acidic proton(s) and nitrogen atoms suitable for forming chelate rings constitute a very important tool in solvent extraction of metal-ions. One of the most representative compounds of this series is 1-(2-pyridylazo)-2-naphthol (PAN), shown in Fig. 1.1, which falls in the group of polydentate organic ligands. This group also includes 1-(2-thiazolylazo)-2-naphthol (TAN), 2-(2-pyridylazo)-4-methylphenol and 2-pyridylazoresorcinol, among others.

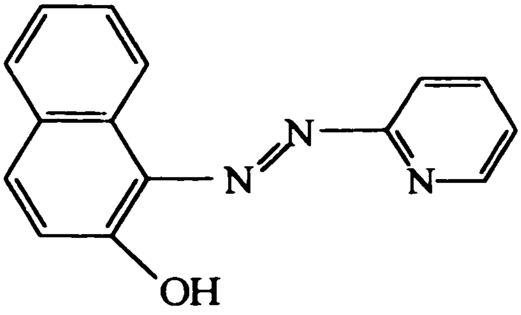


Figure 1.1. Chemical Structure of 1-(2-Pyridylazo)-2-naphthol (PAN) [20]

In 1955, PAN was introduced by Cheng and Bray [21] as a metallochromic indicator for the EDTA titration of Cu(II), Zn, and In. PAN is a very stable reagent in the presence of oxidizing reagents, and is generally used for analytical purposes in 0.1% solution of the reagent in methanol or ethanol [20]. PAN is a very sensitive chelating agent and its metal complexes generally have high stability constants (Table 1.1).

Table 1.1. Stability Constants of PAN Complexes of Selected Transition Metals

Element	log β_2	Ref.
Co	>12	22
Cu	23.9	23
Mn	16.4	22
Ni	25.3	22
Zn	21.7	22

Depending on the pH, PAN may exist in solution in three different forms. Acid solutions (pH<2) contain the water-soluble yellow-green protonated H_2PAN^{-} ion. Between pH 3 and 11, the dissociation equilibrium is displaced to form the neutral HPAN (*a.k.a.* PAN) molecule which is soluble in organic solvents to give a yellow color (colloidal suspensions can be formed in aqueous systems). In alkaline solutions (pH>11), the dissociation of the proton of the *o*-hydroxyl group occurs, and the reagent exists as the water-soluble red PAN^{-} anion (Fig. 1.2). The pK_a of these two equilibria, determined in dioxane (50%) are pK_{a1} = 2.0 and pK_{a2} = 12.3, respectively [20].

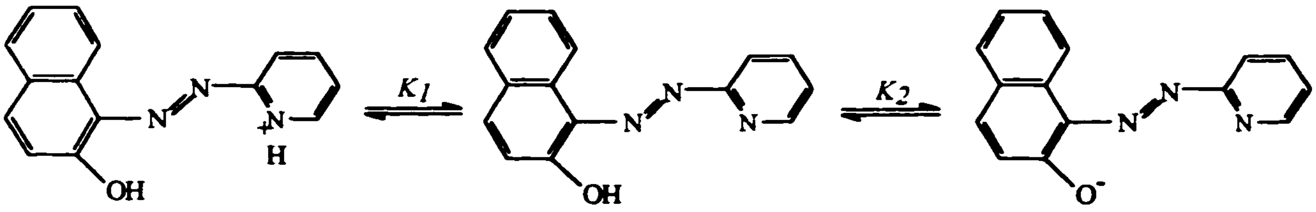


Figure 1.2. Acid-Base Equilibria of 1-(2-Pyridylazo)-2-naphthol in Aqueous Solution [20]

Metal complexes with PAN are sparingly soluble in water, and most of them can be extracted with inert solvents [21, 24, 25, 26]. During extraction, the metal complex and the uncombined PAN pass to the extracting phase. Selectivity in separations can be enhanced by suitable selection of pH and masking agents.

Several studies have been performed to determine the stoichiometry of the PAN:metal chelates. In 1963, Shibata proposed a ratio of 1:2 for most of the lanthanide:PAN complexes [27]. Later on, in 1979, Rao *et al.* reported a 1:3 ratio for some of the lanthanides (Ln) based on IR, UV and visible absorption spectra, electrical conductivity and magnetic susceptibility data in solid state [28]. In these complexes, PAN appears to function as a bidentate ligand. Only one structure of the three possible pairs of combination has been found: the metal bonded to the oxygen and azo nitrogen (linked to the pyridine ring).

Other studies have reported PAN as a powerful chelating agent that reacts with over 30 elements forming highly colored complexes [29]. In most cases, PAN behaves as a tridentate ligand with the heterocyclic nitrogen, the nitrogen of the azo group which is farthest from the pyridine ring, and the hydroxyl oxygen involved in metal bonding. The common metal:ligand ratios are 1:1 (MLX) and 1:2 (ML_2) for metal ions with coordination numbers of 4 and 6, respectively. In the first case, an additional unidentate ligand is included in the metal coordination sphere. For the 1:2 ratio, two PAN anions form a quasi-octahedral arrangement around the central ion as shown in Fig. 1.3 [30].

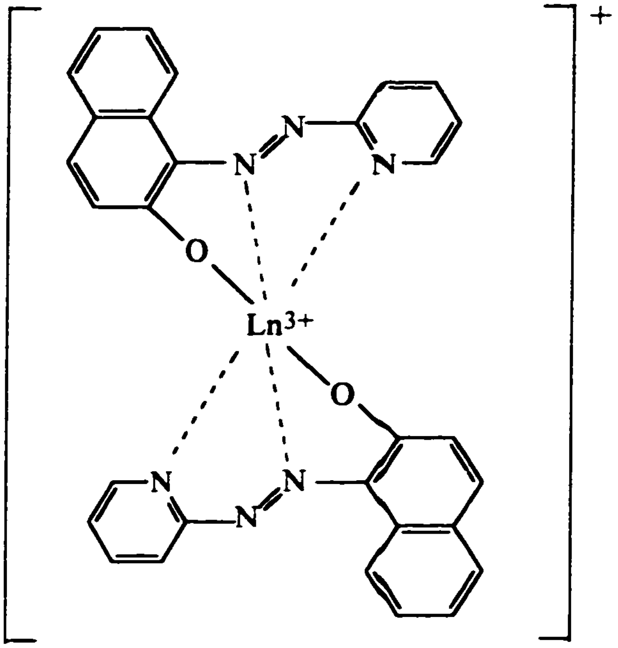


Figure 1.3. Proposed Structure for PAN:Ln Metal Chelates [30]

Although most CPE procedures of metals involve the use of selective chelating agents, PAN has been applied to their determination in several procedures. The uses of PAN as a chelating agent in CPE and in surfactant-related extractions are reviewed in Section 2.2.1.

1.3 Neutron Activation Analysis

Activation analysis is based on the production of a radioactive nuclide in a sample and the subsequent detection and measurement of the radiation emitted during the decay of that nuclide. Neutron Activation Analysis (NAA) is the most common form of activation analysis. Different kinds of nuclear reactions are obtained by activation of target isotopes by neutrons. The type of reaction depends on the neutron energy. For most NAA applications, the analytical response relies on the detection of the γ-ray emitted by the nuclear excited states produced by the capture of thermal neutrons.

Neutron activation techniques can be classified into several categories. A general classification could be instrumental NAA (INAA) and destructive techniques. The second group includes techniques such as preconcentration NAA (PNAA) and radiochemical NAA (RNAA), depending on whether any chemical separations are done before or after the irradiation.

In order to quantify a particular element in a sample, it is necessary to relate the amount of that element to some measurable quantity. In NAA, this quantity is the number of counts, taken as the number of γ-ray occurrences detected at a particular energy value. An irradiated sample is placed on a γ-detector and counted for a given period. The activity is given by the activation equation [31]:

$$A = \sigma\ \Phi\ (m/M)\ N_A\ \theta\ P_\gamma\ \varepsilon\ [1-e^{-\lambda t_i}]\ [e^{-\lambda t_d}]\ [1-e^{-\lambda t_c}] \qquad \textbf{Eq. 1.1}$$

where A is the measured activity (Bq) from the product of an expected reaction,
σ is the activation cross-section of the reaction (cm^2)
Φ is the activating flux ($n\ cm^{-2}\ s^{-1}$)

m is the mass of the element (g),
M is the atomic mass of this isotope (g mol^{-1}),
N_A is the Avogadro constant ($6.022x10^{23}$ molecules mol^{-1}),
θ is the relative natural isotopic abundance of the activated isotope,
P_γ is the probability of emission of a photon with energy E,
ε is the detector efficiency for the measured radiation energy,
λ is the decay constant of the nuclide (units of reciprocal time), and
t_i, t_d, and t_c are the irradiation, decay and counting times, respectively.

The activation equation can be used for elemental assay only if all of these terms are accurately known. This equation can be greatly simplified by using the comparator method. If both sample and standard have the same irradiation, decay and counting times, then equation 1.1 becomes:

$$C_{sample} = C_{standard} (A_{sample}/A_{standard}) \qquad \textbf{Eq. 1.2.}$$

where C_{sample} and $C_{standard}$ are the concentrations of the element in the sample and the standard, respectively, and A_{sample} and $A_{standard}$ are the corresponding activities. In this way, the unknown concentration of the element in the sample can be obtained by comparing the activities of the photopeaks.

Neutron activation analysis represents a convenient option for multielement determinations when the facility is available. Among several advantages that this technique offers, simultaneous multielement determination, possibility of irradiation of solids, liquids and gases, relatively blank-free analyses, and the wide range of elements that can be detected constitute the essential parameters that one should consider when searching for a suitable analytical technique.

Best to the author's knowledge, NAA has not been used as a detection technique in combination with CPE; however, some examples of PNAA with chelating agents as well as the determination of trace metals by NAA are presented in Section 2.3.

For CPE, NAA is particularly advantageous because it is based on the nuclear properties of the element and for its multielement detection ability. In NAA, a sample can be irradiated at any time after its preparation (preconcentration), and it is not dependent on the stability of the chelate.

1.4 Summary of the Objectives

The main objective of this thesis was to develop preconcentration methods based on cloud point extraction for selected trace metals in aqueous samples followed by their determination using NAA. The CPE method was optimized for the preconcentration of two groups of metals, namely transition metals and lanthanides.

Because of the particular characteristics of micellar systems in solution, and the unique character of the cloud point phenomenon, it was first necessary to investigate the conditions for the separation of phases. One of the experimental goals in this research project was to construct the phase diagram of PONPE-7.5 as well as to determine the influence of different additives on the cloud point at a given surfactant concentration. It was also important to obtain the phase ratio after the separation of phases since this parameter defines the preconcentration factor of the extraction.

The distribution of the chelating agent between the surfactant-rich and aqueous phases can have influence on the recovery of the analytes. For this reason, another important objective of this thesis was to evaluate the partition coefficients of PAN between these two phases.

Once the study of the two main processes affecting the CPE, namely the separation of phases and the formation of the chelates, was done, it became necessary to evaluate the influence of several factors for the optimization of the procedure. These factors were solution pH, ionic strength, temperature, and concentrations of PAN and surfactant. After preconcentration of metals, the extracted phase was irradiated and the radionuclides produced by activation were assayed. The versatility of the CPE method using different chelating agents was also examined by using a mixture of TAN and PAN.

Internal and external quality assessments of the CPE method using comparator standards and certified reference materials needed to be done. Discussions on the reagent blanks, possible sources of contamination, sensitivity and detection limits are presented in this thesis. The applicability of the preconcentration methods to the analyses of tap and seawater water samples was evaluated.

2. LITERATURE SURVEY

The surfactant-mediated applications, in particular, cloud point extraction methods, as well as the uses of PAN as a chelating reagent for the determination of trace metals are reviewed in this chapter. A brief description of the literature on PNAA is also given along with some aspects of quality assurance in analytical chemistry.

2.1 Cloud Point Extractions

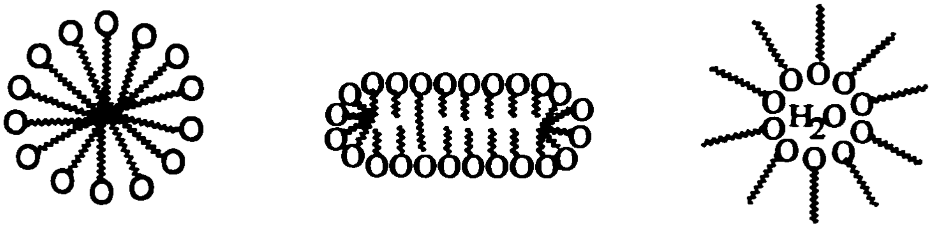
A general explanation of the most important features of the characterization of micellar systems and their specific use in cloud point extractions is summarized below. There are several books and review articles available on the micelle formation process and on cloud-point phenomena [32, 33, 34, 35, 36]. Readers are directed to these materials for more detailed explanations of the physico-chemical phenomena which are not described here.

2.1.1 Classification and Main Properties of Surfactants

Amphiphilic molecules (also called surfactants or detergents) contain distinct hydrophobic and hydrophilic regions. The combination of pronounced hydrophobic and hydrophilic properties within one molecule gives these species unique solubility properties in water [8]. In very dilute aqueous solutions (*e.g.* less than 10^{-4} M), surfactants usually exist as monomers. Aggregation of these monomers and changes of many bulk physical properties of the solution occur at a surfactant concentration known as the critical micelle concentration (*CMC*). The aggregate is termed a micelle and contains from 60 to 100 or more surfactant monomers. Aggregation is a highly cooperative process and occurs over a narrow range of concentrations [8]. Depending on the chemical structure of the surfactants and on the nature of the media, amphiphilic molecules can give rise to different organized structures such as aqueous and reversed micelles, micro-emulsions, monolayers, bilayers and vesicles [37]. These systems, often referred to as organized assemblies, have been shown to exhibit some interesting features. They can be present at very low concentrations in the molecular form in solution and they are capable of solubilizing many water-insoluble substances within and on the surface of the micelles [12]. All of these properties are interrelated and can be applied to analytical chemistry.

Briefly, micelles are dynamic aggregates of amphiphilic molecules. These aggregates assemble in such a way that the tails of the molecules are packed together in the interior or core of the micelle while the polar head groups form a boundary zone between the nonpolar core of the micelle and the external isotropic (polar) aqueous solution. A simplified schematic representation of a few types of micellar aggregates is shown in Fig. 2.1 [38].

Micelle formation is believed to be the result of three primary forces, namely hydrophobic repulsion between the hydrocarbon chains and the aqueous environment, charge repulsion of ionic head groups, and the van der Waals attraction between the alkyl chains. Micelles are generally small enough (3-6 nm diameter) that the macroscopic solution properties approximate those of a truly homogeneous solution [32].



spherical micelle

rod-like micelle

reversed micelle

Figure 2.1. Schematic Representation of Selected Micelles Formed from Surfactants (the circles represent the hydrophilic heads and the lines the hydrophobic tails) [38]

Different models have been proposed to explain and understand micellar structure and behavior. Although the discussion is still open, the existence of a dynamic equilibrium between micelles and their bulk solvent phase is a generally accepted criterion [12, 39]. Two basic processes are thought to occur, namely exchange with the solvent and other micelles of monomers, dimers, trimers, *etc.* occurring over a microsecond to millisecond time scale, and actual "replacement" of a whole micelle by this process over a time frame from milliseconds to seconds. This dynamic nature of micelles has a profound impact on an analyte residing in or on the micelle, and on analytical measurement centered on the associated analyte [13, 32].

Micelle characteristics vary with the nature of the amphiphile as well as with the composition of the solution. Depending on the nature of the polar head groups, surfactants may be categorized as anionic, cationic, non-ionic, and zwitterionic. Examples of amphiphiles with anionic head groups include alkali and alkaline earth metal salts of long-chain carboxylic acids, sulfuric acids, and phosphoric acids. Cationic surfactants usually contain quaternary nitrogen head groups due to the stability of these materials and to their commercial availability. The polar head groups of nonionic micelles generally consist of polyoxyethylene or polyoxypropylene chains [37].

Some basic properties that characterize these systems, and their definitions are given below. (1) The aggregation number (*N*) is the average number of monomer surfactant molecules per micelle. (2) The Krafft point (*KP*) is the temperature at which the solubility of a surfactant is equal to its *CMC*. (3) The fraction of charge (for those micelles composed by ionic surfactants) is the ratio of the number of counter-ions in the Stern layer of the micelle (charged interfacial zone) to the aggregation number, subtracted from one [33].

Most of these properties are dependent on the temperature of the system and are also strongly affected by the presence of organic matter and/or inorganic impurities. For instance, changes in temperature and pressure can impact the process of formation of micelles, and drastic changes in the *CMC* and *N* are observed when additives are present in the surfactant-water systems. The addition of ionic species (*i.e.* electrolytes) usually results in an increase in the aggregation number and a reduction in the *CMC*; the presence of water-miscible organic molecules can either enhance or inhibit micelle formation [34]. These effects must be controlled but can be advantageously used to achieve a better separation.

2.1.2 Critical Separation of Phases (Cloud Point Phenomenon)

There are some distinct differences between ionic and nonionic micelles. In general, micelles composed of nonionic surfactants tend to have lower *CMC* values and higher aggregation numbers than analogous ionic forms [13]. This is thought to be due, in part, to the lack of electrostatic repulsion between the head groups of nonionic surfactants. In ionic micelles, this repulsion tends to limit the size and the *CMC* of the aggregate.

The effects of inorganic salts on the *CMC* of ionic detergents are drastic, with the log of *CMC* decreasing linearly with the log of the total counter-ion concentration. These effects can be accounted for largely in terms of electrostatic interactions, although it has been pointed out that salting out of the hydrocarbon chains of the surfactants may be appreciable [40].

The *CMC* values of nonionic amphiphiles are also lowered by added salts, but to a much lesser degree than those of ionic detergents. Various interpretations have been proposed for the observed effects. According to Horvath and Huie [41], the *CMC* is lowered due to the decreased hydration of the ethylene oxide (EO) chains caused by the added electrolytes, and probably due to the breaking of hydrogen bonds. Calkins *et al.* [42] also attributed the change in *CMC* to a continuous dehydration of the poly(oxyethylene) chains, resulting from an increase in their effective concentrations and due to the volume of added salts, including hydration water.

Possibly the most unusual difference in behavior is that referred to as the phase-separation phenomenon which in the case of aqueous solutions of nonionic and zwitterionic surfactants is known as the cloud-point or turbidity point phenomenon. When an isotropic micellar solution is heated above its Krafft point (the cloud-point temperature for these systems), it suddenly becomes turbid owing to the decrease in the solubility of the surfactant in water. This clouding phenomenon is easily reproducible and readily reversible.

Cloudiness may appear for several reasons in solvent-surfactant mixtures, but often it is due to the separation of small droplets of a second liquid phase in the form of an emulsion. If this emulsion is allowed to stand at a particular temperature, it will separate into two phases of well defined compositions: one rich in surfactant and the other containing a concentration of surfactant close to its *CMC* [32]. Figure 2.2 shows a typical phase diagram of an aqueous nonionic surfactant solution [43], where the composition of the system in each of the coexisting phases at a specific temperature is represented. Above the curve, few micelles are present in the aqueous phase due to the fact that the surfactant molecules are segregated into a concentrated phase. At temperatures much higher than critical temperature, the low-concentration phase becomes more and more dilute whereas the high-concentration surfactant-rich phase may become non-micellar [9, 44].

The most common consolute boundary (curve that defines separation of phases at a specific temperature) found in surfactant systems by far is the lower consolute boundary of polyoxyethylene and other weakly polar surfactants. However, aqueous liquid mixtures of selected zwitterionic and ionic surfactants can also display miscibility gaps in which clouding occurs on cooling their solutions, rather than on heating. Most of the applications of these systems are found in separation procedures for steroidal hormones, vitamins, proteins, and biological compounds in general [39, 45]. The cloud points displayed by some common nonionic and zwitterionic surfactants are shown in

Table 2.1. It is worth noting the drastic dependence of these values on the composition of surfactant solution.

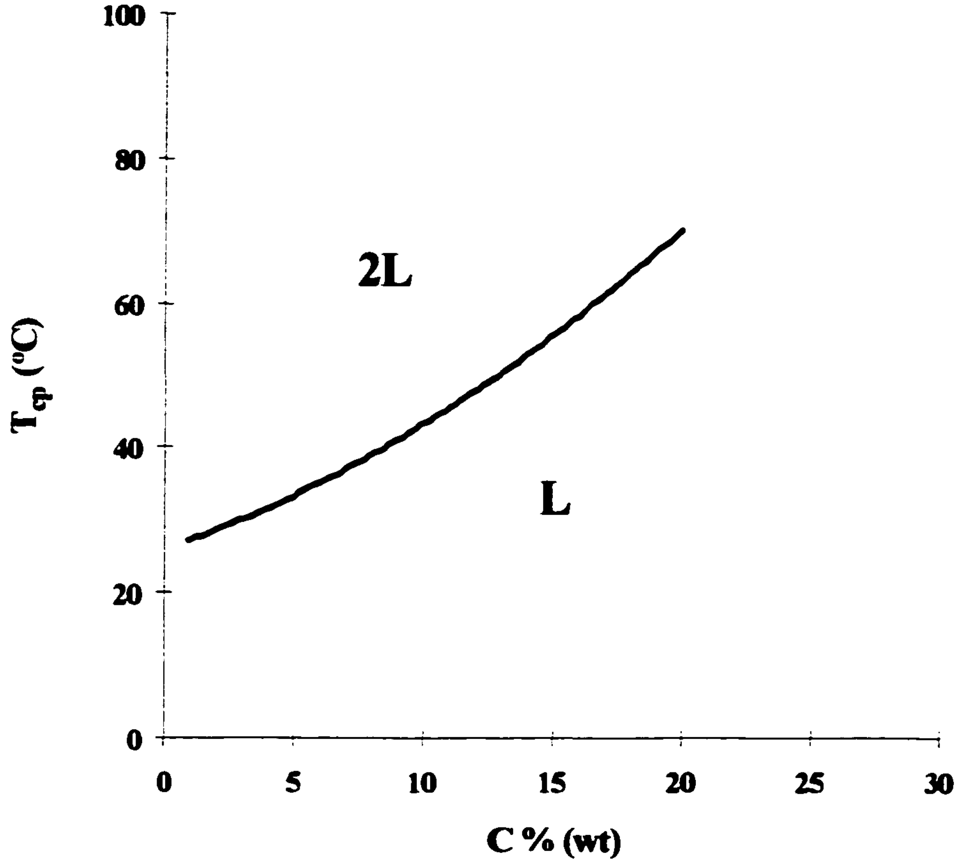


Figure 2.2. Typical Phase Diagram of an Aqueous Nonionic Surfactant Solution. (**L** denotes the single isotropic amphiphilic solution phase and **2L** indicates the presence of two coexisting isotropic phases) [43]

More recently, it has been reported that aqueous solutions of some derivatized cyclodextrins (cyclic oligosaccharides) exhibit critical phase phenomena [46]. In this case, as cyclodextrins are rigid molecules, there is no minimum critical concentration required for the formation of nonionic or zwitterionic surfactant micelles.

On the other hand, not only changes of the temperature of an aqueous micellar solution can produce separation of phases, but also appropriate alteration of conditions, such as pressure change, addition of salt or other additive, *etc.* [47, 48]. Despite the number of applications and studies reported based upon the cloud-point phenomenon, the mechanisms by which the phase separation occurs are not very clear yet, and continue to be source of controversy among different researchers [35, 39, 44, 47, 49]. The most accepted explanation is based on temperature dependence of intermicellar interactions, which are repulsive at low temperatures (where the micelles behave essentially as hydrated hard spheres), but become predominantly attractive at high temperatures with the dehydration of the hydrophilic groups of the amphiphiles [35, 39].

2.1.3 Analytical Procedure for Cloud Point Extractions

From an analytical point of view, the surfactant rich phase can be used to separate and/or preconcentrate different trace elements. There are several advantages of using micellar systems as preconcentration agents based on the cloud-point phenomenon. One of the most important advantages is its high capacity to concentrate a number of elements with high recoveries and concentration factors, since the analytes are extracted into a very small volume (0.2-0.4 mL) of the surfactant-rich phase.

Table 2.1. Cloud Point Values of Selected Nonionic and Zwitterionic Surfactants Commonly Used in CPE

Surfactant	C (wt %)	Cloud Point (°C)	Ref.
Polyoxyethylene nonylphenyl ethers			
PONPE-7.5 (EO = 7-8)	0.1	1	50
	5.0	6	
	10.0	11	
	30.0	30	
PONPE-7.5	10	48	51
	15	53	
PONPE-7.5 in 0.1 M KSCN	3	43	51
PONPE-10 (EO = 10)	-	62-65	52
PONPE-20 (EO = 20)	20	120	53
Polyoxyethylene *p-tert*-octylphenyl ethers			
Triton X-100 (EO = 9-10)	1	65	54, 55
Triton X-114 (EO = 7-8)	0.1	23.6	39
	1.0	24	
	5.0	25	
n-Alkyl-polyoxyethylene ethers (C_xE_y)			
C_6E_3	3	46	44
	11	45.5	
	20	44.8	
$C_{12}E_8$	2	71.1	35
$C_{12}E_8$	10	72	
$C_{14}E_7$	1	57.7	35
$C_{14}E_7$	5	58.6	
Pluronic-L61 ($EO_2PO_{31}EO_2$) (PO: propylene oxide)	-	25	52
3-(decyldimethylammonium) propylsulfate	2	62	45
(C_{10}-APSO$_4$)	5	77	
Tetrabutylammonium tetradecylsulfate	3	37	47
	10	31	
	20	25	

Most surfactants are commercially available. Many of them have lower toxicity than some organic solvents traditionally employed in extraction procedures, and considering the small volumes of surfactants necessary to carry out a separation, cloud point extractions can have extensive applications [39].

Another special feature is the possibility to obtain a suitable cloud point temperature for a given analytical application *via* an appropriate selection of the nonionic micellar-forming surfactant and/or the proper choice of additives. For example, it has been reported that the cloud point of dilute nonionic surfactant solutions can increase upon addition of charged ionic surfactants [56]. Furthermore, the cloud-point of a mixed solution of two nonionic surfactants lies between that of the two pure amphiphiles involved [48, 50, 51, 56, 57].

The general experimental procedure can be summarized in a few steps. The first one consists of the addition of a small volume (typically a few mL) of a concentrated nonionic surfactant solution to a buffered aqueous sample (50-100 mL) containing the element to be extracted and suitable masking agents, if required. The ligand may be dissolved in an aqueous solution or in the added surfactant solution depending upon its solubility in water. The solution is then heated in a constant-temperature bath until a desired fixed temperature, above the cloud-point, is achieved. Because the densities of both phases are not very different, their spontaneous separation is slow and hence centrifugation (between 3 000 and 10 000 rpm) is recommended in order to speed up the separation of the two phases [9].

Watanabe *et al.* [14] published one of the first papers on the application of this micellar-based technique to the analysis of metals in aqueous samples. They reported the determination of Ni(II) by CPE of its 1-(2-thiazolylazo)-2-naphthol (TAN) chelate in a micellar solution of Triton X-100. Since this initial work, the method has been employed to preconcentrate a wide range of compounds. The analytical applications reported include preconcentration of several metal ions forming water-soluble chelates [7, 18, 51, 57, 58, 59, 60, 61, 62], and extraction of organic compounds of environmental concern [9, 44, 45, 53, 63, 64, 65, 66, 67, 68] and of membrane proteins and hormones [45].

Several reviews have also been written on this subject [8, 9, 12, 56, 69], and modifications to the conventional procedure have been reported. For example, the uses of new amphiphilic ligands such as 4-alkylamido-2-hydroxybenzoic acids (PAS-C_n), which possess a chelating group and a tunable hydrophobic moiety, have been reported in several separation procedures for increasing the selectivity of the cloud point separation [56, 70]. Furthermore, they have been employed in environmental clean-up procedures, specifically for organic pollutants, due mainly to the advantages of using surfactants over the classical organic solvents, and to the unique solubilization possibilities that micellar systems have to offer [1].

2.1.4 Factors Affecting Cloud Point Extractions

There are many factors that can affect the cloud-point extraction process. Kinetic and equilibrium studies [6, 56, 71, 72, 73] clearly indicated that the hydrophobicity of ligands and complexes are the fundamental factors which regulate the extraction efficiency. Hinze and Frakewich [63] reported a decrease in distribution coefficients and concentration factors for phenol and 4-chlorophenol, as the amount of extracting surfactant was increased over the concentration range studied.

Favorable conditions for the formation of chelates are highly dependent on the pH of the solution. Most metals will only coordinate with specific species of the chelating agent (organic molecule). Since these species are part of an acid-base equilibrium, the optimization of the pH at which the extraction takes place is necessary. The dependence of extraction yield on pH of complex formation was reported by Moreno-Cordero *et al.* for Cd-PAN complex extraction with Triton X-114 as surfactant [18]. For pH values above 8, the yield is almost constant and close to 100%.

Other important experimental variables that have some effect upon the cloud-point extraction and optimization of systems are equilibration temperature, centrifugation time, concentration of chelating agent, and variations of ionic strength [9, 39, 51, 58, 63].

Another important factor used to explain experimental results and trends is the partitioning equilibrium of analytes between the micellar and the bulk phases [9, 12, 53, 57, 61, 62, 64, 71, 72, 74]. A particular characteristic of the distribution equilibrium of metal chelates solubilized in micellar solutions is the fact that the complex formation constants are generally different from those measured in homogeneous solutions, being largely dependent on the extent of complex-micelle binding [53, 57].

In general, surfactant-based procedures give very good preconcentration of metals [9, 50]. In particular, the method requires a small amount of sample solutions (usually less than 100 mL) and even a smaller amount of surfactant (about 0.2 mL of a 20% solution); in order to obtain the same concentration factor in ordinary liquid-liquid extractions, larger volumes (0.5-1.0 L) of both aqueous solution and organic solvents are required.

The surfactant-rich extracting phase is usually compatible with micellar mobile phases employed in thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) [8, 19, 39, 67, 74], flow-injection analysis (FIA) [58, 59, 65], and pseudo-stationary phase in capillary zone electrophoresis [9]. Improved detection limits of metal complexes can be obtained using spectrophotometric or spectrofluorometric techniques exploiting the surfactant-sensitization effects [8, 12, 13, 65, 67, 15]. More recently, micelle-mediated methods have been applied to more sensitive electrochemical methods [19, 69] and flame absorption techniques [18].

The main disadvantage of cloud-point extractions arises from the relatively low partition coefficients of many neutral metal chelate species. However, the partition coefficients can be increased *via* the use of highly hydrophobic ligands. Because long-chain derivatives are less soluble in aqueous surfactant solutions, this imposes a limit on the amount of excess ligand available in such systems [9].

2.1.5 Multielement Analysis Using Cloud Point Extractions

Some attempts have been made to achieve simultaneous multi-component separations of organic compounds based on cloud point phenomena [63, 67, 72]. For metal ions, the micelle-mediated extraction process is generally used to separate the analyte from other interfering ions that have to be masked in many cases.

The cloud-point applications for preconcentration of metal ions are summarized in Table 2.2. As can be seen, single elements have been determined in general. The list of the elements includes Au, Cd, Co, Cu, Fe, Hg, Ni, Pb, U, V, Zn, and Zr. Most commonly, single element determination techniques such as spectrophotometry and atomic absorption spectrometry (AAS) have been employed.

However, other micellar-mediated techniques have been used for multielement extractions. The most important is Micellar Enhanced Ultra-Filtration (MEUF), a recent technique based upon the extensive binding of the element to be removed to suitable amphiphilic aggregates, coupled with the use of hydrophilic membranes having pore sizes small enough to hinder the passage of micelles and their guest solutes. A sort of phase separation is obtained where the target analyte is accumulated in the micellar-rich retentate [60].

Although the MEUF technique has been mainly used for clean-up of waste waters [1, 10], the preconcentration of single cations for analytical purposes represents a new trend in the last few years [15, 70, 75]. Scamehorn and co-workers are one of the pioneers in this technique. They reported the separation of Cu^{2+} and Zn^{2+} with sodium dodecylsulfate (SDS) as the surfactant, and also separated Zn^{2+} and *o*-cresol from waste water [1]. Divalent Ni, Cu, Co and Zn metal ions were preconcentrated by Pramauro *et al.* with a non-selective enrichment procedure by a similar procedure using AAS for their determinations [60]. In this case, there exists the possibility of adsorption of surfactants on the filter resin.

Table 2.2. Summary of CPE of Metal Chelates

Element	Reagent	Surfactant	T (^oC)	Method	Notes	Ref.
Cu(II) Ni II) Zn(II) Cd(II)	PAP, TAC PAPM TAMP TAEP TAPP	PONPE-7.5 PONPE-20 (0.1 %)	1 50	SPA, AAS	• pH = 2 • ionic strength = 0.1 M ($NaClO_4$) • % extraction ~ 100 %	57
Co(II) Fe(II) Cu(II) Zn(II)	SCN^-	PONPE-7.5 (3 %)	23 to 43.8 (depending on addition of salts)	CS, AAS SPA	• % extraction = 72-97 % • cloud-point of PONPE-7.5 is sensitive to [SCN^-] • KSCN raised the cloud point, and NaCl lower it (anion-effect)	51
Fe(III)	PAS-C_n	Triton X-100 - BL 4.2 system Brij 35 (1-5 %)	26.3 to 28 (in dependence on mixture system)	SPA	• pH-dependence • concentration factor = 3-15 • % of extraction = 39-100 %	56
Fe(II) Ni(II) V(II)	PAP PAPM	Triton X-100 (1%)	25	SPA	• % of extraction~100 % • Ionic strength = 0.1 ($NaClO_4$) • distribution equilibrium study	73
Cd(II)	PAN	Triton X-114 (0.1 %)	23 to 25	FAA	• pH-dependence • applied to tap and sea water • detection limit: 0.4 ppb of Cd • concentration factor = 100	18
U(VI) Zr(IV)	Arsenazo	Tween 40	Room temperature	SPA	• pH = 3 • % extraction > 96 % • salting-out effect • the surfactant-rich phase was a solid rather than a liquid	48

Table 2.2 (*continued*). Summary of CPE of Metal Chelates

Element	Reagent	Surfactant	T (°C)	Method	Notes	Ref.
Zn(II)	PAN	PONPE-7.5 (0.4 %)	1	SPA	• pH = 10 (carbonate) • concentration factor = 40 • % extraction ~ 100 %	50
U(VI)	PAN	Triton X-114 (0.25 %)	23 to 25	SPA	• concentration factor = 10-100 • % extraction = 98 %l • detection limit: 1.1 ppb	39, 76
Cd(II) Fe(II) Ni(II) Zn(II)	TAC TAEP TAMP TAPP	PONPE-7.5 PONPE-20 (0.1 %)	1 50	SPA AAS	• pH (Fe) = 5.5 ; pH (Ni) = 6.8-7 • % extraction ~ 100 % • distribution equilibrium study	61
Cd(II) Cu(II) Hg(II) Pb(II) Zn(II)	H_2Dz	Triton X-100 (5-10 %)	25	SPA	• pH = 5 - 8.6 • % extraction = 93-100 %	7
Cd(II) Cu(II) Ni(II)	PAPM	PONPE-7.5 (0.1 %)	1	SPA	• pH(Ni) = 5.6 ; pH(Cu and Cd) = 7.5 • ionic strength = 0.1 M (NaCl) • concentration factor = 82 • distribution equilibrium study	64
Zn(II)	PAPM	PONPE-7.5 PONPE-20 (0.1 %)	1	SPA	• pH = 7.1 • ionic strength = 0.1 M (NaCl) • distribution equilibrium study	53
Cd(II) Ni(II) Zn(II)	PAPM	PONPE-7.5 PONPE-20 (0.1 %)	~ 0	SPA	• pH (Ni) = 5.7 ; pH (Zn and Cd) = 7.1 • ionic strength = 0.1 M (NaCl) • distribution equilibrium study	62

Table 2.2 (*continued*). Summary of CPE of Metal Chelates

Element	Reagent	Surfactant	T (°C)	Method	Notes	Ref.
U(VI)	5-Br-PADAP	PONPE-2	100	SPA	• pH = 7.5 • ionic strength = 0.5 M ($NaClO_4$) • concentration factor = 20 - 100	16
Ni(II)	TAN	PONPE-7.5	1	SPA	• Watanabe's first work	14
Zn(II)	Xanthate	SDS (1-4%)	N/A	AAS	• pH = 7.5 • salting-out effect (NaCl: 6 – 15%) • back extraction using EDTA	77
Er(III) Gd(III)	3,5-diClDMPAP	PONPE-7.5 (1%)	40	SPA	• pH = 8.5 (Er); pH=8.8-10.5 (Gd) • % extraction > 99.9% • detection limits: 24.8 ppb (Er) and 0.912 ppb (Gd)	78, 79
Ni(II) Zn(II)	PAN	Triton X-114	60	FAA	• pH = 7 – 10 • concentration factor ~ 30 • detection limits: 8 ppb (Zn) and 6 ppb (Ni)	80
Cu(I)	Neocuproine	SDS (3%)	N/A	AAS, SPA	• pH = 3.8 • previous reduction of Cu needed • salting-out effect (NaCl 3%)	81
Pd(II)	Copro-III	Triton X-100	95	Fluorescence	• pH = 4.5 - 6.5 • concentration factor = 10 • detection limit = 0.21 ppb (S/N = 3) • surfactant-rich phase is precipitated • pre-treatment of initial solution	82
Au(III) (in HCl)	-	PONPE-7.5	room temperature	ICP	• concentration factor ~ 7 • recovery > 95% • $C_{HCl} < 3$ M	83

Analytical Methods

AAS- Atomic Absorption

CS- Chromatographic Separation

ICP- Inductive Coupled Plasma

FAA- Flame Atomic Absorption

FIA- Flow Injection Analysis

SPA- Spectrophotometric Analysis

Ligands

H_2Dz- dithizone

5-Br-PADAP- 2-(5-bromo)-2-pyridylazo)-5-diethylaminophenol

3,5-diClDMPAP- 2-(3,5-dichloro-2-pyridylazo-5-dimethylaminophenol)

PAN- 1-(2-pyridylazo)-2-naphthol

PAP- 2-(2-pyridylazo) phenol

PAPM- 2-(2-pyridylazo)-5-methylphenol

PAS-C_n- 4-alkylamido-2-hydroxybenzoic acids

TAC- 2-(2-thiazolylazo)-4-methylphenol

TAEP- 2-(2- thiazolylazo)-4-ethoxyphenol

TAMP- 2-(2-thiazolylazo)-4-methoxyphenol

TAN- 1-(2-thiazolylazo)-2-naphthol

TAPP- 2-(2-thiazolylazo)-4-phenylphenol

Neocuproine- 2,9-dimethyl-1,10-phenanthroline

Copro-III- Coprophorphyrin III

Surfactants (in addition to those in Table 2.1)

BL 4.2- polyoxyethylene (4.2) dodecanol

Brij 35- polyoxyethylene (23) dodecyl ether

Tween 40- polyoxyethylene sorbitan esters of fatty acids (40: sorbitol monopalmitate)

PONPE-2- polyoxyethylene (2) nonylphenyl ether

2.2 Ligands and Solvent Extraction

Solubility in organic solvents is not a property usually attributed to simple metal salts. As can be expected from their highly ionic nature, most metal salts are strong electrolytes with a high solubility in aqueous media, in which the ions are solvated by molecules of water.

In most of the metal extraction systems, some or all of the solvating water molecules must be removed to obtain a species extractable by organic solvents. This species must, however, be uncharged because of the low dielectric constants of the organic solvents that are generally used in extraction procedures [84]. It means that an undissociated complex, ion association compound (ion-pair), or a covalent compound must be formed.

The extraction efficiency is defined in terms of the distribution (or extraction) coefficient, *D*, which is the ratio of total concentration (*i.e.* the concentration of all existing species) of the element in the organic phase (ΣC_O) to that in the aqueous phase (ΣC_{aq}) at equilibrium [84].

$$D = \frac{\sum C_0}{\sum C_{aq}} \qquad \textbf{Eq. 2.1.}$$

The extraction efficiency (*E*) is expressed as

$$E = \frac{100D}{D + \left(V_{aq} / V_0\right)} \% \qquad \textbf{Eq. 2.2.}$$

where V_{aq} and V_O are the volumes of the aqueous and the organic phases, respectively, after separation.

2.2.1. PAN as an Analytical Reagent

In 1951, Liu investigated the reactions of 1-(2-pyridylazo)-2-naphthol (PAN) with metal ions [85]. However, the first major paper to be published was by Cheng and Bray in 1955, who recommended the use of PAN as a complexometric indicator in direct titrations with ethylendiaminetetraacetic acid (EDTA) [21]. In addition, they observed that the chelates of PAN may be extracted into organic solvents, and described a few simple studies on this phenomenon.

PAN has been used for the extraction and determination of many metal ions [24, 27, 86, 87]. However, the low solubility of the reagent and its chelates in water requires the use of non-aqueous media. Surfactants have been used in combination with organic chelating agents, *e.g.* PAN, to obtain stable aqueous solutions and to enhance the analytical signal in different techniques. Early studies of these applications include the determination of Co and Mn with PAN in the presence of a nonionic surfactant (Triton X-100) as a solubilizing agent [88, 89].

The influence of three nonylphenols on the absorptivity of the Cd-PAN chelate at pH 9 was studied by Medina-Esriche *et al.*[15]. The optimized system was applied to the determination of Cd in acetic acid extracts of ceramic enamels. A similar system was reported by Moreno-Cordero and Perez-Pavon, with the use of Triton X-100 to stabilize PAN solutions in a FIA system for the determination of lanthanides [65].

Recently, Agnihotri *et al.* reported the use of Triton X-100 for the determination of the Cu(II)-PAN complex by normal and derivative spectrophotometry [90]. The method gives a detection limit of 4.0 ppb and a wide determination range, and it has been applied to the determination of Cu in commercial samples.

An extensive use of surfactants in analytical chemistry, as well as a more detailed understanding of their solubilizing properties, introduced more interesting and complex applications. The first study using CPE as a preconcentration method and using PAN as a chelating reagent for the determination of Zn was proposed by Watanabe *et al.* in 1978 [50]. PAN was also used in CPE procedures for the determination of Cd, Ni, and U [18, 39, 76, 80], and many of the recent applications include the use of PAN-derivatives as more specific chelating reagents [53, 57, 62, 64, 73].

PAN was used in this work as a non-specific chelating agent, in order to carry out a simultaneous extraction of metals into the surfactant-rich phase. One of the great advantages of PAN is that its solutions and also solutions of its complexes are unusually stable for such a sensitive reagent [91]. This is particularly useful for CPE since the reagent and chelates must remain stable in solution during the heating and centrifugation processes.

2.3 Preconcentration Neutron Activation Analysis

It is generally accepted that neutron activation analysis (NAA) is a very powerful technique for the determination of trace elements in various matrices with high accuracy. Because of the multielemental and simultaneous character of this technique, both qualitative and quantitative information of many elements can be obtained. However, in practice, there could be some difficulties in the determination of several trace elements since their content in the samples may lie below the detection limits of NAA. In order to improve the reliability and quality of the results, a preconcentration step could be used before carrying out the analysis. A chemical preconcentration of the trace elements prior to analysis serves the dual purpose of removal of the interfering major elements and increasing the concentration of the trace elements. This technique is called preconcentration NAA (PNAA).

Different preconcentration procedures have been used for this purpose. These include liquid-liquid extractions, solid-liquid extractions, chromatography, and ultrafiltration. From a general point of view, a good preconcentration procedure should be simple, free of reagent blanks, and should use a minimum volume of the extracting phase in order to obtain high preconcentration factors. The method should also give high extraction efficiency, and the final concentrated solution must be in a physical form that is suitable for the analytical technique to be used for its detection.

Most of the methods for preconcentration used in NAA yield a solid final product, with high enrichments of the trace elements, that can be irradiated in the reactor without further complications. Typical preconcentration methods involve the use of resins, activated carbon, coprecipitants, *etc.*

The preconcentration of trace elements from natural waters by coprecipitation with further determination by NAA has been reported by Lan *et al.* [92]. A mixture of oxine with thionalide together with $Pb(NO_3)_2$ and $Bi(NO_3)_3$ were used as coprecipitants, since there is none or little interference of these elements in the γ-spectrum of the irradiated samples. The detection limits ranged between 8.7 and 0.0004 ppb. Rao *et al.* [93] developed a PNAA method for the simultaneous preconcentration of Cd, Co, Cu, Hg, Mn, Th, U, V, and Zn from water samples by coprecipitation using a combination of TAN, ammonium pyrrolidinedithiocarbamate (APDC) and ammonium salt of N-nitroso-phenylhydroxylamine (Cupferron). For most of the elements, the enrichment factors were of the order of 10^4. The method was applied to sea and drinking water samples and biological materials.

Several authors have reported the use of resins for preconcentration of trace elements in combination with multielement analysis by NAA. Milley and Chatt [94] used a combination of INAA and PNAA methods for multielement determination in acid rain. The trace elements in the soluble fraction were preconcentrated using a Chelex-100 resin which can be directly irradiated. Detection limits between 0.01 and 3 ppb were obtained. Naidu *et al.* [95] preconcentrated traces of Cd, Co, Cu, Zn and Hg in water on a poly(acrylamidoxime) chelating resin with an enrichment factor of 400. The method was applied to spiked and field water samples. The detection limits were in the ppb level.

Yeh *et al.* studied the preconcentration of 8 elements (As, Co, Cr, Fe, Se, Sb, Sm, and Zn) from aqueous solutions by adsorption onto hydrous magnesium oxide [96]. The method was applied to polluted surface waters and extended to the determination of 15 elements by NAA.

The use of CPE as a preconcentration method for NAA does not appear to be reported in the scientific literature yet. However, NAA was used for the determination of the partition coefficients of U(VI) chelates of 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (*a.k.a.* 5-Br-PADAP) in their extraction into a droplet of PONPE-2 suspended on the surface of the aqueous layer [16]. This procedure is similar to the CPE since it is based on the lipophilic character of the nonionic surfactant. The determination of the extraction recovery and the optimization of different factors that influence the extraction were determined by UV/vis spectrophotometry.

2.4 Quality Assurance in Chemistry

"An analytical result cannot be interpreted unless it is accompanied by knowledge of its associated uncertainty" [97]. In order to enhance the quality of analytical results, it is necessary to have a detailed and systematic control of the factors affecting the analytical system, and knowledge of the errors and possible uncertainties associated with the experimental procedure. Within this frame, quality control is defined as the practical activities undertaken to ensure the required freedom from error of analytical results. In a more general concept, quality assurance is the over-all management strategy designed to ensure quality within a laboratory [98].

Different sources of error can be present in an analytical procedure. Among them, errors related to sampling, sample treatment, calibration curves, matrix effects and final determination of the analyte, are most relevant [99].

2.4.1 Internal Quality Assessment

Internal quality assessment (IQA) is suggested to be performed regularly in analytical laboratories on a per batch basis. Generally, the use of a standard prepared in the laboratory may meet the initial requirements for an acceptable control of data from the experimental system. A standard solution used for internal quality control purposes should be of chosen determinand composition, be prepared directly from pure standards, and be usually inexpensive [98]. The results of the analysis of these control materials form the basis of a quality control chart.

The most widely used forms of control charts are the Shewhart charts for mean and range. These take the form of a chart in which the variable of interest is plotted against time or number of standards. The observed values of the variable are compared with either the expected or the accepted true value [98]. The main function of such a chart is to help in the detection of deviations from statistical control, and not primarily as a means of assessing the acceptability of the accuracy of individual results.

An analytical system of adequate accuracy will usually operate with the control limits of the chart within the limits determined by analyzing certified reference materials (CRM). To ensure statistical control, charts should be updated and examined as regularly as possible so that systematic trends and/or various out-of-control conditions may be detected quickly and appropriate corrective actions taken before conducting more analyses.

Nevertheless, it is important to stress that data quality control, even when properly executed, does not exclude the possibility of important errors. Statistical uncertainty, as well as errors arising from particular samples falling outside the scope of the method validation, are some of the common problems that control methods cannot detect rigorously [97].

2.4.2 External Quality Assessment

Although IQA provides with an efficient way of controlling the statistics of the analytical process, a further step in checking the quality and accuracy of results is to analyze certified reference materials. A CRM is considered to be satisfactory when its matrix is similar to the test material and has similar concentration and trace elements pattern [100].

2.4.3 Control of the Blank

Quality assurance also requires the control of the analytical blank. The blank is defined as the signal resulting from a sample which is identical, in principle, to the sample of interest, except that the substance sought is absent (or small compared to its standard deviation) [101].

The blank is usually a reliable indicator of possible contamination from the environment and materials and of the background noise in the measurement. In order to decrease the variability of the blank, it is necessary to evaluate the various sources contributing to the blank value. Quality control must therefore cover blank assessment.

All of the above factors were taken into account in the experiments conducted in this thesis.

3. EXPERIMENTAL

This chapter describes the experimental conditions as well as the chemical reagents and equipment used in this work. It includes the experimental procedures for the determination of the phase diagram of PONPE-7.5, distribution studies of PAN, and the general preconcentration method. The analysis of certified reference materials and the irradiation and counting schemes are also described in this chapter.

3.1 Reagents and Equipment

The surfactants polyoxyethylene nonylphenylether (PONPE) with 7.5 and 20 oxyethylene units used in this work were of analytical grade and were purchased from Tokyo Kasey Industries (TCI). *Puriss* grade 1-(2-pyridylazo)-2-naphthol (PAN) was obtained from Fluka. Analytical grade citric acid, diammonium hydrogenphosphate, tris-(hydroxymethyl)aminomethane (Trizma base), and piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) used for the preparation of buffer solutions were purchased from Sigma Chemicals. Glacial acetic acid American Chemical Society (ACS) *Assured* grade was bought from Caledon, and analytical grade ammonium nitrate and ammonia solution (30%) were from BDH.

All reagents were analyzed by INAA in order to determine possible elemental impurities. The water utilized in all analytical work was de-ionized distilled water (DDW) obtained by passing distilled water through a mixed bed ion-exchange column (Barnstead 9-034-3 from Fisher Scientific Company).

A temperature-controlled water bath model Haake F3 obtained from Fisher Scientific was used for the cloud point measurements, and for keeping solution mixtures at the desired temperature before centrifugation. The precision of temperature measurements in terms of absolute standard deviation was ± 0.1°C.

The pH measurements were done using an Accumet pH meter (Model 820 manufactured by Fisher Scientific) with a precision of ± 0.2 pH units at 25°C (according to manufacturer's data). Samples were centrifuged in a refrigerated Superspeed Centrifuge (Sorvall RC-5B from Du Pont Instruments) using a fixed-angle rotor type SS-34. The centrifuge tubes were made of polycarbonate (Nalgene model #3138-0050). A freeze dryer Modulyo (Edwards) or an infrared lamp Infra-Radiator (Fisher) were used for drying the samples.

The dissolution of the biological certified reference material (CRM), namely *IAEA-H-8*, was carried out by digesting the samples in a conventional microwave oven (675-W solid state Admiral) at full power and using 45-mL Teflon sample cup of a microwave acid digestion bomb (Parr Instrument Company).

The irradiations were performed at the Dalhousie University SLOWPOKE-2 Reactor (DUSR) with a nominal neutron flux of 5×10^{11} cm^{-2} s^{-1} in the inner pneumatic sites. The irradiated samples were counted using a Canberra Ge(Li) semiconductor detector. This detector had a resolution of 1.88 keV at the 1 332-keV photopeak of ^{60}Co, a peak-to-Compton ratio of 35:1, and an efficiency of 9.5%. An Aptec multichannel analyzer (MCA) card built in a PC was used for the analysis of the photopeaks. A second system was used for anticoincidence γ-ray spectrometry [102]. Here the principal detector was an ORTEC HPGe detector and the guard detector consisted of a 10"x10" NaI(Tl) supplied by Harshaw. The peak-to-Compton ratio for the 661.6 keV photopeak of ^{137}Cs was about 600:1.

The absorbance measurements were done in a spectrophotometer (Hewlett Packard model H8452) with a diode array detector. The cells used were made of quartz with 1-cm of optical length.

3.2 Preparation of Comparator Standards

Single element and multielement comparator standard stock solutions were prepared from commercially available ultrapure elemental standards (supplied by SPEX Chemicals). Stock solutions of Cd, Co, Cu, Hg, Mn, Ni, Zn, and lanthanides were prepared at a pH<3 in order to avoid adsorption problems. These solutions were placed in dark glass vials with a Teflon cap and stored at 4°C to avoid evaporation. A suitable amount of these stock solutions was used for evaluating the extraction procedure. The elemental concentrations chosen were close to their expected values in water samples. Diluted solutions of comparator standards were irradiated regularly for internal quality assessment (IQA) purposes.

3.3 Determination of Cloud Point, Phase Ratio and Percent of Water in Surfactant-Rich Phase

The temperature at which the cloud point phenomenon occurs was determined by the method reported by Carvalho *et al.* [103]. The method is based on the visual observation of the separation of phases in the micellar solution. The initial solution was heated in the water bath at a specific temperature which was well above its cloud point (turbid solution). Then the solution was cooled gradually with constant stirring and keeping the temperature stable at selected values. The cloud point was considered as the temperature at which the solution became clear. To verify the results, the opposite process was carried out by gradually heating the clear solution until turbidity appeared. The reported value was the average of these two determinations; in most cases, these two temperatures were identical, within ± 0.5°C.

The phase diagram for PONPE-7.5 was obtained by determining the cloud point temperature of aqueous surfactant solutions at different concentrations, *viz.* between 0.05% and 32% (wt).

In order to determine the influence of the reagents added to the surfactant phase, cloud point determinations were performed with the additions of ethanol, buffer, PAN, inorganic salts, and various mixtures of these reagents. The procedure for the critical temperature determination was the same as above, but using only a surfactant concentration of 0.1% (wt).

The percent of water in the surfactant-rich phase was also obtained using UV-spectrometry for the determination of the surfactant concentration in the surfactant-rich phase. A calibration graph was constructed using PONPE-7.5 solutions with concentrations between 0.005% to 0.035% (wt). After the CPE, suitable aliquots were taken from the surfactant-rich phase. A dilution of up to 10 mL was then done with DDW. Solutions prepared in this way were cooled prior to the measurement to avoid the possibility of clouding. The absorbance of the solutions was measured at 277 nm.

The volumes of the respective surfactant-rich and aqueous phases obtained after the separation of phases were determined using calibrated centrifuges tubes for calculating the preconcentration factor. Surfactant solutions (0.1%) containing typical amounts of the analytical reagents were extracted using the general CPE procedure, followed by the measurement of the respective phase volumes. The results reported are the average of three determinations.

3.4 Spectrophotometric Analysis of PAN

A general study of the influence of pH on the PAN absorbance spectrum was done using the citric acid/diammonium hydrogenphosphate system as a wide range pH buffer. The values of pH tested were 2.15, 4.95, 6.85 and 7.80, and the concentration of PAN in each case was 1.2×10^{-5} M. Because of the low solubility of PAN in water, stock solutions of this reagent were prepared in 95% ethanol. Aliquots from the stock solution were diluted with appropriate amounts of buffer solutions to obtain the desired concentrations of PAN.

The absorption spectrum of the surfactant solution was also obtained in order to evaluate any possible spectral interferences in the PAN absorbance spectrum. A quantitative determination of the influence of surfactant on the sensitivity of PAN at the analytical wavelength was done as well, by comparing the absorbance values of a solution containing the complexing reagent dissolved in ethanol and a solution at the same concentration but dissolved in a surfactant solution at 1% (wt).

In order to understand the behavior of PAN in cloud point extractions, a calibration curve was prepared with concentrations in the range of 4.0×10^{-6} M to 5.2×10^{-5} M. The blank reagent used was ethanol, based on the null influence of the surfactant solution on PAN absorbance at the analytical wavelength.

The effect of PAN concentration on partition coefficients of the reagent between the surfactant-rich and aqueous phases was studied at pH 7.00 (neutral species) using the standard extraction procedure given in Section 3.5. The pH of the aqueous phase was checked after each extraction. The absorbance of the aqueous phase was measured directly. In the case of the surfactant-rich phase, a dilution up to 10 mL was done by adding water and 500 µL of PONPE-20 solution (10%). The reagent was added to increase the cloud point of the final turbid solution, making it clear and able to detect its absorbance.

3.5 Cloud Point Extraction General Procedure

The following reagents were added to a 50-mL centrifuge tube in the following order: 0.2 g of 20% (wt) surfactant stock solution, 0.75 mL of buffer (containing KNO_3 as an electrolyte), suitable amounts of PAN solution, 0.1 mL of the comparator standard solution, and DDW up to 40 mL. This mixture was stirred for 2 min in a shaker, then kept in a constant-temperature bath for 10 min. The tube containing the sample and reagents was centrifuged at 10 000 rpm for 10 min in order to speed up the separation of phases. After centrifugation, phases were separated by pipeting the upper aqueous phase. The pH of the aqueous phase was measured after each extraction. The surfactant-rich phase was transferred to a 1.5-mL polyethylene vial. The centrifuge tube was rinsed with DDW (4x100 µL) and the rinsings were added to the surfactant-rich phase. This general procedure was used in the present work. The degree of extraction (recovery) was calculated as the ratio of the amount of species extracted by the surfactant-rich phase to the amount initially added to the system. To ensure reproducibility of results, all experiments were done in duplicate except when stated otherwise.

The dependence of the extraction efficiency on some experimental conditions was studied. The parameters examined were solution pH, ionic strength, concentration of the chelating agent, concentration of surfactant, and extraction temperature. In these studies, some conditions were kept constant as in the general procedure. These conditions included stirring time, mass of standard, and centrifugation speed and temperature.

These general conditions were optimized depending on the specific application under study. The results of these experiments are discussed in Chapter 4 in Sections 4.4 to 4.7.

3.6 Drying of the Extracted Phase

The volumes of the concentrated samples obtained after CPE were reduced to 0.2 mL by evaporation. The main reason for this extra step was to increase the sensitivity of measurement by concentrating the sample at the bottom of the vial (*i.e.* counting it closer to the detector). This drying step also offers the advantage of ensuring the same geometry for all samples and standards during counting. Moreover, in the case of relatively long irradiation times, it is convenient to avoid the irradiation of large amounts of water because of the possibility of radiolysis of water. This latter process may produce a high pressure in the vial resulting in the leak of radioactive materials in the pneumatic tube.

Initially, the freeze-drying of samples for 20 h in a multiple sample container was evaluated. The method was effective and the volume of sample was considerably reduced. However, some of the KNO_3 spilled out of the vials during drying. The possibilities of cross-contamination and loss of analyte were considered as disadvantages of the freeze-drying method.

As a second option, evaporation was considered. The small polyethylene vials containing the extracts were exposed to infrared light until most of the water had evaporated. However, melting of the bottom part of the vials was observed after the first two hours of exposure to heat, as well as before the water present in the surfactant rich-phase was totally evaporated. A combined method of sequential heating and cooling periods was used with satisfactory results. After nearly three hours, the sample still retained some moisture (to keep the KNO_3 dissolved), but it was reduced to a small volume in the bottom part of the vial.

Both methods were used during the CPE procedures reported in this work.

3.7 Irradiation and Counting of Samples

Samples were irradiated in doubly heat-sealed small polyethylene irradiation vials (1.5-mL size). Different timing schemes were used depending on the elements under study (Table 3.1). Special care was taken to ensure the reproducibility of conditions by selecting the appropriate geometry for counting.

3.8 Quality Assurance

Blank reagents and comparator standards were irradiated along with the samples in each set of measurements, and at least once a day to check for the possible presence of interfering nuclides in the γ-ray spectra.

Several certified reference materials were used in this work. A biological CRM, namely *IAEA-H-8 Horse Kidney*, was analyzed according to the following procedure. About 250 mg of this material was placed in a microwave Teflon cup and digested for 30 s with 2 mL of concentrated HNO_3. The digested sample was diluted with DDW to 500 mL. An environmental CRM, namely *NIST SRM 1643c Trace Elements in Water*, was also analyzed using the optimized CPE procedure. Since this material originally contained a very high percent of HNO_3 (~20%), 100 mL of it were evaporated to dryness under an infrared lamp. The residue was re-dissolved with DDW and the volume was made up to 100 mL. Between 30 and 40 mL of these solutions were used for CPE.

Table 3.1. Timing Schemes Used in this Work

Radionuclide	Half-life [104]	Irradiation Time	Decay Time	Counting Time
^{111m}Cd	48.6 min	10 min	1 min	10 min
^{115}Cd (^{115m}In)	2.22 d	3h	2 d	1 h
^{141}Ce	32.38 d	2 h	5 d	1 h
^{60m}Co	10.47 min	10 min	1 min	10 min
^{66}Cu	5.1 min	10 min	1 min	10 min
^{164}Dy	2.33 h	2 h	1 h	1 h
^{171}Er	7.52 h	2 h	1 h	1 h
^{152m}Eu	9.3 h	2 h	1 h	1 h
^{159}Gd	18.56 h	2 h	1 d	1 h
^{166}Ho	26.6 h	2h	1 d	1h
^{140}La	40.27 h	2 h	1 h	1 h
^{177}Lu	6.71 d	2h	5 d	1h
^{56}Mn	2.57 h	10 min	1 min	10 min
^{147}Nd	11.06 d	2 h	5 d	1 h
^{65}Ni	2.52 h	3 h	1 h	2 h
^{142}Pr	19.13 h	2 h	1 h	1 h
^{153}Sm	1.95 d	2 h	1 h	1 h
^{160}Tb	72.1 d	2 h	1 h	1 h
^{170}Tm	128.6 d	2 h	5 d	1 h
^{175}Yb	4.19 d	2 h	5 d	1 h
^{69m}Zn	13.8 h	3 h	1 h	2 h

4. RESULTS AND DISCUSSION

The phase diagram and additional studies on the PONPE-7.5 micellar system are described in the first sections of this chapter. The other sections contain the results of the optimization of the CPE procedure and of the quality assessment. Each of these sections begins with a brief reference to the analytical aspects of the elements of interest. The last sections include other possible developments using the CPE procedure, as well as an analysis of the influence of the reagent blank.

4.1 Characterization of the Micellar System

4.1.1 Phase Diagram of PONPE-7.5

The phase diagram of the surfactant PONPE-7.5 obtained in this work at different concentrations is shown in Fig. 4.1. The data were fitted using a polynomial equation of fourth degree with the aid of SigmaPlot regression analysis software. The coefficients obtained to fit the data were $a_1 = 21.70920$, $a_2 = 0.81460$, $a_3 = -0.03263$, $a_4 = 0.00293$ and $a_5 = -0.00005$. The correlation coefficient with experimental data was 0.99895. The shape of the curve coincides with that expected for a typical nonionic surfactant [32]. At very low concentrations, *e.g.* 0.01% (wt), the cloud point increases to temperatures higher than 70°C (not shown in Fig. 4.1), due to the residual solubility of the surfactant.

It has been mentioned by several authors that solutions of nonionic surfactants generally can show different critical temperatures at similar concentrations depending upon the experimental conditions and purity of the amphiphiles [44]. Nonionic polyoxyethylene surfactants are usually synthesized as a mixture of oligomers characterized by an average number of oxyethylene units. Furthermore, the presence or addition of other compounds to the amphiphile solution can lead to changes in the clouding process. Thus, one should not expect complete agreement among the results obtained by different researchers unless the experiments have been carefully controlled and all operating conditions are exactly repeated.

Nevertheless, it is interesting to compare the results obtained in this study with those reported in the literature for the same surfactant. As evident from the phase diagram given in Fig. 4.1, the lowest cloud point values determined for PONPE-7.5 were 21°C and 21.5°C, which corresponded to 0.05% (wt) and 0.1% (wt), respectively.

Our data are in good agreement with those of Okada [51] and Hinze [43], who reported cloud point temperatures of 24.8°C (5% wt) and 19.0°C (4% wt), respectively, for micellar solutions of PONPE-7.5 without any additives. Okada also reported an increase in the cloud point of these solutions when KSCN was added in concentrations higher than 0.1M, and a slight decrease (23.0°C) in solutions containing NaCl (0.1M). Much earlier work by Watanabe *et al.* [50] reported cloud point temperatures of 1°C for 0.1% (wt) and 6°C for 5% (wt) micellar solutions of this surfactant under similar experimental conditions. One of the reasons for this difference in cloud points could be due to a different distribution of ethylene oxide (EO) units in the PONPE-7.5 used in this study.

The composition of the surfactant used in our laboratory was determined. This surfactant was analyzed by high-performance liquid chromatography/UV/mass spectrometry (HPLC-MS) in order to determine the distribution of polyoxyethylene chains in the pure solution. The mass spectrometer was operated in atmospheric pressure chemical ionization (APCI) mode.

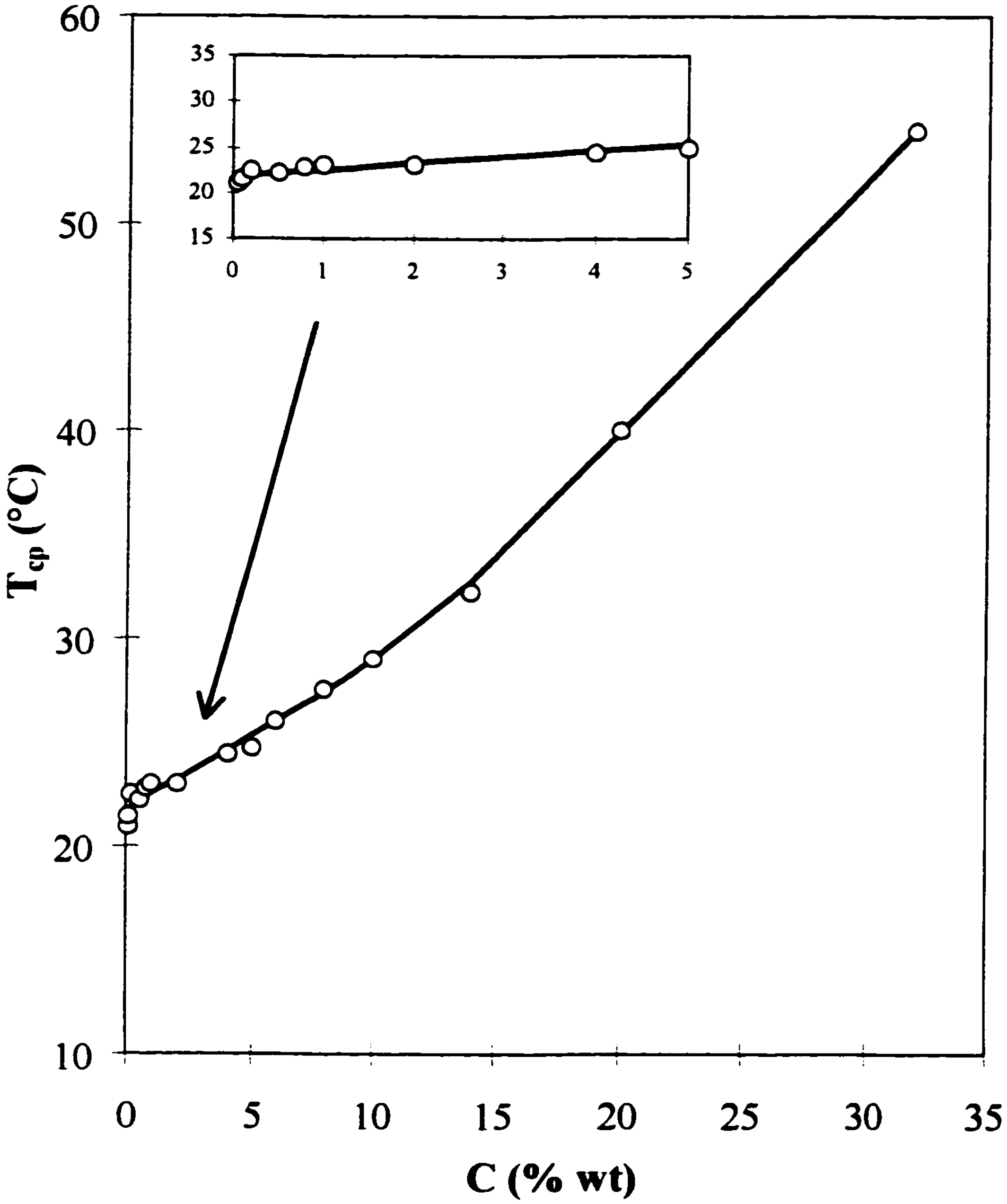


Figure 4.1. Phase Diagram of PONPE-7.5 (T_{cp} is the cloud point temperature of the micellar solution at a concentration C)

The chromatogram (Fig. 4.2) shows a distribution of peaks between 2 and 12 EO units, which are typical of surfactants of the nonylphenyl polyoxyethylene series. The assignment of peaks was done using single ion monitoring (SIM) of the protonated molecular ion corresponding to a given oligomer (Fig. 4.3). The centroid of the SIM chromatogram was then matched in time with the centroid of the peak in the UV chromatogram, thus enabling a direct correlation of a peak with a mass. Once one oligomer was assigned, the rest were simply identified in numerical order. The most intense peaks corresponded to the molecules composed of 6, 7 and 8 EO units, 7 being the most intense followed by 8 and 6, respectively. It corroborated the data from the manufacturer reporting the average EO number as 7.5 units.

4.1.1.1 Influence of Additives on Cloud Point

Several experiments were carried out to ensure that slight changes in some experimental conditions would not drastically influence the cloud point temperature. The critical temperatures of micellar solutions of 0.1% (wt) PONPE-7.5 containing ethanol, buffer, PAN, electrolyte and mixtures of these reagents were determined and are given in Table 4.1. The concentrations of these reagents simulated the conditions used later in this study.

The range of cloud point temperatures with additives varied from 20.0°C to 21.8°C compared to 21.5°C without any additives. This slight variation is within the overall experimental error. Therefore, the experimental conditions were set according to a cloud point temperature of 21.5°C for 0.1% (wt) PONPE-7.5 solutions.

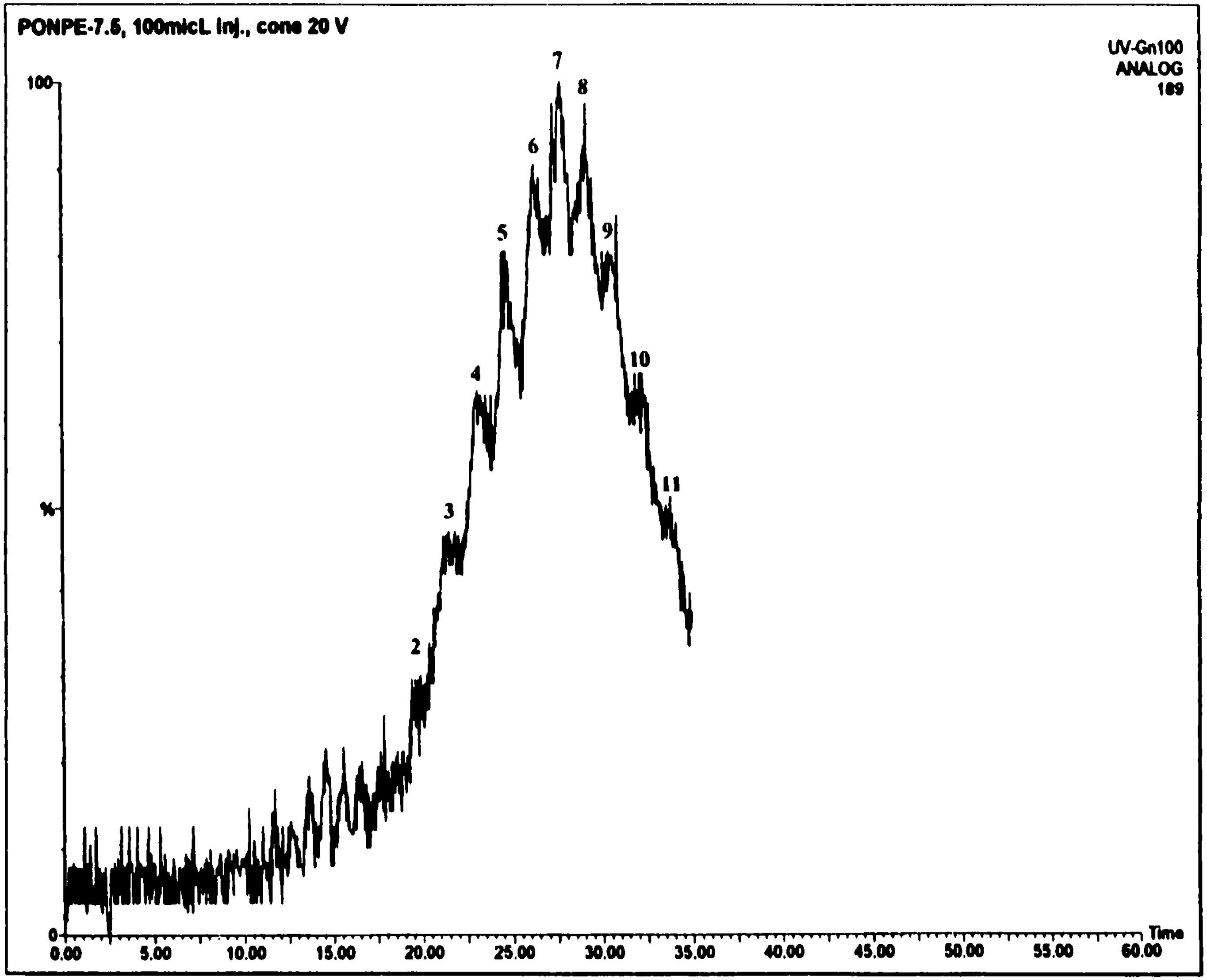


Figure 4.2. UV-Chromatogram of PONPE-7.5

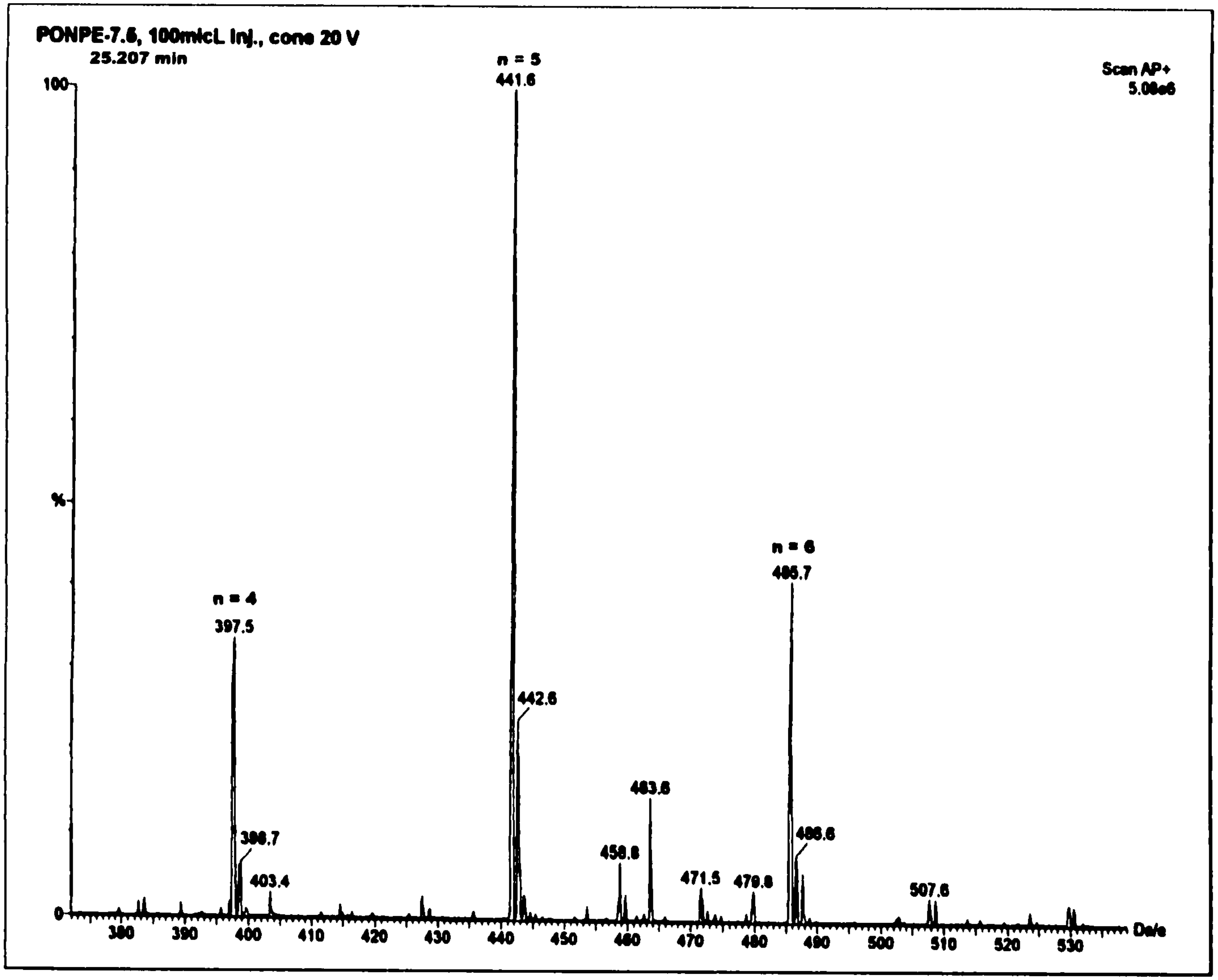


Figure 4.3. Mass Spectrum for Fraction Collected at 25.26 min

Table 4.1. Effect of Different Additives on Cloud Point of 0.1% PONPE-7.5 Solutions

	Without additives	pH buffer (1.6×10^{-4} M)	PAN (2.5×10^{-4} M)	PAN and buffer	NaCl (0.1 M)
T_{cp} (°C)	21.5	20.0	21.5	21.8	21.0

4.1.2 Phase Ratio Determinations

After the separation of phases, the volumes of the surfactant-rich and aqueous phases were determined using graduated centrifuge tubes. The measured volume of the lower surfactant-rich phase was 0.64 mL (± 8%), using the optimized conditions of the CPE method. The volume of the aqueous phase was obtained by subtracting this value from the initial volume of the solution (40 mL). Experiments were done in triplicate. The preconcentration factor (~60) is the volume ratio between the two phases.

The volume of the surfactant-rich phase was also measured under different conditions in order to quantify the volume changes and to evaluate the possible influence of these changes on the recovery. The conditions studied were changes in ionic strength, temperature, concentration of the chelating agent, and concentration of surfactant. These conditions were similar to those used during the optimization of the method. The results are shown in Table 4.2.

Table 4.2. **Variation of the Surfactant-Rich Phase Volume with Different Conditions Compared to Some Optimized Values (I = 0.05 M, T = 40°C, $C_{PAN} = 5\times10^{-5}$ M, and $C_{PONPE\text{-}7.5} = 0.1\%$)**

	Optimized Conditions	I = 0 M	T = 25°C	$C_{PAN} = 2.5\times10^{-5}$ M	$C_{PONPE\text{-}7.5} = 0.2\%$
Volume (mL)	0.64	1.03	0.3	0.60	1.37
Standard Deviation (mL)	0.05	0.04	0.1	0.02	0.02

An analysis of the apparent changes in the volume of the surfactant-rich phase showed that the factors with a greater influence are the ionic strength, the concentration of surfactant in the initial solution, and the temperature at which the clouding occurs. A decrease in the concentration of PAN did not produce any significant change in the value obtained under optimized conditions.

The effect of ionic strength can be conveniently explained in terms of a faster dehydration of the ethoxylated chains due to the presence of inorganic ions competing for the interaction with the water molecules, *i.e.* a "salting-out" effect. In this way, at higher values of ionic strength, the separation of phases is more complete and the volume of the surfactant-rich phase will be smaller. It is important to indicate that this effect is more pronounced for alkaline cations and some anions, since other ions (such as transition-element cations) have been reported to increase the cloud point temperature of certain non ionic surfactants [42, 105, 106, 107, 108, 109].

Another important factor is the concentration of surfactant in the initial micellar solution. The volume of the surfactant-rich phase obtained from a micellar solution of 0.2% (wt) is nearly twice that of the 0.1% (wt) solution (used as optimum). This increase is probably due only to the higher amount of surfactant present in the solution. It is important to note that the cloud point does not change significantly with surfactant concentration in this region of the phase diagram (Fig. 4.1).

The last factor to be mentioned is the influence of the temperature at which the separation of phases occurs. In this case, it should be pointed out that a complete separation of phases after centrifugation is not attained. Since the cloud point temperature of the micellar system at 0.1% (wt) is 21.5°C, at 25°C the phase separation is not yet complete, and the water layer remains slightly cloudy, leading to a higher surfactant concentration in the water phase after centrifugation. This may be the reason why the volume obtained for the surfactant-rich phase is fairly small in comparison to the value used for comparison.

4.1.3 Percent of Water in Surfactant-Rich Phase

It is known that the surfactant-rich phase usually contains a fair amount of water. The quantification of this amount can be very useful, since it may explain to some extent the behavior of solutes concentrated in this phase. The percent of water in the surfactant-rich phase was calculated by determining the concentration of surfactant in this phase.

Standards and samples from the surfactant-rich phase were measured at 277 nm by UV-vis spectrometry.

A calibration curve of PONPE-7.5 in water was constructed using least-squares regression with a correlation coefficient of 0.9963 (Fig. 4.4). The values of the slope and the intercept were 28.1 ± 0.2 and -0.002 ± 0.003, respectively. The results showed that about 81% by weight (± 1% error) of the surfactant-rich phase is formed by water. This value agrees with numbers reported in the literature for surfactant-rich phases obtained by CPE procedures [9, 50].

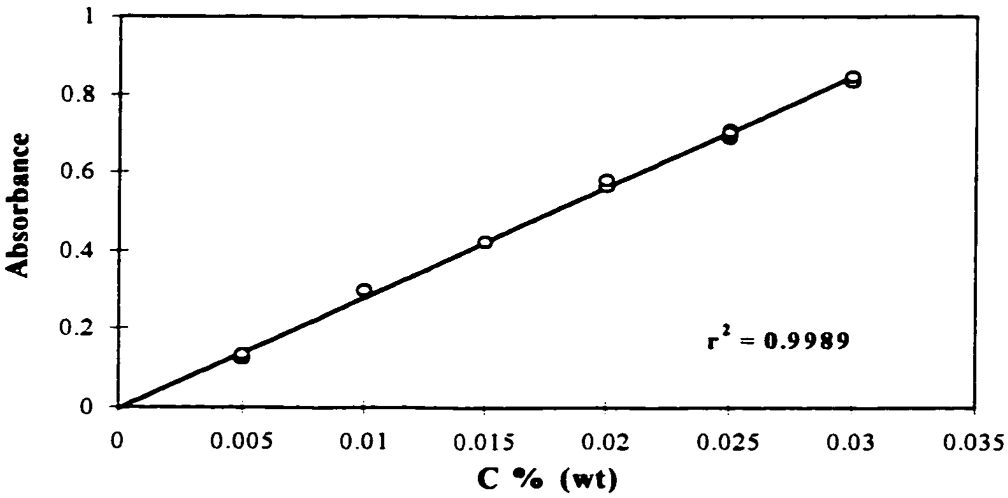


Figure 4.4. Calibration Curve of PONPE-7.5

4.2 Spectrophotometry of PAN

4.2.1 General Spectrophotometric Studies

A general spectrophotometric study of the PAN/PONPE-7.5 system was done prior to the analysis of the distribution equilibrium. This study was performed using UV-vis spectrophotometry to understand the behavior of PAN in aqueous solutions at four different pH values, namely 2.15, 4.95, 6.85 and 7.80. The results for the most important range of wavelengths are shown in Figs. 4.5 and 4.6. A maximum absorbance was obtained at a wavelength of 470 nm at near neutral pH; since the species of interest in our experiments were neutral species, this wavelength was selected for further work.

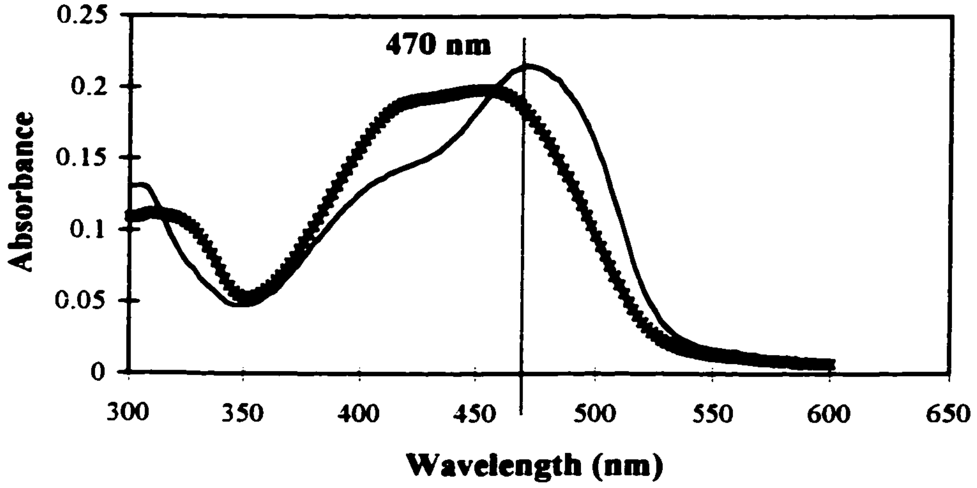


Figure 4.5. Spectra of PAN Solutions at pH 2.15 (xxx) and 7.80 (—)

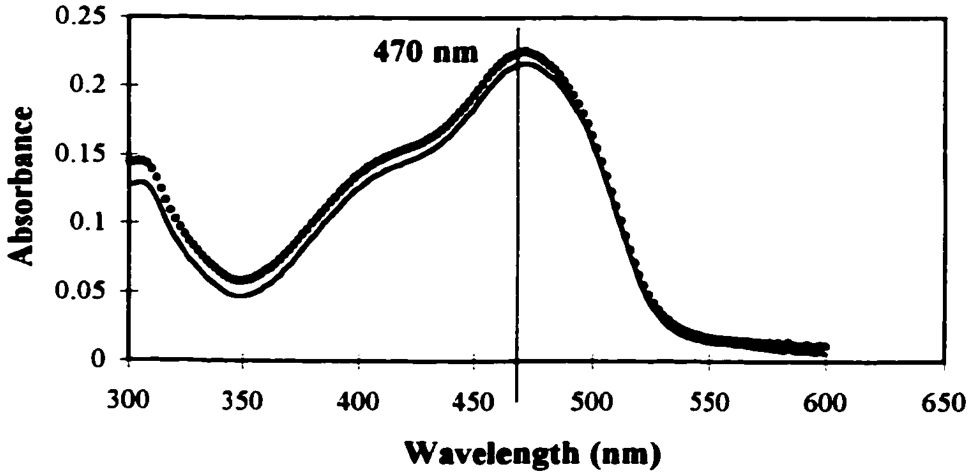


Figure 4.6. Spectra of PAN Solutions at pH 4.95 (—) and 6.85 (···)

The next step was to determine the possible influence of surfactant interactions in the absorption spectrum of PAN. The UV-vis spectra of PAN dissolved in EtOH and in a micellar solution at 0.1% (wt) were recorded. The results showed no interference of surfactant with PAN spectrum at the selected wavelength of 470 nm, as can be seen in Fig. 4.7.

In order to calculate the concentration of PAN, a calibration curve in the range of 4.0×10^{-6} M to 5.2×10^{-5} M of PAN was obtained at 470 nm. A set of three solutions at each concentration was prepared and measured; the results are shown in Fig. 4.8. The data obtained were adjusted by least-squares regression with a correlation coefficient of 0.9957. The values of the slope and the intercept were 15 235 ± 150 and -0.006 ± 0.005, respectively.

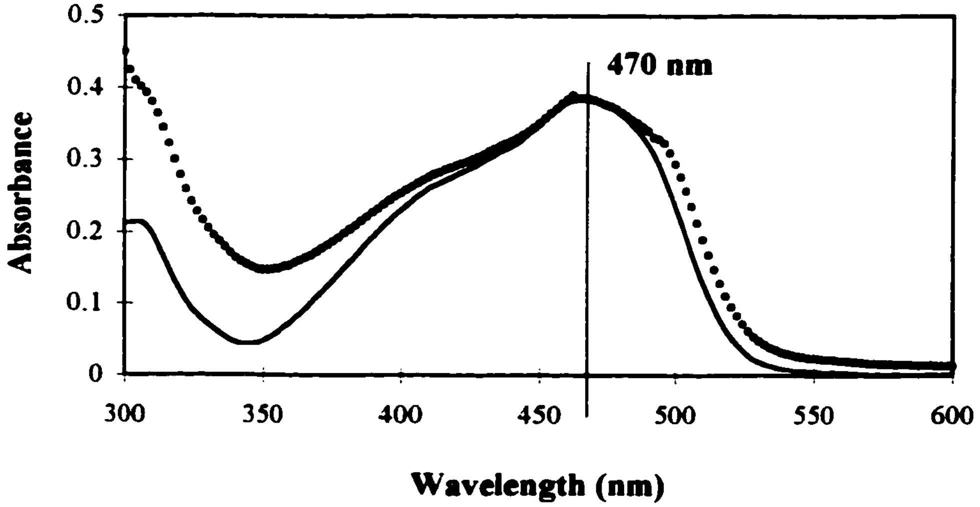


Figure 4.7. Influence of PONPE-7.5 on PAN Absorbance at pH 7.50
(— PAN/EtOH spectrum, ··· PAN/surfactant spectrum)

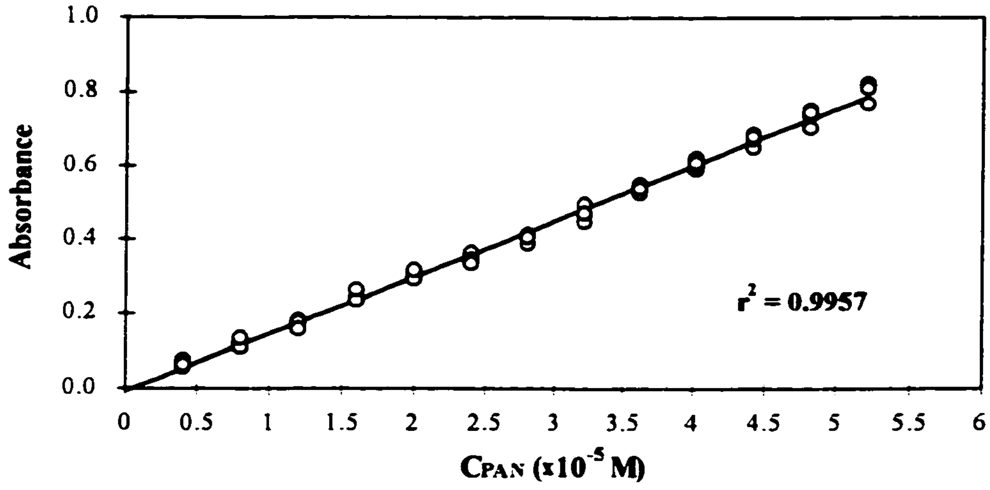


Figure 4.8. Calibration Curve of PAN

4.2.2 Partitioning of PAN in PONPE-7.5 Micellar Solutions

The distribution equilibrium of PAN between the aqueous and surfactant-rich phases after cloud point extraction was studied at different pH values and at different concentrations as part of a study on general distribution equilibrium. Due to the non-polar character of the surfactant-rich phase, only the neutral species (non-ionized) could be extracted to a significant extent. These results can give some insight on the extractability of the species once chelates have been formed, since the major interactions involved in this CPE are between the aromatic rings of PAN and the non-polar tails of the surfactant.

The PAN concentration was calculated for each phase by measuring the absorbance of the two phases and using the calibration curve (Fig. 4.8). The partition coefficients (K_d) of the neutral PAN species were calculated using the following formula:

$$K_d = \frac{[HL]_S}{[HL]_{aq}} \qquad \textbf{Eq. 4.1}$$

where $[HL]_S$ and $[HL]_{aq}$ represent the concentrations of PAN neutral species in the surfactant-rich and aqueous phases, respectively.

These coefficients were calculated at PAN concentrations similar to those used in the CPE experiments. Some of the results are shown in Table 4.3. The values obtained are lower in comparison with the coefficients reported for partitioning in traditional organic solvents, which is reasonable considering the hydrated nature of the extracting surfactant-rich phase. For example, Nakagawa and Wada [23] reported log K_d = 5.1 for PAN in chloroform-water system. Nevertheless, the partition coefficients obtained in this work compare well with typical values for cloud point extractions using PONPE-7.5 and similar chelating agents such as PAP and PAPM [53, 73]. No data on PAN distribution coefficients or distribution studies of chelating agents in CPE were found in the literature.

Based on conventional solvent extraction theory, the partitioning of species between two phases should be independent of the species concentration or the volume ratio of solvents. However, in these extractions a decrease in the partition coefficients with increasing concentration of the reagent has been observed. This unusual trend may be attributed to different factors: a possible change in the micellar structure due to the interaction of the organic species (PAN) and the surfactant molecules, or the formation of dimers of the PAN molecules. However, as the PAN concentration increases, this effect is less pronounced. For example, at a PAN concentration of $5\text{x}10^{-5}$ M, the log K_d obtained was 2.38.

Table 4.3. Some Partition Coefficients of PAN at pH 7, I=0.5 M and 40°C

C_{PAN} ($\times 10^{-6}$ M)	log K_d
2.4	3.73
5.2	2.82
7.6	2.77

Considering the high distribution coefficients obtained for PAN in this study, different concentrations were chosen for the extraction procedure according to the specific cases. The chosen values were always greater by one order than the total analyte concentration. This decision was supported by literature recommendations of using high PAN concentrations compared to the metal concentration to diminish competing reactions with other possible chelating species that might be present in the sample [110]. It also guarantees the chelation of all metal ions in the solution, avoiding the interference by other cations that may form complexes with PAN and can be present in water samples (*e.g.* Ba, Ca, Mg and Sr).

It is important to note that generally the partition coefficients for the chelates are considerably higher than the ones for the chelating agent. Thus, it is expected that the concentration of the species of interest in the surfactant-rich phase will increase when extracting the metal complexes. The chelates will be extracted into the surfactant-rich phase to a greater extent than the PAN molecules because of the decrease in polarity and the rupture of hydrogen bonds between PAN and water.

4.3 Cloud Point Extraction of Copper

Copper was chosen for the initial work because of the high stability constant (log $\beta = 23.8$) of its complex with PAN, and its common presence in the aquatic environment. Another reason for this selection lies in the advantage of using relatively short irradiation and counting times, since Cu produces a short-lived nuclide (5.1-min ^{66}Cu) by neutron activation that can be used in its determination (Table 4.4).

Copper, as many other transition metals, is an essential trace element for most living organisms. However, for many aquatic plants and animals it may become toxic at higher concentrations. Copper(II) ions (the most common species in water) are reported to be more toxic than the organically bound species. In surface waters, Cu may be toxic to aquatic plants and many fish species at concentrations >1.0 mg L^{-1} [111].

Table 4.4. Nuclear Data of Cu [104]

Target Nuclide	Isotopic Abundance (%)	Thermal Neutron Cross Section for Target Nuclide ($\times10^{-24}$ cm^2)	Nuclear Reaction	Half-life of Nuclide Produced	γ-ray Used (keV)
^{65}Cu	30.9	2.2	^{65}Cu (n,γ) ^{66}Cu	5.1 min	1039.6

4.3.1 Optimization of the CPE Procedure

The dependence of the extraction efficiency upon changes in some experimental parameters was studied. These included solution pH, ionic strength, temperature, concentration of the chelating agent, and concentration of the surfactant. In these studies, some conditions such as stirring time, mass of standard, and centrifugation speed were kept constant as in the general procedure. The possible interferences of several metal ions were also evaluated under the optimal conditions selected for the analysis.

4.3.1.1 Effect of pH

For ionizable solutes, the charge of the solute can greatly influence its extent of binding to a micellar assembly [38]. The ionic form of a molecule normally does not interact with and bind the micellar aggregate as strongly as does its neutral form. Thus adjustment of the solution pH is of special importance when controlling experimental variables in CPE.

A general study of the influence of pH on extraction recovery was done keeping other parameters constant. The variation of extraction recovery on pH for Cu is shown in Fig. 4.9. It is evident that the recovery is generally very poor at low pH values, but becomes quantitative at pH values between 7 and 8.5. A pH range of 7.00 to 7.20 was selected to perform further extractions. Three or four measurements were done at each pH value.

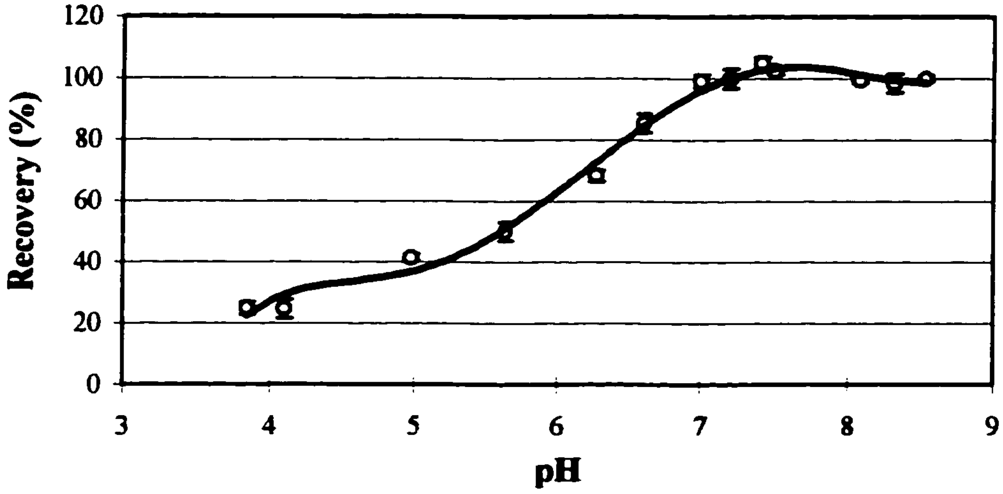


Figure 4.9. Effect of pH on CPE of Cu

4.3.1.2 Effect of Ionic Strength

Different results referring to the influence of ionic strength on CPE have been reported in the literature, but most of them referred to the use of relatively high concentrations (between 1 M and 5 M) of inorganic salts as clouding agents [17, 48]. Many of the reports dealing with CPE of metals by temperature change did not evaluate the influence of electrolytes. In some cases, an ionic strength of 0.1 M was used but its influence on recovery was not reported [53, 56, 64].

A recent CPE paper [83] suggested that, in some cases, the addition of salts may contribute to a poor phase separation. In particular, the authors reported that an upper surfactant-rich phase is formed after the addition of NaCl (1.0 M) to a micellar system containing high HCl concentrations. This inversion of phases made the extraction process more difficult and, as a consequence, lower recoveries were obtained.

The influence of the ionic strength on the cloud point extraction done in this work did reveal an improvement on the extraction efficiency (Table 4.5). A value of 0.05-M concentration of the added electrolyte was chosen for the optimized procedure. At higher values of electrolyte concentration, the recovery was the same. However, the radioactivity of the ^{42}K isotope, obtained by the neutron activation of ^{41}K isotope in KNO_3 present in the sample, started to increase substantially the background of the γ-ray spectrum. Therefore, a concentration of 0.05 M of KNO_3 was chosen as optimum.

The influence of the electrolyte seems to have more effect on the stability of the chelating agent and on the reproducibility of the chelation process than on the phase separation phenomenon. Moreover, from the practical point of view, it was easier to separate the phases when an electrolyte was added. This is in agreement with published works which deal with the effects of salts on non-ionic surfactants and mention that an increase in the polarity of the aqueous phase occurs with the addition of inorganic salts [41, 53, 112].

Table 4.5. Effect of Ionic Strength on CPE of Cu*

C_{KNO_3} (M)	% Recovery	
	pH = 7.00	pH = 7.2
0.01	85 ± 2	85 ± 2
0.05	100.1 ± 0.4	100 ± 2
0.10	101 ± 2	101 ± 7

*results are the average of 3 determinations

The presence of inorganic salts in the micellar solution is an important practical factor to consider in CPE methodologies since it can lead to higher preconcentration factors, shorter centrifugation times, and a better distribution of the neutral species into the surfactant-rich phase.

4.3.1.3 Effect of Temperature

It has been suggested that CPE procedures based on the temperature-driven phase separation typical of nonionic micellar solutions should be done at temperatures well above the cloud point [113]. At these temperatures, the interfacial concentration of amphiphile is maximized and polar groups are still sufficiently hydrated to form a viscous interface structured by lateral hydrogen bonding *via* intermediate water molecules. Nevertheless, these relatively high temperatures required for efficient extractions usually imply a limitation for many practical applications because of the fact that most of the common nonionic surfactants have a minimum in the phase diagram at temperatures above 25°C.

To study the influence of the temperature on the efficiency of the extraction procedure using PONPE-7.5 micellar solutions, three temperature values, namely 25°C, 30°C and 40°C, were chosen in this work. As the results in Table 4.6 show, the recovery of Cu is quantitative and reproducible at 40°C in the pH range selected for the procedure. This temperature is still low enough to obtain an effective separation of phases during centrifugation, without a substantial loss of the analyte due to re-dissolution of the surfactant-rich phase.

Table 4.6. Effect of Temperature on CPE of Cu*

Temperature (°C)	% Recovery	
	pH = 7.00	pH = 7.2
25	72 ± 1	73 ± 3
30	86 ± 3	86 ± 3
40	99 ± 2	100 ± 3

*results are the average of 3 determinations

4.3.1.4 Effect of Concentration of PAN

The influence of the concentration of PAN on the CPE efficiency was evaluated for the Cu chelate using Cu:PAN concentration ratios between 1:10 and 1:75. It is evident from Fig. 4.10 that the recovery of Cu is quantitative and reproducible at a ratio 1:50 or higher. Based on these results, it was concluded that the 1:50 ratio could be used as an optimum value. This high ratio could also avoid, to some extent, the formation of non-extractable hydroxy complexes that can interfere with the extraction mechanism.

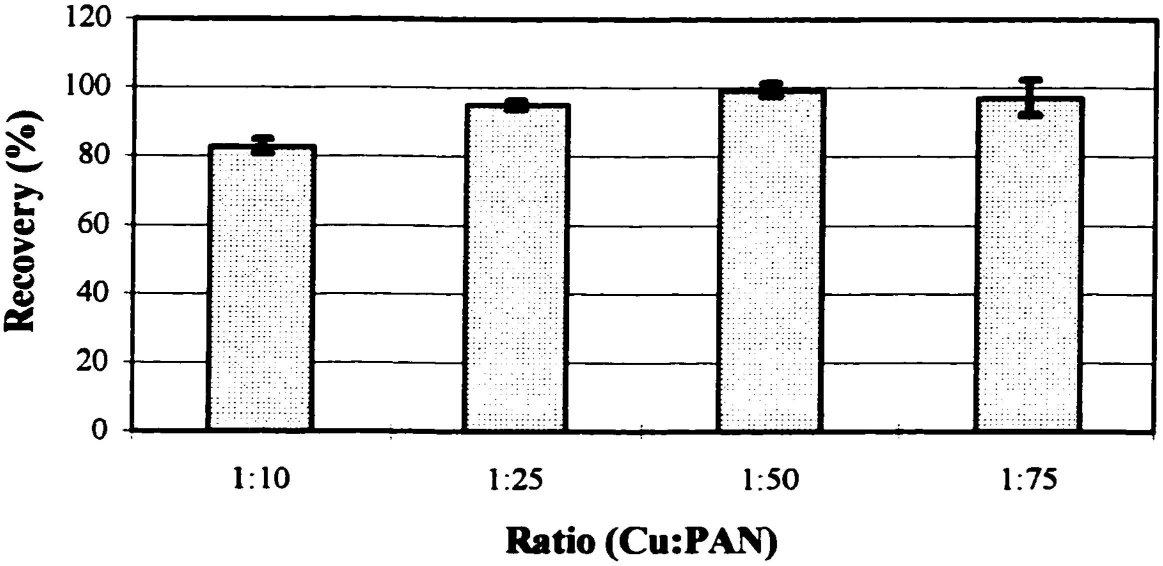


Figure 4.10. Effect of PAN Concentration on CPE of Cu

4.3.1.5 Effect of Concentration of Surfactant

The phase diagram of PONPE-7.5 shows a fairly flat region between ca. 0.05% and 1% (wt) which is very convenient for CPE since small variations in the concentration of the initial micellar solution should not have a significant influence on the cloud point. This observation was advantageously used to evaluate the influence of surfactant concentration on the extraction recovery.

The results of this study for two surfactant concentrations (0.1% and 0.2%) and two metal concentrations (10 μg and 20 μg) are shown in Figs. 4.11 and 4.12. Different concentrations of PAN were also used in the CPE procedure to compare the influence of the chelating agent and the surfactant in the efficiency of the extraction.

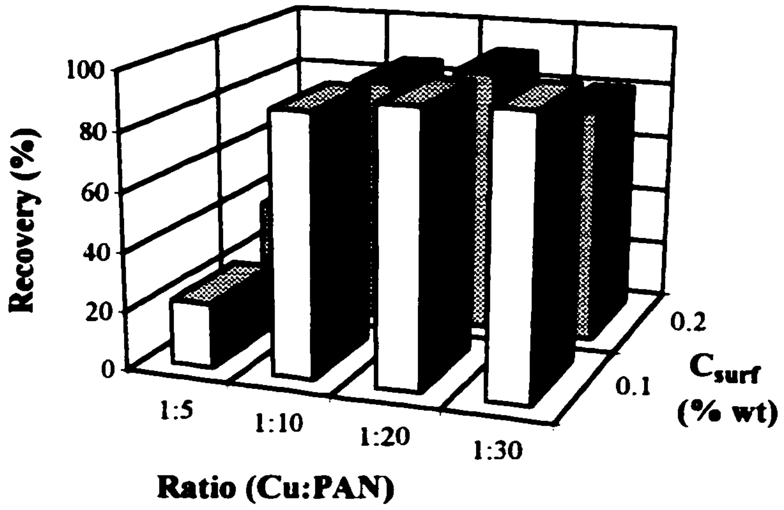


Figure 4.11. Comparison of the Effect of Surfactant and PAN Concentrations on CPE of Cu (10 μg)

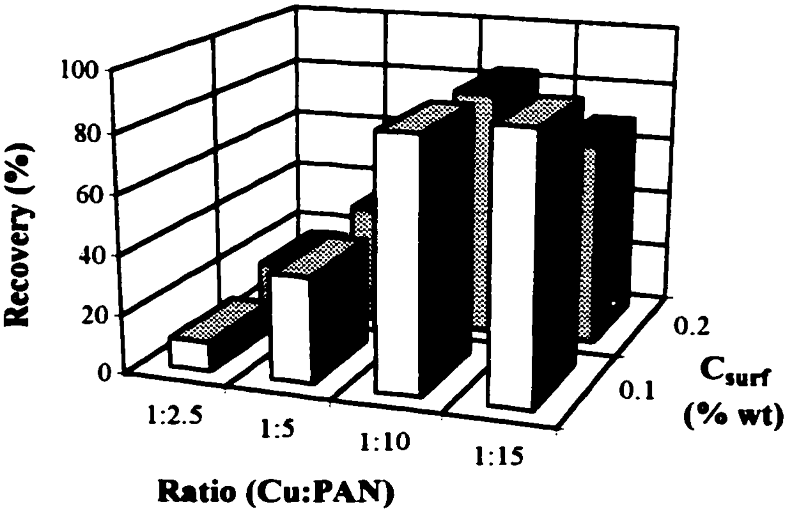


Figure 4.12. Comparison of the Effect of Surfactant and PAN Concentrations on CPE of Cu (20 μg)

It is evident from the graphs that the chelating agent is the factor that has more influence on the efficiency of the extraction procedure. Recoveries >80% can be obtained from a Cu:PAN ratio of 1:10. This is another indication of the importance of selecting the appropriate chelating agent for the metal of interest (*i.e.* high stability constant) in order to attain a quantitative extraction using a minimum amount of the agent at a given metal concentration.

Conversely, the surfactant concentration seems to play a small, if any, role in the extraction under the conditions used here. This result may suggest a large capacity of the micelles and the surfactant-rich phase to accommodate the chelate molecules.

Other researchers have also been interested in determining the role that the surfactant concentration may play in the extent of extraction. Akita *et al.* [83] reported that an increase in the concentration of PONPE-7.5 provides a dramatic increase in the extraction efficiency of Au(III) as $AuCl_4^-$. However, the distribution ratio tends to decrease for surfactant concentrations greater than 1% (wt). The authors explained such a decline in terms of an increase in the volume of the surfactant-rich phase. Since in that experiment the chelating agent and the surfactant were the same reagent, a stronger influence on the recovery can be expected.

4.3.1.6 Effect of Other Metal Ions

Since PAN is a non-selective chelating agent that complexes many other metal ions efficiently, the effect of possible interferences present in the sample was studied. The recovery obtained (99% ± 1%) indicates that under the conditions used in the proposed CPE procedure, the Cu(II) extraction is not affected by the presence of up to 5 μg of Cd, 0.5 μg of Mn, 5 μg of Th and 0.7 μg of U.

4.3.2 Quality Assurance

4.3.2.1 Internal Quality Assessment

In an attempt to evaluate internal quality assessment (IQA), elemental comparator standards were irradiated along with the samples. Irradiations were done on a per batch basis and between samples. Blank solutions were also analyzed to quantify possible impurities that may be present in the preconcentration process.

The Schewart control chart was constructed using the results from 27 comparator standards (Fig. 4.13). The mean (X) and the standard deviation (σ) were calculated, and the control and warning limits were set as the X±3σ and X±2σ intervals, respectively. The results illustrate the statistical control condition of the irradiation-decay-counting process. Generally, "the results falling outside the bounds of X±2σ would be sufficiently unusual (about 5%) to act as a warning of a possible problem" [97]. It is evident from this chart that the system is operating under a fairly good statistical control.

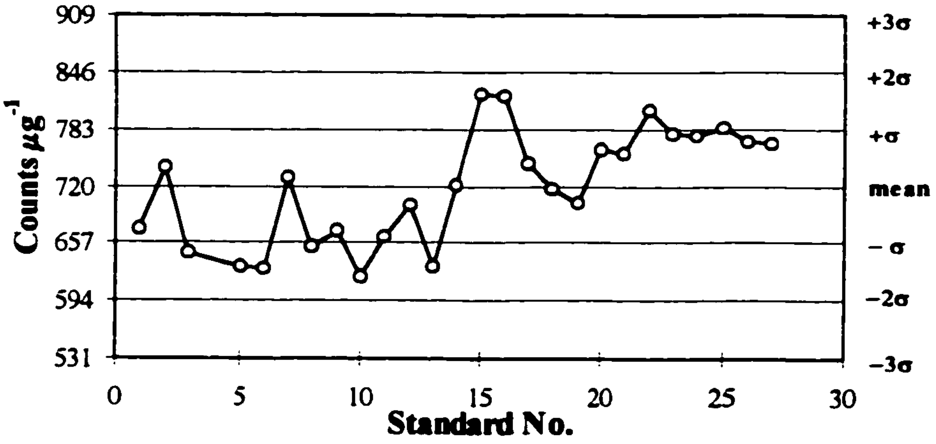


Figure 4.13. Schewart Control Chart for Internal Quality Assessment of Cu

4.3.2.2 External Quality Assessment

Two certified reference materials, namely *NIST SRM-1643c Trace Elements in Water* and *IAEA-H-8 Horse Kidney*, were analyzed in order to evaluate the accuracy of the preconcentration method. The details of the analysis of the water SRM for Cu is described in Sections 3.8 and 4.4.2.2 along with those for Cd and Mn. The experimental details for the dissolution of the *Horse Kidney* is also described in Section 3.8. Suitable aliquots of this solution were taken for analysis. The average of three determinations is shown in Table 4.7.

Table 4.7. Analysis of Certified Reference Materials for Cu*

	Cu Concentration	
	NIST 1643c *Trace Elements in Water* (ppb)	*IAEA-H-8* *Horse Kidney* (ppm)
This Work	21.12 ± 0.04	33 ± 4
Certified Value	22 ± 3	31 ± 2

*results are the average of 6 determinations

4.3.3 Sensitivity and Detection Limits

The sensitivity for Cu by NAA was found to be 720 counts μg^{-1} using a t_i=10 min, t_d=1 min, and t_c=10 min. The detection limit (L_C) for Cu was calculated according to the definitions described by Currie [101] for radiochemical analysis:

$$L_C = 2.33\ B^{1/2} \qquad \textbf{Eq. 1.3}$$

$$L_D = 2.71 + 4.65\ B^{1/2} \qquad \textbf{Eq. 1.3}$$

$$L_Q = 50\ [1 + (1 + B/12.5)^{1/2}] \qquad \textbf{Eq. 1.3}$$

where L_C, L_D and L_Q are the decision, qualitative and quantitative detection limits, respectively. The value of L_c for the determination of Cu was found to be 1.5 ppb.

4.3.4 Preconcentration Factor

A general preconcentration factor of 60 was obtained with this CPE method, which is in good agreement with the literature values reported for cloud point extractions. This value was calculated as the volume ratio between the two phases after the separation of phases. Typical preconcentration factors reported in the literature varied from 10 to 100.

The CPE method gives a better preconcentration factor compared to conventional solvent extraction methods for Cu and other metals studied in this work. In general, high preconcentration factors in CPE can be achieved using small amounts of surfactants which have large capacity to accommodate chelate molecules. The hydrated nature and relative polarity of micelles, on the other hand, limit the extraction of metal-chelates into the surfactant-rich phase.

4.3.5 Analysis of Samples

This CPE method was applied to tap water samples collected at different times and under different conditions. The results, shown in Table 4.8, exhibit the typical trend expected for this kind of water samples. The highest concentrations are found in the water collected early in the morning, which is explained by the fact that Cu-containing pipes could be source for Cu(II) ions, which may dissolve in the water during the night time. There is also a marked increase in the concentration in hot water with respect to cold water, after 1h running, since higher temperatures can accelerate the dissolution process.

Table 4.8. Determination of Cu in Tap Water Samples*

Collection Details	Concentration (ppb)
Cold Water (9:00 a.m., T = 21.5°C)	246 ± 9
Cold Water (1:00 p.m., T = 22.5°C)	146 ± 3
Cold Water (5:00 p.m., T = 26.0°C)	90.0 ± 0.6
Cold Water** (9:15 a.m., T = 8.5°C)	18.2 ± 0.8
Hot Water** (9:30 a.m., T = 63.0°C)	83 ± 3

*results are the average of 3 determinations

**samples collected in the morning after 1 h running

4.4 Simultaneous CPE of Cadmium, Copper and Manganese

Following the successful determination of Cu by CPE combined with NAA, a simultaneous extraction of Cd, Cu and Mn was attempted. These transition metals can be advantageously determined with short irradiation, decay and counting time sequences. The nuclear characteristics of these elements are shown in Table 4.9.

Table 4.9. Nuclear Data of Cd, Cu and Mn [104]

Target Nuclide	Isotopic Abundance (%)	Thermal Neutron Cross Section (x 10^{-24} cm^2)	Nuclear Reaction	Half-life of Nuclide Produced	γ-ray Used (keV)
^{110}Cd	12.4	0.1	$^{110}Cd\ (n,\gamma)^{111m}Cd$	48.7 min	245.4
^{114}Cd	28.8	0.3	$^{114}Cd\ (n,\gamma)^{115}Cd$	53.5 h	336.3
^{65}Cu	30.9	2.2	$^{65}Cu\ (n,\gamma)^{66}Cu$	5.1 min	1039.2
^{55}Mn	100	13.3	$^{55}Mn\ (n,\gamma)^{56}Mn$	2.58 h	846.6

4.4.1 Optimization of the CPE Procedure

The parameters examined here were pH, ionic strength, temperature and concentration of the chelating agent. The stirring time, mass of standard, concentration of surfactant, and centrifugation speed were kept constant as described in the general procedure.

In this study, particular emphasis was placed to develop a method for the simultaneous extraction of all three metals with high recoveries. It was designed so that the method would operate within a range of optimal values of the experimental parameters rather than under limited conditions in order to determine metal concentrations in samples of diverse composition.

4.4.1.1 Effect of pH

As discussed before, the pH study represents the most important step in the optimization of a CPE procedure. This is of particular importance when a simultaneous extraction is preferred, since the equilibrium conditions for the chelating process are frequently different for most of the elements.

A study of the influence of pH on extraction recovery keeping the other parameters constant showed that, in general, the recovery is poor at low pH values but becomes significant at higher pH values. The recoveries of Cd and Mn reached maximum values around pH 9, while quantitative recoveries of Cu were over a wider pH range (Fig. 4.14). In order to obtain a simultaneous extraction of the three metal ions, a pH of 9 was selected as the optimum value for the CPE preconcentration procedure.

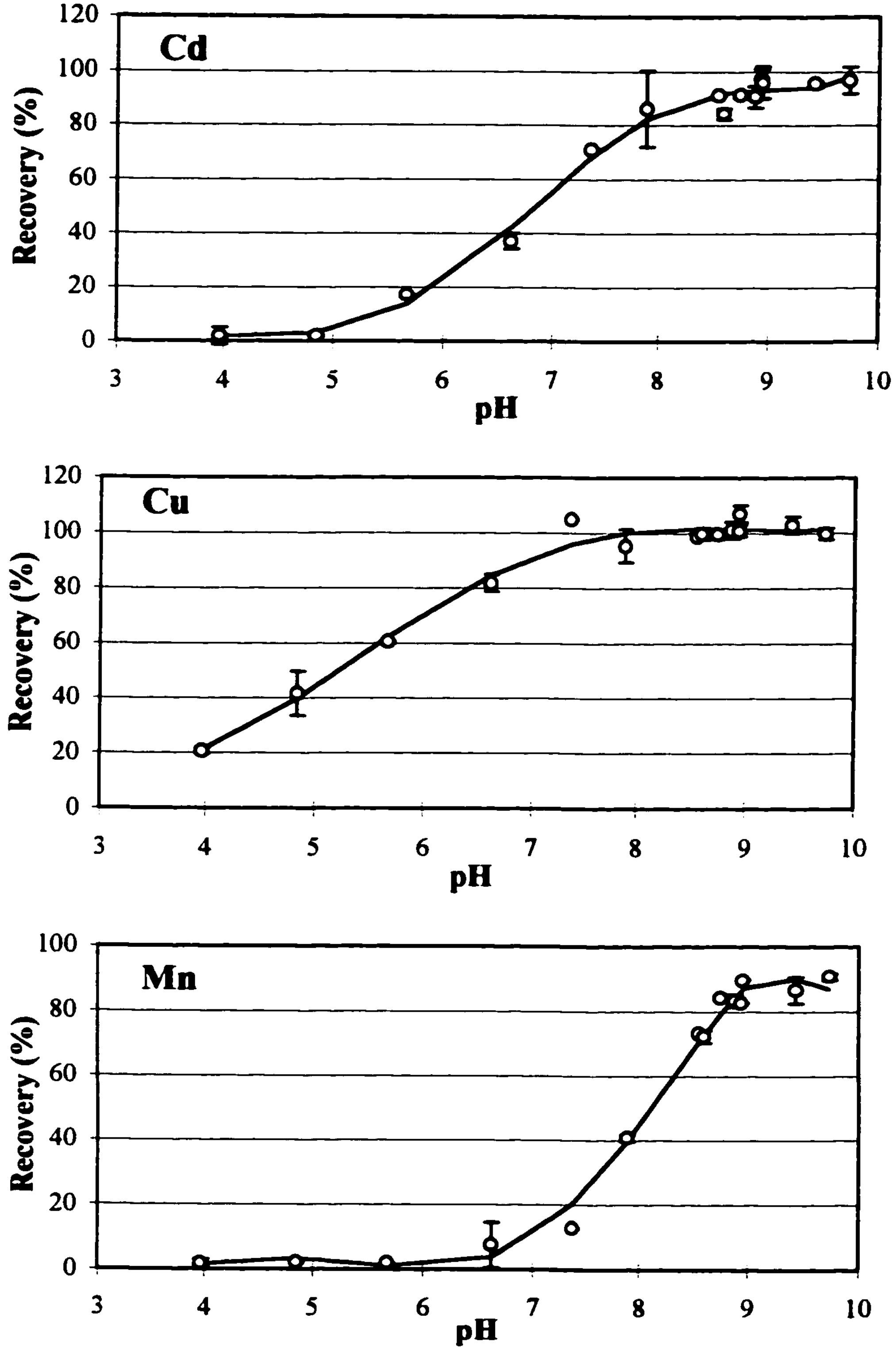


Figure 4.14. Effect of pH on CPE of Cd, Cu and Mn
(results are the average of 3 determinations)

4.4.1.2 Effect of Ionic Strength

The influence of an added electrolyte on the CPE of Cd, Cu and Mn did reveal a general improvement of extraction efficiency as the concentration increased (Table 4.10). At a KNO_3 concentration of 0.05 M and higher, the recovery was quantitative with the exception of Mn which had a maximum recovery at 0.05 M.

Based on the analysis of these results and those obtained for Cu, it was decided that an optimized ionic strength value of 0.05 M (using KNO_3 as electrolyte) can be used in future CPE procedures involving the analysis of transition metals.

4.4.1.3 Effect of Temperature

The temperature study for the simultaneous extraction showed that the recoveries of Cd, Cu and Mn are quantitative and reproducible at 40°C and a pH of 9.00 (Table 4.11).

The substantial increase in the recovery by increasing the temperature at which the CPE takes place coincides with the better separation of phases obtained at temperatures higher than the cloud point (Section 4.1.2). It is evident that an improvement of the separation of phases driven by a decrease in the solubility of this phase will increase the concentration of micelles containing the analyte in the surfactant-rich phase with a consequent increase in the extraction recovery. Considering that this result coincides with the one obtained for Cu (given in Section 4.3.1.3), a temperature of 40°C was selected to carry out the CPE in further experiments.

Table 4.10. Effect of Ionic Strength on CPE of Cd, Cu and Mn (at 40°C and pH = 9.00)*

C_{KNO_3} (M)	Recovery (%)		
	Cd	Cu	Mn
0.01	94 ± 3	94 ± 2	80 ± 2
0.05	97 ± 5	100 ± 2	90 ± 1
0.10	102 ± 1	102 ± 1	81 ± 2

*results are the average of 3 determinations

Table 4.11. Effect of Temperature on CPE of Cd, Cu and Mn (using 5×10^{-5} M PAN and at pH = 9.00)*

T (°C)	Recovery (%)		
	Cd	Cu	Mn
25	48 ± 4	56 ± 1	37 ± 1
30	67 ± 6	89 ± 17	55 ± 1
40	97 ± 5	100 ± 2	90 ± 1

*results are the average of 3 determinations

4.4.1.4 Effect of Concentration of PAN

The influence of the concentration of the chelating agent on the CPE efficiency was evaluated for the metal chelates using different PAN concentrations (Table 4.12).

The recoveries of the metals were found to be quantitative and reproducible at a PAN concentration of 5×10^{-5} M (1:80 metals:PAN ratio). It was then concluded that this concentration could be used as an optimum value.

Table 4.12. Effect of PAN Concentration on CPE of Cd, Cu and Mn (at 40°C and pH = 9.00)*

C_{PAN} ($\times10^{-5}$ M)	Recovery (%)		
	Cd	Cu	Mn
2.5	83 ± 8	94 ± 6	79 ± 11
3.8	87 ± 14	95 ± 1	81 ± 3
5.0	97 ± 5	100 ± 2	90 ± 1
7.6	82 ± 1	96 ± 1	78 ±4
8.8	72 ± 1	100 ± 2	73 ± 2

*results are the average of 4 determinations

Since the highest Mn recovery obtained was nearly 90%, attempts were made to ensure that the recovery was not affected by any other factor than the stability of the complex. The possibility of spectral interference of the 846.6-keV γ-ray of ^{56}Mn by the 843.7-keV γ-ray of ^{27}Mg was considered. An alternative photopeak of ^{56}Mn, namely the 1811.2 keV, was used, and it gave similar recovery values.

Some authors have reported the possible oxidation of Mn(II) to higher oxidation states by dissolved oxygen and air [114]. In the present work, experiments were done using excess amounts of ascorbic acid. Other changes in the experimental conditions included an increase in PAN concentration, use of buffers of higher pH values, and extractions at a higher temperature. Only one or two experiments were done to test each factor. None of these experiments gave any substantial increase in the recovery of Mn by this CPE method (Table 4.13). Therefore, a recovery of 90% was considered to be the highest achievable under the optimized conditions reported here.

Table 4.13. Study of Possible Factors Affecting Mn Recovery

Conditions	Ascorbic Acid		PAN:Mn		T = 50°C	pH = 9.5
	$6.0\text{x}10^{-5}$ M	$2.4\text{x}10^{-4}$ M	2000:1	3000:1		
Recovery (%)	90	89	86	88	85	87

4.4.2 Quality Assurance

4.4.2.1 Internal Quality Assessment

In an attempt to evaluate IQA, multielemental comparator standards were irradiated along with the samples. Irradiations were done on a per batch basis and in between samples. The Schewart control charts were constructed using the results from 29 comparator standards (Fig. 4.15).

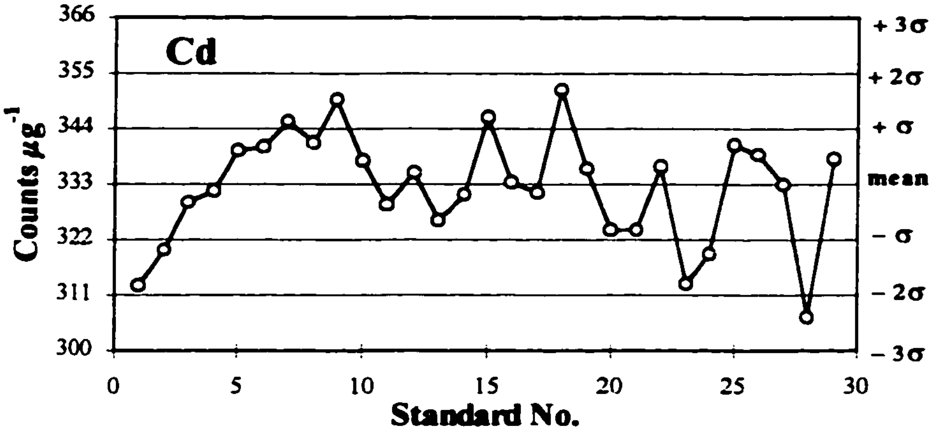
4.4.2.2 External Quality Assessment

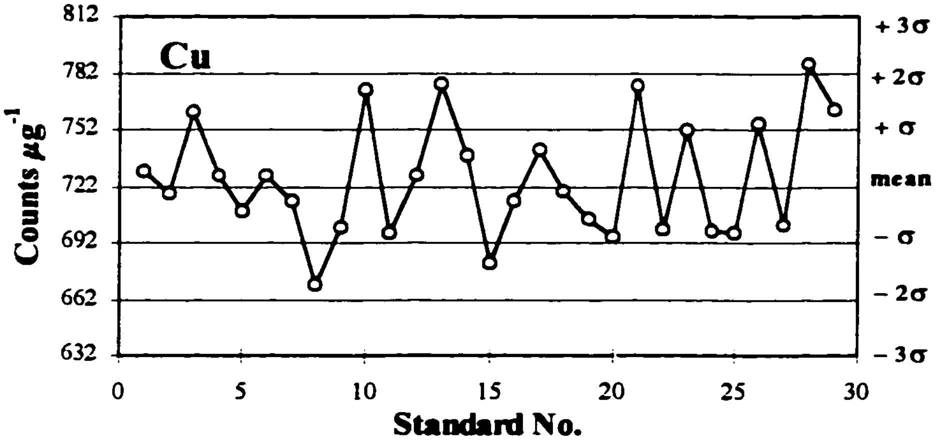
External quality assessment (EQA) was also carried out. An environmental certified reference material (*NIST SRM-1643c Trace Elements in Water*) was analyzed in order to evaluate the accuracy of the preconcentration method. In general, the results are in good agreement with the certified values within the experimental errors (Table 4.14).

Table 4.14. Analysis of *NIST SRM-1643c Trace Elements in Water* for Cd, Cu and Mn[*]

	Concentration (ppb)		
	Cd	Cu	Mn
This Work	13.0 ± 0.3	21.21 ± 0.04	31.7 ± 0.2
Certified Values	12 ± 1	22 ± 3	35 ± 2

[*]results are the average of 6 determinations





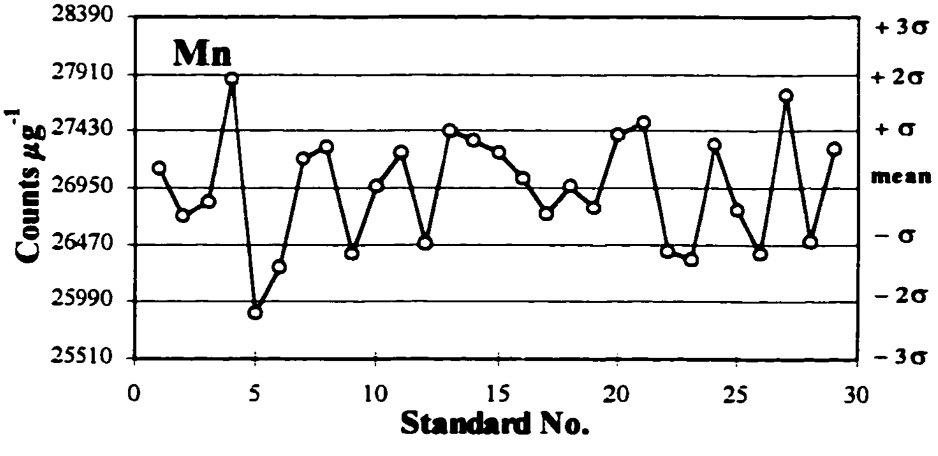


Figure 4.15. Schewart Control Charts for Internal Quality Assessment of Cd, Cu and Mn

4.4.3 Sensitivity and Detection Limits

The sensitivities for Cd, Cu and Mn were calculated based upon the multielemental comparator standards (Table 4.15). Low detection limits [101] for Cd (1.35 ppb), Cu (1.46 ppb) and Mn (0.198 ppb) were obtained by conventional γ-spectroscopy (Table 4.16).

It is evident from this table that even lower detection limits can be achieved by anticoincidence γ-spectroscopy. Since the background is significantly reduced using this technique, detection limits are improved by almost an order of magnitude. There is no advantage of using this technique for the detection of the 245.4-keV γ-ray of ^{111m}Cd.

In general, the detection limits for these elements, except 0.4 ppb for Cd [58], using CPE procedures have not been reported in the literature. Water samples spiked with trace elements at ppb to ppm levels were usually employed by other researchers for evaluating the CPE procedures. When compared with other analytical techniques, the CPE procedure developed here appears to be an efficient and inexpensive analytical method for the simultaneous determination of trace amounts of these metals.

4.4.4 Analysis of Samples

The CPE method developed here was applied to tap water samples collected at different times and under different conditions. The conventional γ-ray spectrum of a water sample preconcentrated using this method is shown in Fig. 4.16.

Table 4.15. Sensitivities of Cd, Cu and Mn Nuclides

Element/Nuclide	^{111m}Cd	^{115}Cd	^{66}Cu	^{56}Mn
Sensitivities (counts μg^{-1})	333 ± 10	2 588 ± 80	722 ± 30	26 950 ± 1890

Table 4.16. Detection Limits of Cd, Cu and Mn (ppb)*

Element/Nuclide	Cd (^{111m}Cd)	Cd (^{115}Cd)	Cu (^{66}Cu)	Mn (^{56}Mn)
Conventional γ-Spectroscopy	1.35 ± 0.03	1.56 ± 0.05	1.46 ± 0.03	0.198 ± 0.007
Anticoincidence γ-Spectroscopy	3.9 ± 0.1	0.783 ± 0.007	0.71 ± 0.04	0.031 ± 0.002

*results are the average of 10 determinations

The results from the analysis (Table 4.17) show that the highest concentrations of Cu, as expected, are found in the water collected early in the morning. However, in the cases of Cd and Mn, a different trend is observed. The detailed discussion of these results is beyond the scope of the present work.

Table 4.17. Determination of Cd, Cu and Mn in Tap Water Samples (collected on 99-04-09)*

Collection Details	Concentration (ppb)		
	Cd	Cu	Mn
Cold Water (9:00 a.m., T = 21.5°C)	2.1 ± 0.2	246 ± 9	10 ± 1
Cold Water (1:00 p.m., T = 22.5°C)	3.8 ± 0.4	146 ± 3	53 ± 3
Cold Water (5:00 p.m., T = 26.0°C)	8.5 ± 0.7	90.0 ± 0.6	25.0 ± 0.4
Cold Water** (9:15 a.m., T = 8.5°C)	4.6 ± 0.3	18.2 ± 0.8	31 ± 1
Hot Water** (9:30 a.m., T = 63.0°C)	7 ± 2	83 ± 3	7.9 ± 0.1

*results are the average of 3 determinations

**samples collected in the morning after 1 h running

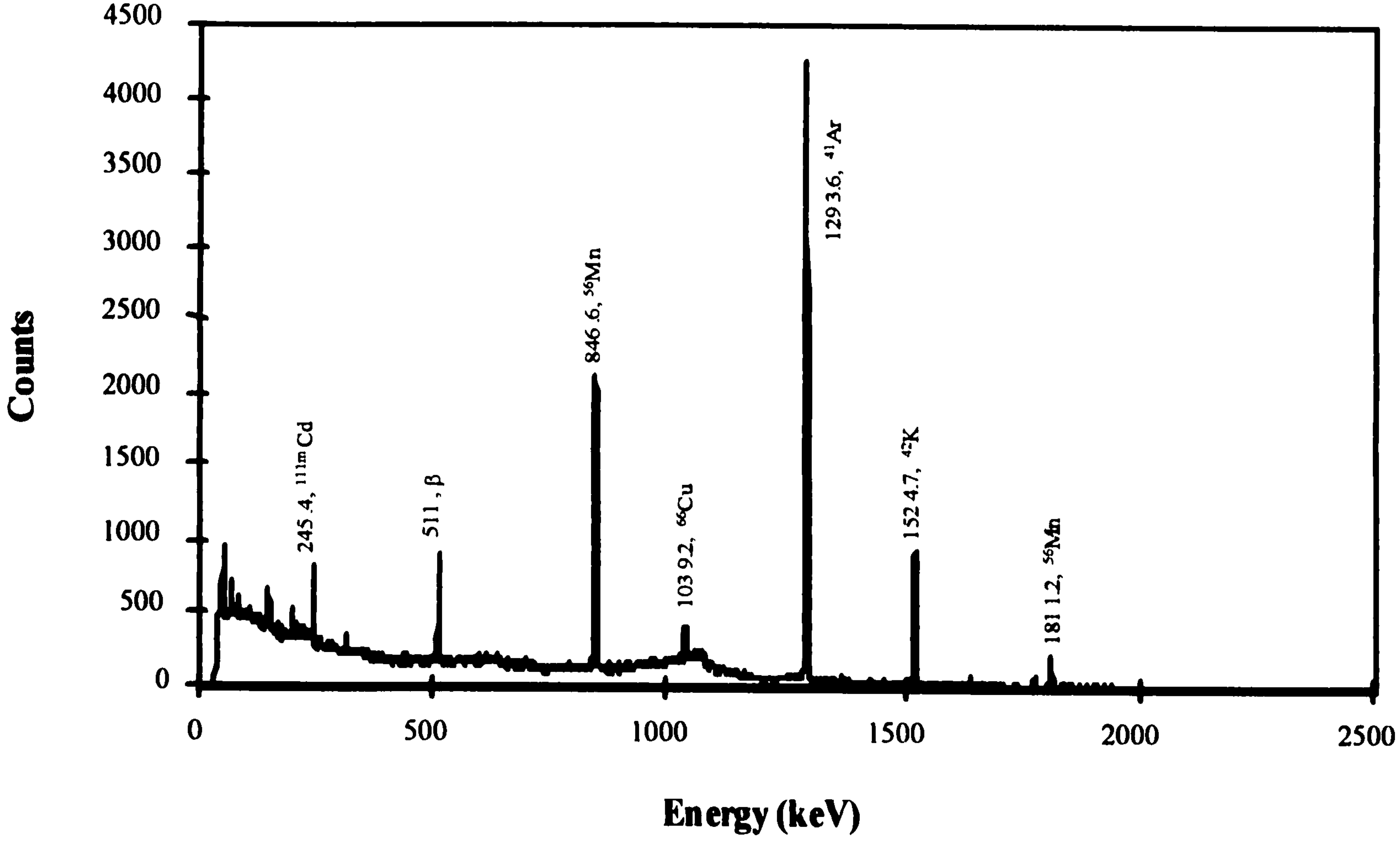


Figure 4.16. Gamma-ray Spectrum of a Tap Water Sample Analyzed in this Work

4.5 Simultaneous CPE of Cadmium, Cobalt, Copper, Manganese, Nickel and Zinc

Based on the successful results obtained for the simultaneous CPE of Cd, Cu and Mn, an attempt was made to obtain a simultaneous extraction of additional transition metals such as Co, Ni and Zn because of their environmental importance. It so happens that Co, Ni and Zn also form chelates of relatively high stability constants with PAN.

Two different irradiation-decay-counting (t_i, t_c, t_d) schemes were necessary considering the different half-lives and sensitivities of the elements of interest: 10 min, 1 min, 10 min for Cd, Co, Cu and Mn, and 3h, 1h, 2h for Ni and Zn. The nuclear characteristics of the nuclides of interest are shown in Table 4.18.

4.5.1 Optimization of the CPE Procedure

It became evident from the experiments for the extraction of Cd, Cu and Mn that it is not necessary to study all the parameters involved in the development of a similar CPE methodology for a new metal. Once the conditions directly related to the surfactant-mediated process have been optimized, a detailed analysis of only the conditions which are likely to be affected by a change in the central atom of the chelates need to be done. The pH and PAN concentration were the parameters selected to be optimized for Co, Ni and Zn.

Table 4.18. Nuclear Data of Cd, Co, Cu, Mn, Ni and Zn [104]

Target Nuclide	Isotopic Abundance (%)	Thermal Neutron Cross Section (x 10^{-24} cm^2)	Nuclear Reaction	Half-life of Nuclide Produced	γ-ray Used (keV)
^{110}Cd	12.39	0.1	$^{110}Cd\ (n,\gamma)^{111m}Cd$	48.7 min	245.4
^{59}Co	100	20	$^{59}Co\ (n,\gamma)^{60m}Co$	10.5 min	58.6
^{65}Cu	30.9	2.2	$^{65}Cu\ (n,\gamma)^{66}Cu$	5.1 min	1039.2
^{55}Mn	100	13.3	$^{55}Mn\ (n,\gamma)^{56}Mn$	2.58 h	846.6
^{64}Ni	1.08	1.49	$^{64}Ni\ (n,\gamma)^{65}Ni$	2.52 h	1481.8
^{68}Zn	18.6	0.074	$^{68}Zn\ (n,\gamma)^{69m}Zn$	13.8 h	438.63

4.5.1.1 Effect of pH

The range of pH studied for the optimization of the CPE procedure was from 6.0 to 9.5 (Fig. 4.17). The results show that the recoveries of all metals except Mn are quantitative (>95%) at high pH values *viz.* between 8 and 9. Manganese has a maximum recovery of 100% at a pH near 9. The same optimum pH was also obtained for a 90% recovery of Mn in the study reported in Section 4.5.

4.5.1.2 Effect of Concentration of PAN

The effect of the concentration of PAN on the CPE of the six transition metals was studied. The results revealed a general improvement in the recoveries of Co, Mn, Ni and Zn at a PAN concentration of 5.0×10^{-5} M (Table 4.19). Quantitative recoveries are also obtained for most of the elements at higher PAN concentrations, but since an increase in the chelating agent concentration tends to decrease the distribution coefficient, a minimum amount of PAN should be used.

Table 4.19. Effect of PAN Concentration*

C_{PAN} ($\times 10^{-5}$ M)	Recovery (%)					
	Cd	Co	Cu	Mn	Ni	Zn
2.5	99.0 ± 0.8	92.3 ± 0.6	101 ± 2	95 ± 1	93 ± 1	93.9 ± 0.1
5.0	99 ± 1	101 ± 3	99 ± 2	98 ± 1	102 ± 14	96.0 ± 6
7.5	102.9 ± 0.8	93 ± 4	100 ± 1	87 ± 3	95 ± 6	96 ± 8

*results are the average of 5 determinations

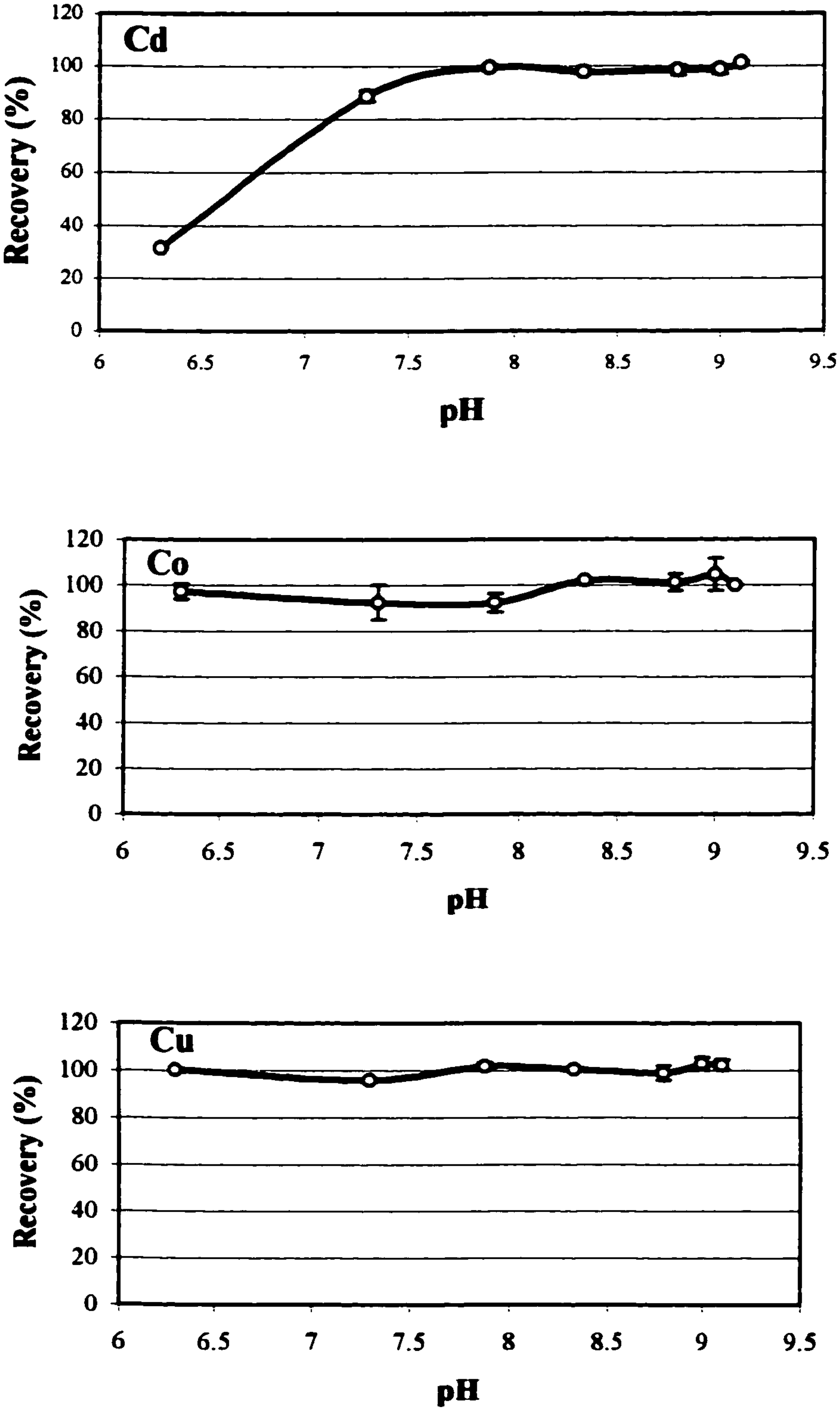
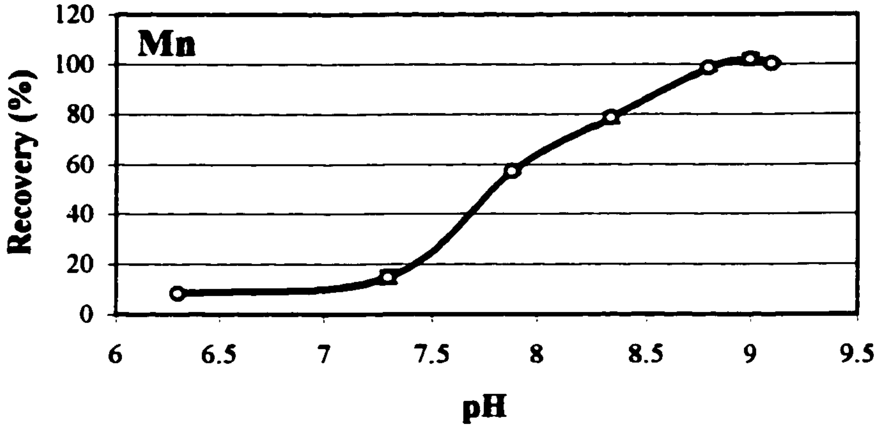
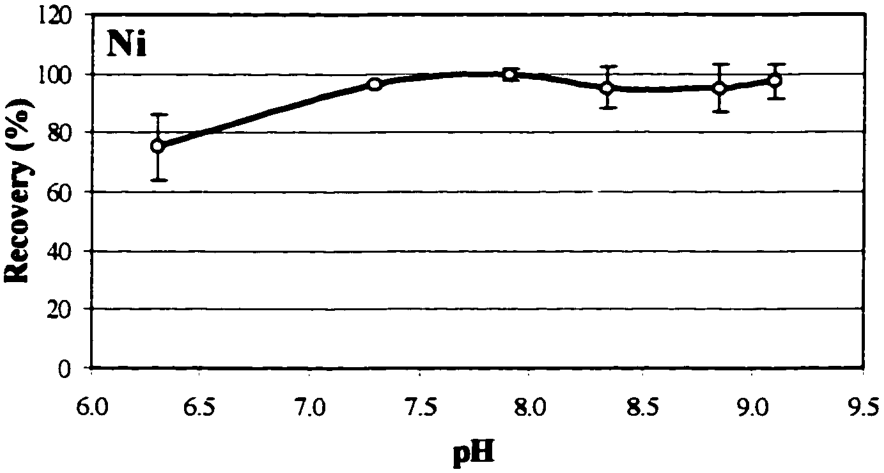


Figure 4.17. Effect of pH on CPE of Cd, Co, Cu, Mn, Ni and Zn (results are the average of 3 determinations)





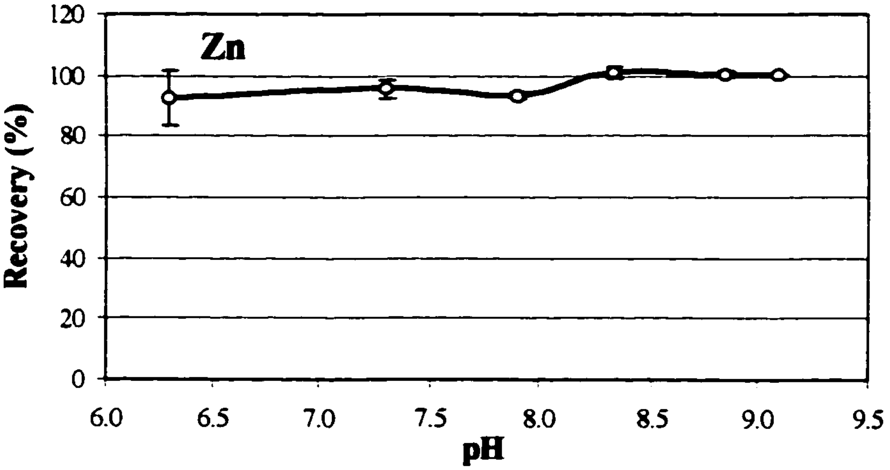


Figure 4.17 (*continued*). Effect of pH on CPE of Cd, Co, Cu, Mn, Ni and Zn (results are the average of 3 determinations)

4.5.2 Quality Assurance

4.5.2.1 Internal Quality Assessment

Multielemental comparator standards were irradiated along with the samples in every experiment for the purpose of IQA. The Schewart control charts constructed using these results show a good statistical control of the process (Fig. 4.18).

4.5.2.2 External Quality Assessment

A biological certified reference material, namely *IAEA-H-8 Horse Kidney* was analyzed in order to evaluate the accuracy of the PNAA method. The results obtained compare favorably with the certified values within the experimental errors (Table 4.20).

Table 4.20. Analysis of Certified Reference Material *Horse Kidney* (*IAEA-H-8)* for Cd, Co, Cu, Mn, Ni, and Zn*

	Concentration (ppm)					
	Cd	Co	Cu	Mn	Ni	Zn
This Work	187 ± 14	0.12 ± 0.02	33 ± 4	5.59 ± 0.03	99.9 ± 0.7	186.4 ± 0.9
Certified Values	189 ± 5	0.13**	31 ± 2	5.7 ± 0.3	N/A	193 ± 6

*results are the average of 4 determinations

**not certified

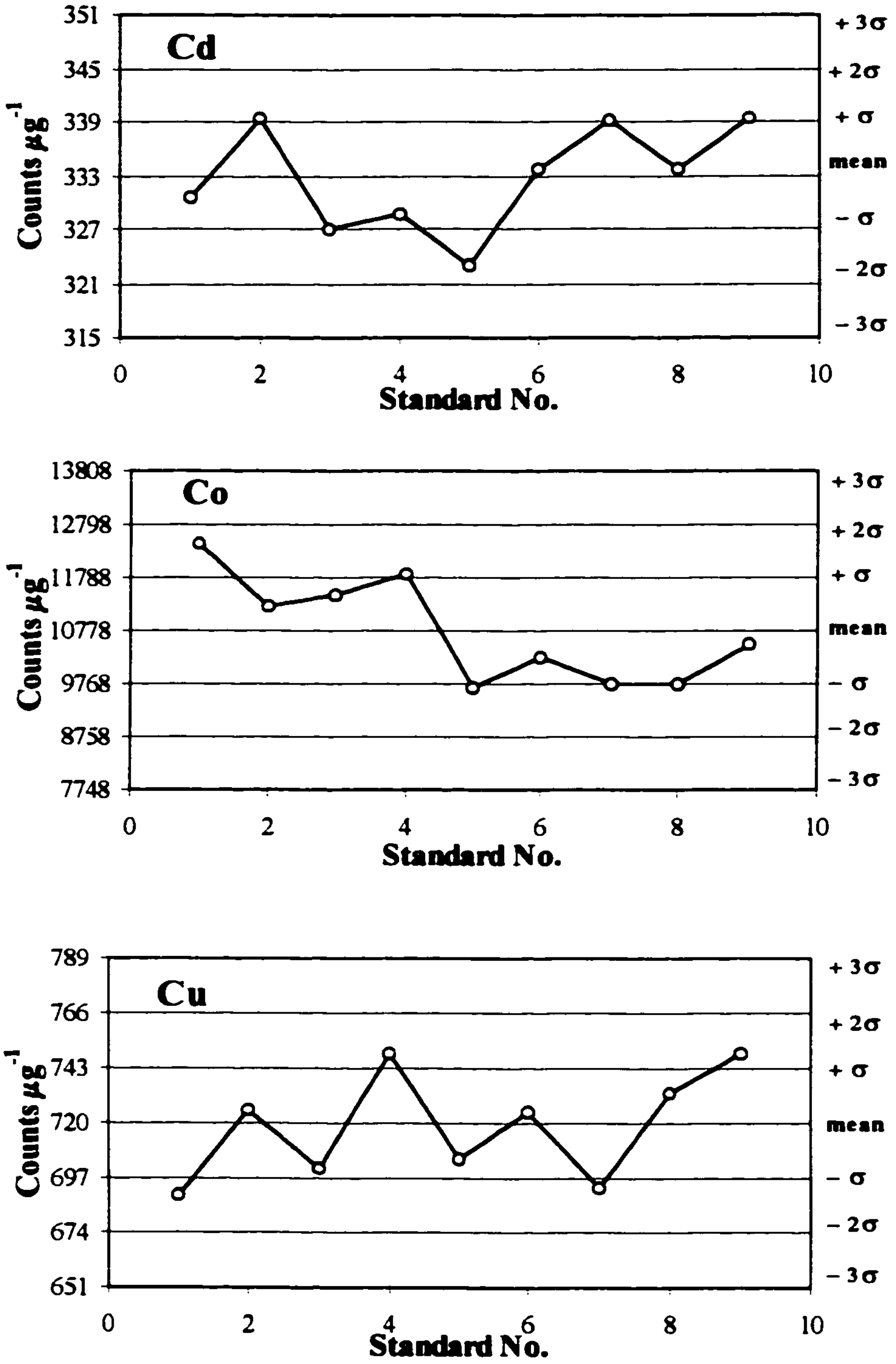


Figure 4.18. Schewart Control Charts for Internal Quality Assessment of Cd, Co, Cu, Mn, Ni and Zn

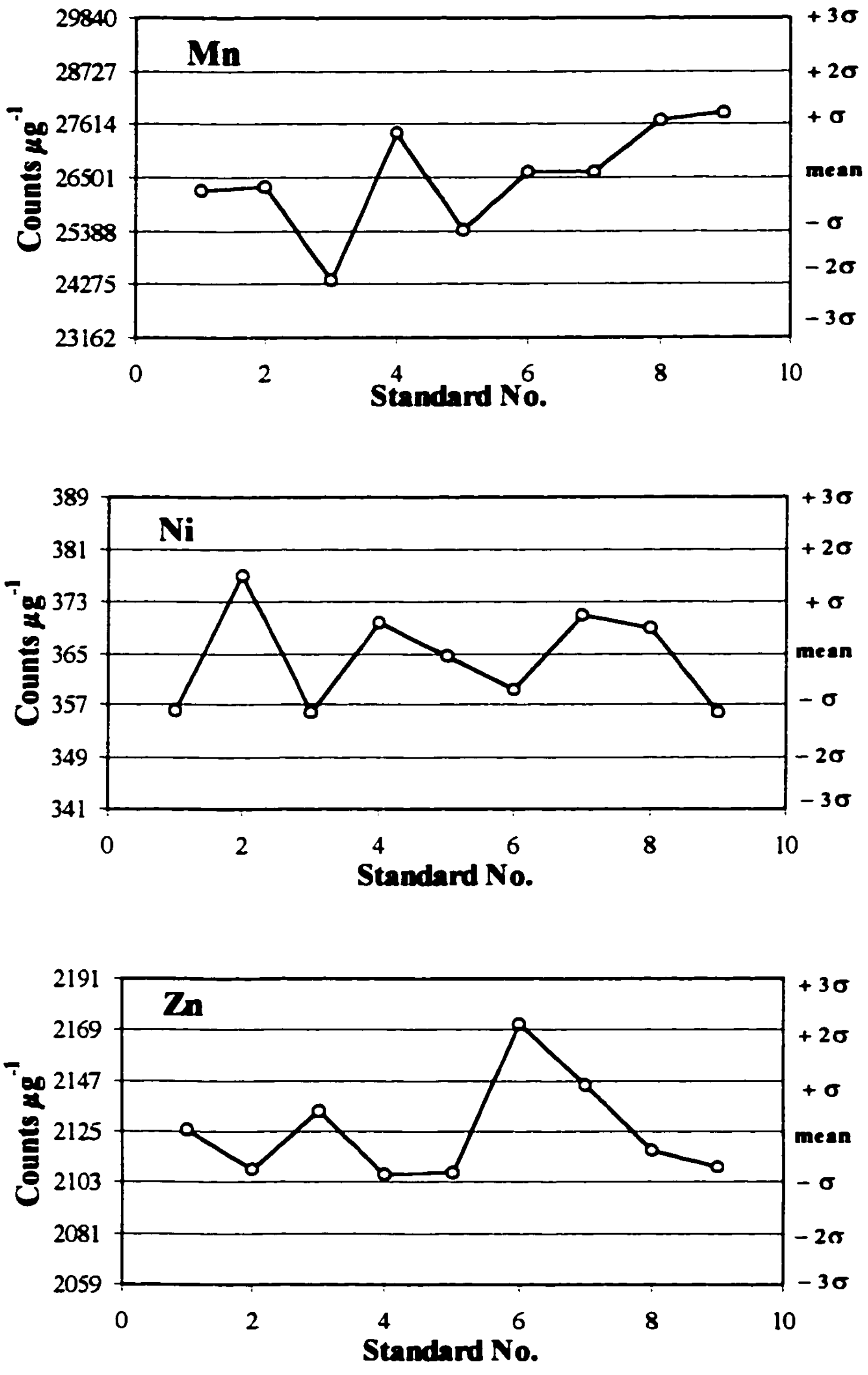


Figure 4.18 (*continued*). Schewart Control Charts for Internal Quality Assessment of Cd, Co, Cu, Mn, Ni and Zn

4.5.3 Sensitivity and Detection Limits

The sensitivities for Cd, Co, Cu, Mn, Ni and Zn were calculated based upon the multielement comparator standards (Table 4.21). The detection limits for these metals were obtained by conventional γ-spectroscopy and following the definitions given by Currie [101] for radiochemical analysis (Table 4.22). The spectra of a spiked solution containing the metals of interest, and determined using the timing schemes given at the beginning of this section, are shown in Figs. 4.19 and 4.20.

It is apparent that the detection limits have a general tendency to increase as the number of elements increases in the sample, which is logical considering the consequent increase in the total background of the spectrum. As mentioned in the Section 4.4, the detection limits for some of these elements using CPE procedures are scarcely reported in the literature, except 0.4 ppb for Cd [58], and 6 ppb for Ni and 8 ppb for Zn [80]. Although a comparison between various analytical techniques, based on different fundamental principles, using non-destructive and preconcentration methods is not an easy task, it is interesting to compare certain parameters. Detection limits recently reported for ICP-AES include 30 ppb for Cu, 0.8 ppb for Mn, and 90 ppb for Zn [115]. Other values reported for similar techniques, such as ICP-MS, are: 8 ppt for Cd, 14 ppt for Co, 29 ppt for Cu, 9.3 ppt for Mn, and 65 ppt for Zn [116].

The results obtained in this work suggest that the CPE procedure can be used for the simultaneous analysis of trace amounts of transition metals in water. It can be particularly useful for Ni and Zn determinations by PNAA, where these elements offer a higher sensitivity than INAA. The detection limits obtained for Ni and Zn (23.6 and 10.8 ppb, respectively) are satisfactory for most fresh water and biological samples.

Table 4.21. Sensitivities of Cd, Co, Cu, Mn, Ni and Zn Nuclides

Element/Nuclide	^{111m}Cd	^{60m}Co	^{66}Cu	^{56}Mn	^{65}Ni	^{69m}Zn
Sensitivities (counts μg^{-1})	333 ± 6	10 778 ± 1 010	720 ± 23	26 501 ± 1 113	365 ± 8	2125 ± 22

Table 4.22. Detection Limits of Cd, Co, Cu, Mn, Ni and Zn (ppb)*

Element/Nuclide	^{111m}Cd	^{60m}Co	^{66}Cu	^{56}Mn	^{65}Ni	^{69m}Zn
Conventional γ-Spectroscopy	8.19 ± 0.13	0.385 ± 0.004	2.42 ± 0.04	0.080 ± 0.001	23.9 ±0.3	10.8 ± 0.6

*results are the average of 10 determinations

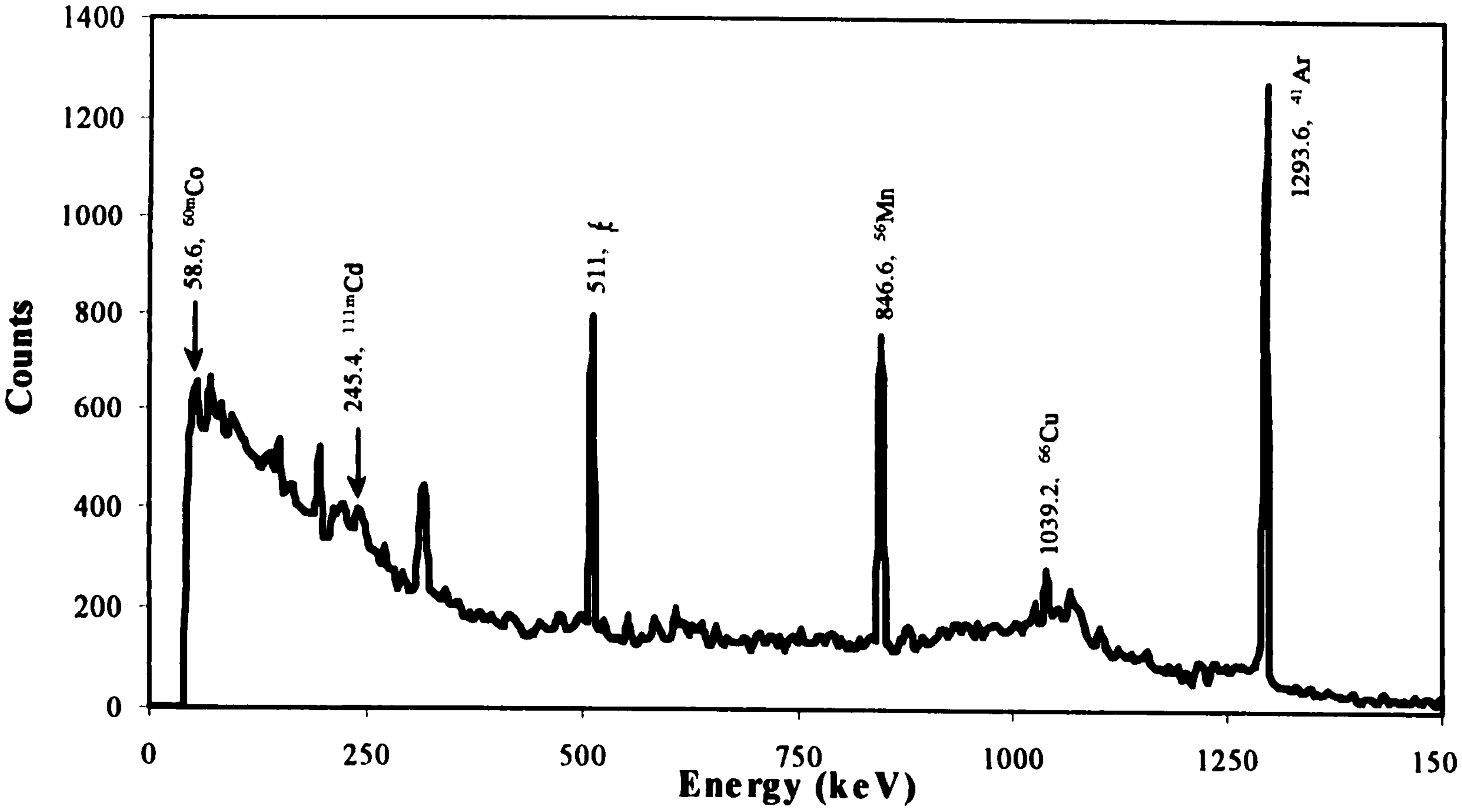


Figure 4.19. Gamma-ray Spectrum of a Solution Spiked with Cd, Co, Cu, Mn, Ni and Zn (t_i=10 min)

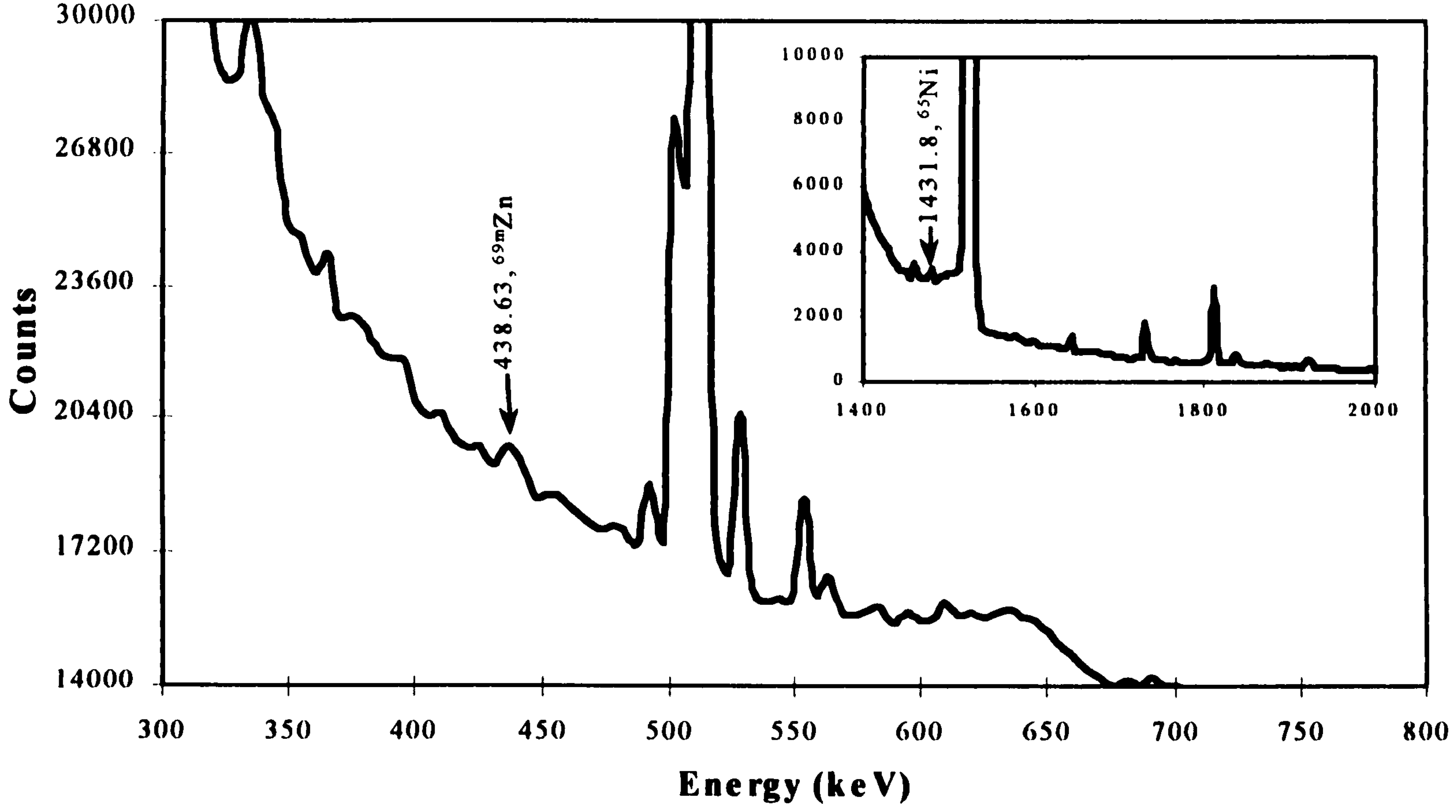


Figure 4.20. Gamma-ray Spectrum of a Solution Spiked with Cd, Co, Cu, Mn, Ni and Zn (t_i=3 h)

4.6 Simultaneous CPE of Lanthanides

Lanthanides (*a.k.a.* rare earth elements, REE) are being increasingly used in many industries including glass and ceramic, metallurgy, nuclear, electronics, and more recently for the preparation of superconductor materials [87]. Combustion of fossil fuels also introduces large amounts of lanthanides in the atmosphere. These activities are continuously increasing the levels of lanthanides in the environment. Their determination has also become of analytical importance. Due to the low concentrations of lanthanides, even modern analytical techniques like ICP-MS and NAA may require a preconcentration step.

Classical spectrophotometric techniques are useful for the determination of the total amount of lanthanides. Simultaneous determination of several individual elements can be very difficult due to the unavailability of selective chromogenic reactants. Other well-known techniques such as AAS and atomic emission spectroscopy (AES) are of restricted use because these elements, besides presenting complex absorption and emission spectra, have a tendency to form refractory oxides as well as to undergo ionization [117].

The determination of lanthanide ions in aquatic samples usually requires a preconcentration step since most of these elements are present in concentrations close to or below the detection limit of the analytical technique usually available. Preconcentration techniques currently used for REE determination in water include coprecipitation [118], ion exchange [119], liquid-liquid extraction [120] and extraction chromatography [121], absorption onto silica-immobilized 8-hydroxyquinoline or immobilized bacteria [122, 123]. Chelating resins have been one of the most popular preconcentration media for trace amounts of REE in the last few years; however, an extra step is also required here to remove Mg^{2+} and Ca^{2+}. These procedures increase sample manipulation and possible contamination [124]. A CPE method has been developed in this work for the simultaneous extraction of 12 lanthanides which were determined afterwards by NAA.

In order to determine the lanthanides with a minimum spectral interference from the overlapping γ-ray peaks and the minimum possible background, three different timing schemes were used: 3h, 1h, 1h (Dy, Er, Eu, La, Pr, Sm, Tb); 3h, 24h, 1h (Ho, Gd); 3h, 5d, 1h (Lu, Tm, Yb). The nuclear characteristics of the lanthanides under study are presented in Table 4.23.

4.6.1 Optimization of the CPE Procedure

The parameters optimized were solution pH, and concentrations of the chelating agent and surfactant. This last factor was included to evaluate the possible influence of the capacity (effective volume) of the surfactant-rich phase on the efficiency of the extraction when a large number of elements is included.

4.6.1.1 Effect of pH

Based on the pH values reported for the extraction of lanthanides with PAN [30, 78], the pH interval tested in the present work was between 5.5 and 9.5. Simultaneous extraction was achieved for 12 elements (namely, Dy, Er, Eu, Gd, Ho, La, Lu, Pr, Sm, Tb, Tm and Yb) at pH 8.5 (Fig. 4.21). Quantitative recoveries were obtained for most of

Table 4.23. Nuclear Data of Lanthanides [104]

Target Nuclide	Isotopic Abundance (%)	Thermal Neutron Cross Section ($\times 10^{-24}$ cm^2)	Nuclear Reaction	Half-life of Nuclide Produced	γ-ray Used (keV)
^{164}Dy	28.2	1000	^{164}Dy (n,γ)^{165}Dy	2.35 h	94.7
^{170}Er	15.0	5.72	^{170}Er (n,γ)^{171}Er	7.52 h	308.3
^{151}Eu	47.8	3300	^{151}Eu (n,γ)^{152m}Eu	9.3 h	121.8
^{158}Gd	24.7	2.5	^{158}Gd (n,γ)^{159}Gd	18.6 h	363.6
^{165}Ho	100	63	^{165}Ho (n,γ)^{166}Ho	26.8 h	49.1
^{139}La	99.91	9.0	^{139}La (n,γ)^{140}La	40.23 h	487.0
^{176}Lu	2.6	2050	^{176}Lu (n,γ)^{177}Lu	6.71 d	208.0
^{141}Pr	100	7.6	^{141}Pr (n,γ)^{142}Pr	19.16 h	1575.9
^{152}Sm	26.7	206	^{152}Sm (n,γ)^{153}Sm	46.5 h	103.2
^{159}Tb	100	25.5	^{159}Tb (n,γ)^{160}Tb	72.3 d	879.4
^{169}Tm	100	103	^{169}Tm (n,γ)^{170}Tm	128.6 d	84.3
^{174}Yb	31.8	19	^{174}Yb (n,γ)^{175}Yb	4.19 d	396.0

the elements except Er, which suggests that the CPE procedure can be effectively used for the analysis of traces of lanthanides.

It is interesting to point out that Dy, La and Pr are not extracted at pH 7, while Eu, Gd, Ho, Tb, Tm and Yb are quantitatively extracted. This feature can be advantageously used as a separation technique between these two groups. This can be particularly useful in the case of La, since most geological and environmental samples contain large amounts of La that can induce interference for the initial equilibrium process and the subsequent irradiation and counting steps.

In this work, special attention was paid to select appropriate counting schemes and γ-ray energies for reliable measurement. However, the large total background obtained as a result of the combined activities of the different radionuclides present in the sample made the integration and definition of the peaks more difficult. This problem can appear when working with many elements of high sensitivity in NAA, such as some of the lanthanides, and is partially responsible for the high standard deviations that some elements show at particular values.

4.6.1.2 Effect of Concentration of PAN

The study on the influence of the concentration of PAN on the CPE of lanthanides showed a quantitative recovery at 1×10^{-4} M for most elements, except for Er whose recovery increases when less PAN is used. Nevertheless, it is possible to get fairly high recoveries at lower concentrations of PAN for Dy, Eu, Ho, Lu, Pr, Sm, Tb, Tm, and Yb (Table 4.24).

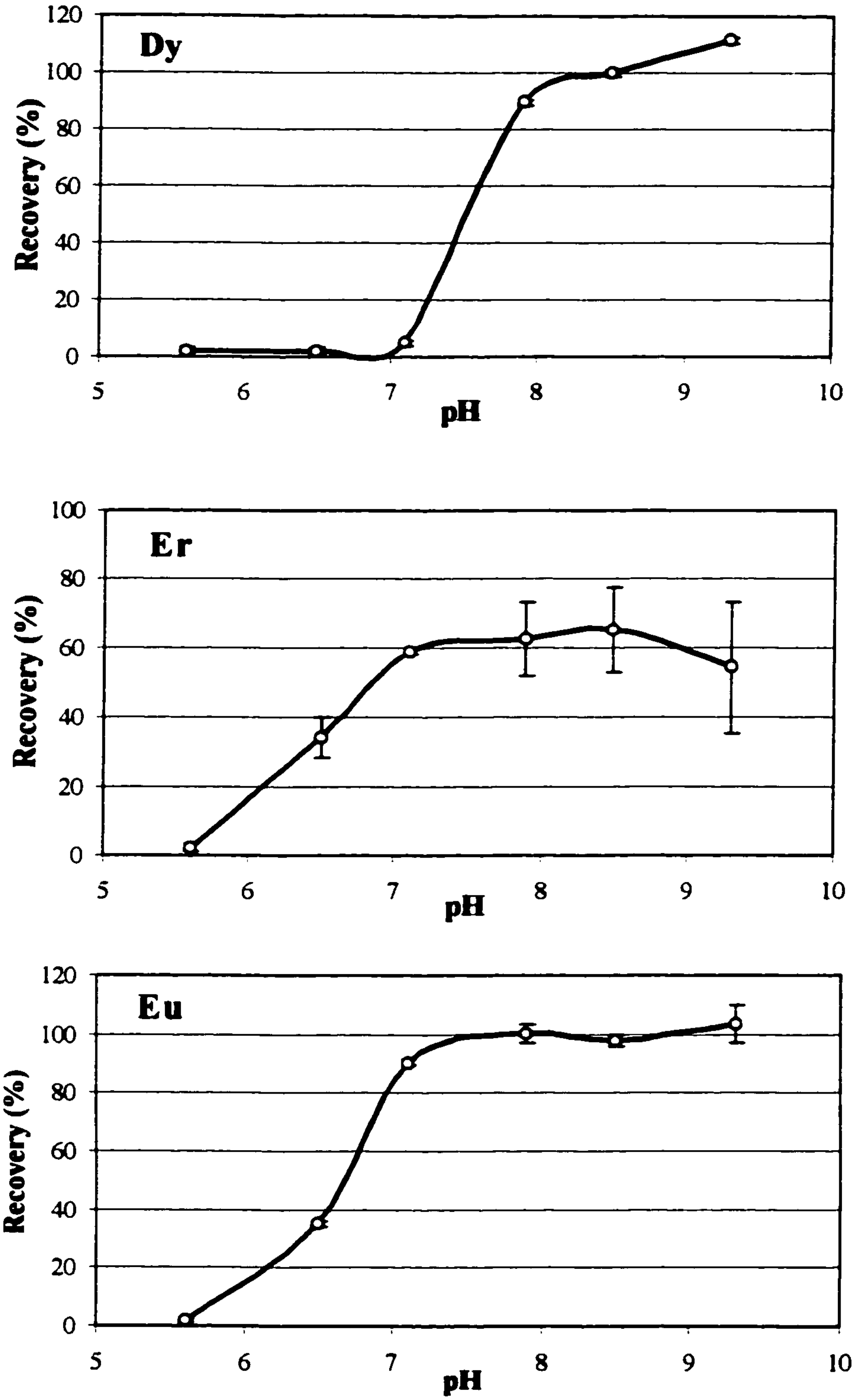


Figure 4.21. Effect of pH on CPE of Lanthanides
(results are the average of 3 determinations)

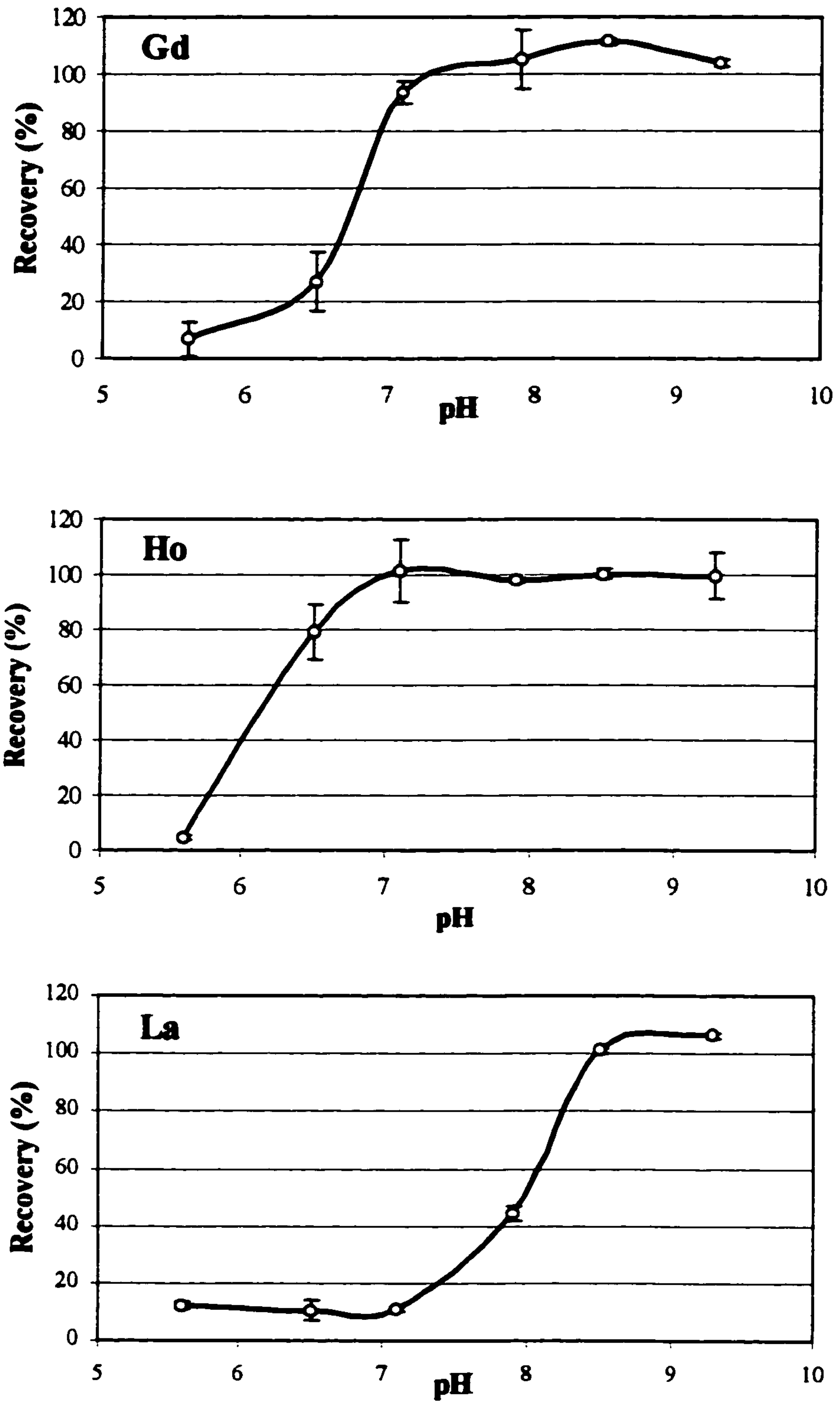


Figure 4.21 (*continued*). Effect of pH on CPE of Lanthanides (results are the average of 3 determinations)

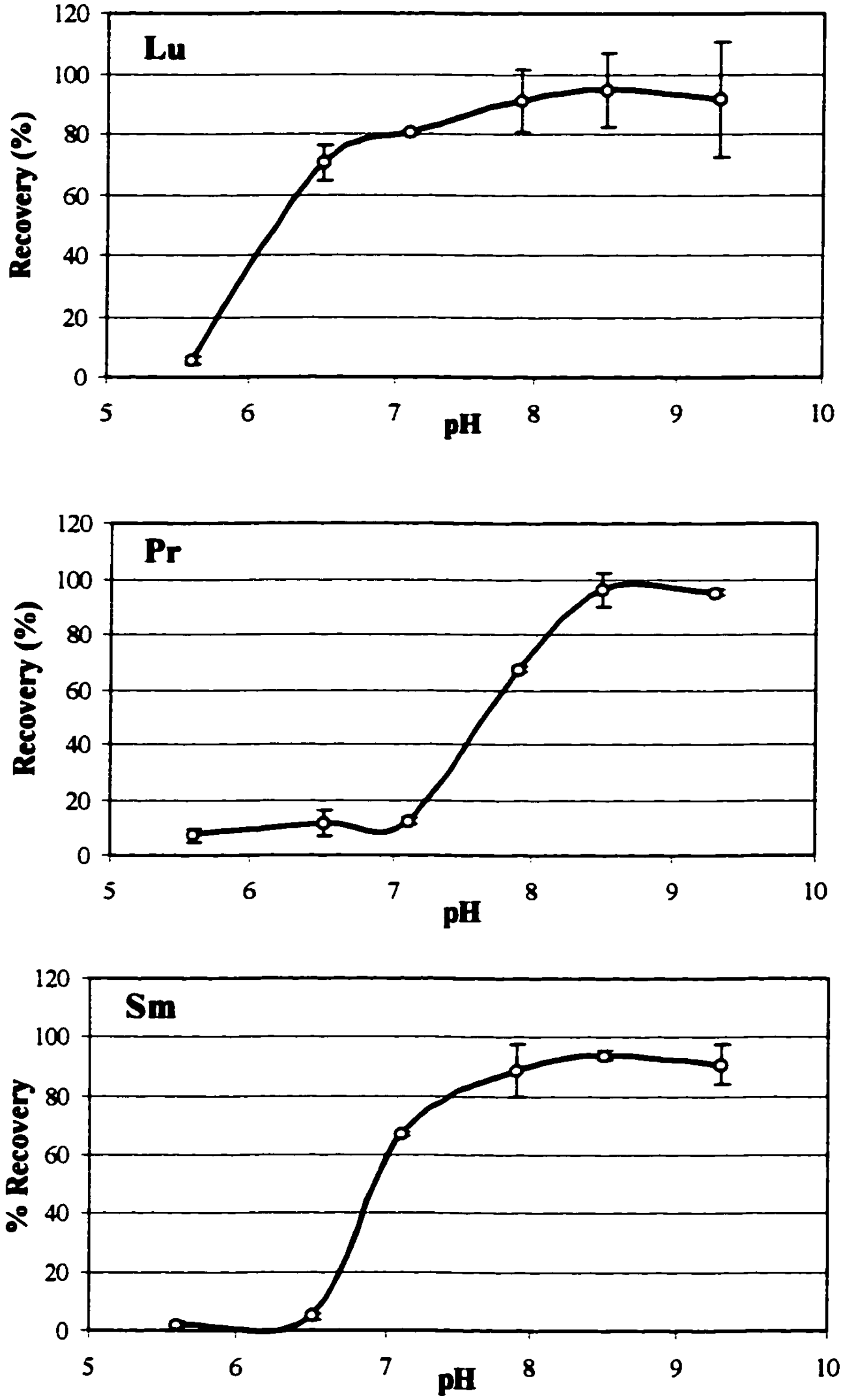


Figure 4.21 (*continued*). Effect of pH on CPE of Lanthanides
(results are the average of 3 determinations)

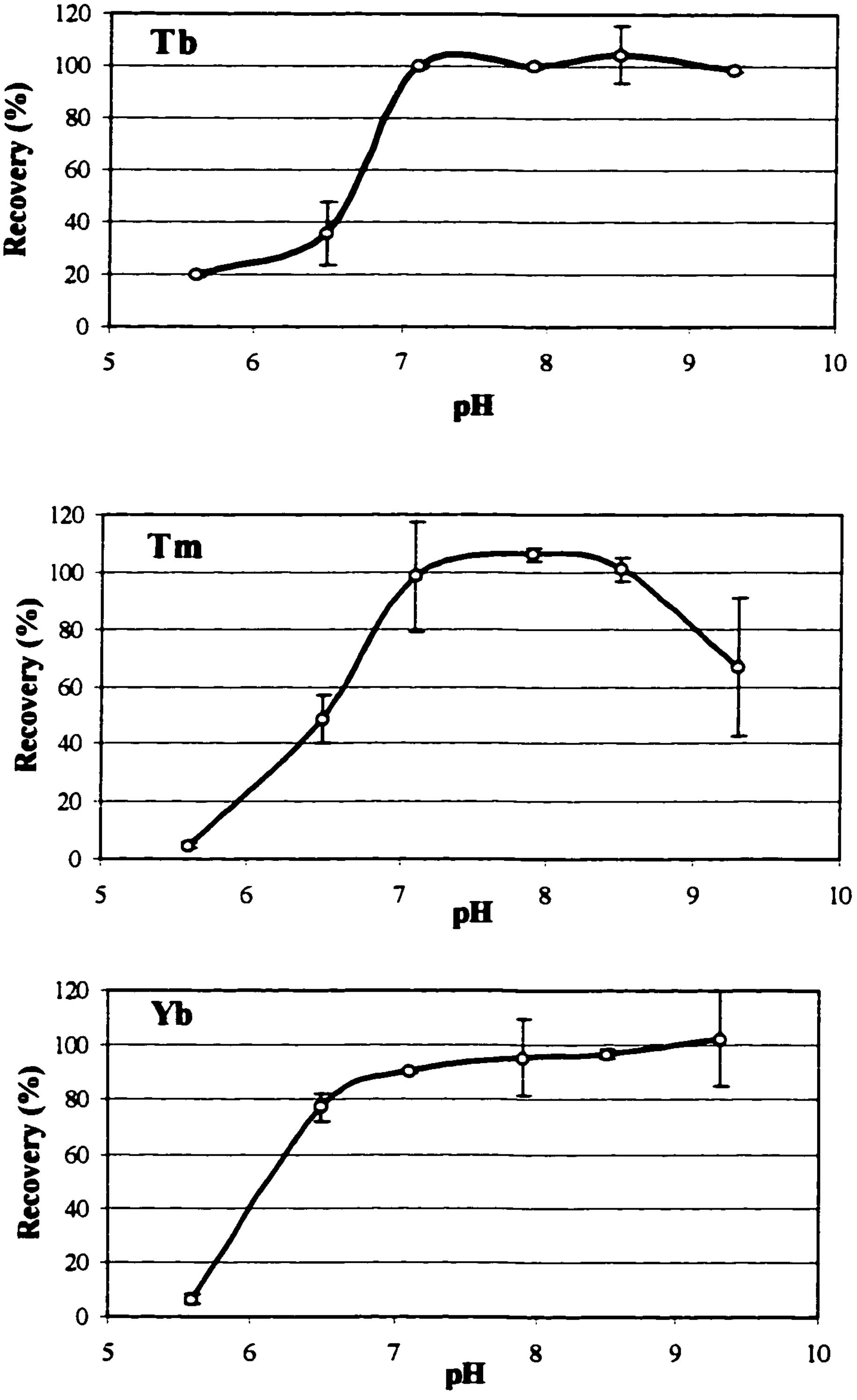


Figure 4.21 (*continued*). Effect of pH on CPE of Lanthanides
(results are the average of 3 determinations)

Lanthanum showed a different behavior, producing a very low recovery at 5×10^{-5} M of PAN, probably due to a lower stability constant in comparison with the rest of the lanthanides. This behavior, in combination with the low recovery at a pH lower than 7, can be useful to extract selectively other elements in the series in presence of large amounts of La. By adjusting the concentration of the chelating agent, spectral interferences can also be minimized and the total background can be decreased.

4.6.1.3 Effect of Concentration of Surfactant

Corroborating the results obtained in Section 4.3.1.5 for Cu, the concentration of the surfactant does not seem to play an important role under the present conditions for the preconcentration of lanthanides by CPE. The recoveries obtained (Table 4.25) are very similar at the three concentrations investigated (0.05%, 0.1% and 0.2%). The slight decrease observed at a concentration of 0.2% is probably due to the increase in the surfactant-rich phase volume at this concentration (reported in Section 4.1.2).

4.6.2 Uranium Interference in Determination of Lanthanides

It has been reported that spectral interferences from U-fission in NAA can cause analytical problems in the determination of some elements [125, 126]. These spectral interferences occur when the product nuclides are identical to the activated nuclides being analyzed or when their characteristic γ-rays have energies very close to the analytical peaks of the activated nuclides.

Table 4.24. Influence of the PAN Concentration on CPE of Lanthanides*

Element	Recovery (%) at Selected PAN Concentrations			
	5×10^{-5} M	7.5×10^{-5} M	1×10^{-4} M	2.5×10^{-4} M
Dy	98 ± 2	96 ± 2	100 ± 1	78 ± 1
Er	84 ± 13	74 ± 9	65 ± 12	41 ± 18
Eu	98 ± 2	107 ± 2	98 ± 2	76 ± 13
Gd	84 ± 1	90 ± 3	111 ± 1	87 ± 14
Ho	99 ± 7	101 ± 1	100 ± 2	86 ± 6
La	15 ± 4	29 ± 6	101 ± 1	89 ± 3
Lu	98 ± 1	97 ± 1	95 ±1	71 ± 1
Pr	94 ± 13	108 ± 1	96 ± 6	82 ± 6
Sm	101 ± 1	95 ± 2	94 ± 2	69 ± 18
Tb	104 ± 7	104 ± 11	104 ± 12	93 ± 2
Tm	89 ± 12	86 ± 8	101 ± 4	64 ± 6
Yb	101 ± 4	101 ± 5	97 ± 2	72 ± 5

*results are the average of 3 determinations

Table 4.25. **Influence of the Surfactant Concentration on CPE of Lanthanides**[*]

Element	Recovery (%) at Selected Surfactant Concentrations (% wt)		
	0.05%	0.10%	0.20%
Dy	90 ± 5	100 ± 1	96 ± 1
Er	66 ± 8	65 ± 12	58 ± 10
Eu	98 ± 3	98 ± 2	89 ± 2
Gd	98 ± 5	111 ± 1	89 ± 5
Ho	96 ± 4	100 ± 2	87 ± 1
La	87 ± 7	101 ± 1	81 ± 9
Lu	89 ± 3	95 ±1	86 ± 1
Pr	93 ± 9	96 ± 6	100 ± 3
Sm	88 ± 4	94 ± 2	88 ± 4
Tb	100 ± 7	104 ± 11	94 ± 3
Tm	97 ± 3	101 ± 4	83 ± 9
Yb	94 ± 5	97 ± 2	92 ± 3

[*] results are the average of 3 determinations

Ila *et al.* reported a detailed study on U and U-fission interferences in multielement analysis of uraniferous rocks by NAA [127]. The elements most affected by the spectral interferences were Ce, La, Nd, and Sm. Most fission product contributions, as well as the content of U, were evaluated by using the 277-keV γ-ray of ^{239}Np, a decay-product of the fission of ^{239}U. The authors recommended that, in routine analysis, the need to irradiate the U standard each time for the purpose of determining the fission interference can be avoided if the experimental conditions are normalized to some preset standard conditions.

Since no U was added to the multielement standards used in the CPE study described here, no spectral interference was considered in the analysis of the results. Nevertheless, the frequent presence of U in materials containing lanthanides suggests the necessity of correction factors to account for these interferences when applying the optimized CPE procedure to the analysis of samples.

About 10 μg of U were extracted using the CPE procedure and the surfactant-rich phase was irradiated for 2 h. The results using different decay times (same as described earlier in this Section) showed a major spectral interference of ^{239}Np with the analytical peak characteristic of ^{153}Sm (103.2 keV). If relatively large amounts of U are present in the sample, this interference may also affect the resolution of the 94.7 keV-photopeak (^{165}Dy). These interference effects can be corrected using correction factors, which will depend on the neutron flux as well as the counting geometry and the timing schemes used for the analysis.

4.6.3 Quality Assurance

4.6.3.1 Internal Quality Assessment

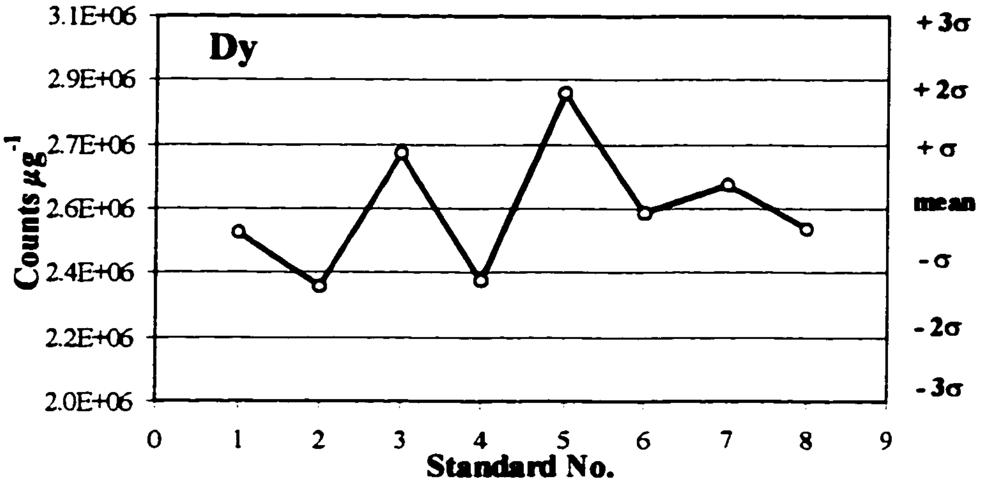
The Schewart control charts were constructed for the multielement standards of lanthanides used in this work (Fig. 4.22). The majority of the experimental points are within the warning limits in all charts and the error was kept below 6%, with the exception of Dy (8%). The 94.7-keV photopeak of ^{165}Dy is located in a high background region. This peak is also very close to the peaks produced by the X-rays from the Pb-shielding of the detector, which makes the integration procedure more difficult. However, the error associated with the counting statistics alone is usually considered to be around 10%.

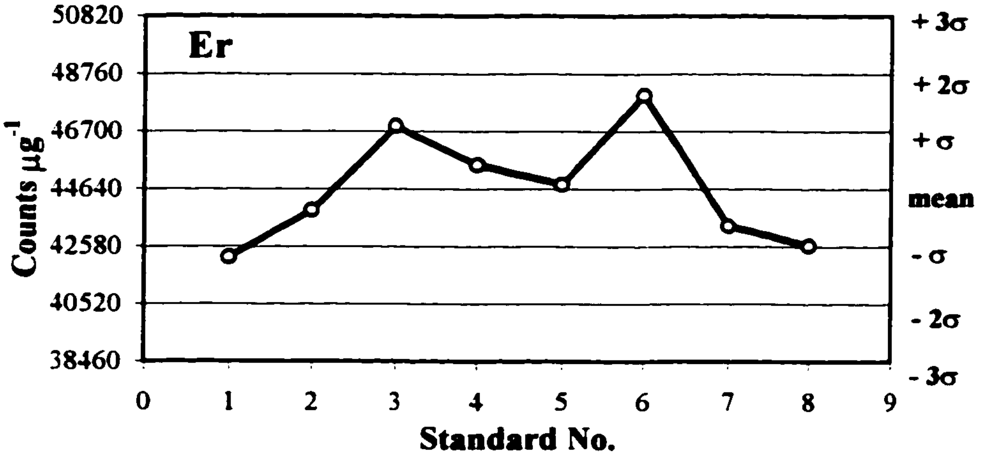
4.6.4 Sensitivity and Detection Limits

Preconcentration of lanthanides using CPE has not yet been reported extensively in the literature. There are only two recent papers found on the use of CPE for the preconcentration of Er and Gd among the lanthanides.

The CPE extraction of Er(III) with 2-(3,5-dichloro-2-pyridylazo)-5-dimethyl aminophenol as the chelating agent and PONPE-7.5 at 0.01% (wt) has been reported by Silva *et al.*[78]. The detection limit of Er was reported as 24.8 ppb.

A similar CPE procedure was reported by Olsina *et al.* for the determination and monitoring Gd in urine samples, following the administration of Gd-based pharmaceuticals [79]. Using a microscale CPE protocol, the total and free Gd(III) content can be determined. The limit of detection for Gd(III) was reported as 0.912 ppb.





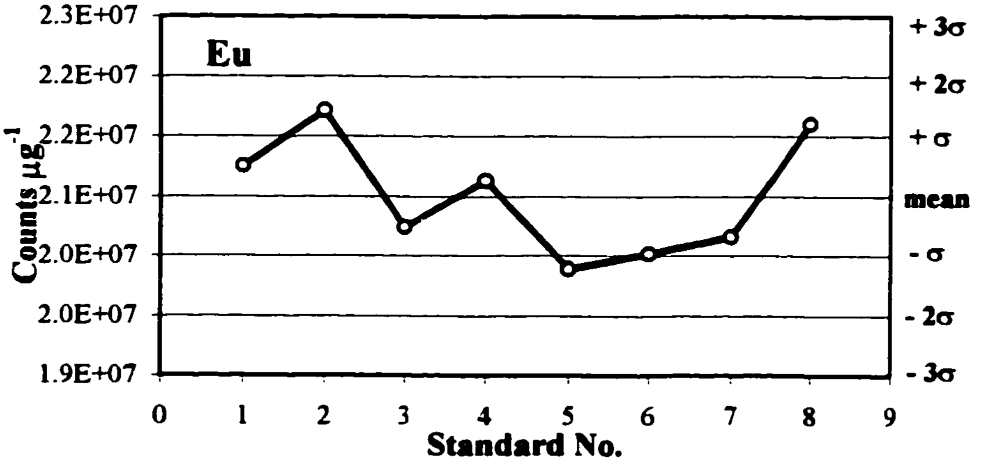
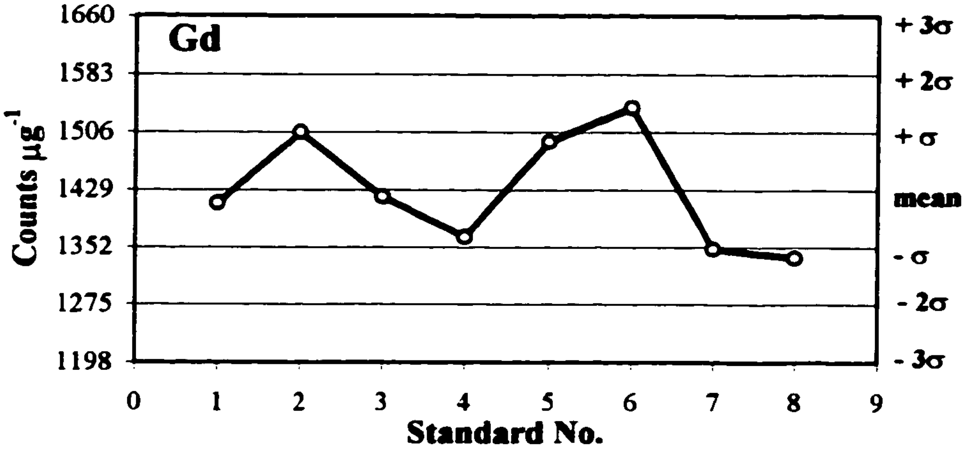
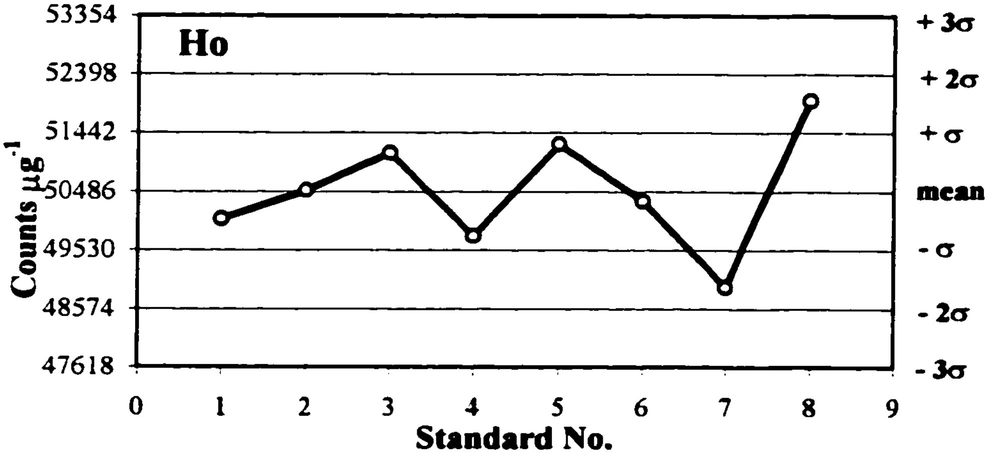


Figure 4.22. Schewart Control Charts for Internal Quality Assessment of Lanthanides





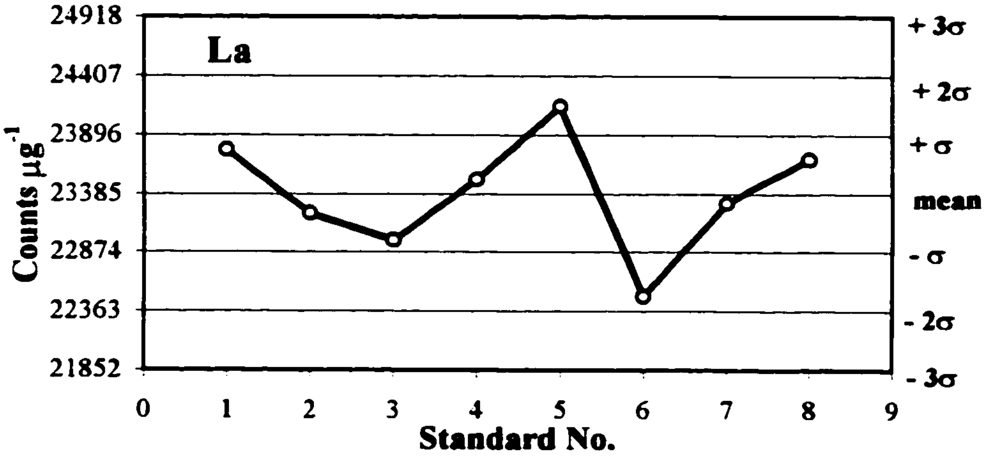
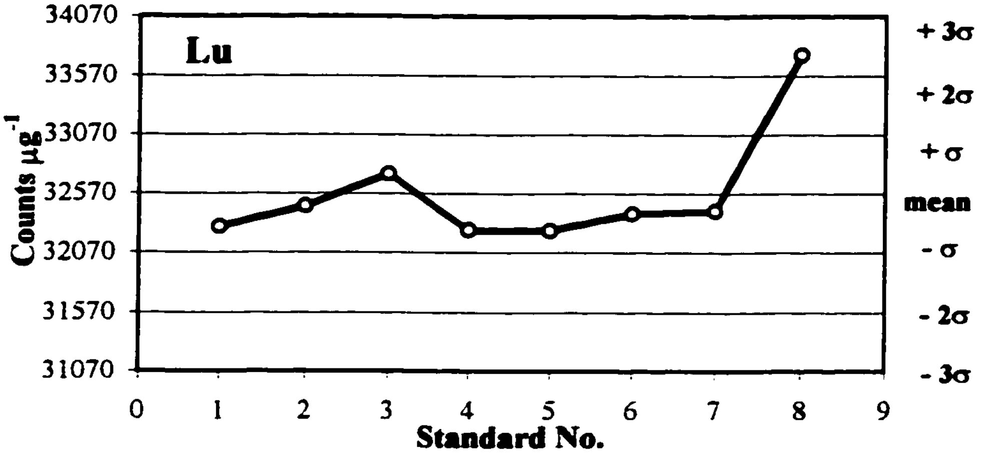
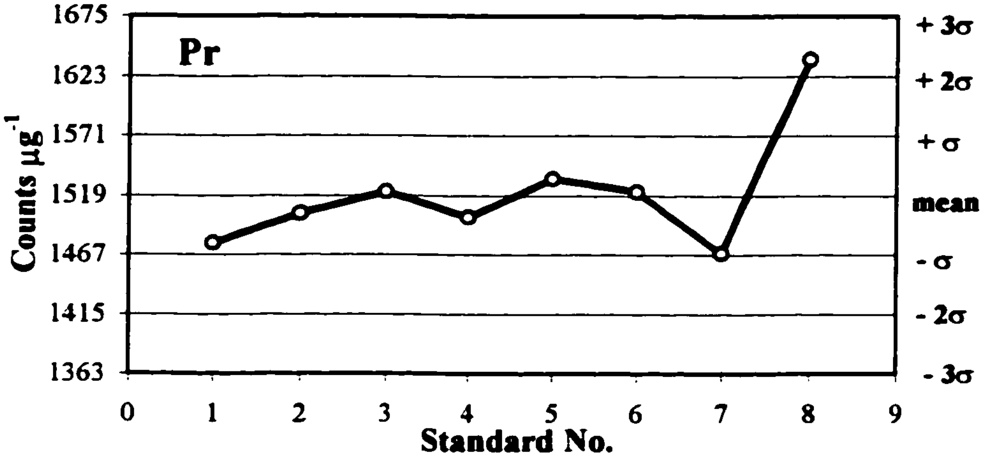


Figure 4.22 (*continued*). Schewart Control Charts for Internal Quality Assessment of Lanthanides





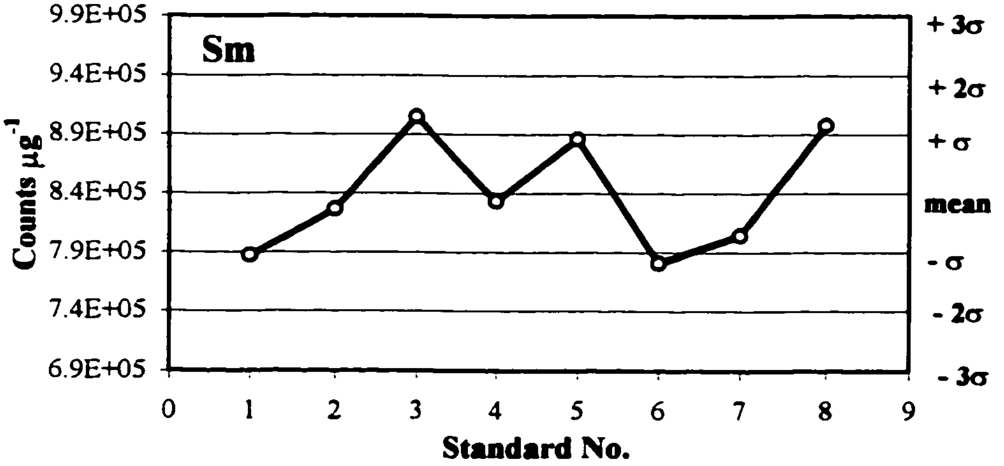
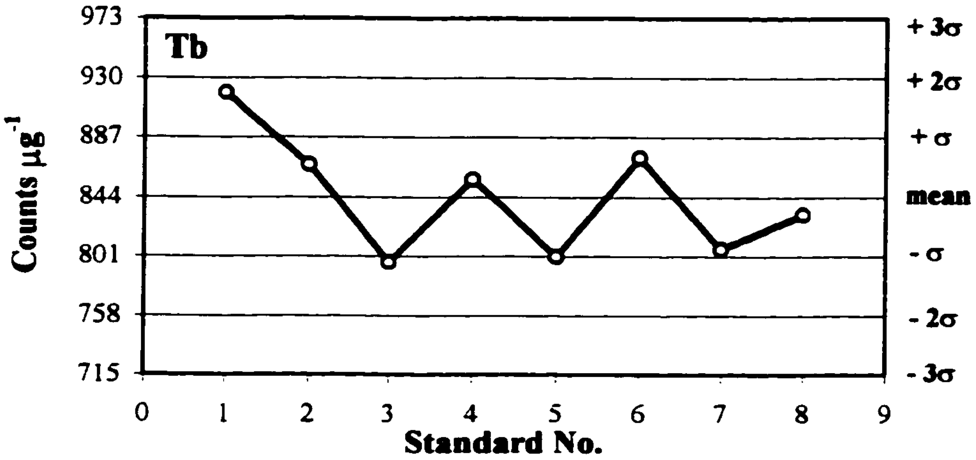
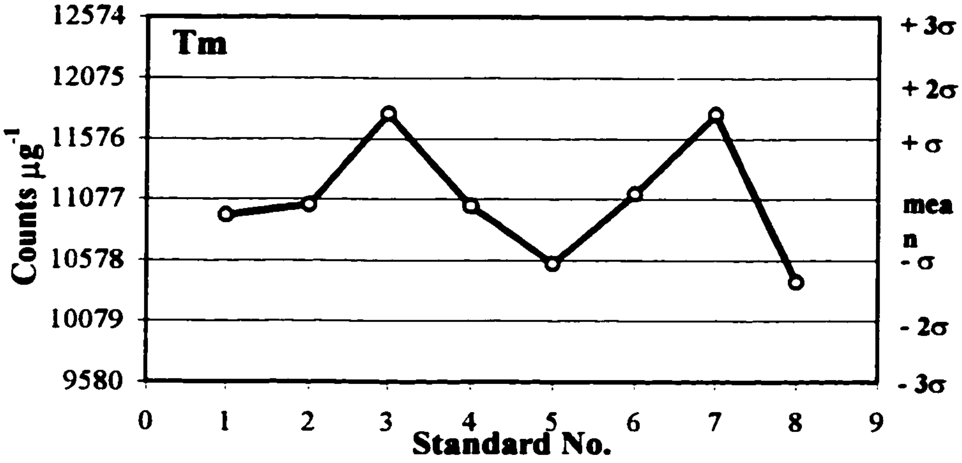


Figure 4.22 (*continued*). Schewart Control Charts for Internal Quality Assessment of Lanthanides





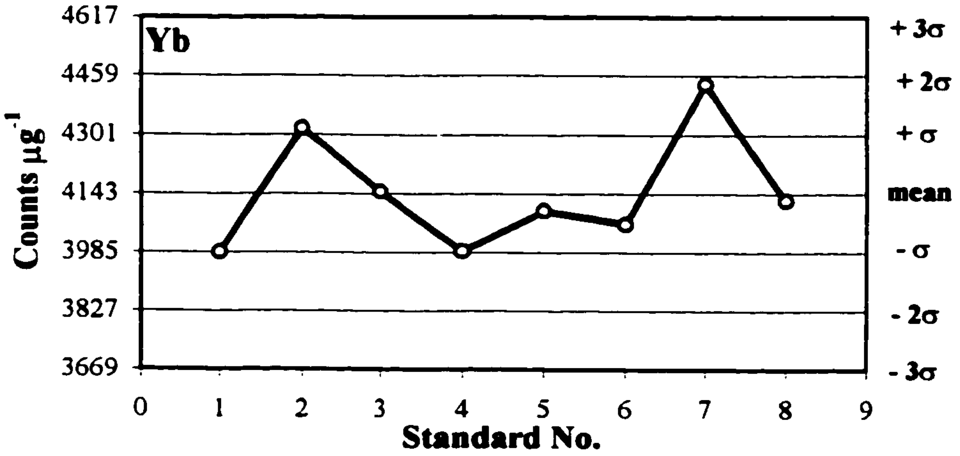


Figure 4.22 (*continued*). Schewart Control Charts for Internal Quality Assessment of Lanthanides

Most procedures used for preconcentration and/or determination of lanthanides usually report the results as the total content in the sample, since the separation and analysis of single elements can be very time-consuming and difficult [128]. The CPE procedure presented here offers the possibility of simultaneous determinations of the individual lanthanides. The sensitivities and the detection limits obtained are presented in Table 4.26. Most of the results are near to the 1-ppb mark, which can be very useful for the determination of the lanthanides in environmental and biological samples. The standard deviations reported represent less than a 3% error. The spectra of a spiked solution used in the optimization of the CPE of lanthanides are shown in Figs. 4.23 to 4.25.

4.7 Other Possible Applications

The use of surfactants as agents for compartmentalization has countless possibilities of applications. The peculiar properties that characterize these systems have been used to extract biomolecules [129] and purify proteins [130] as part of multi-step procedures. Other novel applications include the use of the surfactant-rich phase as a medium for the room temperature phosphorescence (RTP) determination of Pd(II)-complexes due to the high permeability of this phase [82], as well as the direct extraction of organics from soil and sediment samples [67].

In order to explore other possible applications of the CPE procedure reported here, two studies were considered. One involves the use of another chelating agent, namely TAN, to enhance the recovery of low-stability PAN complexes, while the other refers to the possibility of preconcentration metals in seawater by CPE.

Table 4.26. Sensitivity and Detection Limits of Lanthanides*

Element	Sensitivities (counts μg^{-1})	Detection Limit (ppb)
Dy	$(2.6 \pm 0.2) \times 10^6$	1.76 ± 0.03
Er	44 640 ± 2 060	1.72 ± 0.03
Eu	$(2.09 \pm 0.06) \times 10^7$	3.07 ± 0.02
Gd	1 429 ± 77	0.595 ± 0.006
Ho	50 486 ± 956	0.867 ± 0.004
La	23 385 ± 511	1.39 ± 0.05
Lu	32 570 ± 500	0.575 ± 0.007
Pr	1 519 ± 52	0.295 ± 0.003
Sm	$(8.4 \pm 0.5) \times 10^5$	2.91 ± 0.05
Tb	844 ± 43	1.136 ± 0.007
Tm	11 077 ± 499	0.978 ± 0.005
Yb	4 143 ± 158	0.270 ± 0.008

*results are the average of 9 determinations

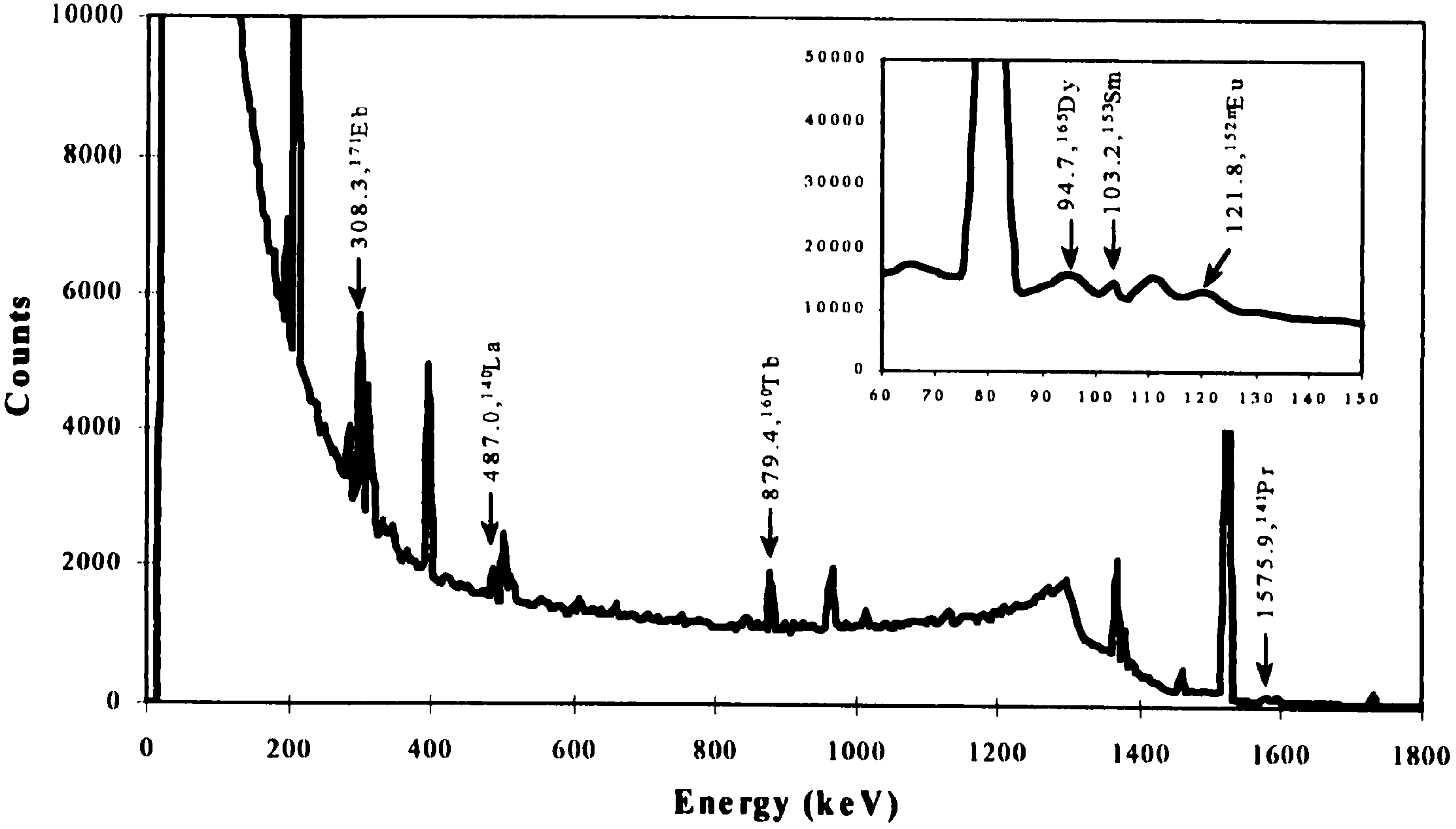


Figure 4.23. Gamma-ray Spectrum of a Solution Spiked with Lanthanides (t_d=1 h)

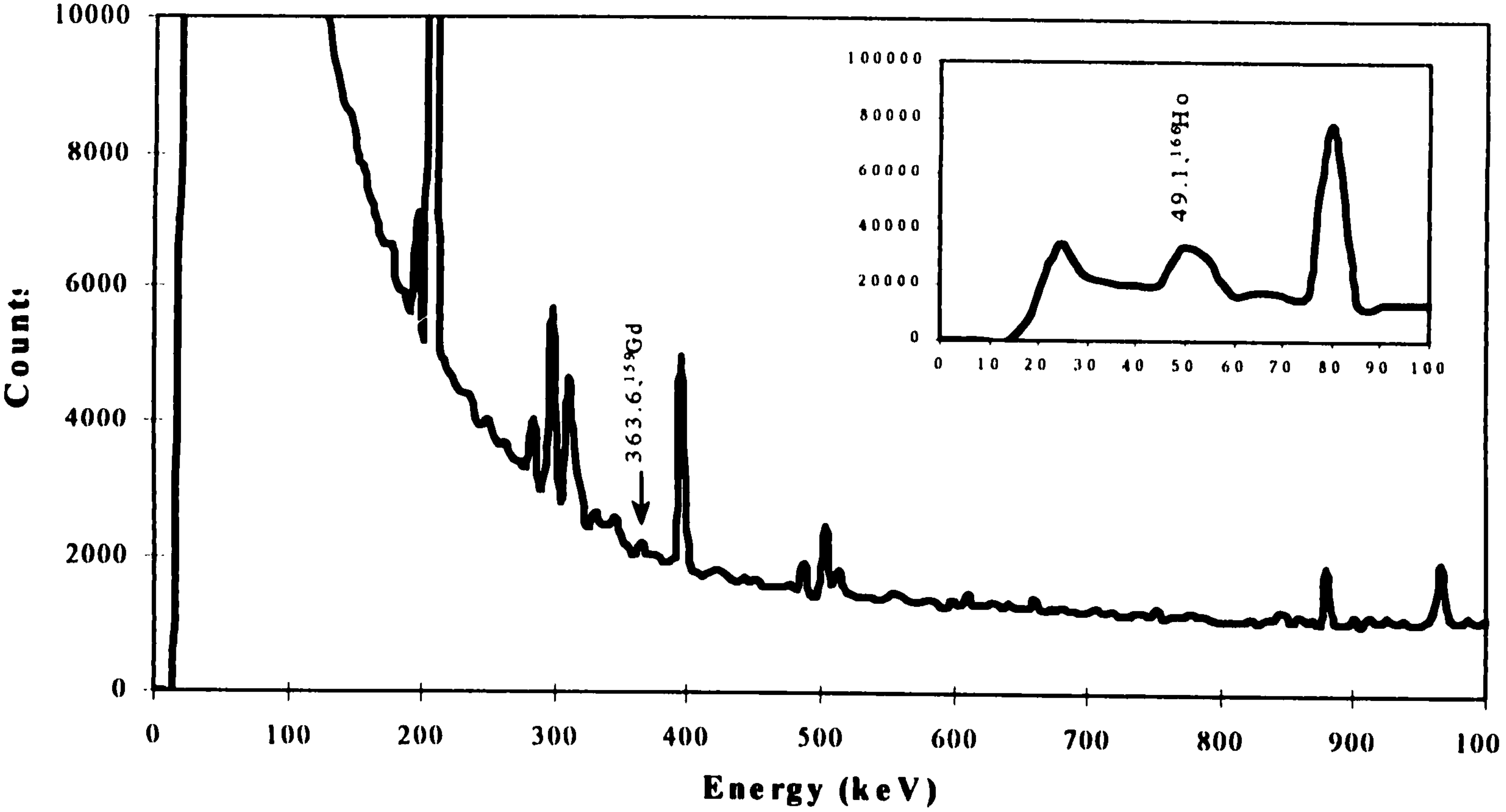


Figure 4.24. Gamma-ray Spectrum of a Solution Spiked with Lanthanides (t_d=24 h)

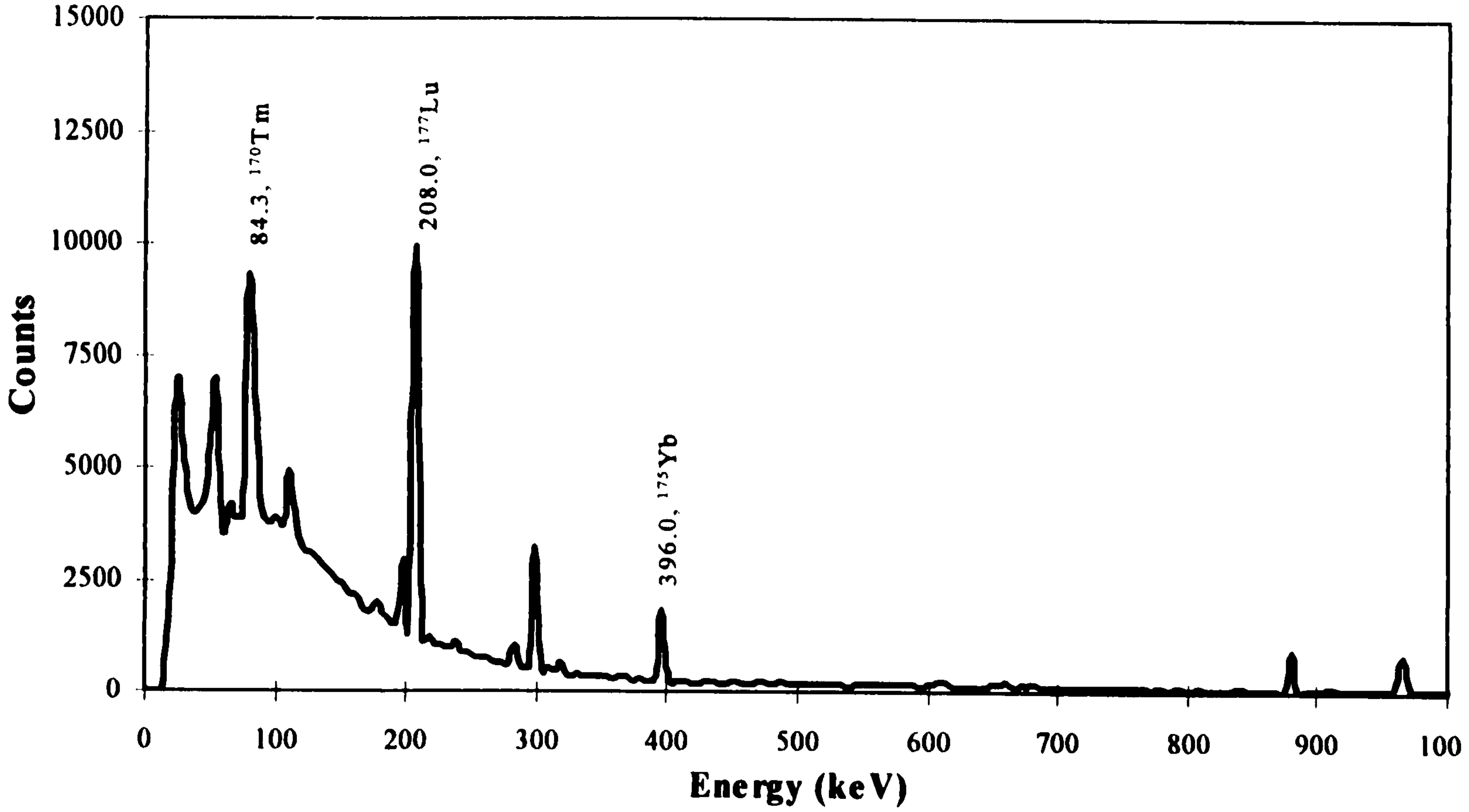


Figure 4.25. Gamma-ray Spectrum of a Solution Spiked with Lanthanides (t_d=5 d)

4.7.1 Use of a Mixture of PAN and TAN in CPE

Despite the variety of metals that can form complexes or precipitates with PAN, not all of them have significantly high stability constants. Some of the heavy metals require large amounts of the reagent in order to quantitatively form chelates that can be extracted into an organic phase. This is particularly inconvenient in the work with aqueous environmental samples since other complexing agents (*e.g.* humic acids, hydroxy ions, *etc.*) can also be present in measurable amounts affecting the conditional formation constant of the chelate [111].

The CPE of Hg(II) was first attempted using PAN as the chelating agent. Recoveries of only 30% were found after the optimization of the experimental conditions. Based on the importance of the complex formation equilibrium in the CPE procedure, a different chelating agent was considered in order to improve the extraction of Hg.

4.7.1.1 TAN as an Analytical Reagent

The chelating agent 1-(2-thiazolylazo)-2-naphthol (TAN) was synthesized first in the mid-1950's by Boni and Hammer [131] as one of a series of azo derivatives of thiazole. The structure of TAN is similar to most of the azo-naphthol-based chelating agents (Fig. 4.26). The centers of coordination of the molecule with metal ions are usually the deprotonated naphthol oxygen, the nitrogen of the thiazole ring, and the azo nitrogen adjacent to the naphthol ring [132], although the sulfur atom of the thiazole ring can potentially act also as a donor.

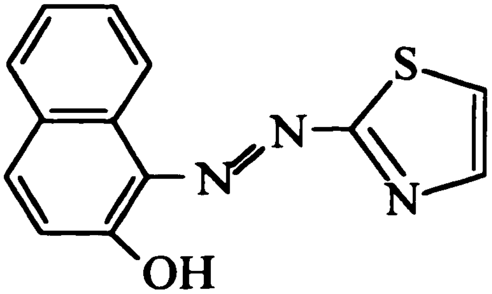


Figure 4.26. Chemical Structure of 1-(2-thiazolylazo)-2-naphthol (TAN) [132]

Although TAN was one of the first chelating agents used for CPE procedures [14], most other applications of TAN deal with the use of thiazolylazo derivatives. These chelating agents appear to be more selective and offer higher stability constants for specific elements [57, 61].

Mercury (I) and (II) have been reported to form stable complexes with TAN at pH>7 [133]. A combined CPE extraction was attempted for Cd, Hg and Zn using PAN and TAN as chelating agents. The characteristics of the nuclides of interest are given in Table 4.27.

Table 4.27. Nuclear Data of Cd, Hg and Zn [104]

Target Nuclide	Isotopic Abundance (%)	Thermal Neutron Cross Section (x 10^{-24} cm^2)	Nuclear Reaction	Half-life of Nuclide Produced	γ-ray Used (keV)
^{114}Cd	28.8	0.3	$^{114}Cd(n,\gamma)^{115}Cd$	53.5 h	336.3
^{202}Hg	29.7	4.9	$^{202}Hg(n,\gamma)^{203}Hg$	46.59 d	279.17
^{64}Zn	48.9	0.8	$^{64}Zn(n,\gamma)^{65}Zn$	243.8 d	1115.5

4.7.1.2 Combined CPE Procedure

The CPE procedure was developed using the same optimized conditions reported in Section 4.5, at pH 8.7 and using a 1:1 TAN:PAN mixture (v/v) with a total ligand concentration of 5×10^{-5} M. Different timing schemes were used in order to achieve maximum sensitivity (Table 4.28).

Table 4.28. Results from Combined CPE of Cd, Hg and Zn with TAN and PAN*

Element	Cd	Hg	Zn
Timing Scheme (t_i, t_d, t_c)	10min, 1 min, 10 min	3 h, 7 d, 2 h	3 h, 1 h, 2 h
Recovery (%)	100.0 ± 0.2	99 ± 3	95 ± 5

*results are the average of 3 determinations

Quantitative recoveries were obtained for the simultaneous CPE of Cd, Hg, and Zn with TAN and PAN. An optimization of the procedure was not necessary since the results were satisfactory in the first attempt. The spectrum of a spiked sample used in the analysis is shown in Fig. 4.27.

This preliminary study on CPE using a combination of chelating agents can be extended to the use of other reagents that complement each other. In this way, a larger number of elements can be extracted in only one preconcentration step. TAN seems to be a superior chelating agent for Hg compared to PAN since the sulfur atom contained in its structure is a soft-type ligand which interacts with a soft-type metal ion like Hg(II) better than a strong-type ligand such as nitrogen.

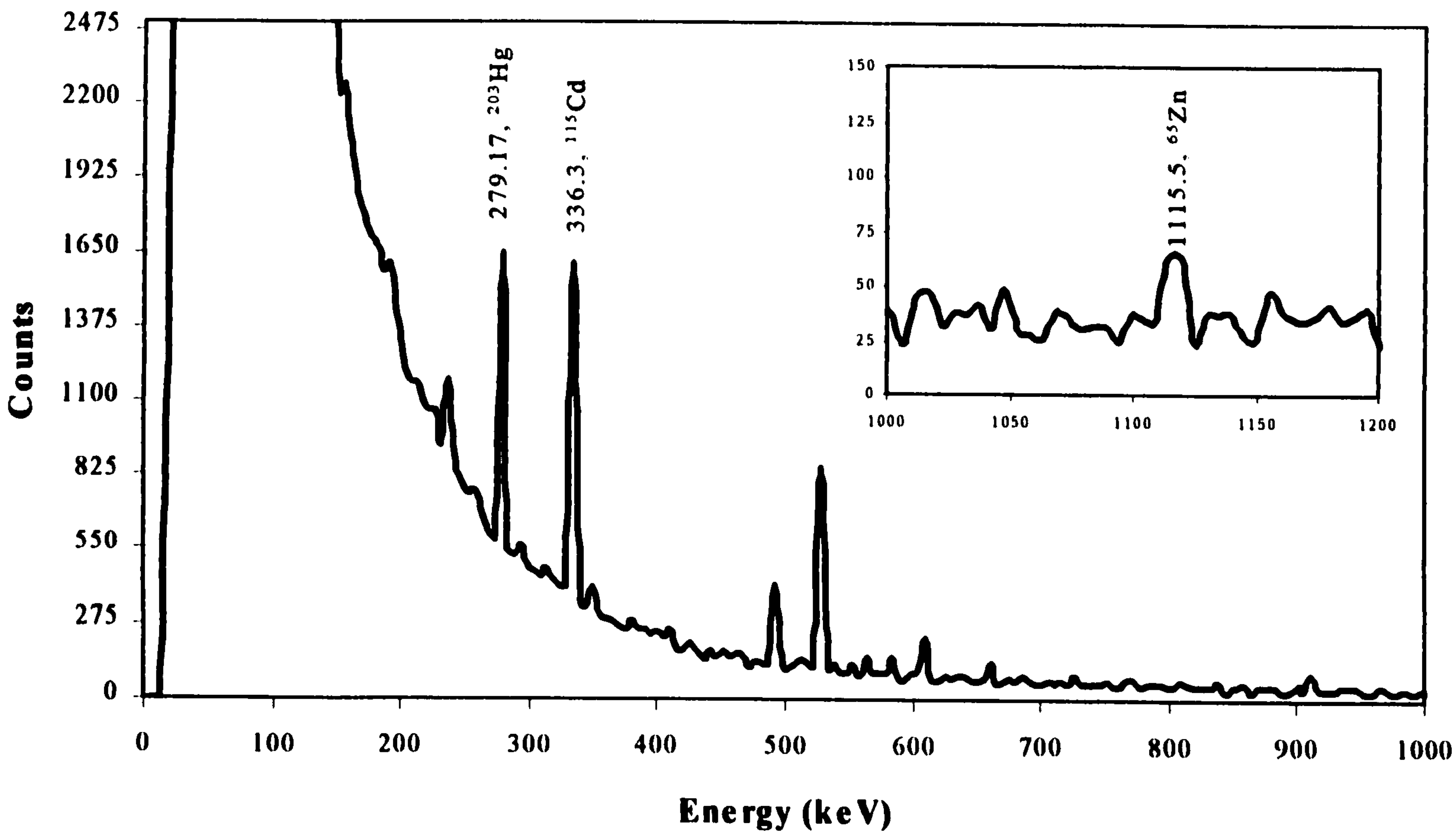


Figure 4.27. Gamma-ray Spectrum of a Solution Spiked with Cd, Hg and Zn

The improvement in the extraction recovery can probably be attributed also to the formation of PAN/TAN mixed complexes with the metal ions. It has been reported that the formation of mixed complexes can be a method to increase the rate of extraction. For example, in the presence of pyridine [134] or 1,10-phenanthroline (phen) [135], the rate of extraction of Ni-dithizone mixed complexes is very different from that of Ni-dithizonate alone; the times required to attain equilibrium were reported to be 30 min and 10 days, respectively.

It has also been shown that mixed complexes may be used in extraction processes not only to increase reaction rates, but also to improve certain other properties of extraction systems. The extraction of complexes of dithiol with low-valence metal ions is very difficult, but in the presence of phenanthroline, the mixed complexes formed with Zn and with other bivalent metal ions are readily extractable [136]. Similarly, mixed complexes of Zn with 8-hydroxyquinoline and 1,10-phenanthroline have higher extraction constants, and their value increase with the number of phenanthroline molecules in the compound extracted [137].

4.7.2 Analysis of Seawater

The analysis of seawater has always been a difficult task due to the very low concentrations of elements of interest in presence of high amounts of interfering elements such as Na and Cl. Particularly in NAA, the high sensitivities of Na- and Cl-nuclides represent a challenge for preconcentration methods.

Sand-filtered seawater was supplied by the Oceanography Department, Dalhousie University, collected from the North West Arm in Halifax, Nova Scotia. The samples were filtered using 0.45-μm Nucleopore membranes and used immediately after the collection. Two samples were irradiated in each study. The conditions used in the extraction are described in Section 4.7.1.2.

The irradiation of the surfactant-rich phase obtained after the first CPE of 40 mL of seawater gave a very high dead time, which made the counting of the sample (t_i=10 min, t_d=1min, t_c=10min) impossible. The main activities found in the γ-spectrum corresponded to the characteristic photopeaks of ^{24}Na and ^{38}Cl nuclides. This relatively high content of inorganic salts in the surfactant-rich phase is probably due to the hydrated nature of this phase.

A double CPE was attempted in order to reduce the amount of salts in the extractant phase and the results were satisfactory in terms of counting requirements. After counting for 10 min, Cu, Cd, and Mn were detected. However, the quantification of these metals was very difficult since only a very few counts were found. A more detailed study must be pursued considering the influence of the re-dissolution process of the chelates in the second CPE on the equilibrium of formation.

A third study was done using only one extraction step and a different timing scheme (t_i=3 h, t_d=6 d, t_c=2h). In this case, the background activities were greatly reduced and Cd (0.3 ppb), Hg (0.05 ppb) and Zn (3.2 ppb) were detected.

In general, the CPE procedure could be useful for the analysis of seawater because of its large preconcentrating capacity. In the case of NAA as a detection technique, future work must include the search for a better decontamination method for Na and Cl combined with the CPE. This method should be simple and should not involve many extra steps that could increase the reagent blank. Some promising possibilities are the use of more hydrophobic surfactants to reduce the amount of water in the surfactant-rich phase, and a previous cleaning of the sample by passing it through a selective ion-exchange column.

4.8 Reagent Blanks

Reagents could be possible sources of contaminants in any preconcentration method. All reagents used in this work were of the highest purity that is commercially available. Nevertheless, blank solutions were routinely analyzed using the same NAA timing scheme to quantify any possible metallic impurities as well as any interfering elements. The total blank solutions contained traces of Na (<90 ppb), Cl (<1 ppm), and Al (<8.5 ppb), and these low levels did not interfere with the analyte signals.

Sample handling could be another source of contamination. Precautions were taken to minimize sample handling as much as possible. It has already been mentioned that all apparatus was thoroughly cleaned prior to use. The CPE method developed here involves only one step where a contamination may possibly occur, namely during the transfer of the extracted sample in the surfactant-rich phase from the centrifuge tube to the irradiation vial. This phase was simply poured into the vial without using any pipette. The centrifuge tube was washed with water using a pipette, which might be a source of contamination. Since the total blank for the elements of interest was below their respective detection limits, the contamination, if any, was considered to be negligible.

5. CONCLUSIONS AND RECOMMENDATIONS

Several methods for the simultaneous preconcentration of trace metals based on CPE were developed in this thesis. The methods involved the extraction of PAN:metal chelates in micellar solutions of PONPE-7.5 followed by the determination of the metals by NAA.

A phase diagram of surfactant PONPE-7.5 was constructed, and the results agreed with the general shape of the curve published by other scientists for a nonionic surfactant. The curve attained a minimum temperature of 21.5°C at 0.1% (wt). This surfactant concentration was selected so that the CPE need not be carried out at very high temperatures. The influence of buffer, chelating agent, and electrolyte on the cloud point of PONPE 7.5 (0.1%) was evaluated. Changes in the critical temperature were within the overall experimental errors.

The variation of conditions in the extraction procedure showed changes in the volume of the surfactant-rich phase. A fairly small volume was obtained when optimized conditions were used. The preconcentration factor, calculated as the phase ratio between the aqueous and the surfactant-rich phases was 60. The high percent of water contained in the surfactant-rich phase (80%) might explain to certain extent the solubilization of ions like K, Na and Cl in this non-polar phase.

The spectrophotometric behavior of the chelating reagent at different pH was examined and a maximum absorbance was observed at 470 nm for pH values near 7. A null influence of PONPE 7.5 on the PAN absorbance at this wavelength was verified. A spectrophotometric study of the distribution of PAN between the aqueous and the surfactant-rich phases was carried out. The partition coefficients (logK_d) for the neutral species were between 2.38 and 3.73. These high values indicated the possibility of obtaining high extraction recoveries of metal-PAN chelates.

The CPE method was optimized first for a single element, namely Cu, in order to study the possible factors affecting the extraction efficiency. After obtaining successful results for this optimization, simultaneous preconcentration of selected transition elements was attempted. The metals were determined in two groups depending on their half-lives: (i) Cd, Cu and Mn; (ii) Cd, Co, Cu, Mn, Ni and Zn. The simultaneous preconcentration and determination of most of the lanthanides (Dy, Er, Eu, Gd, Ho, La, Lu, Pr, Sm, Tb, Tm and Yb) were also achieved using CPE followed by different irradiation, decay and counting time schemes.

The influence of different factors on the metal recoveries after preconcentration by the CPE procedure was evaluated. The solution pH revealed to be one of the most important factors in the optimization of the extraction recovery. Most of the chelates were quantitatively extracted (>90%) at high pH values (between 8 and 9). Another critical factor was found to be the concentration of PAN. Since the extraction recovery was strongly dependent upon the metal chelate stability, higher amounts of PAN were needed at higher total metal concentrations. The PAN concentration was always in excess with respect to the total metal concentration.

Other important factors evaluated were the ionic strength, temperature and the concentration of surfactant. An improvement in the separation of phases was achieved at a KNO_3 concentration of 0.5 M. Not only the recoveries are the highest at this value, but the addition of an inorganic electrolyte also facilitated the separation of phases by means of weakening the water-surfactant hydrophilic interactions. This behavior could lead to higher preconcentration factors, shorter centrifugation times, and a better distribution of the neutral species into the surfactant-rich phase. The optimum temperature at which the extraction took place was 40°C. This temperature was well above the cloud point of PONPE-7.5 (0.1%) and was still low enough to obtain an effective separation of phases without a substantial loss of the analyte due to re-dissolution of the surfactant-rich phase during centrifugation. The concentration of the surfactant appeared to have little influence on the CPE efficiency other than that described in the analysis of the phase diagram.

The main objective of this thesis was to obtain a simultaneous CPE of selected metals. Nevertheless, some selective separation could be attempted using the different behaviors of individual metal chelates at various pH values and PAN concentrations. For example, many of the lanthanides could be selectively extracted from solutions containing large amounts of La by carrying out the extraction at $pH<7$ and PAN concentrations lower than 1×10^{-4} M.

The CPE method was applied to the analysis of metals in tap and seawater samples. The analysis of tap water samples gave reproducible results. Low (ppb) concentrations of Cd, Hg and Zn in seawater were determined by the CPE method in conjunction with NAA. However, better decontamination methods involving the use of more hydrophobic surfactants and/or a pre-cleaning of the samples using chromatographic columns are needed since the radionuclides of Na and Cl can interfere in the quantification step.

Some studies were also done regarding the use of a mixture of TAN and PAN for the CPE of Cd, Hg and Zn. The recoveries were quantitative under the optimum conditions reported here. The possibility of using a mixture of two or more chelating agents can extend the use of simultaneous CPE to other metals or elements that are not efficiently extracted by a single chelating agent.

Comparator standards were used to construct Schewart control charts for internal quality control purposes. The results were within the warning limits ($X\pm2\sigma$) with a few exceptions. The accuracy of the method was assessed by analyzing biological and environmental certified reference materials. Our values agreed well with the certified values in most cases, within the reported errors. Total blank solutions were also regularly irradiated. The levels of trace elements found did not interfere with the analyte signals.

The CPE method developed here can be used to preconcentrate simultaneously trace metals from aqueous samples in only one step which reduces the possibility of contamination. The detection limits obtained are of the order of ppb (0.03-25 ppb). The use of NAA not only allows a multielement determination, but also offers the possibility of attaining even lower detection limits by anticoincidence γ-spectroscopy. The method can be advantageously applied to the analysis of environmental and biological samples containing metals at trace levels.

6. REFERENCES

1. Scamehorn, J.F.; Christian, S.D.; Rex, T. In *Surfactant-Based Separation Processes*; Scamehorn, J.F.; Harwell, J.H., Eds.; Marcel Dekker, Inc.: New York; 1989; 139-152.
2. Einax, J. and Geib, S. *Fresenius' J. Anal. Chem.* **1994**, *350*; 14-17.
3. Beazley, P.I.; Rao, R.R.; Chatt, A. *J. Radioanal. Nucl. Chem.* **1994**, *179(2)*; 267-276.
4. Bubb, J.M.; Lester, J.N. *Environm. Monit. Assess.* **1996**, *41*; 87-105.
5. Saleh, A.I.; Remail, S.W.; Milad, F.M. *J. Radioanal. Nucl. Chem.* **1993**, *168(1)*; 23-27.
6. Perez-Bendito, D.; Rubio, S.; Sicilia, D. *Anal. Chim. Acta* **1993**, *284*; 149–157.
7. Williams, R.R.; Paradkar, R.P. *Anal. Chem.* **1994**, *66*; 2752-2756.
8. McIntire, G.L. *Crit. Rev. Anal. Chem.* **1990**, *21(4)*; 257-278.
9. Hinze, W.L.; Pramauro, E. *Crit. Rev. Anal. Chem.* **1993**, *24(2)*; 133-177.
10. Scamehorn, J.F.; Taylor, R.W.; Christian, S.D.; Dharmawardana, U.R. *Langmuir* **1992**, *8*; 414-419.
11. Watanabe, H. In *Solution Behaviour of Surfactants*; Mittal, K.L.; Fendler, E.J., Eds; Plenum: New York; 1982; 1305-1316.
12. Pramauro, E.; Pelizzetti, E. *Anal. Chim. Acta* **1985**, *185*; 1-29.
13. Armstrong, D.W. *Sep. Purif. Meth.* **1985**, *14(2)*; 213-304.
14. Watanabe, H.; Ishii, H.; Miura, J. *Bunseki Kagaku* **1977**, *26*; 252. Cited in 11.
15. Medina-Eriche, J.; Llobat-Estelles, M.; Bosch-Reig, F. *Talanta* **1983**, *30(12)*; 915-918.
16. Abe, S.; Endo, M.; Ozaki, K. *J. Radioanal. Nucl. Chem.* **1991**, *154(1)*; 41-51.
17. Chen, X.; Li, G.; Hu, Z. *Mikrochim. Acta* **1996**, *122(1-2)*; 143-149.
18. Moreno-Cordero, B.; Perez-Pavon, J.L.; Fernandez-Laespada, M.E. *Analyst* **1993**, *118*; 209-212.

19. Perez-Pavon, J.L.; Moreno-Cordero, B.; Garcia-Pinto, C. *Anal. Chem.* **1992**, *64*; 2334-2338.

20. Shibata, S. *Chelates in Analytical Chemistry*; Marcel Dekker, Inc.; **1972**, Chapter 4; 101-108.

21. Cheng, K.L.; Bray, R.H. *Anal. Chem.* **1955**, *27(5)*; 782-785.

22. Corsini, A.; Yih, I.M.L.; Fernando, Q.; Fresier, H. *Anal. Chem.* **1962**, *34*; 1090-1093.

23. Nakagawa, G.; Wada, H. *Nippon Kagaku Zasshi* **1964**, *85*; 549. Cited in ref. 20.

24. Shibata, S. *Anal. Chim. Acta* **1960**, *22*; 479-484.

25. Anderson, R.G.; Nickless, G. *Analyst* **1962**, *92*; 207-238.

26. Shibata, S. *Anal. Chim. Acta* **1961**, *25*; 348-359.

27. Shibata, S. *Anal. Chim. Acta* **1963**, *28*; 388-392.

28. Rao, I.M.; Satyanarayana, D.; Agarwala, U. *Bull. Chem. Soc. Jpn.* **1979**, *52(2)*; 588-591.

29. Dwyer, F.P.; Mellor, D.P. *Chelating Agents and Metal Chelates*; Academic Press: London; 1964; Chapter 1; 1-50.

30. Gao, J.; Hu, G.; Kong, J.; Bai, G. *Talanta* **1993**, *40(2)*; 195-200.

31. Landsberger, S. In *Chemical Analysis by Nuclear Methods*; Alfassi, Z.B., Ed.; John Wiley & Sons; 1984; Ch. 6.

32. Laughlin, R.G. *The Aqueous Phase Behavior of Surfactants*; Academic Press Inc.; 1994.

33. Hinze, W.L. In *Ordered Media in Chemical Separations*; Hinze, W.L.; Armstrong, D.W., Eds.; ACS: Washington, DC ; 1987; Chapter 7; 48-55.

34. Magid, L. In *Solution Chemistry of Surfactants*; Mittal, K.L., Ed.; Plenum Press: New York; 1979; Vol. I; 427-440.

35. Degiorgio, V.; Corti, M.; Minero, C.; Piazza, R. *J. Chem. Phys.* **1985**, *82(2)*; pp. 1025-1030.

36. Scamehorn, J. F. *Phenomena in Mixed Surfactant Systems*; Scamehorn, J. F., Ed.; ACS Symposium Series; 1986; No.311; 20-25.

37. Berezin, I.V.; Martinek, K.; Yatsimirskii, A.K. *Russ. Chem. Rev.(Engl. Transl.)* **1973**, *42(10)*; 787-799.

38. Fendler, J.H. *Chem. Rev.* **1987**, *87*; 877-899.

39. Moreno-Cordero, B.; Perez-Pavon, J.L.; Garcia-Pinto, C.; Fernandez-Laespada, M.E. *Talanta* **1993**, *40(11)*; 1703-1710.

40. Ricceri, R.; Vila Romeu, N.; Taddei, G. *Langmuir* **1996**, *12*; 913-915.

41. Horvath, W.J.; Huie, C.W. *Talanta* **1992**, *39(5)*; 487-492.

42. Calkins, N.; Komaromy, G.; von Wandruszka, R. *Langmuir* **1996**, *12*; 916-920.

43. Hinze, W.L. *Report No. 92-269*; Wake Forest University, North Caroline; 1992.

44. Degiorgio, V.; Corti, M.; Minero, C. *J. Phys. Chem.* **1984**, *88*; 309-317.

45. Hinze, W.L.; Saitoh, T. *Anal. Chem.* **1991**, *63*; 2520-2525.

46. Armstrong, D. W.; Warner-Schmid, D.; Hoshi, S. *Sep. Sci. Technol.* **1993**, *28*; 1009-1018.

47. Yu, Z.J.; Xu, G. *J. Phys. Chem.* **1989**, *93*; 7441-7445.

48. Buhai, L.; Meng, M. *Talanta* **1990**, *37(9)*; 885-888.

49. Lindman, B. *Surfactants*; Tadros, T. F., Ed.; Academic Press Inc.; 1984; 102-105.

50. Watanabe, H.; Tanaka, H. *Talanta* **1978**, *25*; 585-589.

51. Okada, T. *Anal. Chem.* **1992**, *64*; 2138-2142.

52. Hinze, W., *Ind. Eng. Chem. Res.* (to be published).

53. Watanabe, H.; Kawamorita, S.; Haraguchi, K. *Anal. Sci.* **1985**, *1*; 41-45.

54. Ganong, B. R.; Lu, Ch. *Anal. Biochem.* **1989**, *179*; 66-71.

55. Werck-Reichhart, D.; Benveniste, I; Teutsch, H.; Durst, F.; Gabriac, B. *Anal. Biochem.* **1991**, *197*; 125-131.

56. Pramauro, E.; Minero, C.; Pelizzetti, E. *Ordered Media in Chemical Separations*; Hinze, W.L.; Armstrong, D.W., Eds.; ACS: Washington, DC ; 1987; Chapter 7; 152-161.

57. Watanabe, H.; Saitoh, T.; Kamidate, T.; Haraguchi, K. *Mikrochim. Acta* **1992,** *106*; 83-90.

58. Moreno-Cordero, B.; Perez-Pavon, J.L.; Fernandez-Laespada, M.E. *Analyst* **1993,** *118*, 209-212.

59. Perez-Pavon, J.L.; Moreno-Cordero, B. *Analyst* **1992**, *117*; 215-217.

60. Pramauro, E.; Prevot, A.B.; Zelano, V.; Hinze, W.L.; Viscardi, G.; Savarino, P. *Talanta* **1994**, *41(8)*; 1261-1267.

61. Watanabe, H.; Saitoh, T.; Kimura, Y.; Kamidate, T.; Haraguchi, K. *Anal. Sci.* **1989**, *5*; 577-581.

62. Watanabe, H.; Kawamorita, S.; Haraguchi, K. *Proceedings of Symposium on Solvent Extraction* **1984**; 75-80.

63. Hinze, W.L.; Frakewich, R.P. *Anal. Chem.* **1994**, *66*; 944-954.

64. Watanabe, H.; Kamidate, T.; Kawamorita, S.; Haraguchi, K.; Miyajima, M. *Anal. Sci.* **1987**, *3*; 433-436.

65. Perez-Pavon, J.L.; Moreno-Cordero, B. *Anal. Chim. Acta* **1990**, *234*; 239-245.

66. Johnson, W.E.; Fendinger,N.J.; Plimmer, J.R. *Anal. Chem.* **1991**, *63*; 1510-1513.

67. Moreno-Cordero, B.; Perez-Pavon, J.L.; Garcia-Pinto, C. *Anal. Chem.* **1994**, *66*; 874-881.

68. Stangl, G.; Niessner, R. *Mikrochim. Acta* **1994**, *113*; 1-8.

69. Pramauro, E.; Prevot, A.B. *Pure Appl. Chem.* **1995**, *67(4)*; 551-559.

70. Pramauro, E.; Bianco, A.; Barni, E.; Viscardi, G.; Hinze, W.L. *Colloids Surf.* **1992**, *63*; 291-300.

71. Rubio, S.; Perez-Bendito, D. *Anal. Chim. Acta* **1989**, *224*; 185-198.

72. Pramauro, E.; Pelizzetti, E.; Cavasino, F. P.; Sbriziolo, C. *J. Phys. Chem.* **1989,** *93*; 469-473.

73. Watanabe, H.; Saitoh, T.; Segawa, H.; Kamidate, T.; Haraguchi, K. *Bull. Chem. Soc. Jpn.* **1993**, *66*; 3676-3679.

74. Armstrong, D.W.; Nome, F. *Anal. Chem.* **1981**, *53*; 1662-1666.

75. Pramauro, E.; Prevot, A.B.; Pelizzetti, A. *Anal. Chim. Acta* **1992**, *264*; 303-310.

76. De la Guardia, M.; Peris-Cardells, E.; Morales-Rubio, A. *Anal. Chim. Acta* **1993**, *276*; 173-179.

77. Tagashira, S.; Murakami, Y.; Otobe, S.; Sasaki, Y. *Anal. Sci.* **1998**, *14*; 849-851.

78. Silva, M.F.; Fernandez, L.; Olsina, R.A.; Stacchiola, D. *Anal. Chim. Acta* **1997**, *342*; 229-238.

79. Silva, M.F.; Fernandez, L.P.; Olsina, R.A. *Analyst* **1998**, *123*; 1803-1807.

80. Cerrato-Oliveros, M.C.; Jimenez-deBlas, O.; Perez-Pavon, J.L.; Moreno-Cordero, B. *J. Anal. At. Spectrom.* **1998**, *13*; 547-550.

81. Tagashira, S.; Murakami, Y.; Yano, M; Sasaki, Y. *Bull. Chem. Soc. Jpn.* **1998**, *71(9)*; 2137-2140.

82. Igarashi, S.; Endo, K. *Anal. Chim. Acta* **1996**, *320*; 133-138.

83. Akita, S.; Rovira, M.; Sastre, A.M.; Takeuchi, H. *Sep. Sci. Technol.* **1998**, *33(11)*; 2159-2177.

84. Stary, J. *The Solvent Extraction of Metal chelates*; Pergamon Press; 1964; Chapters 1-4; 1-50.

85. Liu, J.Ch.-I. *Ph.D. Thesis*; University of Illinois, 1951. Cited in ref. 21.

86. Pease, B.F.; Williams, M.B. *Anal. Chem.* **1959**, *31(6)*; 1044-1047.

87. Cornejo-Ponce, L.; Peralta-Zamora, P.; Maretti-Bueno, M.I. *Talanta* **1998**, *46*; 1371-1378.

88. Watanabe, W. *Talanta* **1974**, *21*; 295-302.

89. Watanabe, H.; Goto, K.; Taguchi, S.; Fukue, Y.; Ohta, K. *Talanta* **1977**, *24*; 752-753.

90. Agnihotri, N.K.; Singh, V.K.; Singh, H.B. *Talanta* **1997**, *45*; 331-341.

91. Betteridge, D.; John, D. *Analyst* **1973**, *98(1167)*; 377-389.

92. Lan, C.R.; Sun, Y.C.; Chao, J.H.; Chung, C.; Yang, M.H.; Lavi, N.; Alfassi, Z.B. *Radiochim. Acta* **1990**, *50*; 225-229.

93. Rao, R.R.; Chatt, A. *J. Radioanal. Nucl. Chem.* **1993**, *168(2)*; 439-448.

94. Milley, J.E.; Chatt, A. *J. Radioanal. Nucl. Chem.* **1987**, *110(2)*; 345-363.

95. Naidu, G.R.K.; Rama-Devi, P.; Gangaiah, T. *Anal. Chim. Acta* **1991**, *249*; 533-537.

96. Yeh, S.J.; Wei, J.C.; Ke, C.N.; Yang, C.Y. *J. Radioanl. Nucl. Chem.* **1994**, *179(2)*; 221-229.

97. Analytical Methods Committee *Analyst* **1995**, *120*; 29-34.

98. Analytical Methods Committee *Analyst* **1989**, *114*; 1497-1503.

99. Marchandise, H. *Fresenius' J. Anal. Chem.* **1993**, *345*; 82-86.

100. Broderik, B.E.; Cofino, W.P.; Cornelis, R.; Heydorn, K.; Horwitz, W.; Hunt, D.T.E.; Hutton, R.C.; Kingston, H.M.; Muntau, H.; Baudo, R.; Rossi, D.; van Raaphorst, J.G.; Lub, T.T.; Schramel, P.; Smyth, F.T.; Wells, D.E.; Kelly, A.G. *Mikrochim. Acta* **1991**, *II*; 523-542.

101. Currie, L.A. *Anal. Chem.* **1968**, *40(3)*; 586-593.

102. Zhang, W. Studies on Anticoincidence Gamma-Ray Spectrometry in Neutron Activation Analysis; Ph.D. Thesis, Dalhousie University, 1997.

103. Carvalho, B.L.; Briganti, G.; *J. Phys. Chem.*; **93**; pp.4283 (1989)

104. Erdtmann, G. *Neutron Activation Tables*; Weinheim: New York, 1976.

105. Cross, J. *Nonionic Surfactants. Chemical Analysis*; Cross, J., Ed.; Marcel Dekker, Inc.; 1987; Chapter 11; 375-382.

106. Al-Ghamdi, A.M.; Nasr-El-Din, H.A. *Colloids Surf., A* **1997**, *125*; 5-18.

107. Maeda, H.; Muroi, S.; Kakehashi, R. *J. Phys. Chem.* **1997**, *101*; 7378-7382.

108. Schott, H. J. Colloid Interface Sci.**1997**, 189; 117-122.

109. Schott, H. J. Colloid Interface Sci.**1997**, 192; 458-462.

110. Macczenko, Z. *Spectrophotometric Determination of Elements*; Ellis Horwood limited; Ch. 5; pp.116-121 (1976)

111. Eichenberger, B.A.; Chen, K.Y. In *Water Analysis*; Minear, R.A.; Keith, L.H., Eds., Academic Press, Inc., 1982; Chapter 1.

112. Watanabe, H.; Tachikawa, K.; Hoshino, H.; Saitoh, T.; Taketomi, H.; Yotsuyanagi, T. *Anal. Chim. Acta* **1983**, *147*, 339-345.

113. Hancock, R.I. *Surfactants*; Tadros, T.F., Ed.; Academic Press Inc.; 297-299 (1984)

114. Donaldson, E.M.; Inman, W.R. *Talanta* **1966**, *13*, 489-497.

115. Pomazal, K.; Prohaska, C.; Steffan, I.; Reich, G.; Huber, J.F.K. *Analyst* **1999**, *124*; 657-663.

116. Falk, H.; Geerling, R.; Hattendorf; Krengler-Rothensee, K.; Schmidt, K.P. *Fresenius J. Anal. Chem.* **1997**, *359*; 352-356.

117. Zhu, W.; de Leer, E.W.B.; Kennedy, M.; Alaerts, G.J.F.R. *Fresenius J. Anal. Chem.* **1998**, *360*, 74-80.

118. Kikawada, O.T.; Ossaka, T.; Nozaki, T.; Kakihana, H. *J. Radioanal. Nucl. Chem.* **1990**, *140*, 365.

119. Zachmann, D.W. *Anal. Chem.* **1988**, *60*, 420-427.

120. Panday, V.K.; Becker, J.S.; Dietze, H.J. *Fresenius J. Anal. Chem.* **1995**, *352*, 327-334.

121. Yang, X.J.; Guan, J.S.; Shen, T.J. *Anal. Chim. Acta* **1993**, *279*, 261-272.

122. Esser, B.K.; Volpe, A.; Kenneally, J.M.; Smith, D.K. *Anal. Chem.* **1994**, *66*, 1736-1740.

123. Maquirira, A.; Elmahadi, H.; Puchades, R. *J. Anal. At. Spectrom.* **1996**, *11*, 99-106.

124. Shabani, M.B.; Akagi, T.; Masuda, A. *Anal. Chem.* **1992** *64*, 737-743.

125. Erdtmann, G. *J. Radioanal. Chem.* **1972**, *10*; 137-138.

126. Landsberger, S.; Simsons, A. *Chem. Geol.* **1987**, *62*; 223-226.

127. Ila. P.; Jagam, P.; Muecke, G.K. *J. Radioanal. Chem.* **1983**, *79*; 215-232.

128. Kawasaki, A.; Kimura, R.; Arai, S. *Soil Sci. Plant Nutr.* **1998**, *44(3)*; 433-441.

129. Bodier, C. *J. Biol. Chem.* **1981**, *256*; 1604-1608.

130. Saitoh, T.; Tani, H.; Kamidate, T; Watanabe, H. *Trends Anal. Chem.* **1995**, *14*; 213-217.

131. Boni, I.E.; Hemmeler, A. *Chimica* **1958**, *34*; 443-444.

132. Goodwin, W.E. Preconcentration Neutron Activation Analysis of Trace Elements with 1-(2-Thiazolylazo)-2-Naphthol Using Reversed-Phase Extraction Chromatography; Ph.D. Thesis, Dalhousie University, 1997.

133. Navratil, O. *Coll. Czech. Chem. Commun.* **1964**, *29*; 2490-2506. Cited in *Chem. Abstr.* **1965**, *62*:8365f.

134. Math, K.S.; Bhatki, K.S.; Freiser, H. *Talanta* **1969**, *16*; 412-414.

135. Freiser, B.S.; Freiser, , H. *Talanta* **1970**, *17*; 540-542.

136. Hamilton, H.G.; Freiser, H. *Anal. Chem.* **1969**, *41*; 1310-1315.

137. Woodward, C.; Freiser, H. *Anal. Chem.* **1968**, *40*; 345-348.