DEVELOPMENT OF SPECIATION SCHEME FOR CADMIUM, COPPER, LEAD AND ZINC IN WATER USING ULTRAFILTRATION AND DIFFERENT SOLID SORBENTS

CHARUWAN SUITCHARIT

UNIVERSITI SAINS MALAYSIA

2006

DEVELOPMENT OF SPECIATION SCHEME FOR CADMIUM, COPPER, LEAD AND ZINC IN WATER USING ULTRAFILTRATION AND DIFFERENT SOLID SORBENTS

by

CHARUWAN SUITCHARIT

Thesis submitted in fulfillment of the requirements for the degree of
Doctor of Philosophy

July 2006

ACKNOWLEDGEMENTS

This thesis was completed with support from many people and I would like to express my sincere gratitude to them.

First and foremost, I wish to sincerely thank my supervisor: Associate Professor Dr. Norita Mohamed, co-supervisor: Professor Lim Poh Eng for their supervision, helpful guidance, advice and moral support throughout this work. I am also thankful to Dr. Waraporn Sirinawin, my field supervisor for her understanding, support, encouragement and helpful guidance during my work in Thailand.

Special thanks is extended to Assistant Professor Dr. Kanidtha Hansungnern, Head of Department of Chemistry, Prince of Songkla University, for allowing me to work in the Analytical Chemistry Laboratory at the Department of Chemistry, Prince of Songkla University, Thailand. Without the well-equipped facility, this research work would have been difficult to complete. My thanks are also due to the lecturers of the Analytical Chemistry Division members, Prince of Songkla University, especially Assistant Professor Panit Sherdshoopongsa for his assistance in the use of ASV instrument.

I extend my thanks to Ajarn Peera Tongmee, Department of Statistics, Thaksin University, Thailand, for his valuable discussion and assistance in the statistics for data analysis.

Also, I wish to acknowledge the lab technicians and other members from the School of Chemical Sciences, Universiti Sains Malaysia, and Department of Chemistry, Prince of Songkla University, as well as the librarians of such universities for their assistance.

I wish to express my gratitude to Rajabhat Songkhla University for the scholarship funded through the Rajabhat Songkhla University Affairs as well as the Rajabhat Pranakhorn University for the financial support of this work. I am grateful to the Ministry of Science, Technology and Environment of Malaysia for financial support through an IRPA grant. I would also like to thank the Universiti Sains Malaysia, School of Chemistry Sciences, and Institute of Postgraduate Studies for their support and assistance.

I would also like to thank my friends, Vanitha, Heng Chong, Chieng Hui Yap, Hor Yean Phin, Lim Poh Lee, Jacky, Kitima and Nam, who have been there to offer their friendship and for their contribution either directly or indirectly to the completion of my work.

I cordially wish to thank my beloved parents, sister and brothers who have been very supportive and encouraging. Without their guidance and unconditional support, I would not be the person that I am today. Thanks for always being there for me.

My appreciation is specially granted to my dear husband, Kornthanong and my beloved son, Bhoome, for all their love, patience and understanding during these five years away from home.

Finally, a note of appreciation is also acknowledged to the members of the Examining Committee for approving this thesis.

TABLE OF CONTENTS

CHAPTER 3 : RESULTS AND DISCUSSION 76

APPENDICES

LIST OF TABLES

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

EDTA	Ethylenediaminetetraacetic acid
NTA	Nitrilotriacetic acid
HA	Humic acid
SOX	8-hydroxy-quinoline-5-sulphonic acid or Sulphoxine
OX	8-hydroxyquinoline or Oxine
M	Metal
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
ETAAS	Electrothermal Atomic Absorption Spectrometry
HMDE	Hanging Mercury Drop Electrode
DPASV	Differential Pulse Anodic Stripping Voltammetry
ASV	Anodic Stripping Voltammetry
VLB	Very Labile
SLB	Slowly Labile
INT	Inert
DLB	Dowex Labile
DLLB	Dowex Less Labile
C-18 LB	C-18 Labile
C-18 LLB	C-18 Less Labile
Non-LB	Non-Labile
kDa	Kilo Dalton
MWCO	Molecular weight cut-off
SKL	Songkhla Lake
TLN	Thale Noi
SRW	Synthetic River Water

KAJIAN UNTUK MEMBANGUNKAN SKEMA PENSPESIESAN LOGAM KADMIUM, KUPRUM, PLUMBUM DAN ZINK DI DALAM AIR MENGGUNAKAN KAEDAH PENURASAN ULTRA DAN PELBAGAI PENJERAP PEPEJAL

ABSTRAK

Suatu skema penspesiesan bagi spesies logam terlarut pada paras kepekatan semula jadi telah di bangunkan berdasarkan gabungan pencirian fizik melalui pemecahan saiz menggunakan penurasan ultra dan pencirian kimia melalui kajian penahanan pada pelbagai penjerap pepejal. Penurasan ultra memberi maklumat tentang saiz pada aras jisim-molekul yang berbeza, berdasarkan membran yang mempunyai sekatan jisim molekul yang berbeza. Penjerap seperti resin pertukaran kation Chelex-100, resin pertukaran anion Dowex 1-X8 dan fasa berbalik C-18 telah digunakan dalam sistem turus untuk membezakan kelabilan spesies logam yang ditakrifkan secara operasi mengikut cas atau sifat kimia. Penahanan ke atas penjerap bagi Cd, Cu, Pb dan Zn dalam setiap pecahan saiz pada keadaan pH yang berbeza telah dikaji dengan menggunakan turus individu dan juga sistem tiga turus. Eksperimen telah dilakukan dengan kehadiran ligan model, iaitu asid nitriloasetik (NTA), asid etilenadiamina tetraasetik (EDTA), asid humik (HA), asid 8- hidroksikuinolin-5-sulfonik (SOX) dan 8-hidroksikuinolin (OX). Keputusan yang diperolehi telah dibandingkan dengan taburan spesies daripada pengiraan secara teori bagi semua spesies logam sebagai fungsi bagi pH kecuali untuk kompleks M-HA.

Keputusan menunjukkan bahawa julat pH daripada pH 5.5 hingga 7.5 memainkan peranan yang penting dalam corak penspesiesan yang terhasil bagi spesies logam dengan kehadiran HA kecuali untuk Cd di mana pemendakan kadmium-humate telah diperhatikan. Corak penspesiesan bagi spesies logam dengan kehadiran EDTA menunjukkan bahawa pH tidak berpengaruh di antara 5.5 hingga 7.5. Corak penspesisan yang serupa juga terhasil dengan kehadiran SOX dan OX kecuali bagi Cd dan Zn di mana pH mempunyai kesan yang kecil terhadap corak penspesiesan.

Perubahan pH yang kecil juga mempengaruhi corak penspesiesan dengan kehadiran NTA.

Susunan turus dalam sistem tiga turus telah dikaji dengan mengubah urutan turus. Sebanyak enam jenis gabungan tiga turus telah digunakan untuk mengkaji penahanan logam dengan kehadiran ligan yang berlainan pada pH semulajadi. Kajian mempamerkan bahawa urutan turus memberikan pelbagai corak penspesiesan. Namun, tren keseluruhan bagi penahanan logam dengan kehadiran EDTA, OX dan HA menggunakan sistem tiga-turus tidak menunjukkan perbezaan yang signifikan terhadap enam jenis gabungan tersebut pada aras kepercayaan 95% bagi ujian ANOVA. Jenis C dengan urutan: turus-turus Chelex-100, Dowex 1-X8 dan C-18, telah dipilih untuk diaplikasikan bagi kajian penspesiesan logam terlarut dalam air kolam Songkhla, Thailand. Jumlah pulih-semula bagi semua logam adalah dalam julat antara 96% hingga 110%.

DEVELOPMENT OF SPECIATION SCHEME FOR CADMIUM, COPPER, LEAD AND ZINC IN WATER USING ULTRAFILTRATION AND DIFFERENT SOLID SORBENTS

ABSTRACT

A method for the speciation studies of dissolved metal species at natural concentration levels has been developed. The method is based on a combination of physical characterization by size fractionation using ultrafiltration and chemical characterization by retention studies on different solid sorbents. The ultrafiltration offers information on size at different molecular-weight levels, which are based on the molecular weight cut-off of its membranes. The different sorbents, namely, cation-exchange resin Chelex-100, anion-exchange resin Dowex 1-X8 and C-18 reversed phase were used in column systems for the discrimination of lability of operationally-defined classes of the metal species among its species with different charges or chemical behaviour. The retention of Cd, Cu, Pb and Zn in each size fraction onto the sorbents at different pH conditions was investigated with both individual columns and three-columns systems. Experiments were performed with metals in the presence of model ligands, namely, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), humic acid (HA), 8-hydroxyquinoline-5-sulphonic acid (SOX) and 8-hydroxyquinoline (OX). The results obtained were compared with those computed according to theoretical species distribution of all the metal species as a function of pH except for the M-HA complexes.

Results have shown that the pH range from 5.5 to 7.5 plays an important role in speciation patterns of the metal species in the presence of HA except for Cd in which cadmium-humated coprecipitation was observed. The speciation patterns of the metal species in the presence of EDTA seem to be independent of pH between pH 5.5 to 7.5 and there are similar to patterns in the presence of SOX and OX except for Cd and Zn, which have little effect on speciation patterns. The change of pH slightly affects speciation patterns of the metal species in the presence of NTA.

The arrangement of three-columns systems was investigated by changing the column sequence in series. Six types of three-columns combination were used for the study of retention behaviour of metals in the presence of different ligands at natural pH. The investigation demonstrates that the sequence of column gives speciation patterns of the system from different points of view. However, the overall trend of retention of the metals in the presence of EDTA, OX and HA onto three-columns systems is not significantly different among the six types of combinations at 95% confidence level by ANOVA testing. Type C with the sequence: Chelex-100, Dowex 1-X8 and C-18 columns, was selected to apply for the speciation studies of dissolved metals in Songkhla Lake waters. Total recoveries of all the metals were in the range of 96% to 110%.

CHAPTER 1

INTRODUCTION

1.1 Trace Elements in Natural Waters

Trace elements are ubiquitous in natural waters and present in relatively low concentrations (<10^{-6} M) in fresh waters, oceanic waters, ground waters and interstitial soils, and sediment waters. Trace elements are introduced into natural waters by two main sources, namely, natural sources and anthropogenic sources.

It has been known that trace metals can act as an essential element as well as a toxicant. The role of an essential element in an organism is to build up certain biological compounds such as C, N, H, O, S and P for life growth. Whenever the concentration of the same element is in excess for organism requirements, it may become a toxicant. The actual species of trace metals and its concentration play an important role in the biological systems of natural waters; that is, the effect of trace metals on aquatic organisms (accumulation, nutrition, toxicity, etc.) depends on their chemical speciation. The determination of trace metals in natural waters has been developed over the past decades in terms of the total concentrations of these metals. However, knowledge about their total concentration solely, rarely provides sufficient information to elucidate their bioavailability and toxicity in natural waters. Chemical speciation is the key to better understanding of metal behavior and to predicting their impacts on biota in aquatic systems. Chemical reactivities and biological availabilities are strongly dependent on the individual chemical species of trace metals (Fytianos, 2001). Thus, it is necessary to obtain information about the actual chemical form of an element rather than just its total concentration in a sample.

In natural waters, trace elements may be present in both particulate and dissolved phases. The dissolved phase normally includes hydrated ions, weak and strong inorganic complexes, organic complexes and metal species associated with or adsorbed on colloidal or suspended particles. In some instances, these metals are present in more than one oxidation state. The particulate phase also comprises metals, which are adsorbed onto or within clay particulates and particles of biological origin. The possible physicochemical forms of trace metals in natural waters according to approximate size are shown in Table 1.1.

For the overall dissolved metals in natural waters, the free hydrated ions of metals are present only as a small portion since metal ions are able to form stable complexes with a large number of different inorganic and organic ligands, which influence the bioavailability and toxicity to biota (Mota and Santos, 1995). The dissolved inorganic metal complexes exist mainly as chloro-, carbonato-, sulfato-, oxo-, fluoro- and hydroxo- complexes (Lund, 1990). Besides inorganic ligands, natural waters may also contain both organic matter of biological origin and organic pollutants, which may have complexing properties (Mota and Santos, 1995).

The biogenic ligands in natural waters come from the organic compounds released by living organisms during decomposition. The natural biogenic ligands may be classified into two main categorizes: 1) organic compounds with a well-characterized structure and a low molecular weight (MW) such as amino acids, hydroxyl acids, monosaccharides, and; 2) ill-defined organic compounds, which cannot be fully isolated but can be separated into different fractions of similar properties and/or similar dimensions. They comprise either relatively small compounds with MW <1 kDa such as fulvic acid and peptides or larger ones (MW >10 kDa) such as humic acids, polysaccharides, polypeptides, lipids and proteins (Mota and Santos, 1995).

1.1.1 Definition

The term “speciation” has been used in different ways (Templeton *et al.*, 2000). The geochemists and environmental chemists have often applied the word speciation to describe the species transformations taking place during cycling of elements and their subsequent distribution in the aquatic environment (as in Figure1.1). Another

Table 1.1 The possible forms of metal species in natural waters [a]

Chemical forms	Examples	Approximate diameter of metal species (nm)
Soluble		<5
Simple hydrated metal ion	$Zn(H_2O)_6^{2+}$	0.8
Simple inorganic complexes	$Zn(H_2O)_3Cl^+$	1-2
Stable inorganic compounds	$ZnCO_3$, PbS	1-2
Simple organic complexes	Cu-glycinate	1-2
Stable organic complexes	Cu-fulvate	2-4
Colloidal		10-5,000
Adsorbed on inorganic colloids	Cu^{2+}, Fe_2O_3; Cd^{2+}, MnO_2	
Adsorbed on organic colloids	Pb^{2+}, humic acid	
Adsorbed on mixed colloids, (inorganic/organic)	Cu^{2+}, Fe_2O_3 / humic acid	
Particulate matter		>500
Precipitates, co-precipitates	$PbCO_3$	
Mineral particles	PbS	
Metal adsorbed on soils	M^{2+}, MS, MCO_3 on clay minerals, Mn(IV) oxides	
Metals incorporated with organic material	Metals in algae	

[a] Data from Florence and Batley (1980) and Noller (1992)

meaning of the word speciation has often been used to indicate the analyte activity of identifying chemical species and measuring their distribution in a particular sample or matrix.

In order to avoid confusion, the following definitions have been recommended (Templeton *et al.*, 2000):

(i) *Chemical species*. Chemical elements: specific form of an element defined as isotopic composition, electronic or oxidation state, and/or complex or molecular structure.

(ii) *Speciation analysis*. Analytical chemistry: analytical activities of identifying and/or measuring the quantities of one or more individual chemical species in a sample.

(iii) *Speciation of an element; speciation*. Distribution of an element among defined chemical species (e.g., dissolved, colloidal or particulate forms) in a system.

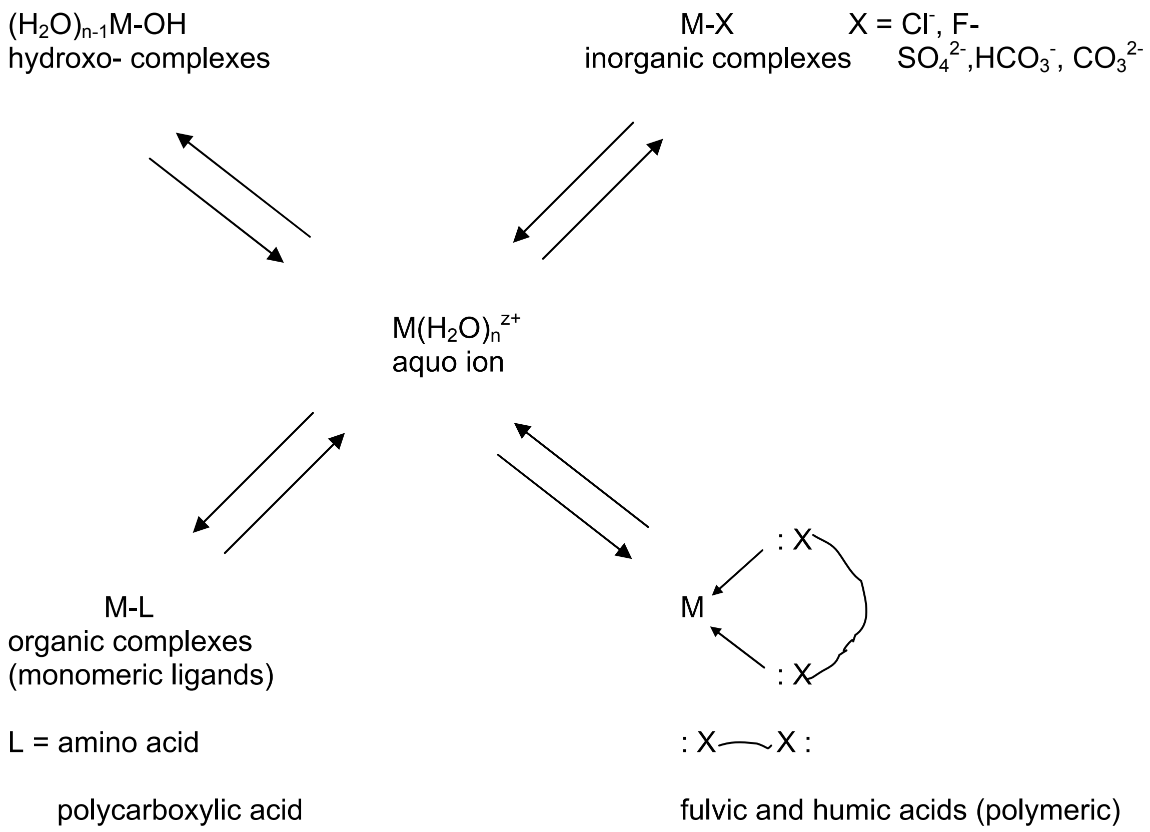


Figure 1.1 General scheme showing metal speciation in solution (Twiss *et al.*, 2000).

(iv) *Fractionation*. Process of classification of an analyte or a group of analytes from a certain sample according to physical (e.g., size, solubility) or chemical (e.g., bonding, reactivity) properties.

For the purpose of this thesis, the process of identifying and quantifying a possible group of metal species present in water according to its operationally-defined speciation was carried out. In operationally-defined speciation, the fractionation process is applied to classify an analyte in the sample according to its physical or chemical properties and the possible fractions obtained are the operationally-defined groupings (Laxen and Harrison, 1981; Ure and Davidson, 1995; Templeton *et al.*, 2000).

1.1.2 Interaction Between Metal and Ligands

When trace metals are introduced into natural waters by natural or anthropogenic inputs, they interact with inorganic ligands and both natural and anthropogenic organic ligands in the waters. In the interactions of trace metals with ligands, complex formation plays an extremely important role in their speciation. For instance, the stability constants of metal-ligand complexes in aqueous solutions are considered in order to classify the types of cation according to its properties.

1.1.2.1 Theoretical Species Distribution

A species distribution diagram is a useful tool for the rapid and accurate assessment of the species present and is known as the “speciation diagram”. The diagram depicts the fractions of species as a function of pH. The construction of a species diagram always starts with the statement of the total concentration of each added component (Martell and Motekaitis, 1992). The calculations require values for the stability constants of all species that may form from the system components. These values must be valid at the ionic strength of the medium concerned. Ionic strength is a factor affecting the stability constants (Glaus *et al*., 2000) due to the dependence on activity coefficient of ionic species present, particularly for solutions with ionic strength of 0.1 M or less (Skoog *et al*., 1992). To correct the stability constants to the required ionic strength, the activity coefficients of all the species in the equilibrium reaction must be calculated (Powell, 2002).

For this study, the theoretical calculation for species distribution of Cd, Cu, Pb and Zn was computed by the procedure as given in Appendix C. The calculations were carried out by taking into account the stability constants of metal-ligand complexes, ligand dissociation, and metal hydrolysis equilibria. The stability constants of metal hydrolysis species were expressed in terms of hydroxo- complex formation. The values of equilibrium constants adopted for the calculations are quoted in Appendix B. The distribution calculations were carried out at pH 0 to 14.

1.1.2.2 Metal and Inorganic Ligands: Classification of Metals

Generally, the majority of metal complexation with inorganic ligands is sufficiently strong interactions (Turner, 1995) resulting from the covalent bonding between metal and ligand. Thus, the term covalent index ($\Delta\beta$) is applied to describe this covalent interaction as shown in this equation:

$$\Delta\beta = \log \beta^0_{MF} - \log \beta^0_{MCl} \qquad (1.1)$$

where β^0_{MF} and β^0_{MCl} are the infinite dilution thermodynamic stability constants for the formation of the mono-fluoro and mono-chloro complexes, respectively. Turner *et al.* (1981) had applied it to classify the cation properties according to covalent interactions based on the difference in stability between the fluoro- and chloro- complexes of a particular element as a guide to its tendency to form covalent bonds. Elements with $\Delta\beta > 2$ are A-type cations, and elements with $\Delta\beta < -2$ are B-type cations that form strong complexes which are largely covalent bound (Stumm and Morgan, 1996a). Nieboer and Richardson (1980) proposed two indexes, i.e. electronegativity interactions (X^2m^r) and electrostatic interactions (Z^2/r). Electronegativity interactions (X^2m^r) is used to describe the character of B-type cations, i.e. the tendency of a metal ion to form covalent bonds whereas electrostatic interaction (Z^2/r) is used to describe the character of A-type cations, i.e. the tendency of a metal ion to form ionic bonds. A plot of X^2m^r versus Z^2/r allows the classification of metal ions into three categories: A-type, borderline and B-type, as shown in Table 1.2.

A-type metal ions prefer to form stronger complexes with ligands containing F, O and N in that order. In contrast, B-type metal ions tend to form stronger complexes with S, Cl, N, F in that order. The borderline cations are able to form stable complexes with various ligands, particularly for chloro-complexes. The A-type cations complex with

Table 1.2 Types of cation elements [b]

Type	Elements
A	Cs, K, Na, Li, Ba, Sr, Ca, Mg, La, Gd, Y, Lu, Be, Sc and Al
Borderline	Mn(II), V, Ti, Zn, Cr(II), Fe(III), Co, Cd, Sn(II), Cu(II), Pb(II), In, Ga, Fe(II), Sb(III), As(III) and Sn(IV)
B	Cu(I), Ti, Ag, Au, Hg(II), Pd, Pt and Pb(IV)

[b] Source: Adapted from Stumm and Morgan (1996b).

organic ligands having oxygen as the donor atom like carboxylate, carbonyl and phosphate groups while the B-type cations prefer to coordinate with bases containing I, S or N as donor atoms like sulphydryl group and purine or pyrimidine bases in proteins (Sirinawin, 1999). Table 1.3 illustrates the concentration range of ligands in natural waters.

There are many inorganic ligands in natural waters such as Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-} and S^{2-}, which are effectively able to form complexes with trace metals. In fresh water, the complexation with sulfate is rather unspecific and corresponds mostly to electrostatic interactions, therefore, the stability constants for sulfate complexes fall within a narrow range as shown below.

$$M^{2+} + SO_4^{2-} \rightleftharpoons MSO_4 \qquad \log K = 1 - 3 \qquad (1.2)$$

Much larger differences for stability constants occur in the hydrolysis constants for the complexation with chloride (B-type cations > A-type cations) and with carbonate. Both bicarbonate and carbonate are the important ligands since they are present in all natural waters. Metal ions such as Cd, Zn, Cu(II) and Pb form quite stable carbonato

Table 1.3 Concentration ranges of some ligands in natural waters; log concentration (M) [c]

Natural ligands	Fresh water	Seawater
HCO_3^-	-4 to -2.3	-2.6
CO_3^{2-}	-6 to -4	-4.5
Cl^-	-5 to -3	-0.26
SO_4^{2-}	-5 to -3	-1.55
F^-	-6 to -4	-4.2
HS^-/S^{2-} (anoxic conditions)	-5 to -3	-
Amino acids	-7 to -5	-7 to -6
Organic acids	-6 to -4	-6 to -5
Particle surface groups	-8 to -4	-9 to -6

[c] Data from Stumm and Morgan (1996b)

complexes while alkali and earth alkali ions tend to form primary ion pairs with carbonate. The stability constants and the species composition of carbonate complexes among different elements have been investigated (Byrne *et al.*, 1988; Bruno, 1990; Millero, 1992). The hydroxo- and carbonato-complexes are predominant under fresh water conditions, while the chloro-complexes are not significant except for Ag^+; this is in contrast to seawater conditions. Stumm and Morgan (1996b) observed that the speciation pictures of metal-inorganic complexes in freshwater and seawater are very similar however, the most important difference occurs for the B-type metals such as Cd(II), Hg(II) and Ag(I), which form complexes with chloride. Sulfide becomes an important ligand under anoxic conditions.

The complexation of metals with inorganic ligands is pH dependent and keeps concentration of free metal ions at low levels at high pH values. For example, in fresh water, the concentrations of Pb(II) and Cu(II) and the fully hydrolyzed metal ions like Al(III) and Fe(III) are found at low levels in the pH range around 8. In contrast, decreasing the pH, as in acidified waters, increases the free metal-ion concentration.

The concentrations of free metal ions increase and contribute to the toxic effects resulting from acidic atmospheric input (Stumm and Morgan, 1996b).

1.1.2.3 Metals and Organic Ligands

Many dissolved biogenic organic ligands especially the humic substances and anthropogenic ligands, are able to form complexes with trace metals in natural waters. The humic substances are a mixture of compounds with different molecular weights (<25 kDa in fresh waters). They are traditionally divided into three operational fractions; the first are humic acids, which are soluble in alkaline solutions but precipitate at pH 2; second are fulvic acids which are soluble in both acidic and alkaline solutions containing more carboxyl and hydroxyl groups than humic acids; and thirdly, humin which is insoluble in water at all pH values (Turner, 1995; Stumm and Morgan, 1996a). Both humic and fulvic acids are mainly comprised of dissolved organic carbons (DOC). However, fulvic acids have relatively lower molecular weights, generally <1 kDa as compared to a several thousands for humic acids. Humic substances in the waters are mainly derived from two sources, namely, leaching of soil organic matter and excretion of organic materials from organic organisms. The aquatic humic substances have a lower mean molecular weight than soil humic substances. Figure 1.2 shows the functional groups involved.

The complexation of metals by humic substances is highly pH dependent (Antelo *et al.*, 2000). Figure 1.3 shows pH dependence on metal complexation by humic substances. At low pH values, hydrogen ion saturates the metal binding sites on humic substances whereas at high pH values, free metal ion is not an important species. A relatively natural pH value is therefore favored for metal complexation by humic substances (Morrison, 1991).

The ability of humic acid for trace metal complexation is significant since it can have a large impact on the metal ion mobility and free-metal ion concentration in

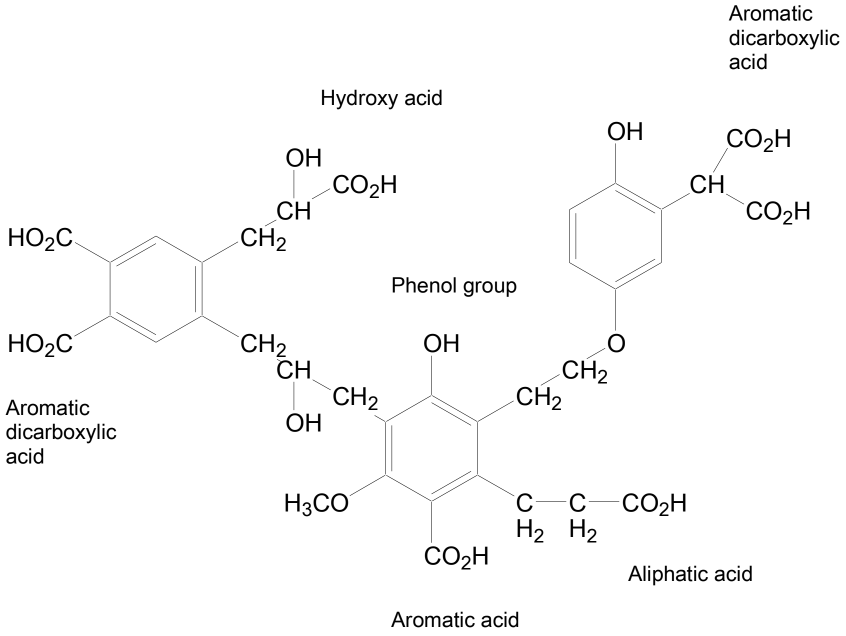


Figure 1.2 Different possible -OH and -COOH groups in a hypothetical humic acid polymer (Stumm and Morgan, 1996a).

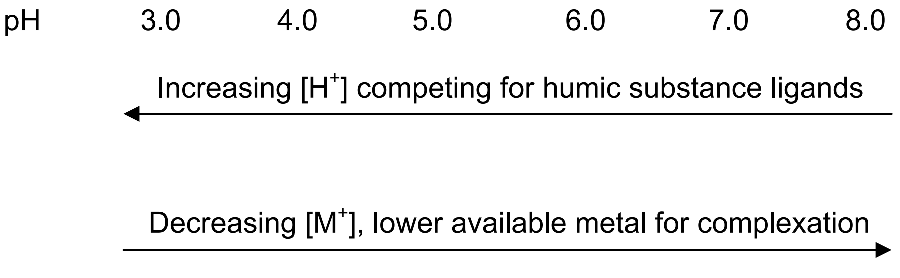


Figure 1.3 Dependence of metal complexation by humic substances on pH.

aquatic systems especially in fresh waters. As indicated above, the metal binding to humic acid is dependent on pH, ionic strength and the presence of other metal ions, especially when the major cations, e.g. calcium and magnesium are present in concentrations many orders of magnitude greater than that of target metal ions (Glaus *et al*., 2000; Christl and Kretzschmar, 2001; Koopal *et al*., 2001; Borrok and Fein, 2004; Chakraborty and Chakrabarti, 2006). The binding process occurs via the chelation of two main neighboring groups of humic acid structures; carboxylic (pKa = 4.8) and phenolic (pKa = 10) groups (Stumm and Morgan, 1996a). Turner *et al*. (1981) proposed that the complexation constants of humic acid complexes obtained for metals correlate well with the stability constants of hydroxide and carbonate complexes. Therefore, the complexation of humic and fulvic acids are most likely for cations which are able to complex with CO_3^{2-} (Hamilton-Taylor *et al*., 2002) and OH^-. It is believed that complexation of metals with humic substances reduces bioavailability of metal, in turn, reducing potential toxicity (Morrison, 1991).

The anthropogenic ligands such as NTA (nitrilotriacetic acid) and EDTA (ethylenediaminetetraacetic acid) are present as anthropogenic pollutants in many natural waters with concentrations above 10^{-8} M (Stumm and Morgan, 1996b). Both EDTA and NTA are multi-dentate ligands. EDTA is a hexadentate ligand, which contains two nitrogen donor atoms from two amine groups and four oxygen donor atoms from the four carboxylic groups. NTA is a tetradentate ligand, which contains one nitrogen donor atom from one amine group and three oxygen donor atoms from the three carboxylic groups. They are very powerful complexing ligands for complex formation by chelation. The ligand exchange reaction is one of the possible pathways for the exchange of metal-multi-dentate ligands with other metals under natural water conditions. For example, EDTA may initially occur as Fe(III)EDTA and the exchange of Fe(III)EDTA with Zn(II) can be demonstrated as follows:

$$Zn^{2+} + \mathrm{Fe(III)EDTA} \longrightarrow Fe^{3+} + \mathrm{Zn(II)EDTA} \qquad (1.3)$$

1.2 Chemistry of Essential and Hazardous Metals

The chemistry of two essential protein and enzyme trace metals like copper and zinc, and two toxic metals like cadmium and lead is discussed.

1.2.2 Copper

Copper exhibits both mono- and divalence. It can form water-soluble cationic simple salts (Cu^+ can only exist in aqueous solution) and also form coordination complexes by association with S- and N-containing ligands with a coordination number of 4. Complexes of cuprous (Cu^+) have a tetrahedral configuration and can coordinate with 2 or 6 ligands. Whereas cupric (Cu^{2+}) coordination complexes are square planar configuration and can coordinate with 6 ligands involving four short and two long metal-ligand bonds. Cuprous ions can also form coordination compounds number of 2 or 3. Therefore, they can form more stable complexes with most ligands than other transition metals. Cuprous ions are oxidized to the stable cupric state by peroxide accumulation in tissue; however, Cu^{2+} ion can bind with SH-containing compounds and reversibly react to form Cu^+ ion and disulfide. Copper oxidase functions through a cyclic shuttling between its valence states, with a continuous uptake of molecular oxygen (Venugopal and Luckey, 1978).

1.2.3 Zinc

Zinc can form stable and water-soluble salts exhibiting a valence of +2 but at higher pH solution. At such pHs, it forms insoluble $Zn(OH)_2$ which is amphoteric in nature and ionizes as follows depending upon the pH of the media:

$$2H^+ + ZnO_2^{2-} \rightleftharpoons Zn(OH)_2 \rightleftharpoons Zn^{2+} + 2[OH]^- \qquad (1.4)$$

Since zinc ions are small size, thus the force of attraction between zinc and oxygen in $Zn(OH)_2$ is as strong as that between oxygen and hydrogen. Zinc forms tetrahedral coordination complexes with a coordination number of 4 whereas zinc complexes with coordination number of 6 are rare. Zinc readily complexes to amino acids, peptide, and proteins in biological media. It has an affinity for thiol and hydroxyl groups and for ligands containing nitrogen as donor (Venugopal and Luckey, 1978).

1.2.3 Cadmium

Cadmium does not occur in the free form in nature. It normally exhibits a maximum valence of +2 to form stable cationic salts. Cd forms tetrahedral complexes with a coordination number of 4. It forms halogen complexes such as $[CdX_3]^-$ and $[CdX_6]^-$. Cadmium is also amphoteric and can form stable salts such as sodium cadmate, $Na_2[Cd(OH)_4]$, in which the hydroxyl groups can be replaced by water and monovalent groups. Cadmium resembles zinc in its electronic configuration and affinity toward organic ligands, but it has greater affinity than zinc to thiol groups and will replace zinc in some metal enzyme complexes. However, zinc is bound more tightly than cadmium to O- and N-containing ligands. The binding of cadmium with S is stronger than that of any essential metals except for copper. Cadmium could not be removed from tissues with chelating agents, which can mobilize zinc from tissues as appeared in the binding very firmly in the tissues (Venugopal and Luckey, 1978).

1.2.4 Lead

Lead exhibits valences of +2 and +4, the more stable form being +2 whereas +4 is shown in organometallic compounds and in basic lead acetate. A divalent Pb ion could be either a hard or soft acid depending upon the biological medium; thus Pb forms stable complexes with free thiol-, carboxylate- and phosphate- carrying ligands of biopolymers and membranes whereas imidazole and amino groups are of very little importance for Pb^{2+} complex formation (Venugopal and Luckey, 1978). Among the toxic metals, Pb binds more strongly with S ligand than Cd. The organic derivatives of Pb are more toxic than are the inorganic salts, because of their greater lipid solubility.

1.3 Biological Implication: Bioaccumulation

Bioaccumulation is the increased concentration of a chemical in tissue compared to the environment. It often occurs with materials that are more soluble in lipids and organics (lipophilic) than in water (hydrophilic) (Landis and Yu, 1999).

Planktons and other aquatic microorganisms directly accumulate metals from solution, however other living organisms may accumulate the metals via food or digestion of non-food particles. The uptake of trace metals by aquatic organisms depends on their chemical speciation. For example, the uptake of the lipid-soluble metal complexes in living organisms is bioconcentrated to a greater extent than the free and weakly complexed metals, and only lipid-soluble metal complexes can magnify through the food cycle (Morrison, 1991). Lipophilic metal species bioconcentrate to a factor of 10^6 to 10^8 in fish, which is a result of the rapid diffusion of lipid-soluble metal species across the biomembrane. Free and weakly complexed metals are transported across the cell membranes at a slower rate and provide bioconcentration factors between 10^2 and 10^5 (Morrison, 1991).

The changes in pH of the environment influence the uptake of the metals by affecting metal speciation directly, and by its effect on the biological surface. Campbell *et al*. (1985) had investigated the influence of acidification on metal speciation in the freshwater ecosystem. They found that Al, Hg, Cu and Pb showed speciation changes, while Ag, Mn, Cd and Zn showed little effect. When the biota is exposed to these metals, there are two sets of responses. For Cd, Cu and Zn, a decrease in pH caused a decreased biological uptake, while for Pb and some other metals, a decrease in pH caused an increase in its availability.

The extent of metal accumulation markedly varies with their chemical species and variety. Therefore, the impact of trace metals on aquatic organisms depends on their chemical speciation.

1.3.1 Biological Interaction

Metals are closely related to biological processes. Some metals are essential to biological activity whereas others, the nonessential metals, may be taken up by cells. Some metal species may be able to bind directly with proteins, enzymes and other biological molecules. Others may also be adsorbed on the cell walls or may diffuse through cell membranes to where they could be assimilated or may influence enzyme reactions or exert a toxic effect.

1.3.1.1 Bioavailability

The total aqueous concentration of a metal is not a good indicator of its bioavailability. The metal's speciation will greatly affect its availability to aquatic organisms (Campbell, 1995). Bioavailable metal may be defined as the fraction of the total metal present in or mobilized from a specific environmental compartment; that is either available or can be made available for uptake by the biota (Langston and Spence, 1995; Peijnenburg and Jager, 2003).

The changes in metal bioavailability are influenced by the types of ligands involved, i.e. organic ligands form lipophilic complexes with metals, low molecular weight organic ligands form hydrophilic complexes, inorganic anions, etc. In the presence of low molecular weight organic ligands such as EDTA and NTA, these ligands form hydrophilic complexes with the metals to which biological membranes are virtually impermeable (Campbell, 1995). The complexation of metals by such ligands is expected to decrease its bioavailability (Campbell, 1995). In the presence of ligands forming lipid-soluble complexes like 8-hydroxy-quinoline (oxine), the toxicity of Cu is enhanced (Florence *et al.*, 1983; Florence and Stauber, 1986). However, the introduction of a sulfonate group as in 8-hydroxy-quinoline-5-sulphonic acid (sulfoxine) caused the enhanced toxicity to disappear (Phinney and Bruland, 1994). For inorganic anions, increasing water hardness generally decreased metal toxicity, possibly due to Ca competition at the cell surface (Rai *et al.*, 1981; Jayaraj *et al.*, 1992). An increase in salinity may affect the toxicity partly because of changes in chemical speciation and in physical characteristics of organisms (Hall and Anderson, 1995).

1.3.1.2 Toxicity

Toxicity is a property or properties of a material that produces a harmful effect on a biological system. A toxicant is the material that produces such biological effects. The main source of toxicants in aquatic systems is produced from anthropogenic origin while toxicants at lower amounts are derived from biological systems (Landis and Yu, 1999). The toxicity effects of different metal ions are closely related to their coordination chemical properties, since the mechanisms often include the binding of metals to sensitive cellular compounds, often enzymes and proteins. Generally, B-type metal ions are more toxic than borderline and A-type metal ions; A-type < borderline < B-type (Whitfield and Turner, 1983). This can be explained by their higher affinity for S- and N-containing ligands (Sigg, 1987; Mason and Jenkins; 1995).

Mason and Jenkins (1995) defined metal toxicity as the impairment of biological function caused by an uptake of a particular metal. The mechanisms of metal toxicity can include an essential metal ion being replaced by a nonessential element with similar chemical properties, blocking essential biological functional groups of biomolecules as well as modifying the active conformation of biomolecules by complexation of metal ions (Sigg, 1987).

The toxicity usually relates to metal speciation; that is, the toxicological effects of metals are strongly dependent on the actual chemical forms of metals in aquatic systems, which may be present as free-metal forms or ligand-metal complexes. Both lipophilic metal complexes and metals in free or weakly complexed forms are potentially able to cross cell membranes, leading to an increased toxicity above its expected values on the basis of the free-ion concentration (Pärt and Wikmark, 1984; Daly *et al*., 1990; Errécalde *et al*., 1998). The free metal ion may exert toxic effects as a result of the competitive inhibition of enzyme systems. In contrast, lipophilic metal complexes are usually very stable and are therefore unlikely to dissociate and provide free ion toxicity (Florence *et al.*, 1983).

The biological responses elicited by a dissolved metal usually depend on the concentration of metal at the cell surface uptake sites and are proportional to free metal ion concentration. The free metal ion concentration is determined not only by the total dissolved metal concentration, but also by the concentration and nature of the ligands present in solution (Campbell, 1995). The free ion activity model (FIAM) used interactions of chemical speciation with organism to predict trace-metal uptake. However, knowledge of the free-metal ion concentration alone is insufficient to predict the biological response. The biotic ligand models (BLM) of acute toxicity have also been developed. The BLM considers the effect of the dissolved metal concentrations on toxicity as well as the metal interactions with organic and inorganic ligands that affect metal speciation and availability (Peijnenburg, 2003).

1.4 Method Development for Speciation Study of Trace Metals in Natural Waters

The analytical methods for the determination of trace metals in natural waters have been developed in the past to measure the total concentration of the metals. However, knowledge of total concentration gives insufficient information about toxicity and bioavailability, which are strongly dependent on the chemical species of trace metals. Thus, it is necessary to obtain more information on the concentrations of the various chemical species of metals.

In speciation analysis, it is necessary to select the analytical technique for measuring individual species, which are often so low that even the total concentration is difficult to determine (Fytianos, 2001). Further information is rarely possible to be obtained except with preconcentration techniques (e.g. ion exchange by specific resins, and solid phase extraction by sorbents) that will not disturb the species distribution.

The main reason for the separation procedures according to physicochemical characterization is to divide the total amount of metals into individual amount of metal forms after being discriminated into operationally-defined metal species. Generally, the combination of physical separation by size fractionation and chemical separation by retention studies onto sorbent has been applied for the characterization of metals in the speciation study.

1.4.1 Physical Characterization: Size Fractionation

The interaction of trace metals with particulate matter is one of the determinants for their dissolved concentrations and the distribution of chemical forms of trace metals between different dissolved complexes is strongly dependent on the dissolved ligands present. Since the distribution of trace metals in solution is governed by contact of dissolved fraction with particulate matter within a period of time, a pretreatment step to separate the two distinguished phases by classical 0.45-µm filtration is necessary prior to studies on metal speciation in natural waters. In general, particulate matters have a size larger than 0.45-µm whereas, the dissolved phase passes through a 0.45-µm membrane filter, that includes both truly dissolved fraction (<1 kDa or <1 nm) (Guéguen *et al*., 2004) and colloidal particles (0.1-µm to 0.001-µm) (Batley, 1991a).

Several techniques for physical characterization of dissolved metals based on its size at the molecular level (molecular filtration) have been employed which includes dialysis, gel permeation, cross flow filtration and ultrafiltration (Burba *et al*., 2001). Among these, ultrafiltration is especially suited for metal speciation with more advantages over the other techniques such as no disturbance of solution equilibrium during determination (Nifant'eva *et al*., 2001), much more reduced surface area for adsorption (Laxen and Harrison, 1981), good reproducibility (Buffle *et al*., 1978), and no concentration polarization of metal at the membrane surface with a pressurized system (Batley, 1991b).

The term ultrafiltration is commonly used to describe the separation of dissolved species based on size at the molecular level or Nominal Molecular Weight Limit (NMWL) of ultrafiltration membrane, although the absolute molecular weight of the material cannot be determined (Singhal *et al.*, 2005). Ultrafiltration membranes having a nominal pore size smaller than 15 nm can be used to discriminate further the size continuum of material in natural waters. Ultrafilters generally consist of a thin organic polymeric hydrous gel film supported on porous polyethylene or cellulose ester bases. They are available with nominal pore sizes ranging from 1.2 to 14 nm. The retentive capability of an ultrafilter is usually designated by a molecular weight cut-off (MWCO) value. This value refers to the molecular weight of a globular solute which is 90% retained. Such assignment is only nominal because fractionation is achieved by molecular size rather than weight (Mora and Harrison, 1983).

Ultrafiltration methods have been used to fractionate both organic materials and trace metal constituents. In trace metal speciation studies, ultrafiltration is often incorporated with studies on chemical behavior of the size groupings to serve as a definitive speciation scheme (Laxen and Harrison, 1981; Chakrabarti *et al.*, 1993; Cheng *et al.*, 1994). Although the absolute molecular weight of the material cannot be determined, suspended matter is crudely fractionated and some trends are evident on the size distribution of trace metals in natural waters.

1.4.2 Chemical Characterization: Different Solid Sorbents

The possibility of using different kinds of solid sorbents for chemical characterization of metal forms has been investigated more recently. Such different sorbents, namely, chelating resins, anion exchange resins and reversed-phase octadecyl silica have been extensively used for simultaneous enrichment of metals and differentiation among its forms, namely, positively-charged species, negatively-charged species and neutral species in water samples with complex matrices. The knowledge obtained from the operationally-defined classes of metal forms by chemical characterization using such different sorbents may provide sufficient information to predict any impact on aquatic systems.

1.4.2.1 Ion Exchange

The ion exchange resin contains numerous fixed positive or negative charges and mobile counter ions of opposite charges, which maintain electrical neutrality and can be replaced reversibly by other ions with similar charges when the resin is immersed in a solution (Mizuike, 1983). An example of a reversible reaction, which takes place on a cation exchange resin, is as follows:

$$\text{Resin-SO}_3^-\text{H}^+ + \text{Na}^+ \text{(solution)} \rightleftharpoons \text{Resin-SO}_3^-\text{Na}^+ + \text{H}^+ \text{(solution)} \qquad (1.5)$$

The reversible reaction for anion exchange takes place similar to cation exchange but with different charges of resin and ions in solution.

The sorption behavior of ions on an ion exchanger greatly depends on the chemical state of such ions, conditions of solution, and the nature of ion exchanger.

a) Cation Exchange Resin: Chelating Resin

Generally, cation exchange is carried out by means of chelating resins such as Chelex-100. This resin is a styrene-divinylbenzene copolymer resin incorporating the paired iminodiacetate groups (see Figure 1.4), which act as chelating groups in binding polyvalent metal ions. This chelating resin is classified as weakly acidic cation exchange resins with carboxylic groups. The resin has a pore size of about 1.5-nm resulting in the exclusion of large molecules in solutions. The Chelex resin effectively exchanges with cations at pH 4 or higher. However, it acts as an anion exchanger at pH 2 or lower. In general, the H^+ form is the basic form of resin and is changed into the Ca^{2+} or NH_4^+ forms due to H^+ enrichment effect on the decreasing retention of metals by Chelex. This effect results from the replacement of H^+ with divalent cations such as

Ca^{2+} or Mg^{2+}, which are the major components of water media (i.e. river waters, lake waters); leading to a liberation of H^+ and thus decreasing pH; resulting in decreasing retention of metals in the samples (Figura and McDuffie, 1977).

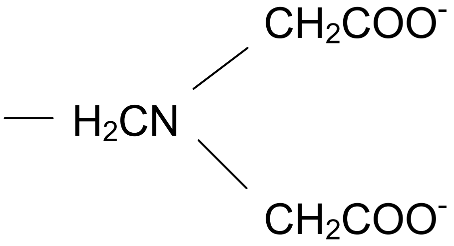


Figure 1.4 Iminodiacetate functional group of Chelex –100.

Chelex-100 resin is widely applied for metal speciation in natural waters. This resin may be used with either a column or a batch method. Both column and batch methods involve metal ion exchange. The time scale of exchange differentiates these two methods. In the column method, a filtered water sample (< 0.45-μm) is directly passed through a column containing the resin to achieve the retention of metal, which depends on flow-rates and column length. Typically in the batch method, the resin is directly added into the sample followed by gentle stirring for a longer time than the exchange time of metal strong complexes. The retained metals by resin from both methods are stripped out by an acid. The fraction of metals retained by Chelex column is commonly defined as the “Chelex labile” fraction (Liu and Ingle, 1989) or “labile metal” (Batley and Florence, 1976) or “Chelex-column-labile” (Chakrabati *et al*., 1993) and the fraction not retained by the resin is defined as the “Chelex nonlabile” (Liu and Ingle, 1989) or “bound fraction” or “bound metal” (Batley and Florence 1976) or “Chelex-column-nonlabile” (Chakrabati *et al*., 1993). The metals retained by the Chelex resin comprise of free aquo metal ions, hydrated metals and weak metal complexes whereas metals not retained by Chelex include the bound metals in strong complexes (or adsorbed on or occluded in colloidal particles).

In 1980, Figura and McDuffie classified the retained metals by this resin based on relative labilities towards Chelex column and batch methods into four types, namely “Very Labile”, “Moderately Labile”, “Slowly Labile” and “Inert”. The fraction defined as “Very Labile” contained ASV active species (see section 1.4.3.1), is determined directly after a classical 0.45 µm filtration. The fraction eluted from Chelex column is defined as “Moderately Labile”. The “Slowly Labile” is the fraction passing through the Chelex column, which is subsequently adsorbed onto Chelex resin by the batch method. The fraction, which is not adsorbed on the Chelex resin in the batch method, is defined as “Inert”.

b) Anion Exchange Resin

The anion exchange resin is an insoluble but permeable synthetic polymer. The resins use crosslinked styrene-divinylbenzene copolymer as resin base containing ionizable functional groups, which acts to bind anionic metal complexes. Dowex 1-X8 is an example of such an anion exchanger. It is classified as a strongly basic anion exchange resin by virtue of its quaternary ammonium group (Mizuike, 1983) as can be seen in Figure 1.5. The conventional form of Dowex 1-X8 available is the chloride form but can be changed into hydroxide (Ou-Yang and Jen, 1993) or acetate forms (Ficklin, 1990; Toda *et al*., 1996) depending on the type of sample used. The Cl^- form of resin may be changed to enrich the distribution coefficient of anion metal complexes by replacing Cl^- with OH^- and converting it back into Cl^- form. However, in saline waters, there is competition between the anionic metal complexes and the chlorides ions leading to a decreased metal uptake by the resin (Groschner and Appriou, 1994).

Both the chemical state of metal and its complexes affect the adsorption behavior of metal complexes on Dowex 1-X8 resin. Groschner and Appriou (1994) had reported that the selective characteristics of the resin were dominated by the ligand rather than by bivalent metal ions. In addition, the enrichment on the resin is greatly

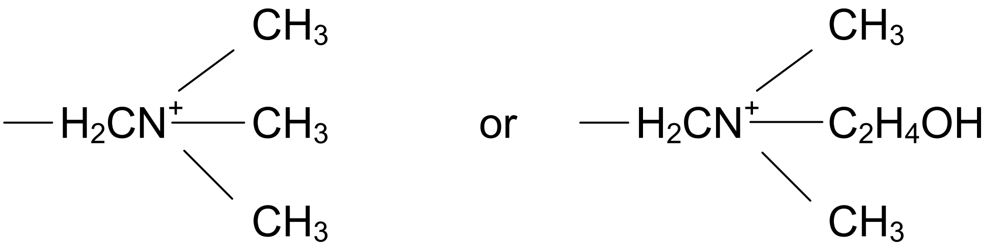


Figure 1.5 Quaternary ammonium functional group of Dowex 1-X8.

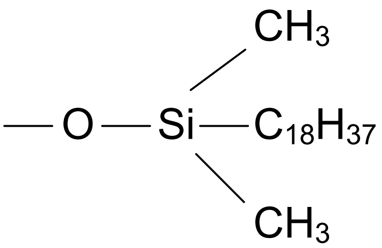
based on the charge interaction law (Ou-Yang and Jen, 1993); that is, the higher the charge on the ion, the greater the interaction between the ion and the bed. Ion exchange is also greater with higher resident times for the sample species in the resin column.

1.4.2.2 Adsorption

The adsorption of neutral metal complexes on nonpolar substrates is basically governed by the principle “like adsorbs like” concept, which is based on solutes having similar chemical properties with the substrate leading to adsorption. However, the interactions between solutes and substrates take place not only through van der Waals forces but also through hydrogen bonding or dipole-dipole attractions as secondary interactions in some cases. In addition, the partitioning between aqueous and solid phase is a low-energy process (5 versus 80 kcal/mol for ion exchange). Nonpolar substrates can also interact with moderately polar compounds in water (Abollino *et al.*, 2000).

a) Reversed-Phase Octadecylsilane

There are several reversed-phase HPLC packing materials used to separate both dissolved organic compounds and metal organic complexes in natural waters. One example of such packing materials is the reversed-phase C-18 (RP C-18). The pre-packed column cartridge contains C-18 (octadecylsilane) moieties bonded to a silica support (see Figure 1.6). The internal volume of one pre-packed column cartridge is approximately 0.5 mL and contains 0.4 g of the reversed-phase packing. It is highly hydrophobic to adsorb hydrophobic species, however, partially hydrophilic ones are also adsorbed by its hydrophobic core made up of free silanol groups through the ion-exchange capacity of the silanol groups (Kremling *et al.*, 1981; Mackey, 1985). Generally, less than 50% of the available silanol groups in the conventional RP C-18 have not reacted after silanization, and only 60% to 70% of these can be eliminated by post-silanization with a short alkyl-chain silane or endcapping (Low, 1986). The adsorbed efficiency for hydrophobic species of RP C-18 depends on the brand since the percentage of carbon loading varies from 12% to 18%. In addition, the pores, which have different sizes and free silanol sites, can interact with polar compounds present at different levels (Abollino *et al.*, 2000). It can be indicated that this particular type of RP C-18 packing does not only adsorb nonpolar fractions, but may also act as ion-exchanger. Although it has been claimed, however, the nonpolar fraction is the most geochemically significant (Becher *et al.*, 1983). Therefore, the application of solid phase extraction in metal speciation still uses reversed-phase C-18 packing materials.



$$—O—Si(CH_3)_2—C_{18}H_{37}$$

Figure 1.6 Octadecylsilane functional group of RP C-18.

In 1993, Haraldsson *et al*. employed three sorbents, i.e. Chelex-100, Sep-Pak C-18 and Fractogel DEAE, to distinguish metal forms of multi-trace elements in fresh