CHAPTER II

EXPERIMENTAL SECTION

2.1 Stability constant [1]

There are two major areas of solution chemistry. The first of these is concerned with the chemical nature and concentration of each of the species present at equilibrium. The second concerns the rate and mechanism by which the species added to the solution come to equilibrium. These two aspects of solution chemistry are, of course, closely related. Consider a system

$$A + B \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C + D \qquad (2.1)$$

where k_f and k_r are the forward and reverse rate constants. The rate at which the reaction proceeds in a forward direction, which equals the rate of loss of A ($-dA/dt$), is given by

$$\frac{-dA}{dt} = k_f\{A\}\{B\} \qquad (2.2)$$

where {X} is the activity of species X. In addition, the rate of the reverse reaction, which equals the rate of formation of A (dA/dt), is given by

$$\frac{dA}{dt} = k_r\{C\}\{D\} \qquad (2.3)$$

At equilibrium the rates of the forward and reverse reactions are equal, and so

$$k_f\{A\}\{B\} = k_r\{C\}\{D\} \qquad (2.4)$$

which may be rearranged to give

$$\frac{k_f}{k_r} = K^\theta = \frac{\{C\}\{D\}}{\{A\}\{B\}} \qquad (2.5)$$

where k_f/k_r, being a constant, is written as a single constant K^θ, and is known as an equilibrium constant. Equation 2.5 is known as Guldberg and Waage's relationship

and can be used as a basis for measuring equilibrium constants provided that the reaction takes place by a one-step mechanism.

Equilibrium constants for the interaction of metal ion (M) and ligands (L) can be written as (charges omitted for the sake of clarity)

$$M + L \overset{K^{\theta}_{ML}}{\rightleftharpoons} ML;\ K^{\theta}_{ML} = \frac{\{ML\}}{\{M\}\{L\}} \tag{2.6}$$

The equilibrium constant K^{θ}_{ML} is known as a stability constant. Its inverse ($1/K^{\theta}_{ML} = \{M\}\{L\}/\{ML\}$) is known as an instability constant.

In many cases, more than one ligand can coordinate to a given metal ion. The formation of those complexes usually occurs in a stepwise manner, i.e. ML is formed first, ML_2 second, and so on. In general, it is not possible to form ML_n without first forming ML_{n-1}. The stepwise reactions and their stability constants are shown in the following equations.

$$M + L \overset{K^{\theta}_{11}}{\rightleftharpoons} ML \quad ; \quad K^{\theta}_{11} = \frac{\{ML\}}{\{M\}\{L\}} \tag{2.7}$$

$$ML + L \overset{K^{\theta}_{12}}{\rightleftharpoons} ML_2 \quad ; \quad K^{\theta}_{12} = \frac{\{ML_2\}}{\{ML\}\{L\}} \tag{2.8}$$

$$ML_2 + L \overset{K^{\theta}_{13}}{\rightleftharpoons} ML_3 \quad ; \quad K^{\theta}_{13} = \frac{\{ML_3\}}{\{ML_2\}\{L\}} \tag{2.9}$$

$$\vdots \qquad\qquad \vdots \qquad\qquad \vdots$$

$$ML_{n-1} + L \overset{K^{\theta}_{1n}}{\rightleftharpoons} ML_n \quad ; \quad K^{\theta}_{1n} = \frac{\{ML_n\}}{\{ML_{n-1}\}\{L\}} \tag{2.10}$$

K^{θ}_{11}, K^{θ}_{12}, K^{θ}_{13}, and K^{θ}_{1n} are known as stepwise stability constants; subscript being stoichiometry of metal ion and ligand, respectively. If equations 2.7 to 2.10 are combined, the stability constant for ML_n formation will be given the Greek letter β and is known as an overall stability constant.

$$M + nL \overset{\beta^{\theta}_{1n}}{\rightleftharpoons} ML_n \; ; \qquad \beta^{\theta}_{1n} = \frac{\{ML_n\}}{\{M\}\{L\}^n}$$

(2.11)

There is a specific relationship between the overall stability constant β^{θ}_{1n} and the stepwise stability constants. In general

$$\beta^{\theta}_{1n} = K^{\theta}_{11} \times K^{\theta}_{12} \times K^{\theta}_{13} \times \ldots K^{\theta}_{1n} = \prod_{i=1}^{n} K_{1i} \qquad (2.12)$$

The overall stability constant for polynuclear complexes can be expressed as

$$xM + yL \rightleftharpoons M_xL_y \; ; \qquad \beta^{\theta}_{xy} = \frac{\{M_xL_y\}}{\{M\}^x\{L\}^y} \qquad (2.13)$$

In all above equations, stability constants have been defined in terms of the activities of the species present. However, in practice many analytical techniques yield concentrations rather than activities. Concentrations are related to activities by the expression

$$\{X\} = [X]\gamma_x \qquad (2.14)$$

where [X] = concentration of X and γ_x = activity coefficient of X. Activity coefficients are in general tedious and difficult to measure. They also depend very significantly on the nature and concentrations of other species present in solution. Theoretical attempts at calculating activity coefficients, based on the Debye-Hückel approach and its extensions, are at best of only limited accuracy.

The most expedient solution to what is a very major problem is to rewrite the thermodynamic stability constant in terms of both concentrations and activity coefficients.

$$K^{\theta}_{ML} = \frac{\{ML\}}{\{M\}\{L\}} = \frac{[ML]}{[M][L]} \times \frac{\gamma_{ML}}{\gamma_M \gamma_L} \qquad (2.15)$$

If the term $\gamma_{ML}/\gamma_M \gamma_L$ remains constant then K defined by Equation 2.16 will also be a constant.

$$K_{ML} = \frac{[ML]}{[M][L]} \qquad (2.16)$$

An overall stability contant, β_{xy}, is known as a stoichiometric stability constant or apparent thermodynamic constant whereas β^{θ}_{xy} is known as a thermodynamic stability constant. The term $\gamma_{ML}/\gamma_M \gamma_L$ in Equation 2.15 may be maintained effectively constant by,

(a) Having a large excess of an inert background electrolyte which does not react with any of the metal, ligand or metal-ligand species.

(b) Using only low concentrations of metal and ligand, so any change in their concentrations as a result of their reaction together has a insignificant change on the overall ionic strength of the medium.

The ionic strength (I) is normally defined by the equation $I = \frac{1}{2}\Sigma c_i z_i^2$, where c_i is the concentration and z_i is the charge of species i; accordingly, ionic strength has the units of concentration (that is, mol/L).

2.2 UV-Vis spectrophotometry

Most UV-Vis spectra are obtained by measuring the intensity of the absorption of monochromatic radiation across a range of wavelengths passing through a solution in a cuvette. The practical wavelength region extends from 190-400 nm (UV range) and from 400-780 nm (Vis range) [23].

In a typical experiment, a light beam of intensity I_0 strikes a sample in a quartz or glass cell. After passing through the cell, the light beam has a reduced intensity I due to reflection losses at the cell windows, scattering at dispersed particles, and eventually absorption in the sample that is only one factor involving chemical substances. It is necessary to measure free solvent so as to compensate for reflection and scattering losses before measuring the interested sample using the same cell. The transmittance T is calculated using the following equation,

$$T = \frac{I}{I_0} \approx \frac{I_{solution}}{I_{solvent}} \tag{2.17}$$

The presentation of transmittance T, as a function of wavelength λ, is the required spectrum of the sample.

The intensity of an absorption band, *absorbance*, is proportional to the number of absorbing species in the illuminated part of the cell. Absorbance, A, is defined by the equation,

$$A = -\log T = \log \frac{I_0}{I} \tag{2.18}$$

and is proportional to the cell thickness, d [cm], the concentration of the solution, c [mol/L]; and a substance-specific proportionality constant ε called the *molar absorptivity*, [L $mol^{-1}cm^{-1}$].

$$A = \varepsilon \cdot c \cdot d \tag{2.19}$$

Equation 2.19 is known as *Beer's law*.

2.2.1 Chromophores

It is a long-recognized fact that colored substances are generated by absorption of light. One or more unsaturated linkages play an important role in this phenomenon. Such linkages or groups were named chromophores by Witt in 1876 [24]. The other groups that by themselves do not confer color to a substance but increase the coloring power of a chromophore are called auxochromes.

Ultraviolet radiation is usually absorbed by a chromophore rather than by the molecule as a whole. Chromophores are, in most cases, covalent unsaturated groups. They are functional groups that usually absorb in the near-ultraviolet or visible region when they are bound to a non-absorbing, saturated residue that possesses no unshared or nonbonded electrons (e.g. a hydrocarbon chain). Auxochromes contain functional groups that have nonbonded valence electrons and exhibit no absorption at wavelengths above 220 nm. They do, however, absorb strongly in the far-ultraviolet region ($n \rightarrow \sigma^*$). If an auxochorme and a chromophore are combined in the same molecule, the chromophore absorption will typically shift to a longer wavelength and show increase in intensity. Shifts to longer wavelengths are called *bathochromic* or *red shifts*; changes to shorter wavelengths, *hypsochromic* or *blue shifts*. An increase in intensity of an absorption band is called a *hyperchromic effect*, whereas a decrease in intensity is termed a *hypochromic effect*.

2.2.2 Factors involved in the formation of absorbing compounds

Reagent concentration The amount of reagent required is dictated by the composition of the absorbing complex formed. An optimum concentration of reagents should be determined, since either not enough reagent or too much reagent can cause deviation from Beer's law. It is proved that reliable spectra should have an absorbance approximately less than 1 [25].

Time The time used for approaching to an equilibrium of each complexes may not equal, so it is essential to estimate appropriate complexation time in order to ensure that the complexes studied are completely formed.

Temperature The relationship between temperature and stability constant of complexes depends on the following equation:

$$\Delta G = -RT\ln K$$

Thus it is necessary to control the temperature steadily in all of the experiments.

Solvent The choice of solvent used in the UV-Vis spectrophotometry is based on two criteria. First, it must dissolve interested compounds completely. Second, the solvent must not absorb the light in the same range as interested substances do. In general, water is the preferred choice whenever the sample is sufficiently soluble. A list of several solvents that can be used for assays in the ultraviolet-visible region is given in Table 2.1.

Table 2.1 Lower transparency limit of solvent in the ultraviolet region [26]

Solvent	Cutoff Point (nm)[a]	Solvent	Cutoff Point (nm)[a]
Water	200	Dichloromethane	233
Ethanol (95%)	205	Buthyl ether	235
Acetonitrile	210	Chloroform	245
Cyclohexane	210	Ethyl proprionate	255
Cyclopentane	210	Methyl formate	260
Heptane	210	Carbon tetrachloride	265
Hexane	210	N,N-dimethylformamide	270
Methanol	210	Benzene	280
Pentane	210	Toluene	285
Isopropyl alcohol	210	*m*-Xylene	290
Isooctane	215	Pyridine	305
Dioxane	220	Acetone	330
Diethyl ether	220	Bromoform	360
Glycerol	220	Carbon disulfide	380
1,2-Dichloroethane	230	Nitrometane	380

[a]Wavelength at which the absorbance is unity for a 1-cm cell, with water as the reference.

2.2.3 Isosbestic point

Occasionally, the absorbance is measured at an *isosbestic point* (or an *isoabsorptive wavelength*), which is a wavelength where the absorbing species in equilibrium have a common value of molar absorptivity (ε). Although points of common ε-value occur also in some irreversible decomposition reactions giving two products, isosbestic points are often taken as criteria for the existence of at least two interconvertible absorbing species, the total quantity of which is constant.

2.2.4 Supporting electrolyte

Supporting electrolytes are required in UV-Vis spectrophotometry to ensure that the interested active species are not perturbed by the uninterested active species in spectrophotometric cell. The supporting electrolyte is often chosen to provide optimal condition for the particular analysis. Solutions of strong acids (e.g. hydrochloric and sulfuric), strong bases (sodium or lithium hydroxide), or neutral salts (e.g. chlorides, perchlorates, or sulfates of either alkali metals or tetraalkylammonium ion) are frequently used. The supporting electrolyte in spectrophotometric studies should be at least 50-fold excess over analyzed ions and its concentration in the system is usually of 0.01-0.05 M [1].

2.3 General procedures

2.3.1 Analytical instrument and equipment

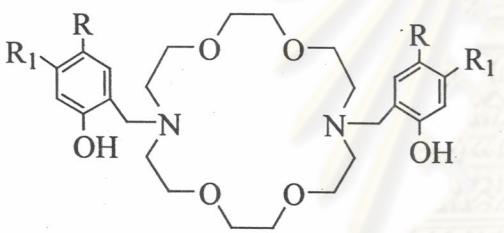
All UV-Vis absorption spectra have been recorded in a 1 cm path length SuperQuartz spectrophotometric cell (STE) by a Varian Cary 50 Probe UV-Vis spectrophotometer at 25 °C using a Julabo F33 refrigerated circulator as temperature controller. All reagents were weighed on the Mettler Toledo model AT 201 balance. Metal solution was transferred into the cell using a 2 mL Gilmont® microburette.

2.3.2 Materials

Methanol, 30% transmission at wavelength of 210 nm, used as solvent was spectroscan grade (LAB-SCAN). Tetraethylammonium chloride (purum, Fluka) was used as supporting electrolyte, except in the case of Pb^{2+} where

Tetrabuthylammonium nitrate (97%, Aldrich) was used instead. Both supporting electrolytes were recrystallized twice in distilled water, rinsed with methanol and vacuum dried overnight. Metal salts, used without further purification, were as follows: $CoCl_2$, $NiCl_2$, and $CuCl_2$ (RPE grade, Carlo Erba); $ZnCl_2$ (purum, Fluka); $CdCl_2$, and $HgCl_2$ (GR ACS grade, Merck); and $PbNO_3$ (puriss, Riedel-de-Haën®). Reagents (all from Riedel-de-Haën®) used in quantitative determinations of metal ions were as follows: EDTA standard solution (Idranal® III 0.1 mol/l) Hexamethylenetetramine (AR grade), Murexide, and Xylenol orange.

All of the ligands studied in this thesis were supplied by Prof. Paul B. Savage, Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah, USA [57]. Their molecular structures are shown in Figure 2.1.



	R	R_1
L1	$-CH_3$	– H
L2	$-(CH_2)_7CH_3$	– H
L3	$-(CH_2)_{11}CH_3$	– H
L4	– H	$-(CH_2)_{14}CH_3$
L5	$-{}^tBu$	– H
L6	$-C(CH_3)_2CH_2{}^tBu$	– H

Figure 2.1 Molecular structure of phenol diazacrown ethers studied in this thesis

2.4 Complexation between phenolic diazacrown ethers and metal ions using UV spectrophotometric titration

Ligand stock solutions, approximately 10^{-3} M, were prepared by dissolution of a weighed amount in MeOH, ionic strength being kept constant at 0.01 M using Et_4NCl, except in Pb^{2+} system where Bu_4NNO_3 was used instead. Typical concentration of ligand solutions for titration, approximately 10^{-4} M, was achieved by dilution of ligand stock solution with 0.01 M supporting electrolyte solution in MeOH. The exact concentration of metal stock solutions in MeOH, approximately 0.03 M, was determined by complexometric titration with EDTA [27,28]. When necessary, Et_4NCl (or Bu_4NNO_3 in the case of Pb^{2+}) was used to control ionic strength of diluted metal solutions at 0.01 M. The concentration of metal solutions used in UV

titration varied from case to case so as to obtain an optimal condition for complexation.

All complexation studies were done by titrating metal into ligand solution. Ligand solution, 2 ml, was placed in spectrophotometric cell of 1 cm path length and its absorbance was recorded from 200 to 400 nm. Metal solution was then added directly and successively into the cell with the help of microburette. The mixture was stirred for 15 seconds after each addition and its spectral variation was recorded. The stability constants were refined from spectrometric data using program Sirko [22]. The cation selectivity evaluated from the resulting stability constant was further performed by program Haltafall [29].

2.5 Refinement of stability constants by program Sirko

Stability constants in this research were refined by program Sirko, developed and written in 1994 by Vetrogon and co-workers [22]. Before this program was released, many other programs such as Superquad [30], and Squad [31] had been used for the equilibrium studies of complexation. However, almost all these programs are inconvenient for the operator because some of these programs are written for scientific computer. On the other hand, Sirko is a user-friendly program that works effectively with a PC computer. It is also modified later to work under window operating systems. In other words, it is easy to copy and paste data between Sirko and other worksheet programs like Microsoft Excel, Origin, or SigmaPlot. Moreover, the previously released programs allow processing of data from one or few types of experiments, whereas Sirko allows processing of a variety of physicochemical experiments, i.e. potentiometry, spectrophotometry, calorimetry, and nuclear magnetic resonance spectrometry. Therefore, Sirko could be considered as an universal personal computer program. Since the first appearance of program Sirko in 1994, it has been used for complexation studies in numerous literatures [32-35].

To obtain reliable stability constants, many involving parameters have to be taken into account. Variables imported into program Sirko can be classified into two groups, i.e. known and unknown variables. The known variables are as follows: UV absorption intensities at chosen wavelengths, initial volumes, concentrations and

molar absorptivities of metal ion and ligand, as well as an extent of titrant added for each titration point. Unknown variables that need to be refined in spectrophotometric method are molar absortivities and stability constant of complexes.

In order to obtain stability constant using Sirko, first, chemically reasonable and possible stoichiometric model must be entered. Then, a refinement between assumed molar absorptivity values and assumed stability constant(s) of complex(es) is alternately done, until they become steady where molar absorptivity values and log β of complexes are obtained. The trustworthiness of the resulting log β and the stoichiometry model rely on titration curves and R-factor value. The titration curves are plotted between consecutive changes of UV absorption, both theoretical and experimental lines, usually as a function of a metal to ligand ratio. The reliable resulting stability constant should be derived from a good fitting of both lines. The R-factor or Hamilton's R-factor is a value used to verify a convergence of a mathematical model of the equilibrium chemical system constructed by imported variables. Checking an agreement of the assumed stoichiometric model with the experimental data is done by a comparison of the R-factor value with the R-limit value which is the errors in the measurement calculated according to the law of error distribution. A fit of the model with the experiment is considered satisfactorily if R-factor is less than R-limit and usually is less than 1.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย