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Equilibrium Studies of Binary and Ternary Complexes Involving 2-Hydroxy-1- Naphthoic Acid and Amino Acids in Dioxane–Water Mixture

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Abstract

Complex formation equilibria of 2-hydroxy-1-naphthoic acid with Cu^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+} and the ternary complexes involving Cu^{2+}, 2-hydroxy-1-naphthoic acid and some amino acids containing different functional groups are investigated. Stoichiometry and stability constants for the complexes are estimated at 25 ^{o}C and 0.1M ionic strength in 50% dioxane-water mixture. The stability of the complexes follows the trend $Cu^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+}$, which is in agreement with the Irving–Williams order of the metal ions. Ternary complexes of amino acids are formed by a simultaneous mechanism. The stabilities of ternary complexes are quantitatively compared with their corresponding binary complexes. The concentration distribution diagrams of the complexes were evaluated.

Keywords: Equilibrium studies; 2-hydroxy-1-naphthoic acid; binary and ternary complexes; amino acids

1. Introduction

There is a growing interest in studying the properties of amino acids and their derivatives with marine natural products **[1-3]**. Amino acids have special importance among molecules since they are the foundation-stones of living organisms. They are not only components of tissues, but are also reactive organic compounds which are important regulators of biological processes. It is obvious that one has to know the physical properties of amino acids in order to explain the behavior and synthesis of proteins and enzymes in organisms. These compounds are usually considered as good model systems to attain a better insight into the characteristics of naturally occurring copper metalloproteins **[4]**. On the other hand, the study of ternary complexes of transition metal ions with amino acids has become the focus of increasing research effort **[5-8].**

These types of complexes are implicated in the storage and transport of metal ions and of active substances through biological membranes. Consequently, it is worthwhile to study their formation, stability, structure, and the mutual influence of two different ligands bound to the same metal ion. Copper(II), among other transition metal ions, plays a vital role in biological processes; several copper complexes containing simple ligand groups have displayed diverse pharmacological activities. For instance, copper complexes with amino acids as ligands show anti-inflammatory and cytostatic activities **[9]**. Mostly, physical constants of bio ligands are studied in aqueous media **[10, 11]**. Despite this, little is known about the chemistry of biomolecules in mixtures of organic solvents and water, either in regard to protonation constants or synthetic applications. One reason for this dearth of knowledge is that in vivo reactions take place in aqueous media, so that interest in bioligand properties in aqueous solution has predominated. However, in the literature **[12-14]**, it has been shown that water is not an ideal model for in vivo reactions. In enzymes, membranes and other biologically important media, the pKa values are far different from those in water, as these media tend to be lipophilic rather than hydrophilic **[12, 15]**. Studies in media other than water should provide some understanding of the chemistry of amino acids in living systems.

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Hydroxynaphthoic acids are important chemicals that find use in numerous applications, including pharmaceuticals, and medical **[16]**. 1-Hydroxy-2-naphthoic **(1)**, 2-hydroxy1-naphthoic **(2)** and 3-hydroxy-2-naphthoic acids **(3)**, are commonly detected in the bacterial degradation of low molecular mass polycyclic aromatic hydrocarbons **[17]**. Diversification of metabolic pathways occurs largely with the degradation of this class of structurally homologous metabolites. Hydroxynaphthoic acids were metabolized by the action of hydroxynaphthoic acid hydroxylase ***via*** dihydroxynaphthalene **[18],** by direct ortho ring-cleavage of ***via*** trans-29-carboxybenzalpyruvate **[19]** or by direct meta ring-cleavage of naphthoic acids **(1,2)** ***via*** 2,2-dicarboxychromene and 2-hydroxychromene-2-glyoxylic acid, respectively **[20, 21]**. In conjunction with our research program directed to the study the binary and ternary complexes of biological significance **[22-27]**, it seems therefore to be of considerable interest to conduct several investigations covering binary complex formation equilibria of 2-hydroxy-1-naphthoic acid with Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+} and ternary complexes of Cu^{2+} with amino acids in 50% dioxane- aqueous medium.

2. Experimental

2.1. *Materials*

The amino acids: glycine, alanine, valine, proline, serine, methionine, histidine and histamine, were provided by the Sigma Chemical Company. 2-hydroxy-1-naphthoic acid (HNA) was obtained from the Aldrich Chemical Company. The metal salts, in the form of nitrates, were provided by BDH. Concentrations of stock solutions of metal ions were determined by conventional analytical methods **[28].** Carbonate-free NaOH (titrant) was prepared and standardized against potassium hydrogen phthalate solution. All solutions were prepared in deionized H_2O.

2.2 *Apparatus*

Potentiometric measurements were made using a Metrohm 751 Titrino. The titroprosessor and electrode were calibrated with standard buffer solutions prepared according to NBS specifications [29]. All potentiometric titrations were carried out at 25 ± 0.05◦C in a double-walled glass cell of 50 ml capacity. The temperature of all the solutions was maintained at 25 ± 0.05◦C by circulation of thermostatic water through the outer jacket of the cell. The solutions were stirred with a magnetic stirrer and all titrations were performed in triplicate at an ionic strength of 0.1 mol·L−1 (NaNO3).

2.3. Equilibrium Measurements

The acid dissociation constants of the ligands were determined potentiometrically by titrating 40 cm3 of the ligand solution (1.25×10−3 mol·dm−3) in 50% dioxane–water mixture. The stability constant of the binary complex was determined by titrating 40 cm3of a solution of M(II) (Cu2+, Ni2+, Co2+, Mn2+) 1.25 × 10−3 mol·dm−3, HNA or amino acid 2.5 × 10−3 mol·dm−3 in 50% dioxane–water mixture. The stability constants of the ternary complexes were determined under the same conditions as those adopted for the binary ones, using potentiometric data obtained from mixtures of Cu(II) (1.25 × 10−3 mol·dm−3), HNA, and amino acid solutions at a concentration ratio 1:1:1. All titrations were performed under a purified N2 atmosphere, using aqueous 0.05 mol·dm−3 NaOH as the titrant at the constant ionic strength of 0.1 mol·dm−3 (adjusted with NaNO3). Value of pKw in dioxane–water mixture was determined as described previously [30]. Various amounts of standard NaOH solution were added to a 0.10 M NaNO3 solution. [OH-] was calculated from the amount of base added; [H+] was calculated from the pH value. The value obtained in this way at 25 oC for - log[H+][OH-], pKw, was 14.92 for 50.0% in dioxane–water mixture.

The general four-component equilibrium can be written as follows (charges are omitted for simplicity):

l(M) + p(HNA) + q(L)+ r(H) = (M)l(HNA) p(L)q(H)r

$$\beta_{lpqr} = \frac{[M_l HNA_p L_q H_r]}{[M]^l [HNA]^p [L]^q [H]^r}$$

Calculations were performed using the computer program [31] MINIQUAD-75 on an IBM computer. The accepted model gave the best statistical fit and was chemically consistent with the titration data without any system bias in residuals [31]. The formation constants of the general species M(HNA)LH, where M, (HNA), L and H refer to metal, 2-hydroxy-1-naphthoic acid, amino acids and proton, respectively are listed in (Table 1). The concentration distribution diagrams were obtained using the program SPECIES [32] (L. Pettit, Personal communication) under the experimental conditions described.

3. Results and Discussion

The acid dissociation constants of the ligands and the formation constants of their binary complexes were determined under the same experimental conditions of solvent, ionic strength and temperature used to study the ternary complexes. The acid-dissociation constants of the amino acids and formation constants of M(II)- amino acid complexes were previously reported. We have been determined these constants **(Table 1)** under the prevailing

experimental conditions as those utilized for determining the stability constants of the ternary complexes. The results obtained are in good agreement with literature values **[33]**.

3.1 *Proton- 2-hydroxy-1-naphthoic acid (HNA) equilibrium*

In order to calculate the stability constants of metal complexes, the acid dissociation constant of the ligand was first determined. Analysis of the potentiometric data of (HNA) in 50% dioxane–water solution at 0.1 mol·dm^{-3} $NaNO_3$ yields two pKa values corresponding to the carboxylate and hydroxyl groups **(Table 1)**. The highest pKa value is attributed to the hydroxyl group (pKa_1 = 10.12) and the lowest one to the carboxylate group (pKa_2 = 4.49) at 25 ^{0}C as shown in **Table 1.** These results are in agreement with previous **[33]**.

Table.1. Formation constants for binary and ternary complexes of HNA(L_1) and amino acids with different metal ions in 50% (v/v) dioxane-water solution, 25 ^{o}C and 0.1 M ionic strength

System	l	p	q	r[a]	Logβ[b]	pK$_a$[c]	ΔlogK[d]
Cu-L_1	0	1	0	1	10.12(0.05)	10.12	
	0	1	0	2	14.61(0.02)	4.49	
	1	1	0	0	9.43(0.02)		
	1	2	0	0	14.32(0.04)		
Ni-L_1	1	1	0	0	7.95(0.02)		
	1	2	0	0	11.96(0.04)		
Co-L_1	1	1	0	0	7.68(0.02)		
	1	2	0	0	11.52(0.03)		
Mn-L_1	1	1	0	0	7.31(0.02)		
	1	2	0	0	10.82(0.03)		
Cu-L_1-Glycine	0	0	1	1	9.72(0.01)	9.72	
	0	0	1	2	12.27(0.04)	2.55	0.17
	1	0	1	0	8.38(0.02)		
	1	1	1	0	17.98(0.03)		
Cu-L1-Alanine	0	0	1	1	9.78(0.01)	9.78	
	0	0	1	2	13.75(0.01)	3.97	0.19
	1	0	1	0	9.32(0.03)		
	1	1	1	0	18.94(0.02)		
Cu-L_1-Valine	0	0	1	1	9.62(0.01)	9.62	
	0	0	1	2	11.57(0.07)	1.95	0.29
	1	0	1	0	8.49(0.01)		
	1	1	1	0	18.21(0.02)		
Cu-L_1-Proline	0	0	1	1	10.65(0.01)	10.65	
	0	0	1	2	13.27(0.06)	2.62	0.59
	1	0	1	0	8.79(0.02)		
	1	1	1	0	18.81(0.03)		
Cu-L_1-Serine	0	0	1	1	9.26(0.01)	9.26	
	0	0	1	2	11.62(0.03)	2.36	
	1	0	1	0	7.95(0.02)		0.21
	1	0	1	-1	2.05(0.03)	5.32	
	1	1	1	0	17.59(0.02)		
	1	1	1	-1	11.50(0.03)	8.11	
Cu-L_1-Methionine	0	0	1	1	9.29(0.01)	9.29	
	0	0	1	2	11.51(0.03)	2.22	0.28
	1	0	1	0	8.11(0.02)		
	1	1	1	0	17.82(0.03)		
Cu-L_1-Histidine	0	0	1	1	9.39(0.01)	9.39	
	0	0	1	2	15.04(0.04)	5.65	0.27
	1	0	1	0	9.97(0.02)		
	1	0	1	1	13.87(0.01)	3.90	

	1	1	1	0	19.67(0.02)		
	1	1	1	1	24.49(0.03)	4.82	
	0	0	1	1	9.87(0.02)	9.87	
	0	0	1	2	15.35(0.03)	5.48	
Cu-L_1-Histamine	1	0	1	0	9.53(0.02)		0.25
	1	0	1	1	12.94(0.02)	3.41	
	1	1	1	0	19.11(0.02)		

[a], p, q and r are the stoichiometric coefficient corresponding to metal ion , HNA(L_1), amino acid(L_2) and H^+, respectively; Standard deviations are given in parentheses, Sum of square of residuals are less than $5e^{-7}$; pK_a of ligands or protonated complex.

3.2. *Metal ion- 2-hydroxy-1-naphthoic acid (HNA) complexes*

The stability of binary complexes of Cu^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+} with 2-hydroxy-1-naphthoic acid was studied applying potentiometric measurements. The stability constants of their complexes are shown in **Table 1**. The potentiometric titration curves for Cu^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+} with HNA are significantly lower than the HNA titration curve, corresponding to formation of a complex through release of a proton.

Equilibrium models have been tried to fit the experimental potentiometric data for the M^{II}-HNA system. The model that best fits the potentiometric data is found to consist of 1 : 1 and 1 : 2 species. Potentiometric titration curves of HNA in presence and absence of Cu(II) ion taken as a representative are shown in Fig.1 The good fit is an indication of the validity of the complex formation model.

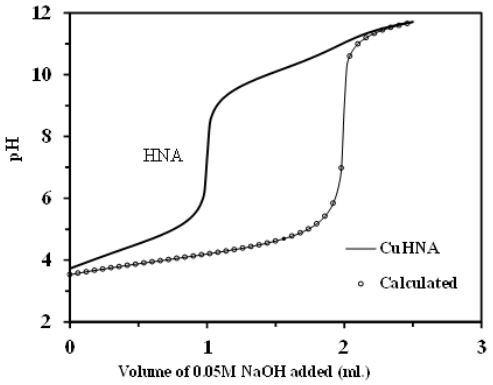

Fig. 1 Potentiometric titration curves of the Cu-HNA system.

From the concentration distribution curves of Cu^{2+} complex, taken as a representative Fig. 2, the species 110 increases with increasing pH, attaining a maximum concentration of 98.95 % at pH≈ 6. Further increase in pH is accompanied by a decrease in the concentration of the 110 species and an increase in the concentration of the 120 species. The main species present under physiological conditions is calculated to be110 species, which can interact with bio ligands such as amino acids.

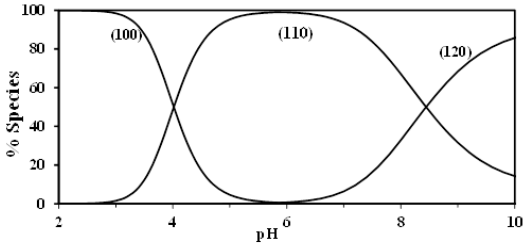

Fig. 2 Concentration distribution of species as a function of pH in the Cu(II)-HNA system.

3.2.1. *Relationships between the properties of central metal and stability of complexes*

In an attempt to explain why a given ligand prefers binding to one metal rather than another, it is necessary to correlate the stability constants with the characteristic properties of the metal ions, such as the ionic radius, ionization energy, electronegativity and the atomic number. Here we have discussed relationships between the properties of central metal ions and the stability constants of complexes.

3.2.1.1. *Atomic number and ionic radius*.

The complex forming ability of the transition metal ions are frequently characterized by stability orders. This is displayed graphically in Fig.3.

It can be seen that log K_1 arranged in the order $Cu^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+}$ is in accordance with Irving–William's order for divalent metals of 3d series **[34]**. Assuming the interaction of the metal ion and the ligand was electrostatic; the stability constants for complexes of metal ions of the same charge should be inversely proportional to metal ion radii. For ions of similar electronic configuration this relationship may be approximately valid, but incompletely fails with metal ions of different groups of the periodic system. The metal chelates of the ligand show more or less linear behavior when their log K values are plotted against the reciprocal ionic radii R, as given in Fig.3.

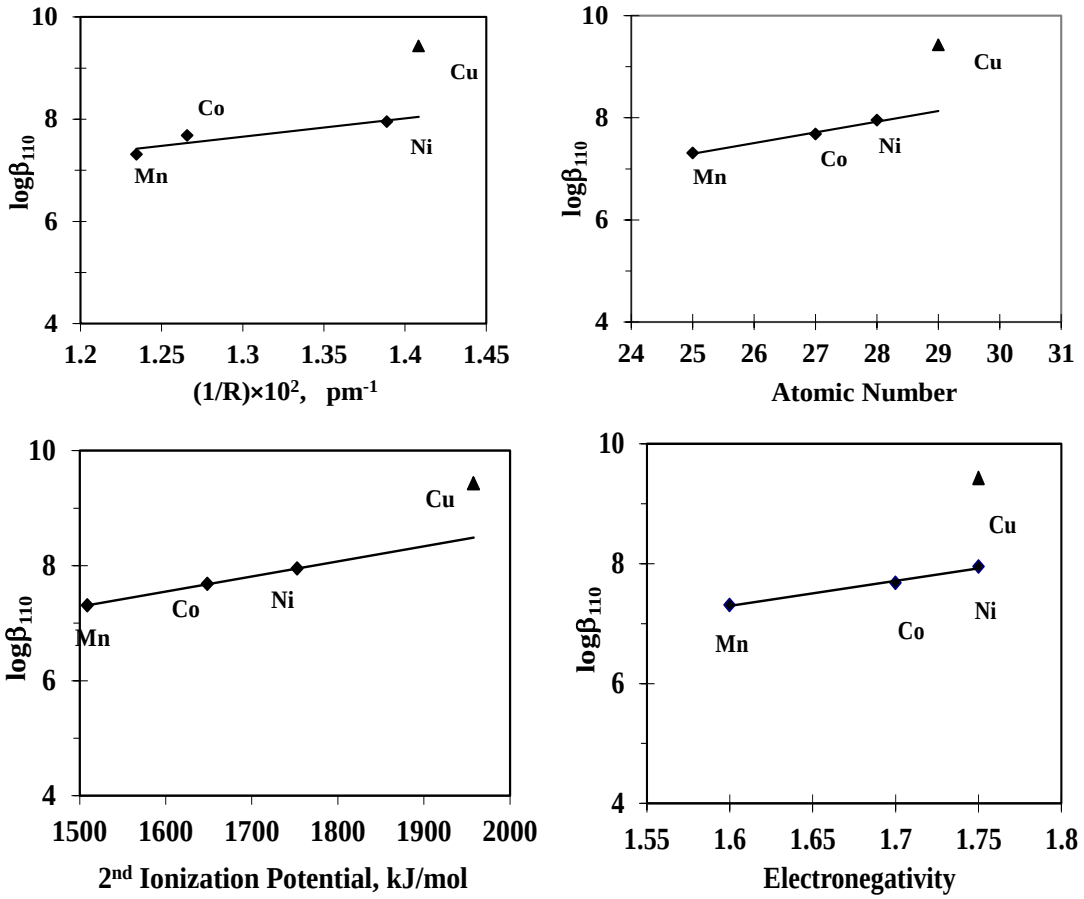

Fig. 3. Effect of metal ion properties on the stability constants of metal complexes.

3.2.1.2. ***Electronegativity***

It is found that the formation constant values for the complexation of HNA with the divalent metal ions follow the order: $Cu^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+}$ which is in agreement with the Irving–Williams series **[34]**. This is in accordance with the fact that increasing electronegativity of the metal ions will decrease the electronegativity difference between the metal atom and the donor atom of the ligand. Thus, the metal–ligand bond would have more covalent character, which may lead to greater stability of the metal chelates. Plotting log K values against electronegativity of metal atoms gives a straight line as shown in **Fig.3**.

3.2.1.3. ***Ionization potential.***

The metal in gas phase is ionized and the metal ion in solution is solvated. Upon complex formation, the coordinated solvent is substituted by the ligand. Therefore, the ionization potential of the metal has an effect on the stability of the formed complex. A linear correlation has been obtained between log K and second ionization potentials of divalent metal ions studied. This confirms the validity of the Van Panthaleon–Van Ech Eq. (1) **[35]**.

$$\log K = P(I - q) \qquad (1)$$

Where, I is the ionization potential for the reaction ($M \rightarrow Mn+ + ne$) in the gaseous phase, P, q are constants depending on the ligand and the experimental conditions but independent of the metal ion. P depends on the number of the donor groups and q is the number of electrons involved. Plotting log K values against the ionization potential of the metal atoms gives more or less straight line as shown in **Fig. 3**. In general, it is noted that the stability constant of the Cu^{2+} complex is quite large compared to the other metals. The ligand field will give Cu^{2+} some extra stabilization due to tetragonal distortion of the octahedral symmetry **[36]**. Thus, log K value for the Cu^{2+}complex deviates significantly when log K values of metal chelates are plotted against properties of the metal ions.

3.3. ***Ternary complexes involving Cu^{II}, 2-hydroxy-1-naphthoic acid (HNA) and amino acids***

Ternary complex formation may proceed either through a stepwise or simultaneous mechanism depending on the chelating potential of HNA and the amino acids. The formation constant of 1 : 1 Cu^{II}-HNA complex is of the same order of magnitude like Cu^{II}- amino acids complex **(Table 1)**. It is reasonable to propose that in presence of both ligands, the

reaction proceeds by simultaneous mechanism. This assumption was supported by comparing the experimental potentiometric data with the theoretically calculated (simulated) curve, (Fig. 4).

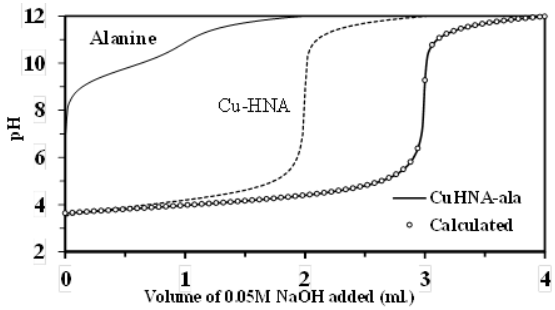

Fig. 4. Potentiometric titration curves of the Cu-HNA-alanine system.

Fig. 4 represents such a comparison for Cu- HNA - alanine system, from which it follows that the experimental data coincide with the theoretical curve. This supports the validity of the ternary complex formation model. The potentiometric data of the ternary complexes involving simple amino acids best fits assuming a complex of stoichiometric coefficient 1110 species for amino acids except for histidine, and histamine, where both 1110 and 1111 species are formed but for serine both1110 and 111-1 species are formed .

Estimation of the concentration distribution of the various species in solution provides a useful picture of metal ion binding. The speciation diagram for Cu- HNA - serine complex (**Fig. 5)**, taken as a representative, indicates that the species 1110 predominates in the physiological pH range. The ionization of the OH group (111-1) starts after pH ≈5 and predominates after pH~8.

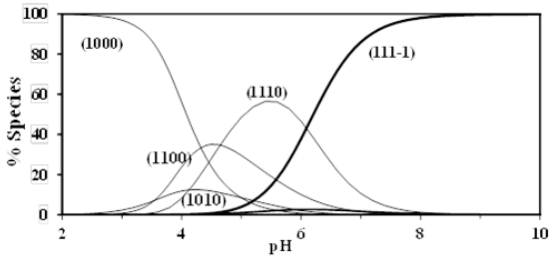

Fig. 5.Concentration distribution of various species as a function of pH in the Cu-HNA-serine system.

The stability constant of the histidine complex is in fair agreement with that of histamine and higher than those of α- amino acids. This indicates that histidine is coordinating in a histamine-like way through the amino and imidazole groups. Histidine and histamine were shown to form both protonated and deprotonated complex species. The acid dissociation constant of the protonated species is given by Eq. (2).

$$pK_a = \log \beta_{1111} - \log \beta_{1110} \quad (2)$$

The proline complex has the highest value. This may be due to the highest basicity of the proline amino group as reflected by the highest pKa value.

Serine has an extra binding center on the β-alcoholate group. This group was reported **[37]** to participate in complex formation. The pKa value of the alcoholate group is given by Eq. (3)

$$pK_a = \log \beta_{1110} - \log \beta_{111\text{-}1} \quad (3)$$

The stability constant value of methionine complex is lower than those of simplest amino acids. This may be explained by the fact that the amino group of methionine is less basic than those of other amino acids as reflected by pK_a values, **Table 1.**

The tendency towards ternary complex formation can be evaluated in various ways. Δ log K has been widely accepted and used for comparing the stabilities of ternary and binary complexes. The Δ logK value for protonated and deprotonated ternary complexes formed through simultaneous mechanism are given by Eqs. (4) and (5)

$$\Delta \log K = \log\beta_{1111} - \log\beta_{1100} - \log\beta_{1011} \quad (4)$$

$$\Delta \log K = \log\beta_{1110} - \log\beta_{1100} - \log\beta_{1010} \quad (5)$$

It is worthy to mention that positive Δ logK values **(Table 1)** for the mixed-ligand complexes indicate that the ternary complexes are more stable than the corresponding binary complexes, and this may be attributed to inter ligand interactions **[38]** occur in the ternary complexes**.**

4. Conclusion

The complex formation equilibria of 2-hydroxy-1-naphthoic acid with some selected metal ions of biological significance has been investigated in the current research. Calculation of the equilibrium distribution of 2-hydroxy-1-naphthoic acid in biological fluids where the metal ions are present, gives a basis for understanding the mode of action of such metal species under physiological condition.

A lower polarity has been detected in some bio-chemical micro-environments, such as active site of enzymes and side chains in proteins, although water has been considered as the traditional solvent to represent biological conditions **[39]**. These properties can be simulated by water/dioxane mixture. Consequently, a study of the M^{+2}-2-hydroxy-1-naphthoic acid complex formation equilibria in dioxane-water solution could be of biological significance. Amino acids form stable ternary complexes with Cu^{II} and 2-hydroxy-1-naphthoic acid. The ternary complexes are formed in the physiological pH range, indicating that interaction

between Cu^{II}, 2-hydroxy-1-naphthoic acid and protein is quite feasible.

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