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STABILITY OF Zn^{2+}, Mn^{2+}, VO^{2+} AND Co^{2+} BINARY AND TERNARY COMPLEXES WITH 2,3-DIMERCAPTOSUCCINIC ACID (DMSA) AS A PRIMARY LIGAND AND SOME BIOLOGICALLY IMPORTANT AMINO ACIDS AS SECONDARY LIGANDS

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ABSTRACT

The mixed ligand complexes of some transition metal ions (Zn^{2+}, Mn^{2+}, VO^{2+} and Co^{2+}) with 2,3-dimercaptosuccinic acid (DMSA) as a primary ligand and some biologically important amino acids (hippuric acid and sarcosine (*N*-methylglycine) as secondary ligands and binary complexes of these metal ions with hippuric acid and sarcosine were studied potentiometrically in aqueous medium. Acid dissociation constants of the ligands used and the formation constants of the binary and the ternary complexes were determined at 30°C, 40°C, 50°C and at ionic strength $\mu = 0.2$ mol dm^{-3} (KNO_3). The stability of ternary complexes was quantitatively compared with their corresponding binary complexes in terms of Δ log K. The thermodynamic parameters like free energy (ΔG^o), enthalpy (ΔH^o) and entropy (ΔS^o) have also been calculated.

Key words: Hippuric acid, Sarcosine, Ternary complexes, Free energy, Enthalpy, Entropy.

INTRODUCTION

Dimercaptosuccinic acid is the organosulphur compound with the formula $HO_2CCH(SH)CH(SH)CO_2H$. This colorless solid contains two carboxylic acid and two thiol groups, the latter being responsible for its mildly unpleasant odour. It occurs in two diastereomers, meso and the chiral *dl* forms. The meso isomer is used as a chelating agent.

Meso 2,3-dimercaptosuccinic acid binds to "soft" heavy metals such as Hg^{2+} and Pb^{2+}, mobilizing these ions for excretion. It binds to metal cations through the thiol groups, which ionize upon complexation.

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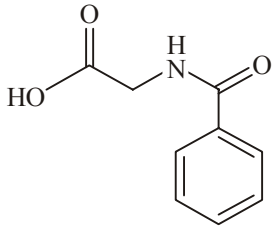
Dimercaptosuccinic acid is indicated for the treatment of lead poisoning in children with blood level measured above 45 µg/dL. The use of dimercaptosuccinic acid is not approved for prophylactic/prevention of lead poisoning in anticipation of exposure in known lead contaminated environments. Its elimination half-life is 2.5-3.5 h. DMSA can cross the blood-brain barrier of mice,[1] but not that of humans, limiting its use in extracting heavy metals from parts of the body other than the central nervous system.[2,3]

Hippuric acid is a carboxylic acid found in the urine of horses and other herbivores. The determination of hippuric acid in urine is important to determine the risk due to the exposition to toluene. The toluene is metabolised mostly in benzoic Acid and subsequently combined with glycine is eliminated by urine as hippuric acid.

An enzyme-linked immunosorbent assay (ELISA) for hippuric acid (HA) was developed using polyclonal anti-HA antibodies. The ELISA system was considered to be useful in the biological monitoring of toluene exposure, and to be more advantageous than time-consuming HPLC, especially when measuring a large number of samples[4].

Patients with non-ketotic hyperglycinaemia excreted significantly more benzoate in the form of hippurate than patients with urea cycle disorders. The plasma concentration of glycine decreased following benzoate treatment only in the patients with non-ketotic hyperglycinaemia[5].



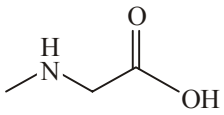
Hippuric acid (N-Benzoyl glycine)

Sarcosine, also known as *N*-methylglycine, is an intermediate and byproduct in glycine synthesis and degradation. Sarcosine is metabolized to glycine by the enzyme sarcosine dehydrogenase, while glycine-*N*-methyl transferase generates sarcosine from glycine. Sarcosine is a natural amino acid found in muscles and other body tissues.

Sarcosine is formed from dietary intake of choline and from the metabolism of methionine, and is rapidly degraded to glycine, which, in addition to its importance as a constituent of protein, plays a significant role in various physiological processes as a prime

metabolic source of components of living cells such as glutathione, creatine, purines and serine.

Sarcosine was shown to activate benign prostate cancer cells and to indicate, the malignancy of prostate cancer cells when measured in urine. Sarcosine was identified as a differential metabolite that was greatly increased during prostate cancer progression to metastasis and could be detected in urine. Sarcosine levels were also increased in invasive prostate cancer cell lines relative to benign prostate epithelial cells[6]. Sarcosine levels seemed to control the invasiveness of the cancer.



Sarcosine (N-methyl glycine)

DMSA seems to be an ideal candidate for chelating toxic metal ions; therefore, a knowledge of the formation constants is essential for exploring its possible use as an antidote for various metal toxicities. Due the ubiquitous presence of various free amino acids in the biological systems, it also becomes necessary to study their interactions as well, and one of the common ways in which they interact is the formation of ternary complexes. Ternary complexes are known to play a very important role in various biological processes including detoxification of toxic cations. Taking these facts into consideration, we are reporting the stability constants of ternary complexes of these metal ions involving DMSA as a primary ligand and some amino acids as secondary ligands.

EXPERIMENTAL

Materials and method

DMSA, hippuric acid (C.D.H.), basic zinc carbonate (Qualigens) and other chemicals of A.R., B.D.H. and Merck were used.

Solutions of cobalt, manganese and zinc nitrate were prepared by dissolving respective metal carbonates in calculated amount of nitric acid till no further salt was soluble. Solution of vanadyl sulphate was prepared in doubly distilled water. Then the solution was filtered through the G-4 crucible of sintered glass and metal ion was estimated as per standard method[7]. DMSA solution was prepared in conductivity water by dissolving calculated weighed quantity of DMSA. Similarly, the solution of hippuric acid and sarcosine were prepared.

Procedure

The potentiometric titrations were carried out with the help of Systronics digital pH meter model 335 with accuracy in the pH range 0.01 unit at 30°C, 40°C and 50°C (± 1°C). Solution temperature was maintained by using refrigerated water bath with circulating system with accuracy ± 1°C (Tanco). Initial volume of each solution to be titrated was kept 50 mL and its ionic strength was kept constant μ = 0.2M (KNO_3). The following solutions were titrated in duplicate against 0.2M KOH at different temperatures using modified Irving and Rossotti method[8,9].

(a) 0.02 M HNO_3 + 0.18 M KNO_3

(b) 0.02 M HNO_3 + 0.002 M Amino acid + 0.18 M KNO_3

(c) 0.02 M HNO_3 + 0.002 M Metal nitrate + 0.002 M DMSA + 0.176 M KNO_3

(d) 0.02 M HNO_3 + 0.002 M Metal nitrate + 0.002 M DMSA + 0.002 M Amino acid + 0.176 M KNO_3

(e) 0.02 M HNO_3 + 0.002 M Metal nitrate + 0.002 M Amino acid + 0.176 M KNO_3

The pKa value of hippuric acid and sarcosine was determined by Martell and Chaberek method[10].

Calculations

The pH titration technique of Irving and Rossotti and its modified form were employed in the present study to determine the stability constants of the ternary complex.

The titration curves were used to evaluate the value of $\bar{n}$ H (the average number of protons attached to the secondary ligand) from the following equation.

$$\bar{n}\ H = \gamma\ T_{CL}^{0} + \frac{(V_1 - V_L)\ (N + E^{0})}{(V_0 + V_1)} / T_{CL}^{0} \qquad \ldots(1)$$

Where,

E^o = Initial strength of acid in the system,

N = Normality of alkali used,

V_o = Total volume of mixture taken initially,

V_1 = Total volume of alkali required in the titration of mineral acid (HNO_3),

γ =Total number of replaceable hydrogen atoms in secondary ligand,

T^o_{CL} = Total concentration of secondary ligand in solution and

V_L = Total volume of alkali required in the titration of secondary ligand.

The values of $\bar{n}$ (average number of secondary ligand molecules attached to the primary complex) were calculated from the following equation[11].

$$\bar{n} = \frac{(V_3 - V_2)(N + E^o)}{(V_0 + V_1)\,\bar{n}\,H\,T^o\,cm} \quad \ldots(2)$$

Where,

T^o_{CM} = Total concentration of metal ion in solution.

V_2 and V_3 are the differences in volume of alkali added between the curves (c) and (d) and between the (a) and (b) respectively (Fig. 1).

The pL values were calculated at $\bar{n}$ = 0.5 from the following equation.

$$pL = \log \frac{\sum_{n=0}^{n=j} \beta_n^H \,(1/anti\ \log\ \beta)^n}{T^o Cl - \bar{n}\,T^o\,cm} \bullet \frac{V_0 + V''''}{V_0} \quad \ldots(3)$$

Where,

β^H_n is overall practical proton ligand stability constant and V"" is the volume of alkali required for curve (d) at the same pH and are summarized in Table 1. The error limit is 0.07 log units.

The thermodynamic parameters like change in free energy (ΔG^o), enthalpy (ΔH^o) and entropy (ΔS^o) have been calculated at different temperatures and at constant ionic strength μ = 0.2M (KNO_3) using following equations.

$$\Delta G^0 = -2.303\ RT\ \log\ K^{MA}_{MAL} \quad \ldots(4)$$

Where,

ΔG^o = Standard free energy change.

R = Gas constant.

T = Absolute Temperature and

K_{MAL}^{MA} = The formation constant of the 1 : 1 : 1 ternary complex.

$$\Delta H^0 = \frac{2.303\ RT_1T_2(\log\ B_2 - \log\ B_1)}{T_2 - T_1} \quad \ldots(5)$$

Where,

ΔH^o = Standard enthalpy change and

B_1 and B_2 are the stability constants at the temperature T_1 and T_2, respectively.

$$\Delta S^0 = \frac{\Delta H^0 - \Delta G^0}{T} \quad \ldots(6)$$

Where,

ΔS^0 = Standard entropy change.

RESULTS AND DISCUSSION

Binary complex systems

The stability constants of 1 : 1 binary complexes of hippuric acid and sarcosine with Mn^{2+}, Zn^{2+}, Co^{2+} and VO^{2+} were calculated using the Irving-Rossotti method. According to Irving-Rossotti, the values of $\bar{n}$ and pL were calculated and the experimental formation curves were constructed by plotting $\bar{n}$ *vs.* pL. The stability constants of binary complexes of Mn^{2+}, Zn^{2+}, Co^{2+} and VO^2 with hippuric acid and sarcosine were calculated from the titration graphs in which the metal to ligand ratio was 1 : 1. These are listed in Table 1.

Ternary complex systems

The potentiometric titration curve for Co^{2+}-DMSA-hippuric acid in a 1 : 1 : 1 molar ratio at 30°C is shown in Fig. 1. The primary complex curve (c) and mixed ligand curve (d) overlap each other up to pH = 3.50. This indicates that in this pH range, when primary

ligand combines with metal, combination of secondary ligand does not take place. Curve (d) diverges from curve (c) after pH = 3.50 showing that at this pH, combination of secondary ligand with primary complex starts. The horizontal distance V_2 between the curve (a) and (b) indicates the protons released due to the self dissociation of secondary ligand and the difference V_3 between the curve (c) and (d) indicates the protons released due to the self dissociation of secondary ligand plus the protons released due to the formation of mixed ligand complex. Thus (V_3-V_2) accounts for the total protons released due to the formation of mixed ligand complex. By using these values, $\bar{n}$ values were calculated. The pL values were calculated at $\bar{n}$ = 0.5 and are summarized in Table 1.

The stabilities of ternary complexes as compared to binary complexes can be quantified by the calculation of Δlog K which is given by the following expressions.

$$\Delta \log K = \log K^{MA}_{MAL} - \log K^{M}_{ML} \quad \ldots(7)$$

Or more appropriately as,

$$\Delta \log K = \log K^{MA}_{MAL} - \log K^{M}_{ML} - \log K^{M}_{MA} \quad \ldots(8)$$

where $\log K^{M}_{MA}$, $\log K^{MA}_{MAL}$ and $\log \beta^{M}_{MAL}$ can be expressed by the following equilibrium reactions (charges have been omitted for simplicity).

$$M + A \leftrightarrow MA \quad \ldots(9)$$

$$K^{M}_{MA} = \frac{[MA]}{[M][A]} \quad \ldots(10)$$

$$MA + L \leftrightarrow MAL \quad \ldots(11)$$

$$K^{MA}_{MAL} = \frac{[MAL]}{[MA][L]} \quad \ldots(12)$$

The overall stability constant for the mixed ligand complex MAL is given by the following equation -

$$M + A + L \leftrightarrow MAL \quad \ldots(13)$$

$$\beta^{M}_{MAL} = \frac{[MAL]}{[M][A][L]} \quad \ldots(14)$$

In the above equations, M represents metal ion, A represents the primary ligand and L is the secondary ligand, which leads to the formation of a ternary or mixed ligand complex MAL.

Table 1: Stability constants and thermodynamic parameters of mixed ligand complexes at different temperatures (μ = 0.2M KNO_3)

System	Temp.	K_{MAL}^{MA}	K_{ML}^{M}	$\Delta \log K$	$\Delta \mathbf{G^0}$	ΔH^0	ΔS^0
VO^{2+} + DMSA + Hippuric acid	30°C	1.79	2.32	-0.53	-2.48	-4.48	-6.64
	40°C	1.68	1.93	-0.25	-2.40		
	50°C	1.59	1.78	-0.19	-2.35		
Co^{2+} + DMSA + Hippuric acid	30°C	1.21	2.02	-0.81	-1.67	-10.75	-30.26
	40°C	0.90	1.87	-0.97	-1.28		
	50°C	0.73	1.63	-0.90	-1.07		
Zn^{2+} + DMSA + Hippuric acid	30°C	1.05	1.92	-0.87	-1.45	-8.28	-22.52
	40°C	0.87	1.77	-0.90	-1.24		
	50°C	0.68	1.63	-0.95	-1.00		
Co^{2+} + DMSA + Sarcosine	30°C	2.76	5.38	-2.62	-3.82	-7.39	-11.60
	40°C	2.63	5.15	-2.52	-3.76		
	50°C	2.43	5.03	-2.60	-3.59		
Zn^{2+} + DMSA + Sarcosine	30°C	3.05	6.13	-3.08	-4.23	-10.75	-21.54
	40°C	2.80	5.93	-3.13	-4.01		
	50°C	2.57	5.75	-3.18	-3.80		
Mn^{2+} + DMSA + Sarcosine	30°C	2.63	3.78	-1.15	-3.64	-8.28	-15.23
	40°C	2.46	3.55	-1.09	-3.52		
	50°C	2.26	3.43	-1.17	-3.34		

CONCLUSION

From Table 1, it can be seen that the stability constants of the binary and ternary complexes with each of the ligands decreases with increasing temperature; and that the complexation is exothermic. For metal-DMSA- hippuric acid system, the stability constants of metal ions follow the order, $VO^{2+} > Co^{2+} > Zn^{2+}$.

For metal-DMSA- sarcosine system, the stability constants of metal ions follow the order, $Zn^{2+} > Co^{2+} > Mn^{2+}$.

It can be observed from the results that log *K* MAL values for all the ternary systems are lower than that of the log *K* ML 1 : 1 complexes resulting in negative value of log K. This may be due to electrostatic repulsion between primary ligand and secondary ligand, since in the ternary complex formation, the secondary ligand L has to approach a neutral or negatively charged complex. The number of coordination position vacant for the coordination of secondary ligand are also less whereas in case of the binary systems, ligand anion has to approach the positively charged M^{2+} ion and more coordination positions are vacant for the attachment. This results in the lowering of stability constants of mixed ligand complexes. The steric hindarance caused by the primary ligand already attached with the metal ion also influences the stability constants.

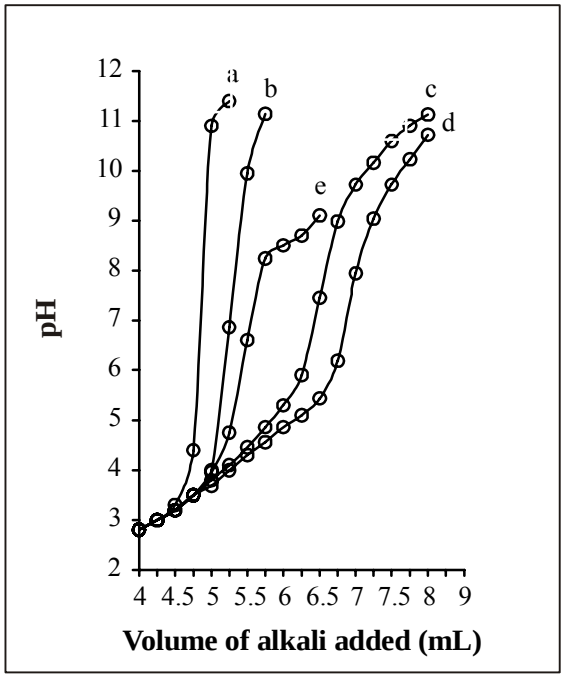


Fig. 1: Titration curve for Co^{2+}-DMSA-Hippuric acid system (30^{o}C)

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REFERENCES

1. Aasath Jan, Dag Jacobsen, Ole Andersen and Elsa Wickstrøm, Treatment of Mercury and Lead Poisonings with Dimercaptosuccinic Acid (DMSA) and Sodium Dimercaptopropanesulfonate (DMPS), Analyst, **120**, 853 (1995).
2. Rooney James, The Role of Thiols, Dithiols, Nutritional Factors and Interacting Ligands in the Toxicology of Mercury, Toxicol., **234**, 145 (2007).
3. Guzzi GianPaolo and Caterina A. M. La Porta, Molecular Mechanisms Triggered by Mercury, Toxicol., **244**, 1 (2008).
4. H. Inagaki and M. Minami, International Archives of Occupational and Environ. Health, **66(2)**, 91 (1994).
5. B. A. Barshop, J. Breuer, J. Holm, J. Leslie and W. L. Nyhan, J. Inherited Metabolic Disease, **12(1)**, 72 (1989).
6. G. Tsai, H. Lane, P. Yang, M. Chong and N. Lange, Biol. Psychiatry, **55(5)**, 452 (2004).
7. A. I. Vogel, A Textbook of Quantitative Inorganic Analysis, Longmans Green, London (1978).
8. H. M. Irving and H. S. Rossotti, J. Chem. Soc., 2904 (1954).
9. M. V. Chidambaram and P. K. Bhattacharya, J. Inorg. Nucl. Chem., **32(10)**, 3271 (1970).
10. S. Chaberek and A. E. Martell, J. Am. Chem. Soc., **77(6)**, 1477 (1955).
11. K. B. Yatsimiriski and V. V. P. Vasile, Instability Constants of Complex Compounds, Pergamon Press, New York (1960).

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