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POTENTIOMETRIC STUDIES OF THE BINARY AND TERNARY COMPLEXES OF MERCURY(II) WITH ACETAZOLAMIDE AND SOME AMINO ACIDS

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ABSTRACT :

Ternary mercury (II) Complexes with 5-acetamide – 1,3,4-thiadiazol-2-sulfonamide (acetazolamide, aza) primary ligand and amino acid L-leucine (leu) or L-serine (ser) or L-cysteine (cys) as secondary ligand have been studied potentiometrically at 25ºC and I= 0.1 M (KNO_3) in 40% (v/v) pure ethanol-water medium. Stoiochiometries and stability constants of binary systems containing the above metal ion in a 1:2 ratio were also determined to compare the effect of the secondary ligand on 1:1 metal – acetazolamide system. The results indicate the preferred formation of Hg (II) ternary complex containing L-cysteine ligand. The result showed that acetazolamide formed both 1:1 in the pH 5.20–7.35 and 1:2 in the pH 7.40 - 9.10 ranges. The optimum conditions for the predominance of the complex forming equilibrium:

Hg (aza) + L $\rightleftharpoons$ **Hg (aza) (L)** **are established**

The difference in stability $\Delta \log k_{Hg} = \log k^{Hg(aza)}_{Hg(aza)(L)} - \log k^{Hg}_{Hg(L)}$ **was found to be positive showing a statistical increase in the value of mixed ligand complex stability constants.**

INTRODUCTION:

Carbonic anhydrase, a Zn(II) metaloenzyme, is an extremely efficient catalyst of the reversible hydration of carbon dioxide. The sulfonamide drugs are well known inhibitors of zinc carbonic anhydrase enzyme and have many applications including use as diuretico, antiglaucoma agents and anti-epileptic drugs among others[1]. Acetazolamide is the active compound in Diamox® and Cidamex drugs. Acetazolamide has shown to be one of the most potent inhibitors[2] and has been used clinically from 1954[3].Through its specific inhibition of that enzyme in the renal tubutes, it decreases tubular reabsorption of bicarbonate in the form of sodium salt of extra cellular fluid, hence diuresis. Acetazolamide contains carbonamido and sulfonamido groups. Most pharmaceuticals contain electron donor groups likely to bind metal ions occurring naturally[4,5]. The acid dissociation constants of acetazolamide and the stability constants of its binary and ternary chelates with copper (II) ion have been reported [6]. This work reports the interaction of Hg (II) with acetazolamyde and L-leucine or L-serine or L-cysteine and investigates the equilibria of acetazolamide in its binary and ternary

complexes. The composition and stability of the complexes have been determined. Another goal of this work is to explore the optimal pH conditions of the reactions. The reactions of mercury (II) with acetazolamide and amino acids were studied since mercury is linked with the most degenerative diseases known to man. The majority of mercury accumulates in human bodies the "silver" amalgam fillings our teeth. In fact, these filling contains about 53 percent of mercury along with other dangerous and toxic metals including copper, nickel, zinc and tin. Mercury poisons many systems in human body leading to dozens symptoms and an equal number of disease states originating in all parts of the body. Mercury is toxic to our cells because it paralyzes the respiratory enzymes, these which make cells enable to use oxygen. Mercury has a phenomenal affinity for sulfhydryl groups, usually designated by the symbol "SH". This binding between mercury and SH groups is strong and stable. Mercury exists in three states in the body: the mercuric ion Hg (II); the mercurous ion Hg (I) and metalillic mercury Hg. No work has been reported so far on the formation constants of ternary complexes of Hg(II) with acetazolamide and amino acids. Acetazolamide have carbonamido and sulfonamido groups. Acetazoamide interacted with Hg(II) througth the nitrogen atom of the deprotonated sulfonamide group, the structural formula of acetazolamide is given in Fig. (1).

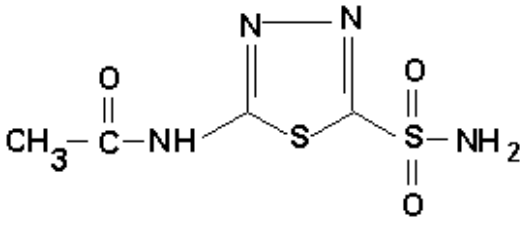

Fig. (1): The structural formula of acetazolamide

Investigations of the stability of ternary complexes will help towards understanding the driving forces that lead to the formation of such complexes in biological systems. In this work, the amino acids studied were a series of C–substituted glycines of general formula R-$CH(NH_2)COOH$, where $R=(CH_3)_2CHCH_2$ [L-leucine], CH_2OH [L-serine], CH_2SH [L-cysteine] all able to form five membered rings. For comparison, complexes of serine and cysteine were investigated. Concurrently complexes of serine showed greater stability than those of leucine due to the presence of OH group in serine. Cysteine form more stable complexes with Hg(II) due to its molecule containing a sulphur atom. All measurements were made at 25ºC, in 0.10 M (KNO_3) ionic strength and in 40% (v/v) ethanol – water medium.

EXPERIMENTAL:

MATERIALS AND METHODS:

All the reagents were of Analytical Grade. Pure ethanol and bidistilled water were used for preparation of the solutions. 5-acetamido-1,3,4-thiadiazol-2-sulfonamide was obtained from Sigma Chemical Company (U.S.A). Mercric nitrate, L-leucine, L-serime and L-cysteine obtained from Sjgma Product of Germany. The aqueous solution of metal ion was standardized according to a well known method[7]. Standard solutions of 0.05 M potassium hydroxide, 0.02M nitric acid and 1.0 M potassium nitrate were prepared in aqueous solutions as usual. Stock solution of the ligand aza (0.005 M) was prepared by direct weighing in pure ethanol because it is very slightly soluble in water, slightly soluble in ethanol (96%) and in acetone; practically insoluble in carbon tetrachloride, in chloroform and in ether. The working solution (0.001M) were prepared by accurate dilution.

PROCEDURE:

pH measurements were performed at 25°C using Corning 215 pH-meter with glass-calomel electrodes. The medium was 40% (v/v) aqueous ethanol and ionic strength 0.1M (KNO_3). The pH reading in partially aqueous media were corrected according to Douheret equation[8]. For the study of binary Hg-aza and Hg-L systems, three mixtures[9] (total volume 50 ml) (A) acid, (B) ligand and (C) chelate were prepared. Mixtures A, B, C were individually titrated against standard alkali and the plots of pH versus volume of alkali gave the titration curves. For the study of the ternary Hg-aza-L system the mixtures prepared (total volume 50 ml) were D acid + aza; (E) mixture D + metal solution, (F) acid + an extra amount of nitric acid equivalent to account for the protons librated as a result of complexation of aza with metal ion; (G) mixture of E + L. The concentrations were: Tc_{L°= Tc_{aza} = Tc_L= 0.001 M, Tc_{M°= Tc_{Hg}= 0.001 M. The mixtures were separately titrated against 0.01M KOH and titration curves D, E, F and G for the system were obtained.

CALCULATIONS:

The pH-titration technique of Irving and Rossotti[10,11] and its modified form[12,13] were employed in the present study to determine the stability constants of the ternary complex.

Binary system: the titration curves were used to evaluate $\bar{n}_A$ (average number of protons associated with the ligand aza); $\bar{n}$ (average number of ligand molecules attached per metal ion) and pL (free ligand exponent). From these data, the proton-ligand and metal –ligand stability constants were obtained as in Table (1).

Hg-aza-L system: The horizantal distancce between curves F and G were measured and used for the calculation of $\bar{n}_{mix}$ (avarage number of secondary ligand (L) molecule attached per (Hg-aza) binary complex using Eq. (1).

$$\bar{n}_{mix} = \frac{(V_G - V_F)\ (N^O + E^O)}{(V^O + V_A)\ \bar{n}_A T_{C\ (Hg\ -\ aza)^O}} \quad (1)$$

where V_G and V_F are the volumes of alkali consumed to reach the same pH value in the curves G and F of the mixed ligand system and $TC_{Hg\text{-}aza}$ is the initial concentration of Hg-aza complex which is equivalent to the initial metal ion concentration. The symbol Nº is the intial concentration of KOH and Eº is the initial concentration of nitric acid. From the values obtained for $\bar{n}_{mix}$ and pL_{mix} free secondary ligand exponent was calculated using Eq. (2).

$$pL_{mix} = \left[\frac{\sum_{n=0}^{n-i} \beta_n^H \left(\frac{1}{anti\ \log\beta} \right)^n}{T_{C_L 0} - \bar{n}_{mix}\ T_c\ (Hg - aza)^o} \times \frac{V^o + V_G}{V^o} \right] \quad (2)$$

Where B_n^H is the proton-ligand stability constant, B is the metal-ligand stability constant. By plotting $\bar{n}_{mix}$ against pL_{mix}, the formation curve was obtained and the formation constant of the ternary system was evaluated.

RESULTS AND DISCUSSION:

Proton Ligand Systems:

Maximum number of protons can be released from acetazolamide was two protons on titration with strong base in the pH range 2.5-12. Acetazolamide behaves as diprotonic acid [H_2-aza], the protonation centres are the amino group of sulfonamide and imido group of acetamide. The acid-base properties of aza in 40%(v/v) ethanol-water mixture and in ionic strength (I=100 mmol dm-3) indicate that one proton only from sulfonamide group was ionized in the pH range 6.50-8.35.The predominant form of this reagent aza within the

pH range 8.40-8.80 is the mono-anionic species ($H\text{-}aza^-$), which undergoes stepwise ionisation on increasing the pH of solution. The transformation of the latter species ($H\text{-}aza^-$) to a diionic form (aza^{2-}) corresponding to the ionisation of imino group proton take place in the pH range 8.85-10.55. The dissociation constant of aza pK_1 corresponding to the ionisation of sulfonamide group was found to be 7.75±0.015 whereas the value of pK_2 for the imido proton was 9.54±0.02. The values of the dissociation constants of aza in this work (Table 1) agree well with the values as reported by Eduardo *et al*[14].

The equilibria established from this study can be shown in the following forms:

$$H_2\text{-aza} \rightleftharpoons H\text{-}aza^- + H^+, \quad \text{pH 6.50-8.35} \quad \text{.....(3)}$$

$$H\text{-}aza^- \rightleftharpoons aza^{2-} + H^+, \quad \text{pH 8.40-10.55} \quad \text{.....(4)}$$

The potentiometric titration curve for aza (Fig. 2) shows a moderate inflection at a=1 followed by a steep inflection (a = moles of base added per mole of ligand).

It can be assumed that the first deprotonation of acetazolamide, which just present in a protonable group (sulfonamido) and the second deprotonation was the proton of the carbonamido group. This is because the presence of the 1,3,4- thiadiazole ring linked to the carbonamido group markedly enhances its acidity via π delocalization.

Althougth the pK values of the amino acids studied have already been reported[15]. These values are determined for the sake of uniformity in experimental conditions, the results are presented in Table (1). The pK_1 and pK_2 values of amino acids under investigation agree well with those reported in the literature[16].

Table (1): Negative Logarithms of the acidity constants of the ligands and Logarithms of its stability constants of the binary Hg(II) complexes

Ligand (L)	$^{H}PK_{H_2L}$	$^{H}pK_{HL}$	$^{Hg}Log\ pK_{HgL}$	$^{Hg}Log\ K_{HgL_2}$
Aza[14]	7.75±0.015	9.54±0.02	4.99	3.23
Leu[16]	9.61	11.509	4.37	-
ser[16]	9.12	11.50	4.71	-
cys[16]	8.30	10.50	4.85	-

Binary Systems:

The stability constants of mercury (II) chelates with acetazolamide or amino acid L-leucine (leu) or L-serine (ser) or L-cysteine (cys) were calculated from the titration graphs in which the metal to ligand ratio was 1:2 are listed in Table (1). The conditions for the measurements were the same as for the acidity constants. The potentiometric titration curve of mercury (II)–acetazolamide is proven in Fig. (2). The potentiometric results of the binary system reveals the formation of 1:1 and 1:2 complexes. The formation of the last binary complex depends on the pH value.

The equilibria in the binary system containing aza is presented below:

$$Hg^{2+} + H_2\text{-aza} \overset{K_{Hg\text{-}aza}^{Hg}}{\rightleftharpoons} [Hg(H\text{-}aza)]^{+} + H^{+}, \quad pH\ 5.0 - 7.4 \quad \ldots.(5)$$

$$[Hg(H\text{-}aza)]^{+} + H_2\text{-aza} \overset{K_{Hg(aza)_2}^{Hg(aza)}}{\rightleftharpoons} [Hg(H\text{-}aza)_2] + H^{+}, \quad pH\ 7.5 - 9.1 \quad \ldots..(6)$$

The titration curve of mercury (II) with the amino acids lec, ser or cys showed that they form 1:1 complexes. This indicates that the amino acids bind througth the amino and carboxylate groups. The stability constants of the complexes formed with Hg (II) decrease in the order cys > ser > leu. In case of leucine the bulky methyl groups at α – carbon atom cause steric hindrance and make the chelate less stable[17] so, serine complex is more stable. In contrast to serine, cysteine form more stable complex which showed greater stability because the molecule contain a sulphur atom. The presence of SH on cysteine ligand showed an affinity to form an extra bond with mercury ion. The constants corresponding to the following equilibria were determined:

$$HL \overset{K_{HL}^{H}}{\rightleftharpoons} L^{-} + H^{+} \quad \ldots\ldots\ldots\ldots\ldots\ldots.(7)$$

$$Hg^{2+} + HL \overset{K_{HgL}^{Hg}}{\rightleftharpoons} [Hg(L)]^{+} + H^{+} \quad \ldots\ldots\ldots\ldots\ldots.(8)$$

Ternary Systems:

The stability constants for the ternary systems were computed from the titrations in which the concentrations of Hg (II) : aza : L were kept in the ratio 1: 1 : 1, listed in Table 2. The experimental data (Fig. 2) show that the formation of the ternary complex Hg (aza) (L) shifts the buffer region of the ligands to lower pH values, which indicates that the ternary complex is more stable than the binary complex. According to the results, the complex equilibria of Hg–aza–L can be represented by the following Scheme :

$$[Hg(H\text{-}aza)]^{+} + HL \rightleftharpoons [Hg(H\text{-}aza)L] + H^{+} \quad \ldots\ldots(9)$$

In order to compare the stabilities of the ternary complex species with those of the parent binary complexes the value ΔlogK, the difference between the stabilities of the binary and the ternary complexes, were determined. The parameter ΔlogK is determined by Equations 10 to 15[18] :

$$Hg + (L) \rightleftharpoons Hg\,(L) \qquad \text{..... (10)}$$

$$[Hg(aza)] + (L) \rightleftharpoons [Hg(aza)\,(L)] \qquad \text{..... (11)}$$

$$\Delta \log k_{Hg} = \log k^{Hg(aza)}_{Hg(aza)(L)} - \log k^{Hg}_{Hg(L)} \qquad \text{..... (12)}$$

$$Hg + (aza) \rightleftharpoons Hg(aza) \qquad \text{..... (13)}$$

$$HgL + (aza) \rightleftharpoons Hg\,(L)(aza) \qquad \text{..... (14)}$$

$$\Delta \log k_{Hg} = \log k^{Hg(L)}_{Hg(L)(aza)} - \log k^{Hg}_{Hg(aza)} \qquad \text{..... (15)}$$

The value of $\Delta \log K_{Hg}$ is the logarithm of the equilibrim constant due to equation (16):

$$Hg(L) + Hg(aza) \rightleftharpoons Hg\,(aza)(L) + Hg \qquad \text{..... (16)}$$

Hg

The overall constant, $\beta_{Hg(aza)(L)}$, which was determined experimentally (Table 2) is connected with $K_{Hg(aza)(L)}$ and $K_{Hg(aza)(L)}$ by equations (14) and (15) respectively.

$$Hg + aza + L \rightleftharpoons Hg\,(aza)(L)$$

$$\beta^{Hg}_{Hg\,(aza)\,(L)} = [Hg\,(aza)\,(L)]/\,[Hg]\,[aza]\,[L] \qquad \text{.....(17)}$$

$$Hg\,(L) + aza \rightleftharpoons Hg\,(L)\,(aza)$$

$$K^{Hg(L)}_{Hg\,(L)\,(aza)} = [Hg\,(L)(aza)]/\,[Hg\,(L)]\,[aza]\,[L] \qquad \text{.....(18)}$$

$$Hg\,(aza) + L \rightleftharpoons Hg\,(aza)(L)$$

$$K^{Hg(aza)}_{Hg\,(aza)\,(L)} = [Hg\,(aza)(L)]/\,[Hg\,(aza)]\,[L] \qquad \text{.....(19)}$$

$$\text{Log } K^{Hg(L)}_{Hg\,(aza)\,(L)} = \text{Log } \beta^{Hg}_{Hg\,(L)\,(aza)} - \text{Log } K^{Hg}_{Hg(L)} \qquad \text{.....(20)}$$

$$\text{Log } K^{Hg(aza)}_{Hg\,(aza)\,(L)} = \text{Log } \beta^{Hg}_{Hg\,(aza)(L)} - \text{Log } K^{Hg}_{Hg\,(aza)} \qquad \text{.....(21)}$$

Table (2) :Logarithms of the stability constants of the ternary Hg(II)-aza-L complexes and some related data [I=0.1, 40% (v/v) ethanol, 25°C]

Ligand	$\log \beta^{Hg}_{Hg(aza)(L)}$	$\log K^{Hg(aza)}_{Hg(aza)(L)}$	$\log K^{Hg(L)}_{Hg(aza)(L)}$	Δlog K
leu	11.66	6.67	7.29	2.3
ser	11.88	6.89	7.17	2.18
cys	12.34	7.35	7.49	2.50

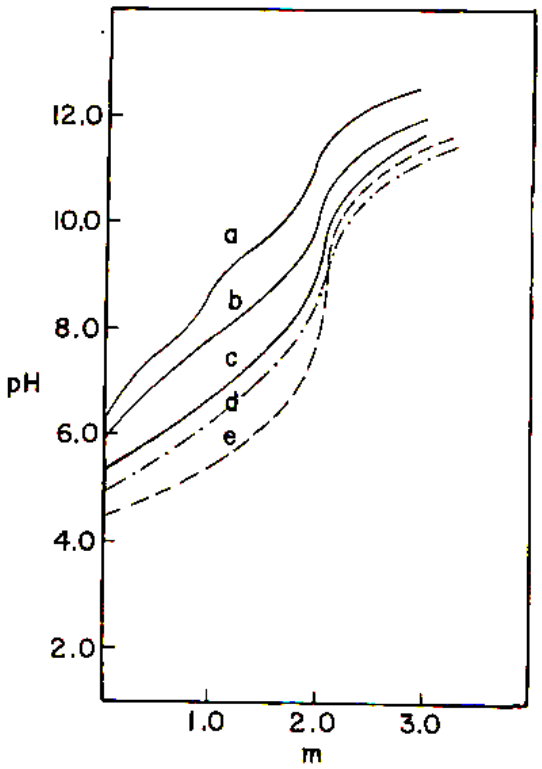

Fig. (2): Potentiometric titration curves of binary and ternary complex systems of Hg(II) [I=100 mmol dm^{-3} (KNO_3), 25°C, 40% (v/v) ethanol]. For curves (a) aza, the abscissa represents the number of moles of base added per mole of ligand.; (b) 1:2 Hg(II)-aza; (c) 1:1:1 Hg(II)-aza-leu; (d) 1:1:1 Hg(II)-aza-ser ; (e) 1:1:1 Hg(II)-aza-cys.

The formation constant values of the three ternary complexes (Table 2) show that in all cases, the Hg(II)-acetazolamide –amino acid ternary complexes are more stable than the 1:1 Hg(II)-acetazolamide or Hg(II)-amino acid binary complexes. Thus the 1: 1 mercury(II)-acetazolamide complex has a greater tendency toward combination with α-amino acid molecule can form five-membered metal chelate ring. The stability of cysteine complexes may be due to the preference of Hg(II) ion to form an extra band with SH group.

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دراسات جهدية على المتراكبات الثنائية والثلاثية للزئبق الثنائى مع اسيتازولاميد وبعض الأحماض الأمينية

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درست فى هذا البحث بالطريقة الجهدية اتزانات تفاعلات التراكب بين أيون الزئبقيك ومركب ٥-اسيتاميد-١و٣و٤-ثياديازول-٢وسلفوناميد (اسيتازولاميد) المادة الفعالة فى دواء ديامكس وسيدامكس المستخدم فى علاج الجلوكوما (المياه الزرقاء)، ويستخدم أيضا فى علاج الصرع، ويستخدم أيضا فى إدرار البول بفعله على انزيم الكربونيك انهيدريز فهو بذلك يقلل من امتصاص بيكربونات الصوديوم بنسبة عالية فى قنوات الكليتين والأحماض الأمينية ل- ليوسين ول- سيرين ول-سيستين وتم التعرف على ظروف وجود اتزانات التراكب الذى يتضمن تفاعل متراكب الزئبقيك واحد هذه الأحماض الأمينة فى نسبة تكوينية (١:١:١) وعين ثابت التكوين للمتراكبات المختلطة، وأمكن تقييم ثبات هذه المتراكبات بالمقارنة بثبات المتراكبات الثنائية مع الكواشف المستخدمة فى محاليل حاوية على٤٠% (بالحجم).

وقد وجد أن المتراكب المختلط مع اسيتازولأميد وحمض السيستين يعطى أكبر ثابت استقرار وذلك يرجع الى وجود مجموعة الميركبتو فى الحمض الأمينى السيستين، وذلك يرجع أيضا إلى ميل الزئبقيك إلى الترابط مع ذرة الكبريت، وأمكن التعرف على الظروف المثالية لتكوين المتراكبات المذكورة وتحديد مدى درجة الرقم الهيدروجينى المناسب للتفاعل.