

RESEARCH ARTICLE

## Study on mixed ligand complexes of Pb(II) with EDTA and dietary ligands to investigate the applicability on chelation therapy

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**Abstract:** Lead is the most distributed toxic metal in the world and chelation therapy is a remedy for removing it from the body, if a person is intoxicated. Stability constants of both binary and mixed ligand complexes of Pb(II) with salicylic acid, ascorbic acid, vitamin B<sub>1</sub>(thiamine), vitamin B<sub>3</sub> (niacin) and Na<sub>2</sub>EDTA were potentiometrically studied, in aqueous media at the temperature of  $37.0 \pm 0.2$  °C and the ionic strength of  $0.15 \text{ mol dm}^{-3}$  in order to identify better mixed ligand systems suitable for chelation therapy of lead. Both metal-ligand and proton-ligand formation constants were calculated using Irving-Rossotti equations. The Formation of ternary complexes was further confirmed theoretically by plotting composite curves.  $\Delta \log K$  values calculated revealed that all mixed complexes were more stable than the complexes of corresponding binary systems. EDTA-Vitamin B<sub>1</sub> and EDTA-Ascorbic mixed complexes with Pb(II) disclosed very high stability constants, which were two times greater than that of EDTA binary complex. Vitamin B<sub>1</sub> revealed a remarkably high ability in forming mixed ligand complexes with all other four ligands. Results of this work clearly indicate that (EDTA-Vitamin B<sub>1</sub>), (EDTA-Ascorbic), (Vitamin B<sub>1</sub>-Ascorbic), (Vitamin B<sub>1</sub>-Salicylic) and (Vitamin B<sub>1</sub>-Vitamin B<sub>3</sub>) combinations can be used for detoxification of lead more effectively using dietary ligands.

**Keywords:** Stability constants, *in vitro* conditions, chelation therapy, thiamine, niacin, vitamin C, salicylic acid.

### INTRODUCTION

Today, lead (Pb) is considered as the most widely distributed toxic metal ion in the world due to many of its industrial applications (Das, 1990; Morose *et.al.*, 2008; Turner & Fader, 1962). Meanwhile, a number of serious health problems are associated with lead toxicity (Das, 1990; Branica *et.al.*, 2006; Chisolm,1968). The only way of curing lead toxicity is chelation therapy, which comes in two modes; single

ligand and mixed ligand chelation therapy. This involves the formation of chelate complexes of toxic metal ions with administered drugs which can be easily excreted via urine or feces (Chisolm,1968; Goyer & Cherian, 1978; May and Williams, 1979; Bulman *et al.*, 1979; Das, 1990;Wang *et.al.*,2006). On the other hand, single and mixed ligand chelates of metals with various ligand molecules have been studied extensively related to various aspects quantitatively (Nakayama *et.al.*, 1989; Florence 1982; Mildvan *et al.*,1967; Shelke & Jahagirdar,1976; Maktell and Carey,1967; Moustafa, 2005) and many of the mixed chelate systems of bio-molecules with biologically important metals like Cu, Ni and Zn investigated were used as models of biochemistry to understand more complicated biological reactions (Mildvan & Cohn, 1966; El-Haty *et al.*,1995; Brumas *et al.*,1995; Kleszczewska, 1999; Bucci, 2000).

Though quantitative studies and stability constants of mixed ligand complexes of Pb(II) with some ligands have been reported (Gupta and Sharma,1982; Joshaghani *et al.*, 2008; Padmaja & Rao, 2012; Thanavelan *et al.*, 2014), the information related to the quantitative investigations on mixed ligand complexes of Pb(II) with biologically important molecules is scarce, especially under the physiological conditions (Chandrathilaka *et al.*, 2013). Thus, the present work was mainly undertaken to study the stability of mixed ligand complexes of EDTA with biologically significant molecules entering into our body via common drugs and food, as an attempt to introduce more effective and less toxic mixed ligand systems for the use in chelation therapy of Pb(II). In order to have more reliable results, physiological conditions were applied during these experiments to study the aqueous

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coordination chemistry of selected ligands; ascorbic acid (AsC), salicylic acid (Sali), vitamin B<sub>1</sub> (Vit B<sub>1</sub>), vitamin B<sub>3</sub> (Vit B<sub>3</sub>) and Na<sub>2</sub>EDTA with Pb(II) using potentiometric pH titration data and UV Visible spectroscopic data.

## MATERIALS AND METHODS

### Materials

Following chemicals of analytical grade were used as received for the potentiometric experiments:

HNO<sub>3</sub> (Sigma), NaOH (Sigma), NaNO<sub>3</sub> (Sigma), salicylic acid (Aldrich), vitamin B<sub>1</sub> (Hebei Medipharm Co. Ltd.), Ascorbic acid (Sigma), vitamin B<sub>3</sub> (Western Drugs Pvt. Ltd.), Pb(NO<sub>3</sub>)<sub>2</sub> (Aldrich), Na<sub>2</sub>EDTA (Aldrich).

1×10<sup>-2</sup> mol dm<sup>-3</sup> standard solutions of the metal and all ligands were freshly prepared using double distilled deionized water. Standardizations of NaOH (0.03 mol dm<sup>-3</sup>) and HNO<sub>3</sub> (0.03 mol dm<sup>-3</sup>) solutions prepared were carried out with primary standards, potassium hydrogen phthalate and sodium carbonate respectively.

### Potentiometric studies

The pH measurements of the potentiometric titrations were carried out with a MARTINI MI 151 pH meter equipped with a MA 917 combination electrode and a temperature probe.

Following mixtures were prepared in a total volume of 40.00 mL by adding required amounts of double distilled deionized water at the ionic strength of 0.15 mol dm<sup>-3</sup> of NaNO<sub>3</sub> for potentiometric titrations. (Note: L, X and M denote ligand 1, ligand 2 and metal ion respectively)

- (i) HNO<sub>3</sub> (10.00 mL); (ii) HNO<sub>3</sub> + L (10.00 mL); (iii) HNO<sub>3</sub> + X (10.00 mL); (iv) HNO<sub>3</sub> + L/X (10.00 mL) + M (10.00 mL); (v) HNO<sub>3</sub> + L(10.00 mL) + X (10.00 mL) + M (10.00 mL)

Each solution prepared was titrated against the standardized NaOH solution at 37.0 ± 0.2°C (a YCW – 05 model thermostatic water bath was used to maintain the temperature) and the pH was recorded after the addition of 0.20 mL aliquots of NaOH. Nitrogen was purged through the reaction mixtures to ensure the absence of oxygen and carbon dioxide in reaction mixtures.

## Computation of stability constants

### Binary systems

According to the method described by Irving and Rossotti (1953 and 1954), the degree of formation of proton-ligand complex can be defined by a parameter  $n_A$  which is the average number of protons associated with a ligand molecule.

$$n_A = \frac{\text{total conc. of protons bound to ligand}}{\text{total conc. of ligand not bound to metal}}$$

Similarly, for the metal ligand complex, average number of ligand molecules attached per metal ion,  $n$  can be defined using the relationship,

$$n = \frac{\text{total concentration of ligand bound to metal}}{\text{total concentration of metal}}$$

Values of  $n_A$ ,  $n$  and the free ligand exponent ( $pL$ ) can be obtained using following expressions derived by Irving and Rossotti (equations 1, 2 and 3).

$$n = \frac{(V_3 - V_2) \times [N + E + T_L(Y - n_A)]}{(V_0 + V_2)n_A T_M} \quad (1)$$

$$n_A = Y + \frac{(V_1 - V_2) \times (N + E)}{(V_0 + V_1)T_L} \quad (2)$$

$$pL = \log \left[ \frac{\sum_{n=1}^n \beta_n \cdot \{anti \log(pH)\}^{-n} \cdot (V_0 + V_3)}{(T_L - \bar{n} \cdot T_M) \cdot V_0} \right] \quad (3)$$

The titration curves of the mixtures, (i), (ii) and (iv) were used to calculate  $n_A$ ,  $n$  and  $pL$ ,  $V_1$ ,  $V_2$ ,  $V_3$  are the volumes of alkali consumed to reach the same pH value in the mixtures (i), (ii) and (iv) respectively and  $V_0$  is the total initial volume of the titration mixture.  $N$ ,  $E$  and  $T_L$  are the total initial concentrations of NaOH, HNO<sub>3</sub> and ligand respectively.  $Y$  is the number of dissociable protons attached per molecule of each ligand. Values of  $Y$  are 1, 3, 2, 0 and 2 for the ligands: salicylic acid, vitamin B<sub>1</sub>, ascorbic acid, vitamin B<sub>3</sub> and Na<sub>2</sub>EDTA respectively. From these data, the proton-ligand and metal-ligand stability constants were calculated using Microsoft Excel. Formation curves for the

proton-ligand and metal-ligand complex systems were obtained by plotting  $n_A$  Vs pH and  $n$  Vs  $pL$  respectively. To obtain the value of  $K_H^1$ , all the  $K_H^1$  values were averaged in the range  $0 < n_A < 1$ . For  $K_H^2$  a similar procedure was adopted taking values in the range  $1 < n_A < 2$ . Calculations of  $K_H^j$  for each  $n_H$  value were carried out using equation 4. Similarly,  $K_f^1$  and  $K_f^2$  were calculated from equation 5. To ensure accuracy, each titration was carried out in triplicate.

$$K_j^H = \frac{(n_H - j + 1)}{\{(j - n_H) \times [H]\}} \quad (4)$$

$$K_n^f = \frac{(\bar{n} - n + 1)}{\{(n - \bar{n}) \times [L]\}} \quad (5)$$

### Ternary systems

Considering the Irving and Rossotti equations derived for binary complexes, a new set of equations was obtained for the calculation of the stability constants of ternary complexes (Chidambaram & Bhattacharya 1970a; Chidambaram & Bhattacharya 1970b). Expressions for average number of secondary ligand (X) molecules attached per complex ML,  $n_{mix}$  and free secondary ligand exponent,  $p_{mix}^L$  are given in equations 6 and 7.

Titration curves (iii) and (v) were used to calculate  $n_{mix}$  and  $p_{mix}^L$ .  $V_X$  and  $V_5$  are the volumes of alkali consumed to reach the same pH in mixtures (iii) and (v) respectively.  $n_A^X$  is the average number of protons of the secondary ligand X. Stability constant of the ternary complex MLX formed by reacting binary complex ML with free ligand X, has been obtained by averaging formation constant values obtained in the range  $0 \leq n_{mix} \leq 1$ . The ligand that exhibited a higher formation constant in binary complex formation was considered as the primary ligand of the mixed ligand system depending on the ligand combinations.

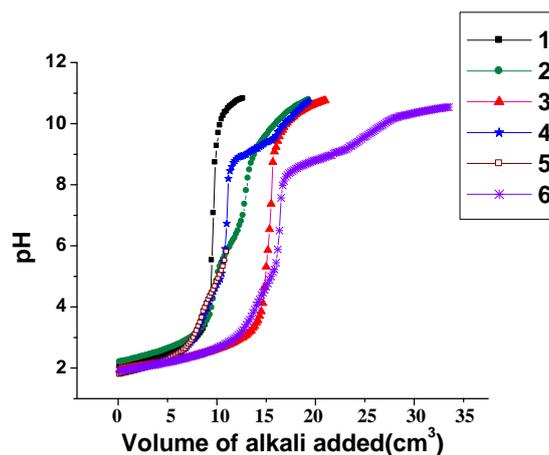
$$n_{mix} = \frac{(V_5 - V_X) \times (N + E + T_X (Y - n_A^X))}{(V_0 + V_X) \cdot n_A^X \cdot T_{ML}} \quad (6)$$

$$p_{mix}^L = \log \left[ \frac{\sum_{n=1}^n \beta_n \cdot \{\text{anti log}(pH)\}^{-n} \cdot (V_0 + V_5)}{(T_L - n_{mix} \cdot T_{ML}) \cdot V_0} \right] \quad (7)$$

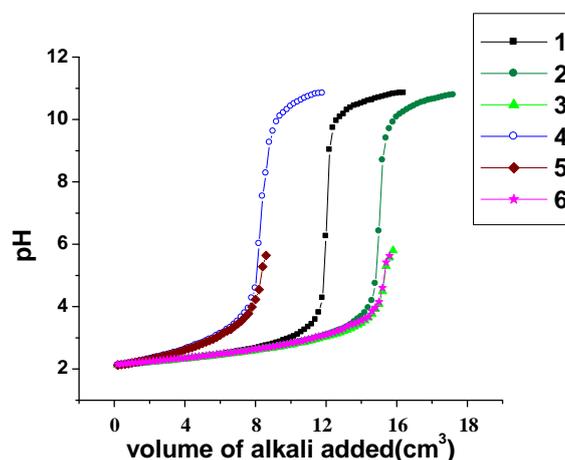
By most of the lead complexes found in literature is octahedral in shape (Battistuzzi *et al.*, 1996; Evans *et al.*, 2003; Najafpour *et al.*, 2007; Davidovich *et al.*, 2009). Therefore, assuming that lead complexes formed in aqueous medium under physiological conditions are also in octahedral shape, stabilities of mixed ligand complexes over single ligand complexes were compared by calculating  $\Delta \log K$  values according to the literature (Sigel, 1975; Maktell & Carey, 1967; Shelke & Jahagirar 1976).

## RESULTS AND DISCUSSION

Variations in the pH values according to the volumes of alkali added are given in Figure 1 for a system, which formed a mixed ligand complex and in Figure 2 for a system which did not form a mixed complex, as two representatives. In Figure 1, it can be clearly seen that the ternary system titration curve has deviated from two binary system titration curves significantly, where as in Figure 2 it has superimposed with the titration curve of the primary ligand, salicylic acid. Similarly, titration curves of other systems forming mixed ligand complexes under the physiological conditions, revealed the same behavior providing preliminary information about complexation. As each titration was carried out until a permanent turbidity was observed in the reaction mixture, at that pH value, it was considered that the complex formation was disturbed by the formation of insoluble lead hydroxide.



**Figure 1:** Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of  $\text{Na}_2\text{EDTA}$  and Vitamin  $\text{B}_1$  ( $I=0.15$  M  $\text{NaNO}_3$  at  $37.0 \pm 0.2$  °C); **1.**  $\text{HNO}_3$ ; **2.**  $\text{HNO}_3 + \text{Na}_2\text{EDTA}$ ; **3.**  $\text{HNO}_3 + \text{Na}_2\text{EDTA} + \text{Pb}(\text{NO}_3)_2$  **4.**  $\text{HNO}_3 + \text{Vitamin B}_1$ ; **5.**  $\text{HNO}_3 + \text{Vitamin B}_1 + \text{Pb}(\text{NO}_3)_2$  **6.**  $\text{HNO}_3 + \text{Na}_2\text{EDTA} + \text{Vitamin B}_1 + \text{Pb}(\text{NO}_3)_2$ .



**Figure 2:** Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of Salicylic acid and Vitamin  $\text{B}_3$  ( $I=0.15$  M  $\text{NaNO}_3$  at  $37.0 \pm 0.2$  °C); **1.**  $\text{HNO}_3$ ; **2.**  $\text{HNO}_3 + \text{Salicylic}$  **3.**  $\text{HNO}_3 + \text{Salicylic} + \text{Pb}(\text{NO}_3)_2$  **4.**  $\text{HNO}_3 + \text{Vitamin B}_3$  **5.**  $\text{HNO}_3 + \text{Vitamin B}_3 + \text{Pb}(\text{NO}_3)_2$  **6.**  $\text{HNO}_3 + \text{Salicylic} + \text{Vitamin B}_3 + \text{Pb}(\text{NO}_3)_2$ .

Protonation constants calculated for five ligands are given in the Table 1 and the overall formation constants of binary and ternary systems and pH values, at which the permanent turbidity was observed are illustrated in Table 2. Accordingly, all ligands have formed ML type binary complexes with  $\text{Pb}(\text{II})$  within the studied pH ranges before the formation of  $\text{Pb}(\text{OH})_2$ . Observation of permanent turbidity at higher pH values in the mixed ligand systems compared

with binary systems implies that,  $\text{Pb}(\text{II})$  interacts more strongly with ligand mixtures than with a single ligand, by forming more stable mixed ligand complexes. Results reveal that among ten mixed ligand systems investigated, in M:L:X in 1:1:1 ratio, only five ternary complexes were formed. Mixed ligand complex formation was further confirmed by recording UV-Visible spectra for binary and ternary systems at pH 4, 5 and 6 under the physiological conditions.

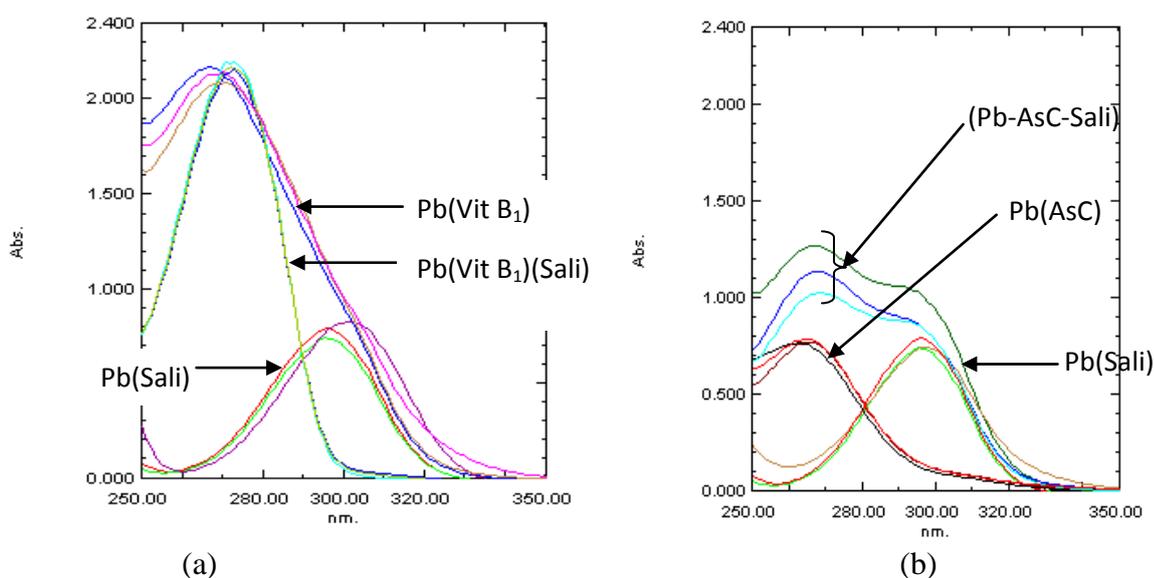
**Table 1:** Protonation constants of ligands.

| Ligand                 | Log $K_H^1$ | Log $K_H^2$ | Log $K_H^3$ |
|------------------------|-------------|-------------|-------------|
| Salicylic acid         | 2.76        | -           | -           |
| Vitamin B <sub>1</sub> | 9.13        | 8.85        | 4.32        |
| Ascorbic acid          | 10.00       | 5.28        | -           |
| Vitamin B <sub>3</sub> | -           | -           | -           |
| Na <sub>2</sub> EDTA   | 9.19        | 5.69        | -           |

**Table 2:** Overall stability constants (log  $\beta$ ) for binary (1:1) and mixed ligand systems (1:1:1) at concentration 0.0025 M.

| L                      | X                      | pH at permanent turbidity | log $\beta_1^a$ | log $\beta_2^a$ |
|------------------------|------------------------|---------------------------|-----------------|-----------------|
| Salicylic acid         | -                      | 5.80                      | 2.67            | -               |
| Ascorbic acid          | -                      | 6.6                       | 3.53            | -               |
| Vitamin B <sub>1</sub> | -                      | 6.8                       | 3.83            | -               |
| Vitamin B <sub>3</sub> | -                      | 5.64                      | 2.29            | -               |
| Na <sub>2</sub> EDTA   | -                      | -                         | 13.53           | -               |
| Na <sub>2</sub> EDTA   | Vitamin B <sub>1</sub> | -                         | -               | 26.47           |
| Na <sub>2</sub> EDTA   | Ascorbic acid          | -                         | -               | 25.42           |
| Vitamin B <sub>1</sub> | Ascorbic acid          | 7.12                      | -               | 7.39            |
| Na <sub>2</sub> EDTA   | Salicylic acid         | -                         | 13.53           | -               |
| Vitamin B <sub>1</sub> | Salicylic acid         | 7.74                      | -               | 7.08            |
| Ascorbic acid          | Salicylic acid         | 7.04                      | 3.53            | -               |
| Na <sub>2</sub> EDTA   | Vitamin B <sub>3</sub> | -                         | 13.53           | -               |
| Vitamin B <sub>1</sub> | Vitamin B <sub>3</sub> | 7.56                      | -               | 6.9             |
| Ascorbic acid          | Vitamin B <sub>3</sub> | 5.81                      | 3.53            | -               |
| Salicylic acid         | Vitamin B <sub>3</sub> | 6.13                      | 2.67            | -               |

<sup>a</sup>  $\beta_n = K_1 \cdot K_2 \cdot K_3 \dots K_n$



**Figure 3:** (a) UV absorption spectra for binary complexes; Pb(Vit B<sub>1</sub>), Pb(Sali) and Pb(Vit B<sub>1</sub>-Sali) systems at three different pH values; 4, 5 and 6. (b):UV absorption spectra for binary complexes; Pb(AsC), Pb(Sali) and Pb(AsC-Sali) systems at three different pH values; 4, 5 and 6.

According to the Figure 3a, mixed ligand system has a  $\lambda_{\max}$  value in between the  $\lambda_{\max}$  values of the two binary systems. This indicates that the mixed system contained a species different from either of binary systems. Thus, the new species must be the mixed complex of Pb(II) with two ligands. Similarly, rest of the systems which formed mixed complexes showed the same behavior by proving the formation of ternary complexes under the physiological conditions.

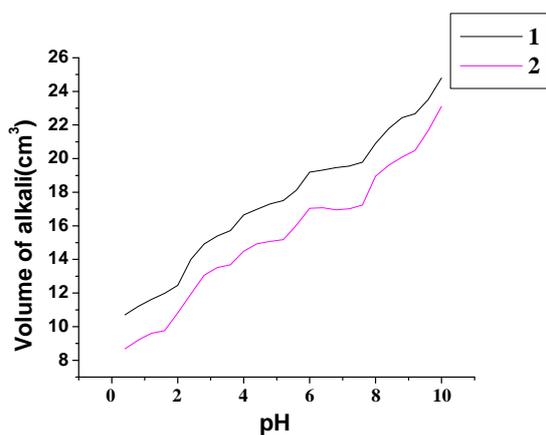
In Figure 3b, a new peak did not appear for a mixed ligand system at a different  $\lambda_{\max}$ ; instead a broader peak area appeared with two maximum regions having their  $\lambda_{\max}$  values closer to the  $\lambda_{\max}$  values of each parent binary complex. This gave a clue for a system consisting of two different binary complexes. Thus, such a system cannot be expected to form any mixed ligand complex under the experimental constitutions.

On the other hand, theoretically, by plotting composite curves, which were obtained by adding the horizontal distance (volume of alkali consumed) of the secondary ligand curve (X) to the horizontal distance of the Pb(II)-L curve at the same pH (Maktell and Carey, 1967; Shelke and Jahagirdar, 1976) mixed ligand complex formation was proved. Volumes

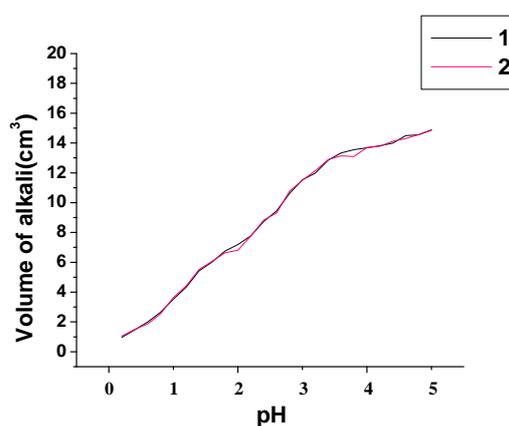
consumed by the ternary system titration curve were compared with the corresponding volumes of composite curve at the same pH by plotting them in a graph. Figure 4a shows a representative composite curve drawn for (EDTA+Vit B<sub>1</sub>+Pb) system forming a ternary complex, in which the composite curve does not overlap with its titration curve of the mixed ligand system (mixture v) and which proves the formation of a mixed ligand complex in the considered system. Here, the titration curve of the mixed ligand system is on the left hand side of the composite curve within the pH range 2-10. Hence, the complexation can be assumed to take place by removing all possible protons in both ligands as given bellow (eq. 8). Similar types of composite curves were given by the other systems forming ternary complexes.

(8)

Figure 4b is the composite curve of a system, which did not form a ternary complex. In this figure, two curves are overlapped by proving the inability of developing a ternary complex between Pb(II) and the ligand combination used.



(a)



(b)

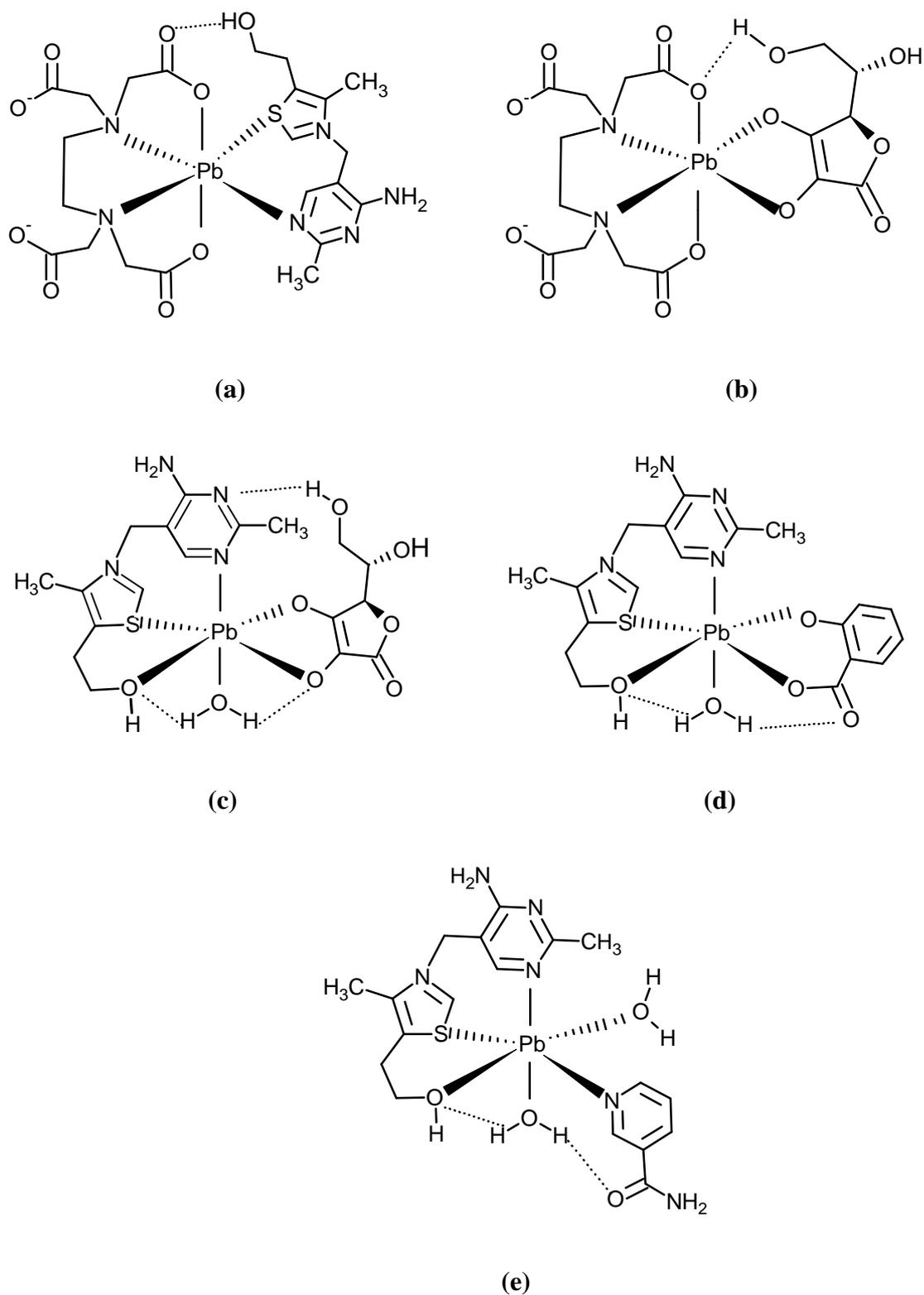
**Figure 4:** (a): Composite curve (2) and mixed ligand titration curve (1) for Pb(EDTA-Vit B<sub>1</sub>) ternary system.(b) Composite curve (2) and mixed ligand titration curve (1) for Pb(Sali+Vit B<sub>3</sub>) ternary system.

Being a borderline metal, lead is presumed to have strong interactions with the donor atoms S and N preferably and as well as with O (Das, 1990; Chisolm, 1968). Thus, one can expect considerable interactions between Pb(II) and any molecule containing at least one of the above mentioned donor atoms. A chelate ring can be formed, when several donor atoms in the same molecule to bind with lead. According to the aspects in coordination chemistry, five membered chelate rings in complexes are more stable than six membered rings (Chisolm, 1968). When taking into account the geometrical and electronic factors affecting the stabilities of complexes, structures with several chelate rings are the most stable and those with monodentate ligands are the least stable. Thus, it is obvious that hexadentate chelator, EDTA, forms the most stable complex with Pb(II) with the highest formation constant, when compared with other ligands. Vitamin B<sub>1</sub>, which carries specially S and N atoms as main ligating sites and O atom as the third ligating site, forms the next strongest chelate ring with Pb(II). Ascorbic acid is liable to form a five membered ring with Pb(II) through its two oxygen atoms and salicylic may forms a six membered ring with Pb(II) through its carboxylic and hydroxyl groups. Thus, Asc-Pb(II) complex should be more stable than Sali-Pb(II) complex. Values obtained for these two complexes obey with this theoretical aspect. Vitamin B<sub>3</sub> the only monodentate ligand in the set has formed the least stable complex with Pb(II). The structure of vitamin B<sub>1</sub> reveals its importance as a chelating agent in medical applications (Sasser *et al.*, 1984; Cheong, 1999). It is interesting to see that only vitamin B<sub>1</sub> has

formed mixed complexes with all the other ligands under the conditions used. It is clear with regard to stability constants that ternary systems of Pb(II) forming with EDTA-Vit B<sub>1</sub> and EDTA-AsC are more than two times stable than EDTA binary system. On the other hand, mixed ligand combinations of Vit B<sub>1</sub>-AsC, Vit B<sub>1</sub>-Sali and Vit B<sub>1</sub>-Vit B<sub>3</sub> also have formed fairly stable mixed complexes with Pb(II). Intermolecular interactions arising between two species keep them very closer to each other forming stable molecular combinations in solutions and this phenomenon lead to form more stable mixed ligand complexes in solutions (Sigel, 1975; Cheong *et al.*, 1999; Luth *et al.*, 1999). Yamauchi and Odani (1981) have extensively studied these interactions using acidic and basic amino acids and have proven that two ligands together can contribute remarkable stability for mixed complexes compared with their binary systems. According to the Table 3, it is clearly understood that all the ternary systems formed are much more stable than their parental binary systems attribute to the strong non bonded interactions with in the mixed systems formed. As given in Figure 5, some ligand-ligand interactions due to hydrogen bonding can be expected within each mixed ligand system formed and they can increase the stability of mixed complexes. The most suitable coordinating sites of Vit B<sub>3</sub> are its pyridine nitrogen atom and carbonyl oxygen atom (Najafpour *et al.*, 2007). Even though, less stable binary complexes are formed through either of these sites, more stable Pb(Vit B<sub>1</sub>)(Vit B<sub>3</sub>) mixed complex can be formed, as a result of some favorable ligand-ligand interactions between them.

**Table 3:**  $\Delta \log K$  values obtained for mixed complexes.

| Mixed complex system                         | $\Delta \log K$ |
|--|-----------------|
| Pb(EDTA)(Vit B <sub>1</sub> )                | 9.11            |
| Pb(EDTA)(AsC)                                | 8.36            |
| Pb(Vit B <sub>1</sub> )(Sali)                | 0.58            |
| Pb(Vit B <sub>1</sub> )(Vit B <sub>3</sub> ) | 0.78            |
| Pb(Vit B <sub>1</sub> )(AsC)                 | 0.03            |



**Figure 5:** Proposed structures for mixed ligand complexes in *in-vitro* conditions; (a) Pb(EDTA)(Vit B<sub>1</sub>) (b) Pb(EDTA)(AsC) (c) Pb(Vit B<sub>1</sub>)(AsC) (d) Pb(Vit B<sub>1</sub>)(Sali) (e) Pb(Vit B<sub>1</sub>)(Vit B<sub>3</sub>)

Since all experiments were carried out at physiological conditions, these results are more applicable for biological systems while enhancing the importance of the combining dietary ligands with EDTA or utilization of dietary ligand mixtures in the Pb(II) chelation therapy for more effective metal detoxification.

Further, parallel to these findings based on stability constants, it was reported that when a combination of Vitamin B<sub>1</sub> and ascorbic acid is given to lead poisoned mice their liver damage can be reduced significantly (Wang *et al.*, 2007). It has also been clinically proven with mice that EDTA-Pb-AsC (Goyer & Cherian, 1978) and EDTA-Pb-Vit B<sub>1</sub> (Kim, 1992) ternary systems were more effective than EDTA-Pb binary system to reduce and control the organ distribution of lead effectively. Therefore, the findings of this work have quantitatively proved the clinical investigations reported.

## CONCLUSIONS

The potentiometric data obtained on the lead complexes support that in aqueous medium, Pb(II) can interact with natural compounds like salicylic acid, ascorbic acid, vitamin B<sub>1</sub> and vitamin B<sub>3</sub> which very often enter into the body through food and nutrient supplements. Remarkable involvement of vitamin B<sub>1</sub> in the single and mixed complex formation with Pb(II) observed during the research can contribute to raise its applications in clinical chemistry.

Very stable ternary complexes of EDTA formed with ascorbic acid or vitamin B<sub>1</sub> along with the other stable combinations; ascorbic acid-vitamin B<sub>1</sub>, salicylic acid- vitamin B<sub>1</sub> and vitamin B<sub>1</sub>-vitamin B<sub>3</sub> must be able to administer to remove body Pb<sup>2+</sup> ions more effectively and this will lead scientists to an interesting path way to find more effective and less harmful ligand combinations to treat metal toxicity.

## ACKNOWLEDGEMENTS

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