

RESEARCH ARTICLE

Spectrophotometric and pH-metric studies on Pb(II), Cd(II), Al(III) and Cu(II) complexes of paracetamol and ascorbic acid

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Abstract: Spectrophotometric and pH-metric studies of the aqueous binary and mixed ligand complexes of Pb(II), Cd(II), Al(III) and Cu(II) were carried out with ascorbic acid and paracetamol. The study was conducted at an ionic strength of 0.15 mol dm^{-3} and a temperature of $37.0 \pm 0.2^\circ\text{C}$. The protonation constants of the free ligands and the stability constants of the binary and mixed ligand complexes were determined using pH titration data. Proton dissociation of free ligands and complex formation were also qualitatively confirmed with UV data. The stabilities of mixed ligand complexes were quantitatively compared with those of the corresponding single ligand complexes in terms of $\Delta \log K$. Aluminium formed the most stable binary complex; Al (paracetamol)₂, with a high overall stability constant ($\log \beta_2 = 16.74$). The stable mixed ligand complexes; Pb (ascorbic acid) (paracetamol), Cd (ascorbic acid)(paracetamol) and Cu (ascorbic acid) (paracetamol) were observed with positive $\Delta \log K$ values showing the ability of using them in less harmful chelation therapy of these metals. Further, the results revealed that the bioavailability of both ligands will be lower for a metal intoxicated person.

Keywords: Ascorbic acid, chelation therapy, mixed ligand complexes, paracetamol, stability constants, toxic metals.

INTRODUCTION

Vitamin C (ascorbic acid), being a good electron donor and an essential molecule in biological systems, acts as an effective antioxidant, protects living cells from damaging effects of reactive species such as reactive oxygen radicals and plays a major physiological role in the body (Correa, 1992; Byers & Guerrero, 1995; Carr & Frei, 1999; Wintergerst *et al.*, 2006).

However, the body is incapable of synthesizing ascorbic acid. Therefore, there is a need to take vitamin C daily from external sources such as vegetables,

fruits and vitamin supplements to maintain good health (Haytowitz, 1995). Paracetamol is the most popular analgesic all over the world today and it is widely used as a non-prescriptive drug, to relieve common health problems such as fever, and various aches and pains in the body (Prescott, 1996).

On the other hand, metal toxicity in humans is a serious issue worldwide, as it can cause many illnesses. In the recent years, attention has been paid to the interactions of biologically active molecules with various metal ions in order to determine the possibility of using these molecules in chelating therapy (Masłowska & Owczarek, 1983, 1988; Kleszczewska, 1999). However, there are only a few reports available on the interactions of paracetamol and vitamin C with toxic metals, although both these molecules have functional groups which can form bonds with metal ions.

As ascorbic acid enters the body almost daily and paracetamol is taken frequently, if there is a metal intoxicated person, there can be several favourable and unfavourable interactions inside the body. In this study the ability of vitamin C and paracetamol to form complexes with Pb(II), Cd(II), Al(III) and Cu(II) under physiological conditions when each ligand is present alone and when the two ligands are present together with each metal was investigated by considering the formation of binary and ternary complexes, respectively.

METHODS AND MATERIALS

Materials

The following chemicals were used as received for the potentiometric and spectrophotometric experiments:

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HNO₃, NaOH, NaNO₃, Pb(NO₃)₂, Cd(NO₃)₂, Al(NO₃)₃, Cu(NO₃)₂, ascorbic acid, paracetamol. All chemicals used were of analytical grade. 1×10^{-2} mol dm⁻³ standard solutions of the metals and ligands were freshly prepared using double distilled deionized water. The NaOH (0.03 mol dm⁻³) and HNO₃ (0.03 mol dm⁻³) solutions were standardized with primary standards, potassium hydrogen phthalate and sodium carbonate, respectively.

Potentiometric studies

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

The following mixtures were prepared in a total volume of 40.00 cm³ by adding required amounts of double distilled deionized water at a constant ionic strength of 0.15 mol dm⁻³ using NaNO₃ for potentiometric titrations (L, X and M denote ligand 1, ligand 2 and metal ion, respectively).

- (a) HNO₃ (10.00 cm³)
- (b) a + L (10.00 cm³)
- (c) a + X (10.00 cm³)
- (d) b/c + M (10.00 cm³)
- (e) a + L (10.00 cm³) + X (10.00 cm³) + M (10.00 cm³)

Each solution was titrated against a standardized NaOH solution at 37.0 ± 0.2 °C (YCW – 05 model thermostatic water bath was used to maintain the temperature) and the pH was recorded after the addition of 0.20 cm³ aliquots of NaOH. Nitrogen was purged through the reaction mixtures to ensure the absence of oxygen and carbon dioxide. Each titration was carried out in triplicate until a permanent turbidity was observed in the reaction mixture. The protonation constants and the formation constants of binary complexes were determined using Irving - Rossotti equations (Irving & Rossotti, 1953, 1954) by calculating the values for average number of ligand molecules attached per metal ion ($\bar{n}$) and free ligand exponent (pL).

With the modifications given by Chidambaram and Bhattacharya (1970a, b), constants for the mixed ligand systems were computed. The comparison of stabilities of mixed ligand complexes over single ligand complexes were carried out according to the literature (Sigel, 1975; Luth *et al.*, 1999; Moustafa, 2005).

Spectrophotometric studies

Spectral measurements were recorded on a SHIMADZU, UV-1800 UV-Visible spectrophotometer. Metal-ligand binary and ternary mixtures identical to the samples used for titrations were prepared for the spectroscopic experiments as well. For each mixture, UV spectra were recorded with increasing pH values until a permanent turbidity was obtained. Resulted spectra at different pH values were analyzed.

RESULTS AND DISCUSSION

Protonation constants for ligands

Protonation constants calculated from the pH titration data are given in Table 1.

Table 1: Protonation constants (^HK_j) of free ligands

Ligand	Calculated ^a		Reported ^{b,c}	
	Log ^H K ₁	Log ^H K ₂	Log ^H K ₁	Log ^H K ₂
Ascorbic acid (Asc)	10.00	5.28	11.57	4.17
Paracetamol (Para)	9.24	-	9.50	-

^a -The values determined at 37 °C

^b -The values determined at 25 °C

^c - O'Neil *et al.*, 2006; Edict Consulting Ltd., 2008

Binary and mixed ligand complexes

The two sets of titration curves plotted for the copper system, which formed a mixed ligand complex with paracetamol and ascorbic acid, and for the aluminium system that did not form a ternary complex with the two ligands are shown in Figures 1 and 2, respectively.

The binary system titration curves deviate from their respective free ligand titration curves showing the formation of binary metal complexes (Figure 1). On the other hand, the mixed system titration curve shows a different behaviour from either of the two corresponding binary systems indicating the formation of a mixed ligand complex. The other two systems that formed mixed ligand complexes also show a similar behaviour.

In the Al-Asc-Para system given in Figure 2, the mixed ligand titration curve superimposes with the titration curve of Al-Para binary system above pH 4.

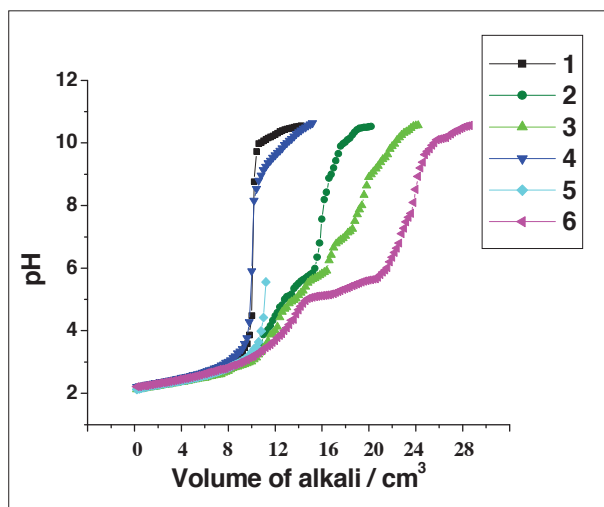


Figure 1: Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of Cu^{2+} with ascorbic acid and paracetamol; 1) HNO_3 ; 2) HNO_3 + ascorbic acid; 3) HNO_3 + ascorbic acid + $\text{Cu}(\text{NO}_3)_2$; 4) HNO_3 + paracetamol; 5) HNO_3 + paracetamol + $\text{Cu}(\text{NO}_3)_2$; 6) HNO_3 + ascorbic acid + paracetamol + $\text{Cu}(\text{NO}_3)_2$

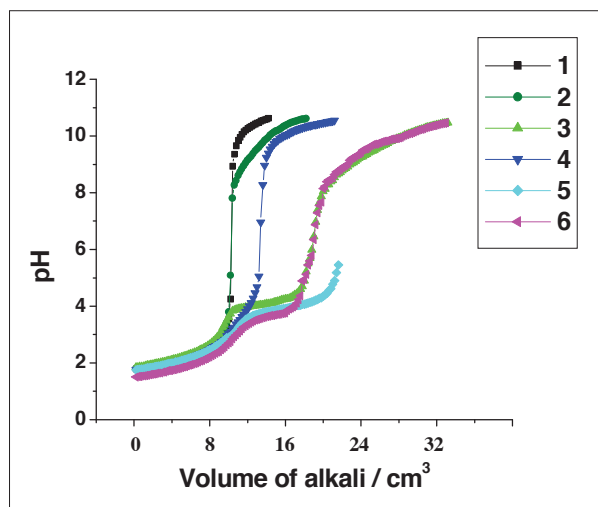


Figure 2: Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of Al^{3+} with ascorbic acid and paracetamol; 1) HNO_3 ; 2) HNO_3 + paracetamol; 3) HNO_3 + paracetamol + $\text{Al}(\text{NO}_3)_3$; 4) HNO_3 + ascorbic acid; 5) HNO_3 + ascorbic acid + $\text{Al}(\text{NO}_3)_3$; 6) HNO_3 + paracetamol + ascorbic acid + $\text{Al}(\text{NO}_3)_3$

However, below pH 4, the mixed ligand titration curve is closer to the Al-Asc titration curve implying the absence of mixed ligand complex formation.

According to Table 2, it is clear that all four metal ions form binary complexes with the ligands used under the experimental conditions. However, most of the complexes are of ML type with low stability constant values. Exceptionally, aluminium and copper form considerably more stable ML_2 complexes. $\text{Al}(\text{Para})_2$ has a comparably high overall formation constant of ca. 10^{17} and $\text{Cu}(\text{AsC})_2$ has an overall stability constant of 10^{13} .

It is possible for Pb^{2+} to coordinate with more than one ascorbic or paracetamol molecule, since it can have a coordination number of six. However, only ML type complexes were observed here for binary systems at acidic pH values with smaller formation constants, and when the pH reaches the basic region, a precipitate of $\text{Pb}(\text{OH})_2$ appeared. In both systems, complexes dissociate during the formation of the lead hydroxide precipitate. Thus, only $\text{ML}(\text{H}_2\text{O})_n$ type binary complexes of lead are possible with either ligand. Although similar type of results were observed with Cd^{2+} , its formation constants and precipitation pH values with two ligands were higher than those of Pb^{2+} . This is possible as the solubility product of $\text{Cd}(\text{OH})_2$ is higher than that of $\text{Pb}(\text{OH})_2$ (Ebbing & Gammon, 2007). However,

it is obvious that both metal complexes have higher stabilities with ascorbic acid, as ascorbic acid is capable of forming chelate complexes through its dienol group (Kleszczewska, 1999).

Cu^{2+} starts to form a ML_2 type complex with ascorbic acid at pH 6.50. Due to the higher stability constant of ML_2 ($\log \beta_2$ is 13.89), copper hydroxide precipitate was not observed for this system. Although Al^{3+} formed a ML_2 complex ($\log \beta_2$ is 7.99) with ascorbic acid, it is not stable, as $\text{Al}(\text{OH})_3$ having a very low solubility product precipitates around pH 5.25. As Al^{3+} prefers to form complexes with organic-oxygen and nitrogen containing compounds, a very high stability was observed for $\text{Al}(\text{Para})_2$ complex ($\log \beta_2$ is 16.74) (Robinson, 1993).

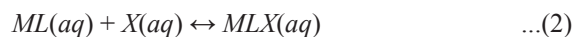
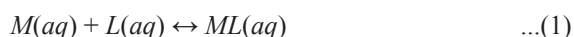
In the calculation, mixed ligand complexes were assumed to form in two steps. Initially, the primary ligand (L), with a higher formation constant in the binary complex, reacts with the metal ion to form a binary complex with formula ML, which then coordinates to the second ligand (X) to form a mixed ligand complex of the form MLX . The primary ligand can also be decided by comparing the titration curves of binary systems. The complex that reached the highest pH before precipitation of the metal hydroxide was considered to be the first complex formed in mixed ligand systems and the corresponding ligand was the primary ligand in a mixed ligand complex.

Table 2: Stability constants of binary complexes

Binary system	pH of permanent turbidity	Log K_1^f (pH range)	Log K_2^f (pH range)
Pb-Asc	6.60	3.53 (2.80 – 5.50)	-
Pb-Para	5.52	2.25 (2.10 – 5.00)	-
Cd-Asc	8.27	3.74 (6.50 – 8.00)	-
Cd-Para	8.05	2.84 (6.75 – 8.00)	-
Al-Asc	5.82	3.86 (3.00 – 3.75)	4.13 (4.25 - 5.25)
Al-Para	No turbidity	9.12 (2.75 – 4.00)	7.62 (4.00 - 6.00)
Cu-Asc	No turbidity	7.56 (4.80 – 6.50)	6.33 (6.50 - 7.20)
Cu-Para	5.56	1.54 (4.75 – 5.50)	-

Table 3: Stability constants for mixed ligand complexes

Mixed ligand complex system	pH of permanent turbidity	log K_1 (pH range)	log K_2 (pH range)
(Pb-Asc-Para)	7.48	3.53 (2.80 – 5.50)	3.70 (5.50 – 7.00)
(Cd-Asc-Para)	No turbidity	3.74 (6.50 – 8.00)	5.45 (8.00 – 9.22)
(Cu-Asc-Para)	No turbidity	7.56 (4.80 – 6.50)	4.91 (6.60 – 9.60)



Equilibrium constant for reaction (1) is the K_1^f value of the primary ligand, and that of reaction (2) was calculated according to the modified method (Chidambaram & Bhattacharya, 1970a, b) taking the values of n_{mix} in the range of 0 and 1. The calculation of n_{mix} was commenced at the pH at which the calculation of K_1^f of complex ML was completed. The overall stability constant of the mixed complex is the product of stability values of reactions (1) and (2).

According to the results given in Table 3, ligand combination Asc-Para forms mixed ligand complexes with all metal ions except aluminium. The overall stability constant of Cu(Asc)(Para) ternary complex is much higher than that of the other two mixed ligand complexes formed. Hence, ascorbic acid alone or ascorbic acid-paracetamol mixture appears to exhibit a strong attraction towards copper under physiological conditions.

Spectroscopic evidences for complex formation

In Figures 3a-3c, mixed ligand system λ_{max} value lies in between the λ_{max} values of the two binary systems, M(Para)

and M(Asc). This indicates that the mixed ligand system contains a species different from either of the binary complexes. This new species must be M(Asc)(Para) mixed ligand complex of the two ligands (Dewitt & Watters, 1954). However, in Figure 3d, UV spectrum of the ligand mixture with Al^{3+} does not contain any new peak. Thus, Al(Asc)(Para) mixed ligand complex cannot be expected and instead, a larger percentage of Al(Para)₂ binary complex can be expected even in the presence of a mixture of ligands.

Further, evidence for the formation of mixed ligand complexes was obtained by comparing the mixed ligand system titration curve with the corresponding composite curve. The composite curve can be drawn by adding the horizontal distance (volume of alkali consumed) of the secondary ligand (X) curve to the horizontal distance of the ML curve at the same pH (Maktell & Carey, 1967; Shelke & Jahagirdar, 1976). Alkali volumes consumed by the ternary system were compared with the corresponding alkali volumes of composite curve at the same pH by plotting them in a graph.

In Figures 4a, 4c and 4d, the mixed ligand titration curves deviate from the corresponding composite curves significantly by showing the ability of forming mixed ligand complexes of Cu^{2+} , Pb^{2+} and Cd^{2+} with the ascorbic acid paracetamol combination. On the other hand, in Figure 4b, two curves are nearly superimposable, indicating the absence of a mixed ligand complex of Al^{3+} with this ligand combination.

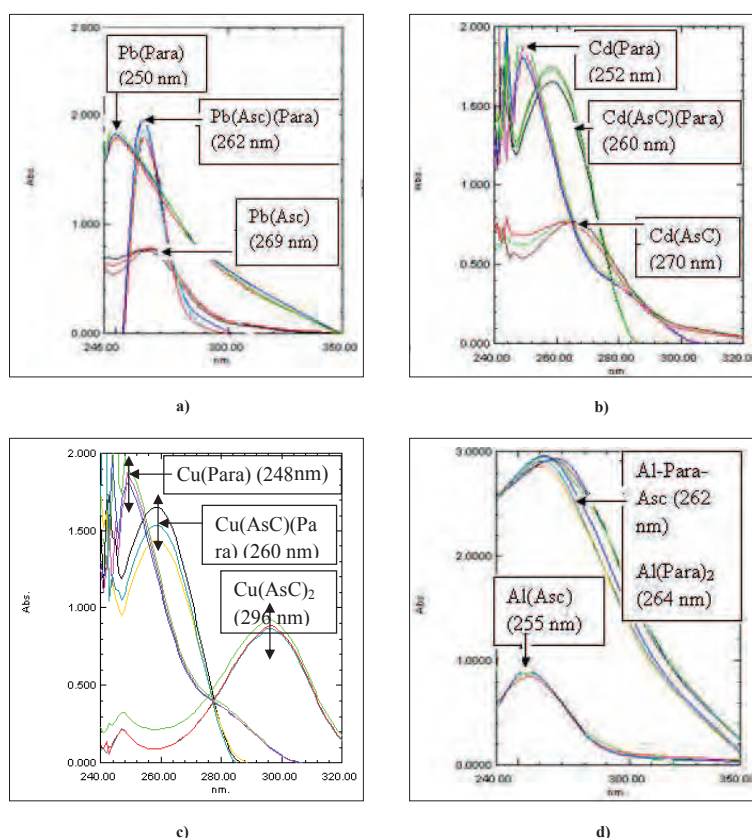


Figure 3: Comparison of UV absorption spectra of binary and mixed ligand systems: (a) Pb(Para), Pb(Asc)(Para), Pb(Asc) (b) Cd(Para), Cd(Asc)(Para), Cd(Asc); (c) Cu(Para), Cu(Asc)(Para), Cu(Asc)₂; (d) Al(Para)₂, Al-Asc-Para, Al(Asc) at pHs of 4.50, 5.00 and 5.50.

The stability of mixed ligand complexes formed over their corresponding binary complexes was evaluated by calculating the parameter $\Delta \log K$ according to the equation 3 (Sigel, 1975; Luth *et al.*, 1999; Moustafa, 2005).

$$\Delta \log K = \log K_{MLX}^{ML} - \log K_{ML}^M \quad \dots(3)$$

K_{MLX}^{ML} = formation constant for the complex MLX formed by the reaction of complex ML with free ligand X

K_{ML}^M = formation constant for the complex ML

In general, if mixed complexes are more stable than their corresponding binary complexes, the value of $\Delta \log K$ is positive (Sigel, 1980; Moustafa, 2005; Thanavelan *et al.*, 2011).

Values given in Table 4 clearly reveal that all mixed ligand complexes formed are more stable than their corresponding binary complexes. Therefore, these mixed

ligand complexes can have a wider range of applications over binary complexes.

To form a more stable mixed ligand complex, there should be favourable acid-base electrostatic interactions (Suzuki *et al.*, 1975; Schwarz & Gilligan, 1977; Lohman *et al.*, 1980) or/and hydrogen bonding (Marzilli & Kistenmacher, 1977; Chiang *et al.*, 1978) in solution due to the presence of several ligating sites in each molecule. According to the literature, electron density of the metal-ligand bonds in ternary systems are redistributed to increase the polarity and hence, mixed ligand complexes are not easily hydrolyzed by metal hydroxides even at high pH values (El-Haty *et al.*, 1995).

Table 4: Calculated $\Delta \log K$ values for mixed ligand complexes

Mixed ligand complex	$\Delta \log K$
Pb(Asc)(Para)	1.45
Cd(Asc)(Para)	2.61
Cu(Asc)(Para)	3.37

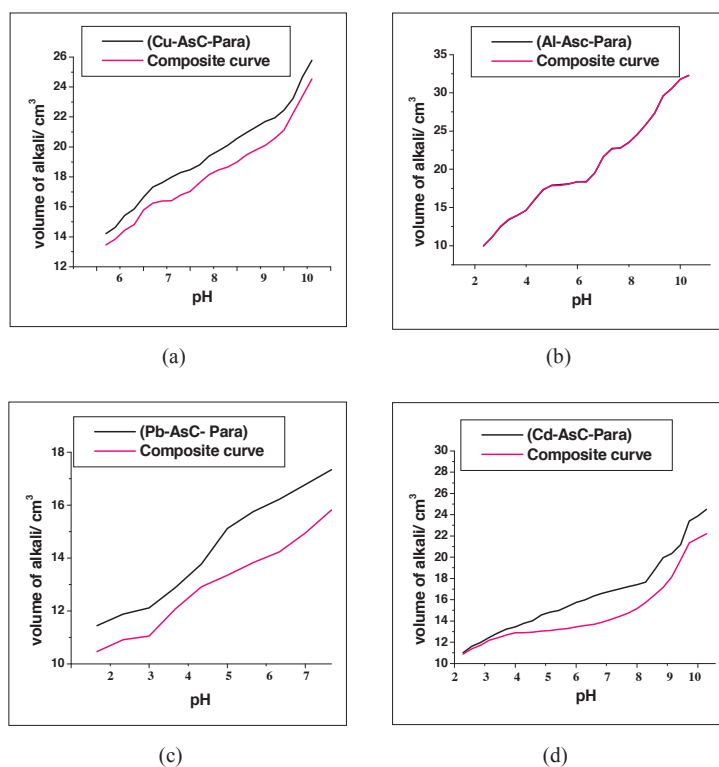


Figure 4: Composite curves and ternary system titration curves of four metal ions :

a) Cu^{2+} ; b) Al^{3+} ; c) Pb^{2+} ; d) Cd^{2+} ;

However, it was found that Al^{3+} did not form a mixed ligand complex under the conditions used according to the titrimetric, spectrophotometric and theoretical data, and only Al(Para)_2 was formed owing to its high stability.

Further, considering the hard-soft nature of metal ions (Das, 1990) and ligating sites of the two ligands (Kleszczewska, 1999; Lawal & Obaleye, 2007), the following structures can be proposed for the complexes obtained (Figure 5).

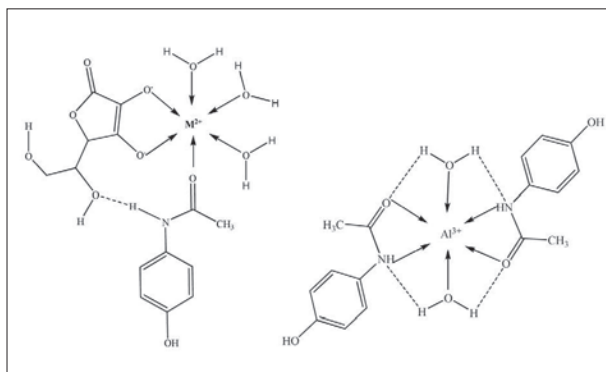


Figure 5: Structure of M(Asc)(Para) mixed ligand complex ($\text{M} = \text{Pb}^{2+}$, Cd^{2+} , Cu^{2+}) and Al(Para)_2 binary complex

Generally, metal complexes with overall formation constants greater than 10^6 are stable over a long range of pHs including high pH values (Furia, 1972). Therefore, the complexes identified in this study having stability constants over 10^6 are suitable for the use in chelation therapy. Such complexes do not dissociate in the gastrointestinal tract or inside any organ, and ultimately they can be excreted from the body as metal complexes. Thus, both binary and ternary complexes having overall stability values of 10^6 or more are suitable for chelation therapy of the respective metals. Accordingly, Pb(Asc)(Para) , Cd(Asc)(Para) , Al(Para)_2 and $\text{Cu(Asc)}_2/\text{Cu(Asc)(Para)}$ complexes are good chelators for Pb(II) , Cd(II) , Al(III) and Cu(II) , respectively. Further, due to the less harmful effects of these biologically important molecules, such ligand combinations will be more useful compared to other ligands or ligand combinations that are already used clinically.

Besides, these findings give important information on the bioavailability of paracetamol and ascorbic acid inside the body of a metal intoxicated person. Taking paracetamol with beverages containing ascorbic acid will reduce the bioavailability of free paracetamol of a metal intoxicated person and will affect the complete medicinal

activity of paracetamol. Therefore, metal intoxicated people may need to have higher dosages of paracetamol in order to have a desirable activity. On the other hand, such a person should consume more ascorbic acid amounts than a normal person. However, to get more accurate information regarding these interactions, further experiments should be carried out under *in-vivo* conditions using animal studies.

CONCLUSION

Potentiometric, spectrophotometric and theoretical data obtained on metal complexes show that a mixture of ascorbic acid and paracetamol form mixed complexes with Pb^{2+} , Cd^{2+} and Cu^{2+} in aqueous media, and these will have applications in chelation therapy. It was also found that paracetamol itself is a good chelator of Al^{3+} , having a high stability in both acidic and basic pHs.

Further, for a person intoxicated with metal ions like Pb^{2+} , Cd^{2+} , Al^{3+} and Cu^{2+} , actual dosages of paracetamol should be more than that for a normal person. Moreover, it is important for such people to take more ascorbic acid from diets when compared to a normal person.

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REFERENCES

- Byers T. & Guerrero N. (1995). Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. *American Journal of Clinical Nutrition* **62**(6 Suppl): 1385S – 1392S.
- Carr A.C. & Frei B. (1999). Towards a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *American Journal of Clinical Nutrition* **69**: 1086 – 1107.
- Chiang C.C., Sorrell T., Kistenmacher T.J. & Marzilli L.G. (1978). Preparation and crystal and molecular structure of sodium [bis(inosine 5'-monophosphate) (diethylenetriamine)copper(II)] decahydrate. Possible implications for intrastrand cross-linking of polynucleotides by copper(II) and platinum (II) complexes. *Journal of the American Chemical Society* **100**: 5102 – 5110.
DOI: <http://dx.doi.org/10.1021/ja00484a032>
- Chidambaram M.V. & Bhattacharya P.K. (1970a). Studies in amine-amino acid mixed ligand chelates – I. *Journal of Inorganic and Nuclear Chemistry* **32**: 3271 – 3275.
DOI: [http://dx.doi.org/10.1016/0022-1902\(70\)80210-X](http://dx.doi.org/10.1016/0022-1902(70)80210-X)
- Chidambaram M.V. & Bhattacharya P.K. (1970b). Studies in some amino acid chelates: Part II- Ni (II), VO (II) & Zn (II) chelates of leucine, isoleucine, norleucine & valine. *Indian Journal of Chemistry* **8**: 941 – 942.
- Correa P. (1992). Human gastric carcinogenesis: a multistep and multifactorial process. *Cancer Research* **52**: 6735 – 6740.
- Das A.K. (1990). *A Text book on Medicinal Aspects of Bio-inorganic Chemistry*. CBS Publishers, New Delhi, India.
- Dewitt R. & Watters J.I. (1954). Spectrophotometric investigation of a mixed complex of copper (II) ion with oxalate ion and ethylenediamine. *Journal of the American Chemical Society* **76**: 3810 – 3814.
- Ebbing D. & Gammon S.D. (2007). *General Chemistry*. Charles Hartford, Belmont, USA.
- Edict Consulting Ltd. (2008). Public Assessment Report: *Paracetamol 250mg/5ml Oral Solution*. Available at <http://www.mhra.gov.uk/home/groups/pl-a/documents/websiteresources/con015073.pdf>. Accessed 12 May 2011.
- El-Haty M.T., Amrallah A.H., Mahmoud R.A. & Ibrahim A.A. (1995). pH-metric studies on ternary metal complexes of some amino acids and benzimidazole. *Talanta* **42**: 1711 – 1717.
DOI: [http://dx.doi.org/10.1016/0039-9140\(95\)01641-4](http://dx.doi.org/10.1016/0039-9140(95)01641-4)
- Furia T.E. (1972). *CRC Handbook of Food Additives*. CRC Press, Boca Raton, Florida, USA.
- Haytowitz D.B. (1995). Information from USDA's Nutrient Data Bank. *Journal of Nutrition* **125**: 1952 – 1955.
- Irving H.M. & Rossotti H.S. (1953). Methods for computing successive stability constants from experimental formation curves. *Journal of the Chemical Society*: 3397 – 3405.
DOI: <http://dx.doi.org/10.1039/jr9530003397>
- Irving H.M. & Rossotti H.S. (1954). The calculation of formation curves of metal complexes from pH titration curves in mixed solvents. *Journal of the Chemical Society*: 2904 – 2910.
DOI: <http://dx.doi.org/10.1039/jr9540002904>
- Kleszczewska E. (1999). The spectrophotometry determination of chelate complex: L-ascorbic acid with cuprum (II) and mercury (II) in alkaline solution. *Polish Journal of Environmental Studies* **8**(5): 313 – 318.
- Lawal A. & Obaleye J.A. (2007). Synthesis, characterization and antibacterial activity of aspirin and paracetamol metal complexes. *Biokemistri* **19**(1): 9 – 15.
- Lohman T.M., De Haseth P.L. & Record M.T. Jr. (1980). Pentalysine-deoxyribonucleic acid interactions: A model for the general effects of ion concentrations on the interactions of proteins with nucleic acids. *Biochemistry* **19**: 3522 – 3530.
DOI: <http://dx.doi.org/10.1021/bi00556a017>
- Luth M.S., Kapinos L.E., Song B., Lippert B. & Sigel H. (1999). Extent of intramolecular stacking interactions in the mixed-ligand complexes formed in aqueous solution by copper (II), 2,2'-bipyridine or 1,10-phenanthroline and 2'-deoxyguanosine 5'-monophosphate. *Journal of the Chemical Society (Dalton Transactions)* **3**: 357 – 365.
DOI: <http://dx.doi.org/10.1039/a808479c>
- Maktell A.E. & Carey G.H. (1967). Mixed Ligand Chelates

- of Uranium (IV). *Journal of the American Chemical Society* **89**: 2859 – 2865.
DOI: <http://dx.doi.org/10.1021/ja00988a600>
21. Marzilli L.G. & Kistenmacher T.J. (1977). Stereoselectivity in the binding of transition-metal chelate complexes to nucleic acid constituents: bonding and nonbonding effects. *Accounts of Chemical Research* **10**(4): 146 – 152.
DOI: <http://dx.doi.org/10.1021/ar50112a007>
 22. Maslowska J. & Owczarek A. (1983). Studies on ascorbate complexes of metal ions of beryllium group by the method of potentiometric surfaces. *Polish Journal of Chemistry* **57**: 719 – 726.
 23. Maslowska J. & Owczarek A. (1988). Potentiometric studies on mixed complexes of alkaline earth group metals with ascorbic and tartaric acid. *Polish Journal of Chemistry* **62**: 75-82.
 24. Moustafa M.H. (2005). Potentiometric studies of the binary and ternary complexes of mercury (II) with acetazoleamide and some amino acids. *Assiut University Bulletin for Environmental Researches* **8**(2): 81 – 88.
 25. O'Neil M.J., Heckelman P.E., Koch C.B. & Roman K.J. (2006). *The Merck Index*, 14th edition, pp. 242. Merck & Co., Inc., Rahway, USA.
 26. Prescott L.F. (1996). *Paracetamol (acetaminophen)*, 2nd edition. Taylor & Francis Ltd., London, UK.
 27. Robinson G.H. (1993). *Coordination Chemistry of Aluminium*. VCH Publishers Inc., New York.
 28. Schwarz G. & Gilligan III T.J. (1977). Dual-mode cooperative binding of adenosine 5'-triphosphate to poly (L-lysine). *Biochemistry* **16**: 2835 – 2840.
DOI: <http://dx.doi.org/10.1021/bi00632a005>
 29. Shelke D.N. & Jahagirdar D.V. (1976). Ternary complexes: equilibrium studies of mixed-ligand complexes of neodymium ion with carboxylic and phenolic acids in aqueous medium. *Bulletin of the Chemical Society of Japan* **49**(8): 2142 – 2147.
DOI: <http://dx.doi.org/10.1246/bcsj.49.2142>
 30. Sigel H. (1975). Ternary Cu²⁺ complexes: stability, structures and reactivity. *Angewandte Chemie International Edition* **14**(6): 394 – 402.
DOI: <http://dx.doi.org/10.1002/anie.197503941>
 31. Sigel H. (1980). *Coordination Chemistry*. Pergamon Press, Oxford, UK.
 32. Suzuki Y., Hara K. & Hirahara T. (1975). Some properties of the salt between inosine and L-lysine. *Bulletin of the Chemical Society of Japan* **48**: 2149 – 2152.
DOI: <http://dx.doi.org/10.1246/bcsj.48.2149>
 33. Thanavelan R., Manikandan G., Ramalingam G. & Thanikachalam V. (2011). Mixed ligand chelates of Cd²⁺ with 2-(1-(aminomethyl)cyclohexyl) acetic acid and dicarboxylic acids. *Pelagia Research Library* **2**(4): 90 – 98.
 34. Wintergerst E.S., Maggini S. & Hornig D.H. (2006). Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. *Annals of Nutrition and Metabolism* **50**: 85 – 94.
DOI: <http://dx.doi.org/10.1159/000090495>