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ON

EQUILIBRIUM STUDIES ON TOXIC METAL ION-MIXED LIGAND SYSTEMS UNDER PHYSIOLOGICAL CONDITIONS

Grant Number: RG/2008/BS/01
Principal Investigator:
Champika V. Hettiarachchi

NSF FINAL REPORT

Section 1

Information regarding project/project personal:

i) Grant Number: RG/2008/BS/01
ii) Title of the Project : **Equilibrium studies on toxic metal ion-mixed ligand systems under physiological conditions.**
iii) Principal Investigator : Dr. Champika V. Hettiarachchi, Department of Chemistry, University of Peradeniya
iv) Co-Investigator : Prof. O.A. Ileperuma, Department of Chemistry, University of Peradeniya.
v) Institute where research was being carried out : Department of Chemistry, Faculty of Science, University of Peradeniya.
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vii) Date of completion of project : 31st of August, 2011.
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xii) Number of technical assistants and/labours employed and period of service : none
xiii) Publications/ communications arising from the project during the reporting period : 4

Section 2

Executive summary of the project:

The study of toxic metal complexes has become a worldwide interest related to various aspects such as, investigation of effective metal chelators for chelation therapy and removal of toxic metals from water reservoirs, etc. However, the research done on interactions between toxic metals and mixed ligand systems is very rare especially with ligands entering to our body more frequently. The main focus of the study was the investigation of mixed ligand complexes of toxic metals to find out their bioavailability and to discover less harmful ligand combinations for chelation therapy.

Thus, this project explored the equilibrium study on Pb^{2+}, Cd^{2+}, Al^{3+} and Cu^{2+} toxic ions and ascorbic acid, paracetamol, salicylic acid, citric acid, uracil, aspirin, vitamin B_1, vitamin B_3, caffeine and Na_2EDTA using pH titrations and UV-Visible spectroscopy under the physiological conditions of 37.0 ± 0.2 ^oC and 0.15 M ionic strength. The analysis revealed that all four metal ions have significant interactions with each ligand alone and with ligand mixtures. Though, Na_2EDTA was the strongest ligand bound to four metal ions in binary complex formation, there were several mixed complexes for each metal, which were more stable having several hundred times larger stability constants than M-EDTA complexes. Specially, $Pb(Na_2EDTA)(VitaminB_1)$, $Pb(Na_2EDTA)$(Ascorbic acid) and $Pb(Na_2EDTA)$(Citric acid) had remarkably high stability in mixed complexation with four metals. As most mixed ligand complexes had stability constants greater than 6, they were all good for chelation therapy with fewer side effects, especially with non EDTA ligand combinations. Further work revealed

that, the bioavailability of paracetamol, Ascorbic acid, Vitamin B_1 will be lower for a metal intoxicated person.

Section 3

Report in detail:

Introduction /background:

More than half of the elements found in nature are metals. Out of them, some of the heavy metals like lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr) and antimony (Sb) etc. are toxic to the biological systems as they tend to make undesirable effects in the biological systems if they are present in significant amounts. Therefore, they are known as toxic metals.

Today entire earth; soil, Water and air consist of at least several toxic metal ions in considerable concentrations due to various human activities. Hence, many food items and beverages contain toxic metal ions and ultimately enter into the human body[1-3]. Once they are adsorbed in both bronchial tubes and gastrointestinal tract, they will be transported by the blood stream into the cellular fluids, where toxic metals exert their toxic effect[1]. Toxic metals can make several imperfections in many systems in the human body including central nerves system, the cardiovascular system, respiratory system and the hematopoietic systems[1&4], due to very high affinity for certain active groups on plasma proteins; especially macromolecular fractions of the protein[5,6,7,8].

Among all the toxic metals, lead is the one distributed largely. Lead can be absorbed into the biological systems either as an organic molecule or as an inorganic one. Hence, lead is absorbed in both water soluble part and water insoluble lipid part of biological systems. Because of that, lead can be found in blood as well as in skeleton[4]. However frequent exposure to lead for a long time will result in diseases like impaired kidney functions, multiple sclerosis, anaemia, neurological diseases and encephalitis[1,7,8]. More common issue found in developing countries like Sri Lanka is that the toxicity arises due to the use of aluminium and metal coated utensils, paints and pigments containing heavy metals. Cadmium can be found very often in commercial zinc and can be entered to the living systems by the use of galvanized material. On the other hand, cadmium poisoning can frequently be seen due to consumption of vegetable and fruits contaminated by cadmium because of the agricultural use of cadmium containing fertilizers and soil contaminated by sewage sludge. Each cigarette contains significantly higher amount of cadmium and more than half of that may enter into the lungs during smoking. After a period of time, such an individual can suffer from chronic renal failure leading to renal diseases[9] and a painful disease due to the erratic bone metabolism known as "*itai-itai byo*" [1]. Being the most abundant element in the earth's crust, aluminium can be found alone and in many organic and inorganic molecules. There are several roots like, from the environment, from foods and beverages, from health care agents, from various administrations as an antacid, anti- diarrheal or as anti-phosphate absorbers through which aluminium can enter into humans. There is a risk of aluminium toxicity as a result of using aluminium utensils in cooking [10]. Dialysis encephalopathy[11], fractures and high levels of bone aluminum[11,12] and Aizheimer's disease[12] are some of those commonly identified diseases due to excessive intake of aluminium. By the definition, copper is an essential element for the activity of different enzymes such as tyrosinase, amine oxidase, cytochrome oxidase, ceruloplasmin and ascorbic acid oxidase etc. Hence, without copper some essential biological functions will not take place. Diseases like Anemia, kinky-hair syndrome can be seen due to copper deficiency. However, if uptake level of copper is too high, then there will be a toxic effect due to excess copper. One more common issue of that is known as Wilson's disease [1,13-15] . Therefore, like any other essential element, copper displays two distinct concentration ranges, one is the zone of biotic effect, which corresponds to optimum physiological range of copper and the other is the zone of pharmacotoxicological effect, where poisonous effects take place[16].

The most severe problem of toxic metals over organic poisons is that they can't be converted into harmless forms by any of the biological process occurring in the body. Toxic metals remain unchanged in the body for several hundreds of years. Hence, the only cure for metal toxicity is the excretion of the metal from the body either via urine or faeces. Ligands binding strongly with toxic metal ions can be used to remove them from the biological systems and this method is called chelation therapy[1]. Hence, agents using in chelation therapy should have high ability to form more stable complexes with toxic metal ions than the interactions of metal with biological molecules and could be easily excreted via urine or faeces as well[1,4,9]. Mostly polydentate chelating agents like EDTA, BAL, Penicilamine etc. are used for this therapy and EDTA is the most commonly used chelating agent due to its ability to act as a hexadentate ligand in complex formation. However, synthetic chelating drugs used in chelation therapy have several harmful side effects.

Meals, drugs and vitamins consist of organic molecules containing reactive functional groups, such as hydroxyl groups, thiol groups, carboxylic groups etc. As they are rich in electron density, metal ions tend to interact with them. Some of these compounds are Ascorbic acid, salicylic acid, citric acid, oxalic acid, etc.. On the other hand, caffeine intake is very high among the people, as most of the beverages including tea, coffee, chocolate and certain soft drinks contain caffeine. Also there are some painkillers containing caffeine. Further, acetylsalicylic acid (Aspirin) is a very common analgesic among the people. In general, people get used to have non-prescription aspirin to reduce fever, headache, toothaches, muscle aches and cold[17]. Acetaminophen also known as paracetamol has been in use over a few decades as an analgesic in home medication. It is believed that paracetamol is a good treatment for the relief of pain and fever. Therefore, it is recognized both as an analgesic and antipyretic medicine which has effects very similar to those of aspirin[18]. People are used to taking several vitamin supplements like vitamin C, vitamin B_1(thiamine), Nicotinic acid (Vitamin B_3) daily to meet the vitamin requirement. Further, the nucleic bases; adenine, guanine, uracil, thymine, and cytosine with chelating properties are also present in biological systems.

Today the investigation of the extent of complex formation of essential metal ions with various organic molecules has become an important and interesting topic to bioinorganic chemists, as information on the bioavailability of the considered drugs or dietary ligands in the presence of a metal[19-23], toxic effects of metals with ligand and pharmacological activity of the metal complexes[24-30] can be obtained. Though scientists in the world, talk about the adverse effects of metal toxicity, much information is not available on the interactions among toxic metals and common drugs and dietary liagnds in biological systems, especially when ligand mixtures are present together with metals[31-34]. However, there can be various favourable and unfavourable interactions among the toxic metals and those different ligands present together in the biological fluids. These interactions can support to identify the bioavailability of these ligands and to discover new mixed ligand systems to be used in chelation therapy with less side effects. As such a situation has not been considered extensively so far, under the physiological conditions, this study was carried out to investigate followings.

Scientific scopes of the project (overall and specific objectives)

To study the interactions between toxic metals; Pb^{2+}, Cd^{2+}, Al^{3+} and Cu^{2+} and

commonly used analgesics; aspirin and paracetamol in the presence of a nucleic base; uracil, / common vitamin supplements; vitamin C, vitamin $B_{1,}$ and vitamin B_3/ dietary ligands; caffeine, citric acid, salicylic acid

dietary ligands in the presence of nucleic base/ vitamin supplements/ analgesics

two dietary ligands/ two analgesics/ two vitamin supplements/

EDTA in the presence of nucleic base/ vitamin supplements/ dietary ligands/ analgesics

to obtain following objectives,

i) to determine the stability constants of each binary and ternary complex available to find out the best chelating agents or mixtures suitable for metal detoxification.

ii) to identify the harmful aspects, bioavailability and therapeutic activity, when taking drugs and natural chelators together by a metal intoxicated person

iii) to compare of the effectiveness of the use of dietary and drug chelators for chelation therapy over EDTA or along with EDTA

Materials and methods

HNO_3 , $NaNO_3$, NaOH, Na_2EDTA , salicylic acid, citric acid, ascorbic acid, uracil, caffeine, metal nitrates were of analytical grade from Aldrich. Acetaminophen, Acetylsalicylic acid, Vitamin B_1 and Vitamin B_3 were all from State Pharmaceutical Manufacturing Corporation and were of analytical grade. NaOH and HNO_3 solutions were standardized with primary standards potassium hydrogen phthalate (KHP) and sodium carbonate (Na_2CO_3) solutions respectively.

pH titrations

To determine the protonation and stability constants of ligands and complexes respectively, following titrand mixtures were titrated with a 0.03 mol dm ^{3} solution of NaOH at physiological conditions. Temperature and ionic strength of each reaction mixture were maintained at 37.0 ± 0.2 ^{0}C (using a thermostat) and at 0.15 M $NaNO_3$ respectively. Further nitrogen gas was purged through the reaction mixture throughout the titration to avoid interference from CO_2 in the atmosphere. The pH measurements were recorded with a temperature controlled digital pH meter (MARTINI, MI151). Three trials were carried out for each titrand mixture to minimize experimental errors and they were averaged as the pH readings of each trail were almost the same. Stock solutions of metals and ligands were prepared freshly for each set of titrations in 1×10^{-2} or 1×10^{-3} mol dm ^{3} concentrations. All titrations were carried out until a permanent turbidity was observed due to the formation of metal hydroxides. Only values up to permanent turbidity were used for calculations of stability constants.

Titrand mixtures used;

a) 10.00 cm^3 HNO_3 (0.03 mol dm ^{3}) + 30.00 cm^3 deionized water.
b) 10.00 cm^3 HNO_3 + 10.00 cm^3 solution of ligand L + 20.00 cm^3 deionized water.
c) 10.00 cm^3 HNO_3 + 10.00 cm^3 solution of ligand X + 20.00 cm^3 deionized water.

d) 10.00 cm^3 HNO_3 + 10.00 cm^3 solution of ligand L + 10.00 cm^3 metal nitrate solution + 10.00 cm^3 deionized water.
e) 10.00 cm^3 HNO_3 + 10.00 cm^3 solution of ligand X + 10.00 cm^3 metal nitrate solution + 10.00 cm^3 deionized water.
f) 10.00 cm^3 HNO_3 + 10.00 cm^3 ligand, L + 10.00 cm^3 ligand, X + 10.00 cm^3 Metal solution.

Calculation of Protonation constants (K_j^H) of ligands

Protonation constants were calculated according the method given by Irving Rossotti[35,36]. Average number of protons attached per ligand molecule (n_H) was calculated with the equation (1). Values for K_j^H were calculated for a particular range of n_H using the equation (2) and they were averaged to get final K_j^H for the considered j value.

$$n_H = Y + \frac{(V_1 - V_2) \times (N + E)}{\{(V_0 + V_1) \times T_L\}} \qquad (1)$$

Y - number of dissociable protons for a given ligand molecule (Table 1)
V_1, V_2 - volumes of NaOH consumed by titrand mixtures a) and b) or c) respectively to reach a particular pH value
N, E - initial concentrations of NaOH and HNO_3 respectively
V_0 - initial total volume of titration mixture (40.00 cm^3)
T_L - total initial concentration of ligand in titration mixture; 2.5 $\times 10^{-3}$ mol dm^3 and 2.5x $\times 10^{-4}$ mol dm^3 for 1.0 $\times 10^{-2}$ mol dm^3 and 1.0 $\times 10^{-3}$ mol dm^3 stock solutions respectively

Table 1: Y values for selected ligands

Ligand	Abbreviation	Y value
Ascorbic acid	AsC	2
Salicylic acid	Sali	1
Paracetamol	Para	1
Aspirin	AsP	1
Citric acid	Cit	4
Uracil	Ura	1
Caffeine	Caf	0
Vitamin B_1	Vit B_1	3
Vitamin B_3	Vit B_3	0
Na_2EDTA	EDTA	2

$$K_j^H = \frac{(n_H - j + 1)}{\{(j - n_H) \times [H]\}} \qquad (2)$$

j - an integer 1,2,3,..........
K_j^H - j^{th} protonation constant
[H] - concentration of proton at a particular pH considered

Calculation of stability constants of metal single ligand complexes

It was assumed that complex formation took place in stepwise as mentioned in reactions (3) and (4) respectively (charges on species were omitted for simplicity). Using Irving and Rossotti method, average number of ligand molecules attached per metal ion ($\bar{n}$) and free ligand exponent (pL) were calculated using the equations (5) and (6)[35,36]. Values of $\bar{n}$ within the range of $0<\bar{n}<1$ and $1<\bar{n}<2$ for the calculation of the first formation constant, K_1^f and the second formation constant, K_2^f were considered respectively. K_1^f and K_2^f were calculated using the equations (7)a and (7)b within the corresponding ranges of $\bar{n}$ and all K_1^f values were averaged to get the first formation constant of the complex ML and all K_2^f values were averaged to get the second formation constants of the complex ML_2.

$$M_{(aq)} + L_{(aq)} \rightleftharpoons ML_{(aq)} \qquad (3)$$

$$ML_{(aq)} + L_{(aq)} \rightleftharpoons ML_{2(aq)} \qquad (4)$$

$$\bar{n} = \frac{(V_3 - V_2) \times (N + E + T_L(Y - n_H))}{(V_0 + V_2) n_H . T_M} \qquad (5)$$

$$pL = \log\left[\frac{\sum_{n=0}^{j} \beta_j . \{anti\log(pH)\}^{-j} . (V_0 + V_3)}{(T_L - \bar{n}.T_M).V_0}\right] \qquad (6)$$

$$K_1^f = \frac{(\bar{n})}{\{(1-\bar{n}) \times [L]\}} \qquad (7)a$$

$$K_2^f = \frac{(\overline{n-1})}{\{(2-\bar{n}) \times [L]\}} \qquad (7)b$$

$[L] = 10^{-pL}$
V_3 – volume of NaOH consumed by mixtures d) or e) to reach the same pH in samples a) and b) or a) and c).
βj – $K_1^H . K_2^H . K_3^H .... K_j^H$; overall protonation constant of the ligand
T_M – total initial concentration of metal ion in the titration mixture

K_1^f – 1st formation constant or the formation constant for the reaction (3)
K_2^f – 2nd formation constant or the formation constant for the reaction (4)

Calculation of stability constants of Metal mixed ligand complexes

In mixed ligand complex formation, one ligand was considered as the primary ligand. The parameter used in selecting primary ligand was values of their formation constants of binary complexes with the same metal ion. One with the highest value was considered as the primary ligand. In mixed systems too, a stepwise complex formation was considered [37-40]. In the reaction mixtures, primary ligand (L) first reacted with free metal ion to form ML type of binary complex similar to its binary system as given in reaction (3) and then to that binary complex ML, second ligand (X) bound to form ternary complex (MLX) as given in the reaction (8).

$$ML_{(aq)} + X_{(aq)} \rightleftharpoons MLX_{(aq)} \qquad (8)$$

Average number of X molecules, bound to the complex ML, $\bar{n}_{mix}$ and free mixed ligand exponent, pL_{mix} were calculated using the equations (9) and (10).

$$\overline{n}_{mix} = \frac{(V_5 - V_{2(X)}) \times [N + E + T_X(Y_X - n_{H(X)})]}{(V_0 + V_{2(X)}) n_{H(X)} . T_{ML}} \qquad (9)$$

$$p^L_{mix} = \log\left[\frac{\sum_{n=0}^{j} \beta_{j(X)} . \{anti\log(pH)\}^{-j} . (V_0 + V_5)}{(T_X - \overline{n}_{mix} . T_{ML}) . V_0}\right] \qquad (10)$$

$V_{2(X)}$ - volume of NaOH consumed by mixture c) to reach the same pH as mixture a)
V_5 - volume of NaOH consumed by mixture f) to reach the same pH as mixtures a) and c)
$n_{H(X)}$ - average number of protons bound per molecule X at the same pH.
Y_X - number of dissociable protons per ligand X
$\beta_{j(X)}$ - j th overall protonation constant of the ligand X

T_{ML} - total initial concentration of the complex ML (this is assumed to be equal to the concentration of metal ion)
T_X - total initial concentration of ligand X

Using the values of $\bar{n}_{mix}$ within the range $0 < \bar{n}_{mix} < 1$, mixed formation constant (K_{mix}^f) was calculated by means of the equation (11). Results obtained were averaged to get the final value of the mixed complex formation constant for a particular complex.

$$K^f_{mix} = \frac{\overline{n}_{mix}}{\{(1 - \overline{n}_{mix}) \times [L_{mix}]\}} \qquad (11)$$

$$[L_{mix}] = 10^{-pL_{mix}}$$

Composite curve

Mixed complex formation was further confirmed theoretically by comparing mixed system titration curve with corresponding composite curve. Composite curve was drawn by the graphical addition of the titration curve of (M + L) system to the titration curve of the free ligand X [41]. This was simply obtained by the term (V_3+$V_{2(X)}$-V_1). Two curves were compared by plotting two variables, V_5 and (V_3+$V_{2(X)}$-V_1) against the corresponding pH. If there was a significant deviation of the composite curve from the titration curve, it gave the evidence theoretically that there was a mixed complex formation in the considered system.

Comparison of the stabilities of single and mixed ligand complexes

According to Sigel[42], stabilities of binary and ternary complexes can be compared quantitatively in different ways when the complexation reaction occurs in steps. The most suitable way of comparison is in terms of Δlog K[43-46] which can be calculated by considering the logarithmic values of stability constants of binary and ternary complexes. Both positive and negative values can be obtained for Δlog K depending on several factors such as the geometry of the complex, availability of coordination sites etc. In general, negative values can be resulted due to less coordinating sites available and low flexibility of ligand molecules. When consider the geometry of the complex, for the square planar complexes the statistical value of Δlog K is -0.6. For the regular and distorted octahedral geometries it is -0.4 and -0.9 respectively. However, ternary complexes are considered to be significantly more stable than corresponding binary complexes when the value of Δlog K is positive [42-44]

For the stepwise reactions of mixed complex formation, Δlog K can be expressed as given in equation 12.

$$\Delta \log K = \log K_{MLX}^{ML} - \log K_{MX}^{M} \qquad (12)$$

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

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

For all the ligand systems forming mixed ligand complexes with four considered metals, Δlog K values were computed using the equation 12 and stability of mixed octahedral complexes were considered.

Determination of complex formation by UV-Visible spectroscopy

UV Visible spectroscopic data were recorded on a UV-1800 SHIMADZU spectrophotometer.

Complex formation was confirmed spectrophotometrically as well considering the UV absorption peaks of ligand molecules. λ_{max} values in nm for ligands: ascorbic acid, salicylic acid, paracetamol, caffeine, acetylsalicylic acid, vitamin B_1, vitamin B_3, and uracil are 271, 297, 243, 273, 224, 267, 214 & 262 and 259 respectively[47]. Shifting of λ_{max} values of each ligand with pH from 2-12 were studied using aqueous solutions of ligands by adjusting the pH of solutions with a NaOH solution.

Determination of M : L ratio in single ligand complex systems

M : L ratios of binary complexes formed were spectroscopically determined using the method given by Kolayli *et al*[48]. Accordingly, percentage chelation or the chelating activity of the ligand was calculated with,

$$\% \, Chelating\ activity = \frac{(A_0 - A)}{A_0} \times 100$$

A_0 – Absorbance of the ligand in the absence of metal in solution
A – Absorbance of the ligand in the presence of the metal in solution

In order to find the ratio between metal and ligand, a series of reaction mixtures was prepared as given in Table 2. Both the ligand and metal nitrate solutions used were in the concentrations of 0.01 mol dm^3. Ionic strength of the solution was adjusted to 0.15 mol dm^3 by adding required mass of $NaNO_3$ and to each experimental mixture 2.50 cm^3 of 0.03 mol dm^3 HNO_3 was added to have UV experimental mixtures similar to the titration mixtures. Before running the spectrum, pH of each sample was adjusted to a value very closer, but little lower to the pH of permanent turbidity using NaOH or a buffer solution.

Table 2: Complex mixtures used for UV experiments

Sample No	**Vol. of ligand/cm^3**	**Vol. of metal ion/cm^3**	**Vol. of water added to keep constant volume/cm^3**
I	1.00	2.00	4.50
II	2.00	2.00	3.50
III	3.00	2.00	2.50
IV	4.00	2.00	1.50
V	5.00	2.00	0.50
VI	6.00	2.00	0.00

UV absorption data obtained using samples I to VI were taken as A, and solutions prepared similar to Table 2 without adding metal solutions were used to get values of A_0.

Investigation of mixed ligand complex formation

Mixtures identical to the titrand mixtures used in pH titrations were prepared to investigate further the mixed ligand complex formation using UV visible spectroscopic method. UV spectra of these mixtures were recorded at several pH values until a precipitate was observed. Shifts in absorbance maximum, λ_{max} and changes in the shape of the ligand peak (whether a broader peak or a narrower peak), were closely examined and mixed ligand system spectra were compared with those of binary complex systems at corresponding pH values [47,49,50]. Absorbance spectra of each mixed ligand system and two corresponding binary systems were recorded at three similar pH values before the precipitation was observed. In each solution M: L, M: X and M: L: X ratios were 1:1, 1:1 and 1:1:1 respectively with ionic strength of 0.15 mol dm^{-3}.

Results

Protonation constants of ligands

Protonation constants of the dissociable protons calculated using pH titration data are given in Table 3. As the titrations carried out in the pH range of 2-12, only the protons dissociating within that range were taken into account. Hence, Y values of ligands were different from their total number of dissociable protons (Table 1).

Table 3: Protonation constant values obtained for ligands together with literature values

ligand	**Log $^{H}K_1$**	**Log $^{H}K_2$**	**Log $^{H}K_3$**	**Log $^{H}K_4$**
Salicylic acid	2.76 2.79[16]	-	-	-
Ascorbic acid	10.00 11.57[51]	5.28 4.17[51]	-	-
Paracetamol	9.24 9.50[52]	-	-	-
Citric acid	- -	5.81 5.40[53]	4.03 4.74[53]	2.59 3.08[53]
Uracil	8.80 9.34[53]	-	-	-
Na_2EDTA	9.19 10.30[54]	5.69 6.18[54]	-	-
Aspirin	2.78 3.46[16]	-	-	-
Vitamin B_1	9.13 13.25[55]	8.84 10.69[55]	4.32 5.02[55]	- -
*Caffeine	-	-	-	-
*Vitamin B_3	-	-	-	-

*Caffeine and Vitamin B_3 do not have removable protons.

As some of the reported values given in the above table were taken at different temperatures and ionic strengths, there was a significant difference between the two values.

UV spectroscopic data for protonation

Molecules consisting of conjugated double bond systems, conjugated systems with carbonyl groups or aromatic rings only showed the absorption peaks in the UV region (λ_{max}>200 nm)[56]. Except Na_2EDTA and citric acid, other eight ligands considered have the systems capable of absorbing energies from the UV region. Ascorbic acid, salicylic acid, aspirin, vitamin B_1 and uracil carrying considerably high acidic or basic groups showed significant shifts in their absorption peaks at the points of ionization. A red shift (bathochromic shift) was observed when conjugation increased[47]. On the removal of protons, a red shift appeared as conjugation became stronger with negative charge[47],[56]. Spectral changes of each ligand with pH are given in Figures 1(a) to 1(h)

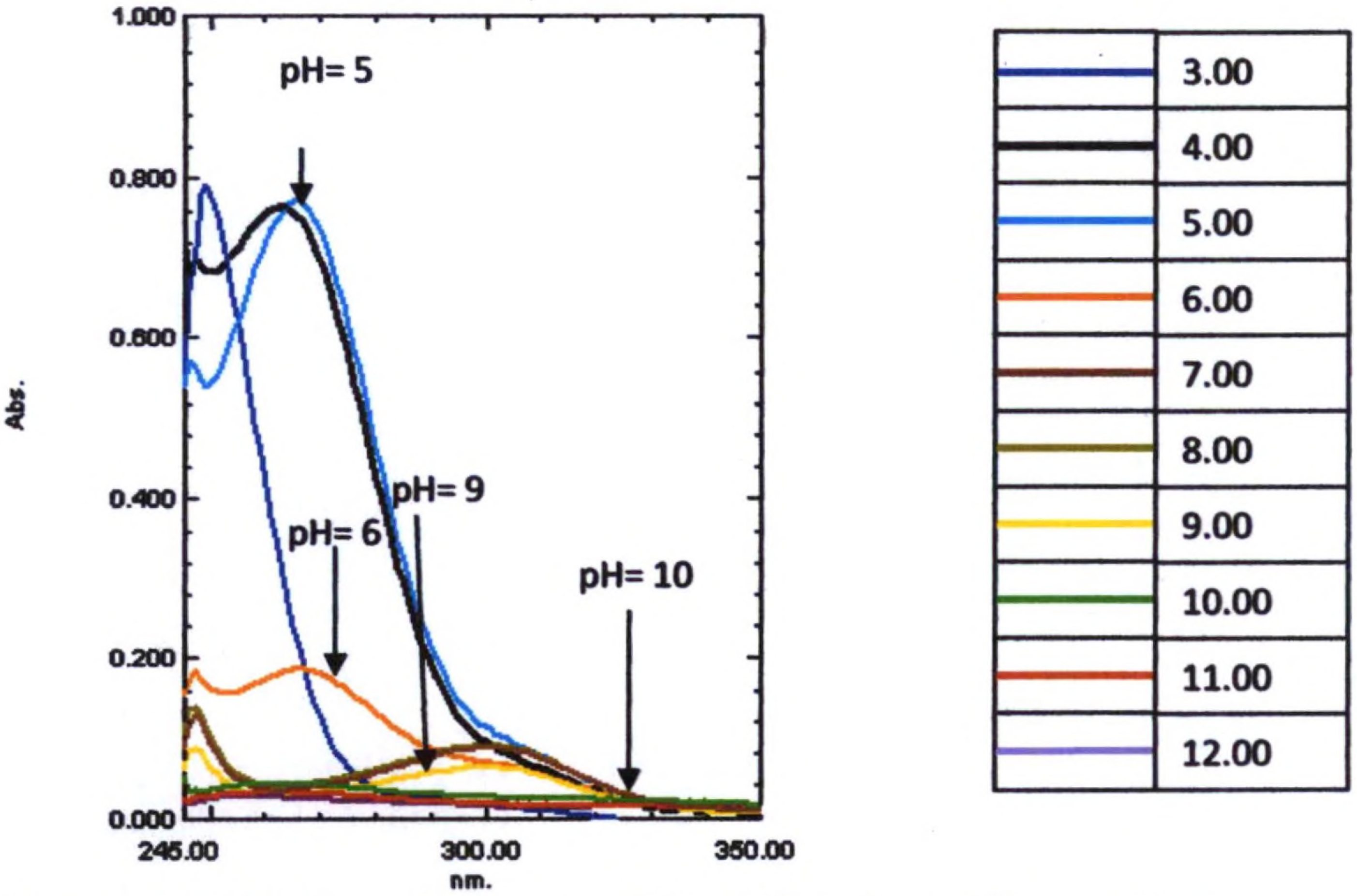


Figure 1 (a): UV absorption spectra of Ascorbic acid at different pH values.

According to the Figure 1(a), significant changes in the values of both λ_{max} and absorbance appeared in pH ranges; 5 to 6 and 9 to 10. Therefore, within these ranges a change in the electron conjugation must have taken place. According to the protonation constants calculated, observed conjugation increment can correspond to the dissociation of protons. Thus proton dissociation can be clearly observed with UV spectra. However, ring structure of ascorbic acid was not stable when the pH reached the basic range. At high basic pHs, ring is broken. Thus, the probability of $n \rightarrow \pi^*$ transitions become lower and therefore, towards basic region absorbance get decreased.

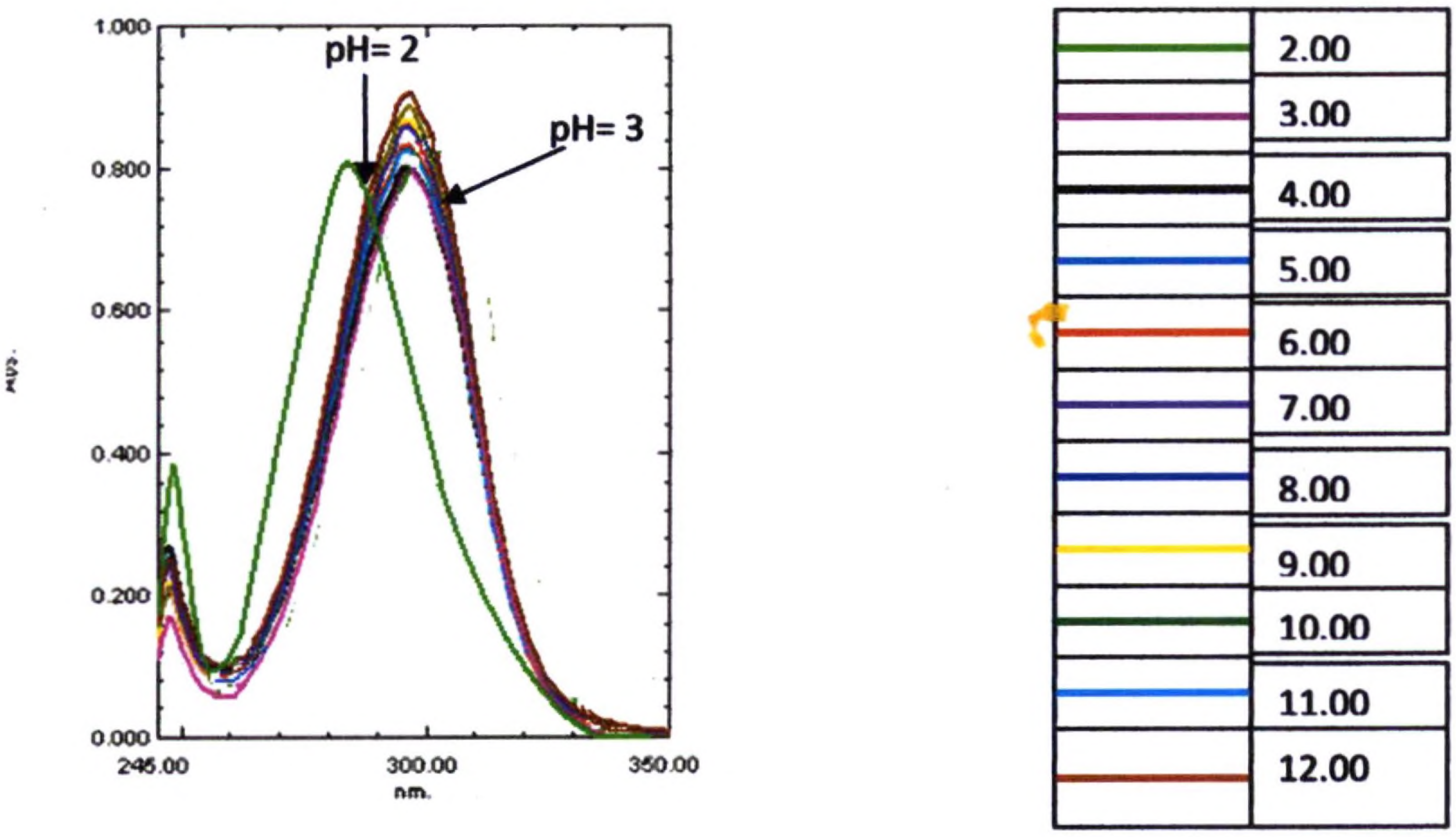


Figure 1(b): UV absorption spectra of Salicylic acid at different pH values.

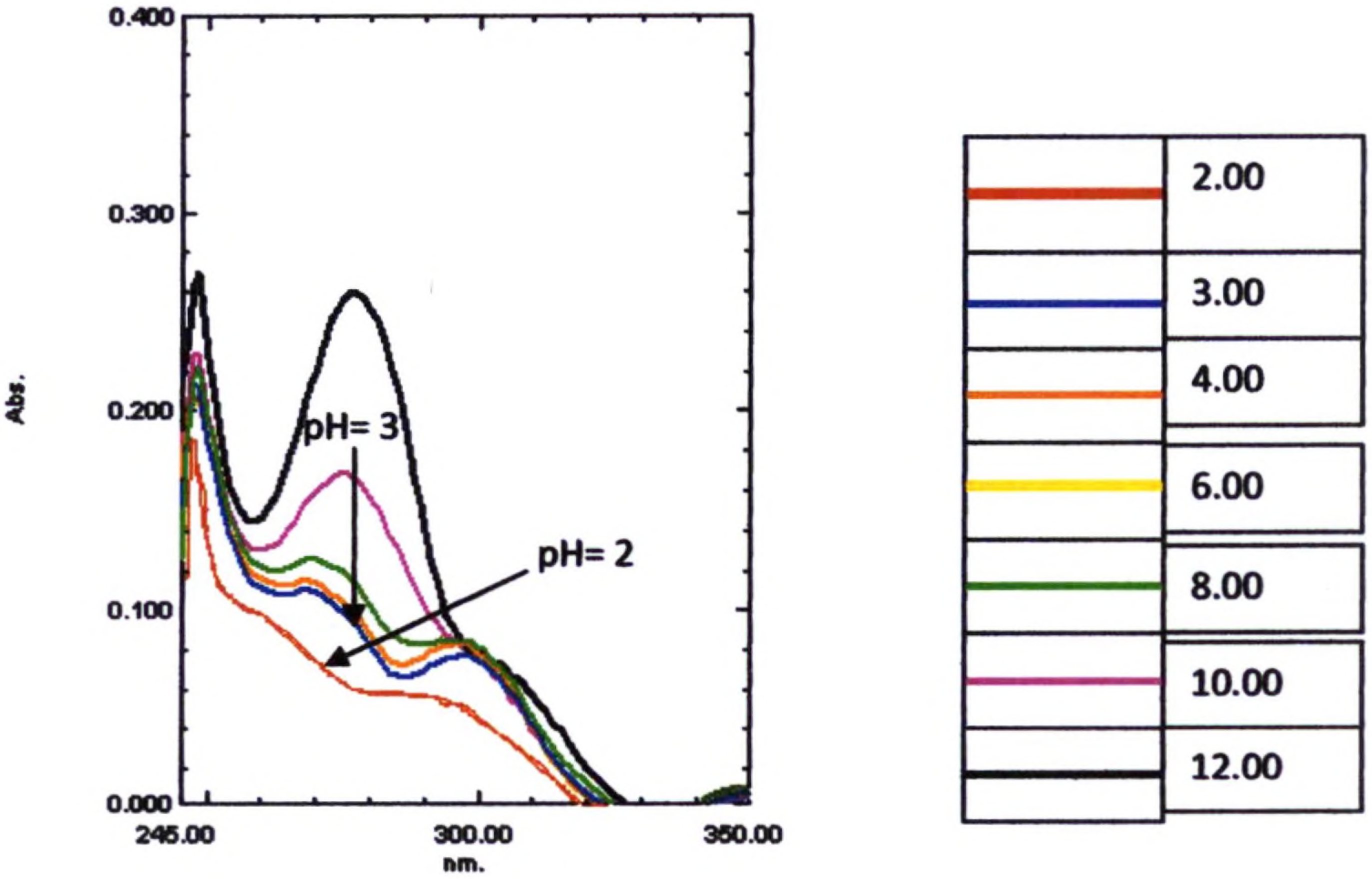


Figure 1(c): UV absorption spectra of Aspirin at different pH values.

In the UV spectra of salicylic acid, a significant change was observed within the pH range; 2 to 3. According to the pH titration data, this corresponds to the dissociation of carboxylic proton having log k value 2.76. However, according to the literature data salicylic acid has two acid dissociation constants (protonation constants); 2.79 and 13.00[16]. But, we could see only one as we studied only up to pH 12.00.

Not like in ascorbic spectra, in salicylic spectra both λ_{max} and absorbance values have increased with pH., as benzene ring is more stable than the five membered ring in ascorbic.

According to the Figure 1(c), aspirin showed a significant change in electron conjugation, when moving from pH 2 to 3. This change correlated with deprotonation of carboxylic group of aspirin similar to salicylic within that pH range. Therefore, both have closer protonation values. After pH 3, electro conjugation in aspirin is stronger than that of salicylic acid. Hence, the spectra of two molecules are different from each other. Aspirin has two recognizable peaks after pH 3.

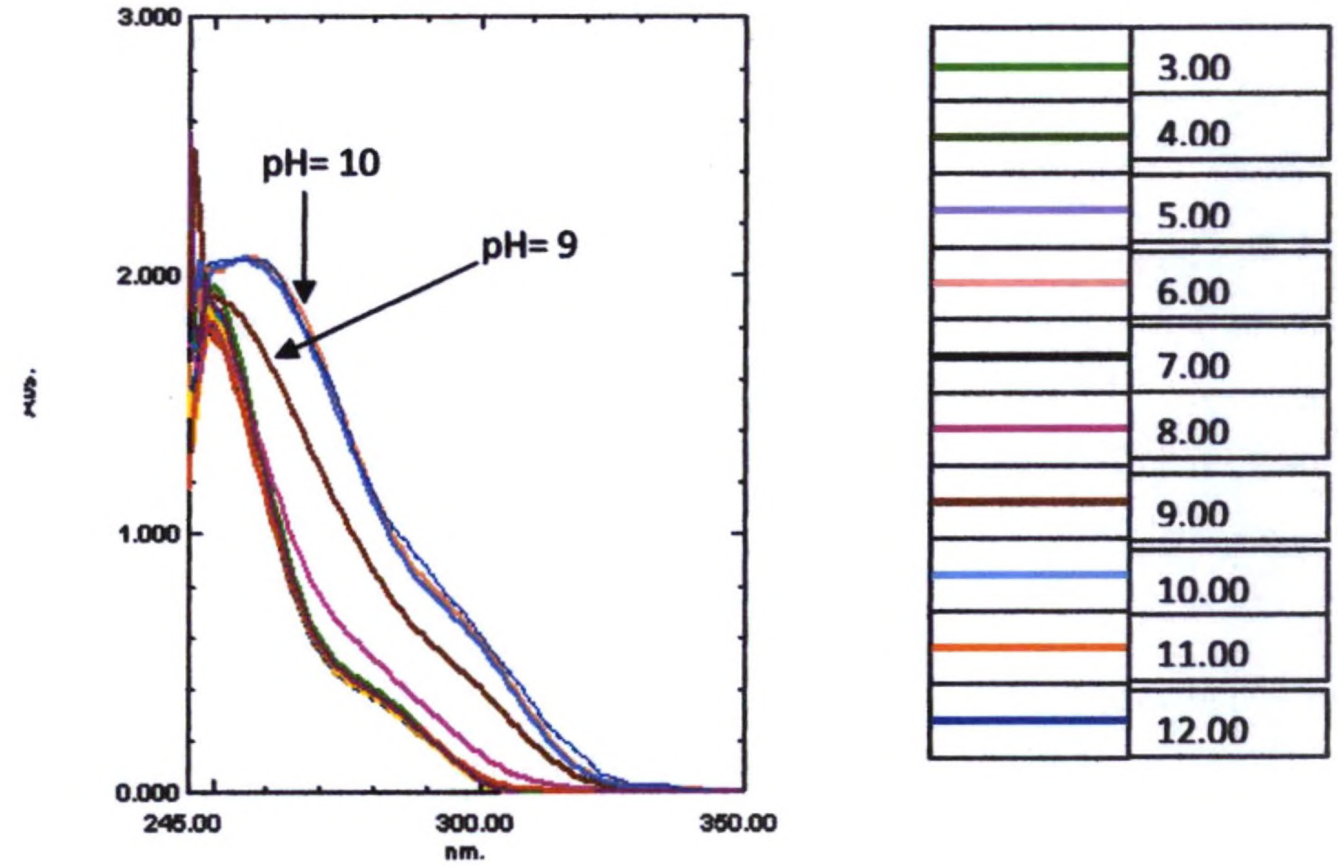


Figure 1(d): UV absorption spectra of Paracetamol at different pH values.

Figure(d) illustrates the UV absorption spectra of paracetamol within the pH range from 3 to 12. In that figure, a significant red shift was observed between pH 9 and 10. Therefore, change in electron conjugation should be significant in that range. According to the titration data, protonation/deprotonation constant of paracetamol is 9.24 and observed significant change of λ_{max} must be due to deprotonation.

In the UV spectra of vitamin B_1 (Fig 1(e)) two significant shifts were present at pH ranges from 4 to 5 and from 9 to 10. However, absorption peak became sharper in the basic pH range. A similar, behaviour has been reported in the literature also[51]. These two significant points corresponded with protonation constant values of vitamin B_1.

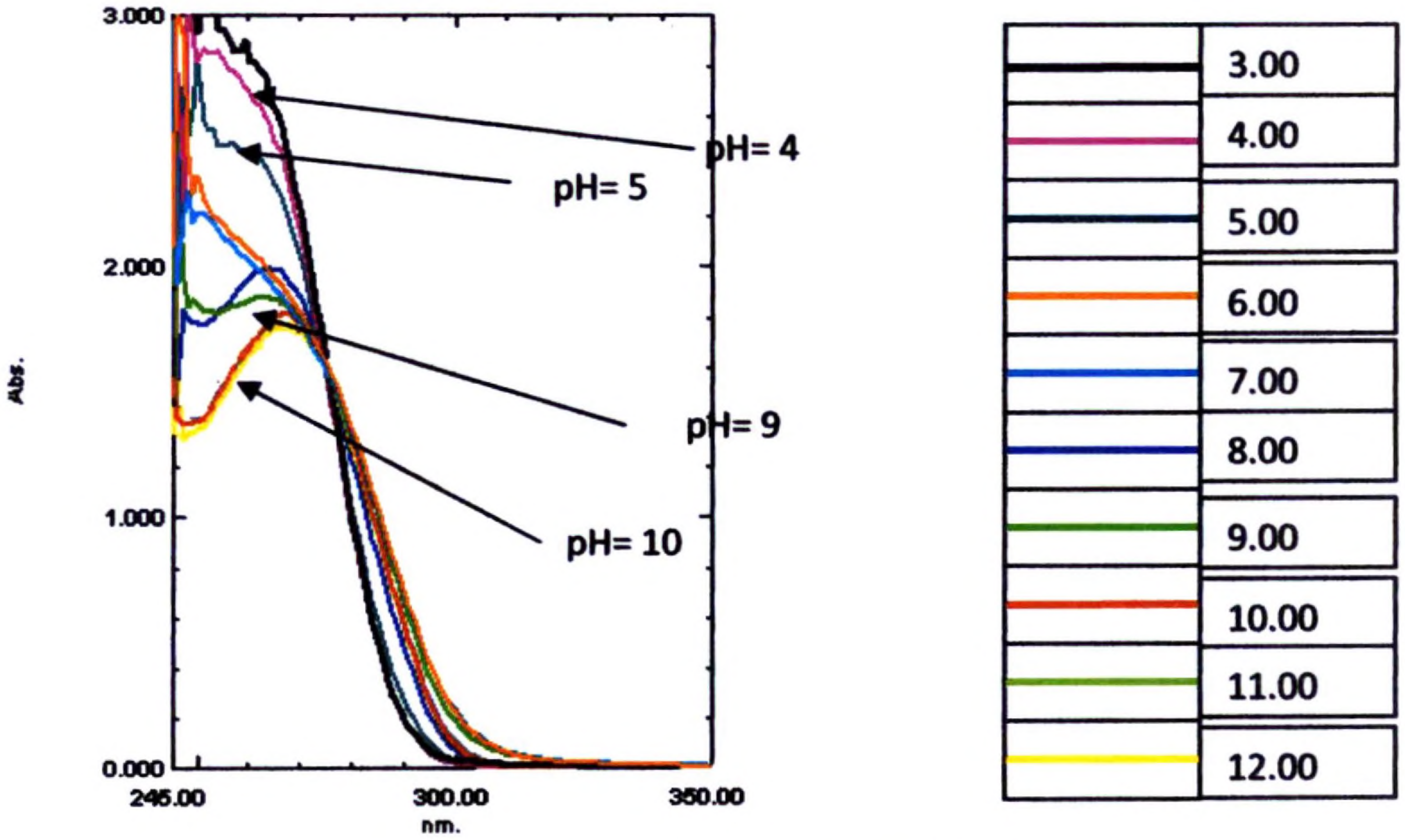


Figure 1(e): UV absorption spectra of Vitamin B_1 at different pH values.

In the UV spectra of uracil ranging from pH 2 to 11(Fig 1(f)), a significant change showed between pH 8 and 9. After pH 8, sharpness of the peak decreased and instead a broader peak appeared. When the medium pH increased to 10, it became much broader and shifted. Hence, within this range, significant change in the structure increasing electron conjugation has happened. When spectra of caffeine and vitamin B_3 are considered, any significant change in λ_{max} cannot be seen when the pH of the system increased(Figure 1g and 1h respectively). This suggests the absence of any structural changes which make significant changes in electron conjugation. Hence, spectroscopic evidence too confirmed that both do not have any removable protons attached to their structures.

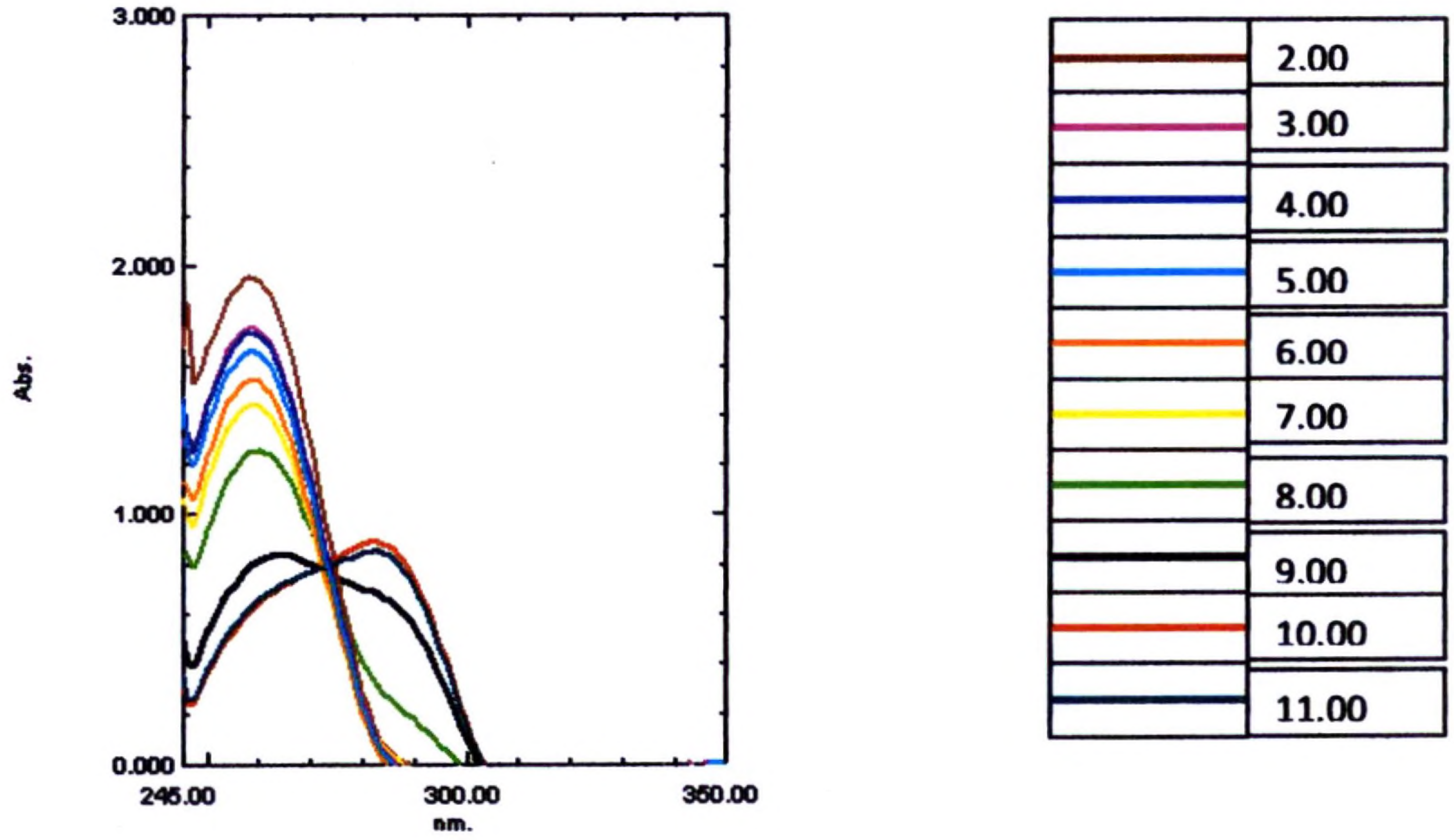


Figure 1(f): UV absorption spectra of Uracil at different pH values.

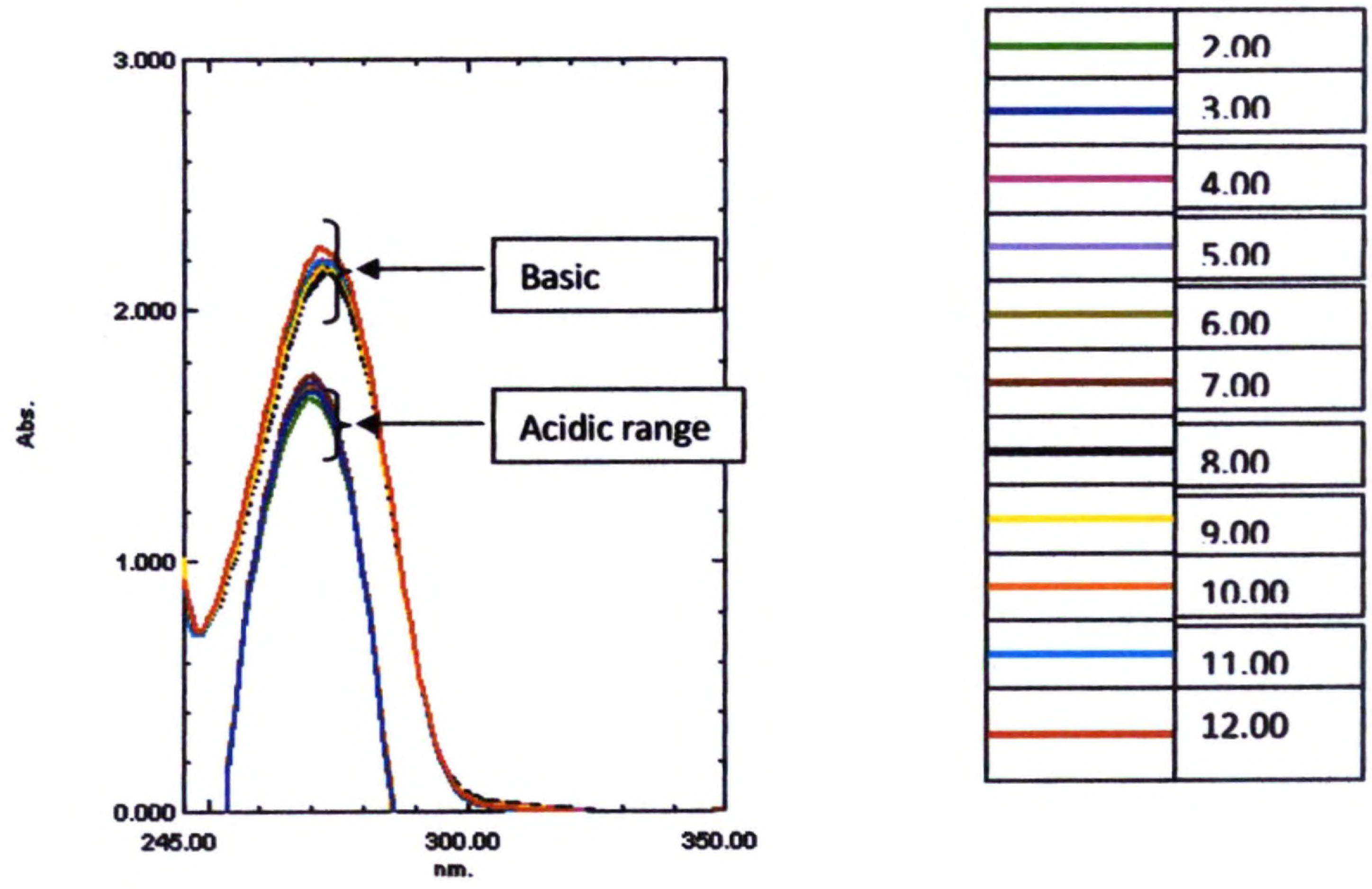


Figure 1(g): UV absorption spectra of Caffeine at different pH values.

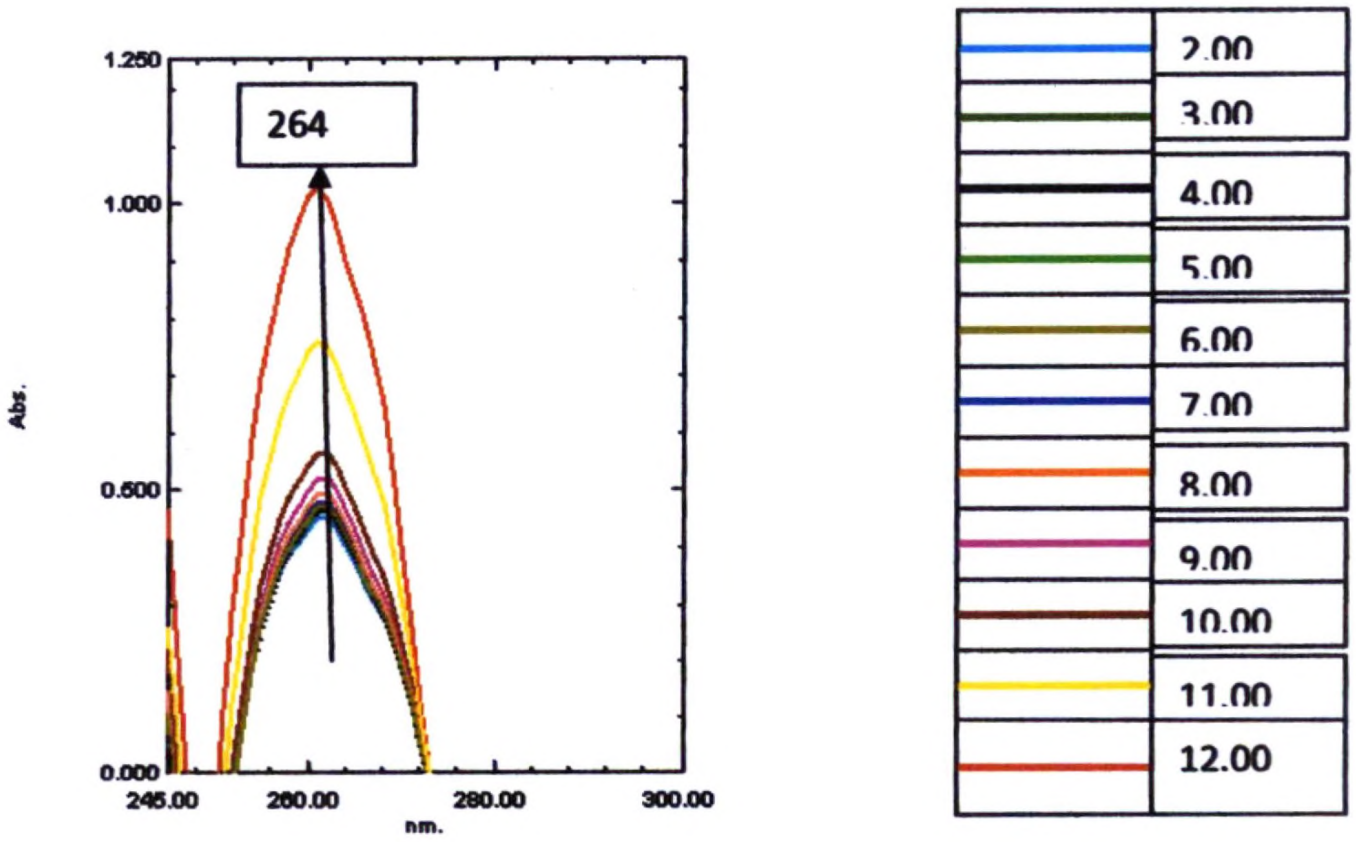


Figure 1(h): UV absorption spectra of Vitamin B_3 at different pH values.

Complexes of lead

Single ligand complexes-pH titration data

Stability constants of lead binary complex systems studied at the concentration of 0.01 mol dm^3 taking 1:1 and 1:2 M: L ratio mixtures are listed in Tables 4 and 5 respectively together with pH values, at which permanent turbidity appeared.

Table 4: Stability constants for lead binary complex systems (1:1 ratio) together with the pH at which permanent turbidity appeared

Binary system with lead	pH of permanent turbidity	Log K_1^f
AsC	6.60	3.53
Cit	5.67	2.61
AsP	6.01	3.00
Caf	5.00	1.85
Ura	5.72	2.64
Sali	5.80	2.67
Vit B_1	6.80	3.83
Para	5.52	2.25
Vit B_3	5.64	2.29
Na_2 EDTA	No turbidity	13.53

Table 5: Stability constants for lead binary complex systems (1:2 ratio) together with the pH at which permanent turbidity appeared.

Binary system with lead	pH of permanent turbidity	Log K_1^f	Log K_2^f
Asc	7.12	4.12	3.14
Cit	6.39	5.56	3.44
AsP	6.45	3.06	-
Caf	6.29	1.94	-
Ura	6.43	2.74	-
Sali	6.82	2.69	-
Vit B_1	7.54	3.55	-
Para	6.32	2.67	-
Vit B_3	6.25	2.30	-
Na_2 EDTA	No turbidity	12.85	-

Results given in Tables 4 and 5 reveal that, except ascorbic and citric acids, all the other ligands form ML type of binary complexes with lead in both ratios. Ascorbic formed an ML_2 type of binary complex in M:L 1:2 ratio, in which the pH of the mixture reached the slightly basic region. Literature also reported that at basic pHs, ascorbic acid tends to form 1:2 complexes with some metals[16]. Table 6 reveals the formation constants for the lead binary systems at the concentration of 0.001 mol dm^3.

Table 6: Stability constants for lead binary complex systems (1:1 ratio) together with the pH at which permanent turbidity appeared at the concentration of 0.001 mol dm^3

Binary system with lead	pH of permanent turbidity	Log β_1	Log β_2
Asc	8.00	6.31	4.14
Sali	6.45	4.86	-
Para	6.30	4.39	-
Cit	7.25	5.40	-
Ura	6.65	5.12	-
Vit B_1	8.38	6.38	-
Vit B_3	5.40	-	-
Asp	7.50	4.85	-
Caf	4.42	-	-
Na_2EDTA	No turbidity	14.64	-

UV experimental results

Percentage chelation calculated based on UV data for each binary system is given in the Table 7.

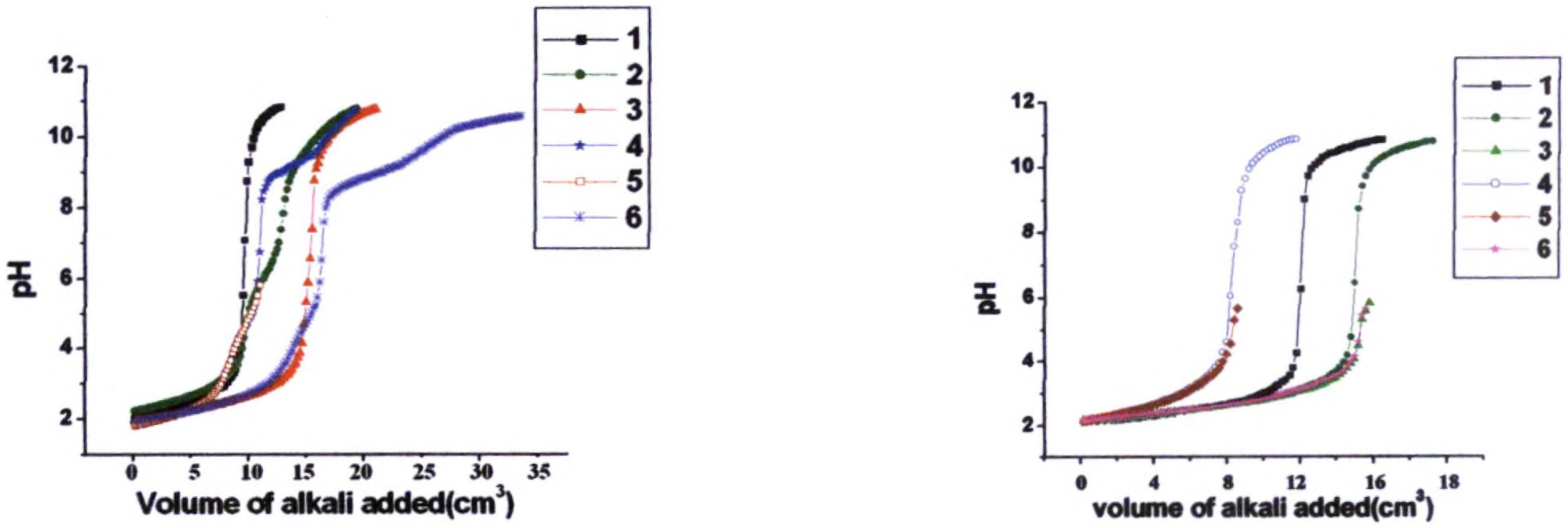
Table 7: Percentage chelation with lead at different pH values

Binary system	**pH**	**% chelation at different M:L ratios**					
		1:0.5	**1:1**	**1:1.5**	**1:2**	**1:2.5**	**1:3**
AsC-Pb	6.50	73.72	81.63	81.90	81.68	81.74	81.75
Sali-Pb	5.70	65.17	77.82	77.48	77.75	77.18	77.28
Para-Pb	5.40	31.11	45.67	45.55	45.68	45.67	45.74
Caf-Pb	4.90	15.06	18.22	18.21	18.16	-	-
Ura-Pb	5.60	57.51	76.87	76.90	76.79	76.78	-
Vit B_1-Pb	6.70	68.50	83.24	83.72	83.82	83.68	-
Vit B_3-Pb	5.50	48.96	60.97	61.00	60.95	61.09	-
Aspirin-Pb	5.90	60.36	79.59	79.48	79.30	79.20	-
AsC-Pb	8.00	72.08	76.64	79.10	82.00	82.10	82.21

According to this table, it is clear that results obtained from potentiometric titrations were further confirmed by UV experiments. Except ascorbic acid, all the other ligands formed ML type of metal complexes in both acidic and basic media. In the presence of excess ligand in the solution, ascorbic acid formed a very stable ML_2 type complex more stable in basic pH values.

Titration data for lead mixed ligand systems

pH titrations were carried out for each ligand pair in the presence of $Pb(NO)_3$ in order to study the mixed complex formations (0.01 mol dm^3). Sample plots for two systems, in which one shows curves for a system forming a mixed ligand complex and the other shows curves for a system that did not form a mixed ligand complex are given in Figures **2a** and **2b** respectively.



(a) **(b)**

Figure 2a: Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of Pb^{2+} with Na_2EDTA and Vitamin B_1 **1.** HNO_3; **2.** HNO_3+Na_2EDTA; **3.** HNO_3+Na_2EDTA+ $Pb(NO_3)_2$; **4.** HNO_3 + Vitamin B_1; **5.** HNO_3 + Vitamin B_1 + $Pb(NO_3)_2$; **6.** HNO_3+Na_2EDTA+Vitamin B_1+ $Pb(NO_3)_2$

Figure 2b: Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of Salicylic acid and Vitamin B_3 **1.** HNO_3 **2.** HNO_3 + Salicylic acid **3.** HNO_3 + Salicylic acid + $Pb(NO_3)_2$ **4.** HNO_3 + Vitamin B_3 **5.** HNO_3 + Vitamin B_3 + $Pb(NO_3)_2$ **6.** HNO_3 + Salicylic acid + Vitamin B_3 + $Pb(NO_3)_2$

Titration curve of mixed ligand system given in Figure 2a is totally different from the titration curves of the two binary systems implying the formation of a new species in the mixed system, whereas that change was not observed in Figure 2b. In the latter plot, titration curves of the ternary system and binary system of primary ligand are more or less similar to each other implying that in mixed system too binary complex of primary ligand formed instead of a mixed complex.

Results of the ternary complex systems studied are given in the Table 8. According to the results, only 17 ligand combinations out of 45 possible pairs formed mixed complexes with Pb^{2+}.

Table 8: Stability constants for lead ternary complex systems with the pH at which permanent turbidity appeared (0.01 mol dm^3)

L(primary ligand)	X(secondary ligand)	pH at permanent turbidity	log β_1	log β_2
EDTA	B_1	No turbidity	13.53	26.47
EDTA	AsC	No turbidity	13.53	25.42
B_1	AsC	7.12	3.83	7.39
EDTA	AsP	No turbidity	13.53	-
B_1	AsP	6.67	3.83	-
AsC	AsP	5.89	3.53	-
EDTA	Sali	No turbidity	13.53	-
B_1	Sali	7.74	3.83	7.08
AsC	Sali	7.04	3.53	-
AsP	Sali	6.32	3.00	-
EDTA	Ura	No turbidity	13.53	-
B_1	Ura	7.60	3.83	7.14
AsC	Ura	5.45	3.53	-
AsP	Ura	7.38	3.00	7.13
Sali	Ura	No turbidity	2.67	11.41
EDTA	Cit	No turbidity	13.53	20.06
B_1	Cit	9.03	3.83	9.22
AsC	Cit	4.14	3.53	-
AsP	Cit	4.02	3.00	-
Sali	Cit	No turbidity	2.67	9.09
Ura	Cit	5.58	2.64	-
EDTA	B_3	No turbidity	13.53	-
B_1	B_3	7.56	3.83	6.90
AsC	B_3	5.81	3.53	-
AsP	B_3	6.74	3.00	-
Sali	B_3	6.13	2.67	-
Ura	B_3	5.74	2.64	-
Cit	B_3	5.74	2.61	-
EDTA	Para	No turbidity	13.53	-
B_1	Para	5.36	3.83	-
AsC	Para	7.48	3.53	7.23
AsP	Para	7.71	3.00	7.83
Sali	Para	8.92	2.67	6.12
Ura	Para	No turbidity	2.64	8.08

Cit	Para	5.66	2.61	-
B_3	Para	8.59	2.29	5.79
EDTA	Caf	No turbidity	13.53	-
B_1	Caf	5.09	3.83	-
AsC	Caf	5.83	3.53	-
AsP	Caf	4.77	3.00	-
Sali	Caf	5.38	2.67	-
Ura	Caf	No turbidity	2.64	5.87
Cit	Caf	5.97	2.61	-
B_3	Caf	5.30	2.29	-
Para	Caf	5.05	2.25	-

Table 9: Stability constants for lead ternary complex systems with the pH at which permanent turbidity appeared (0.001 mol dm^3)

L	X	pH at permanent turbidity	log β_1	log β_2
EDTA	B_1	No turbidity	14.64	24.90
EDTA	AsC	No turbidity	14.64	22.84
B_1	AsC	8.72	6.38	-
EDTA	Cit	No turbidity	14.64	18.36
B_1	Cit	No turbidity	6.38	11.89
AsC	Cit	9.37	6.31	12.38
EDTA	Ura	No turbidity	14.64	-
B_1	Ura	7.83	6.38	-
AsC	Ura	9.36	6.31	14.44
Cit	Ura	No turbidity	5.40	-
EDTA	Sali	No turbidity	14.64	-
B_1	Sali	8.91	6.38	-
AsC	Sali	8.57	6.31	-
Cit	Sali	No turbidity	5.40	-
Ura	Sali	7.31	5.12	-
EDTA	AsP	No turbidity	14.64	-
B_1	AsP	6.70	6.38	-
AsC	AsP	9.01	6.31	11.89
Cit	AsP	No turbidity	5.40	-
Ura	AsP	6.75	5.12	-
Sali	AsP	6.35	4.86	-
EDTA	Para	No turbidity	14.64	-
B_1	Para	6.33	6.38	-
AsC	Para	9.11	6.31	11.63
Cit	Para	No turbidity	5.40	-
Ura	Para	7.50	5.12	-
Sali	Para	9.21	4.86	9.42
AsP	Para	6.30	4.85	-
EDTA	B_3	No turbidity	14.64	-
B_1	B_3	8.40	6.38	-
AsC	B_3	8.17	6.31	-
Cit	B_3	No turbidity	5.40	-
Ura	B_3	6.94	5.12	-
Sali	B_3	6.52	4.86	-
AsP	B_3	5.38	4.85	-
Para	B_3	6.25	4.39	-

EDTA	Caf	No turbidity	14.64	-
B_1	Caf	6.40	6.38	-
AsC	Caf	6.60	6.31	-
Cit	Caf	No turbidity	5.40	-
Ura	Caf	6.87	5.12	-
Sali	Caf	6.50	4.86	-
AsP	Caf	5.41	4.85	-
Para	Caf	6.30	4.39	-
B_3	Caf	5.97	-	-

Table 9 gives the formation constants of lead ternary complexes investigated at the concentration of 0.001 mol dm^3.

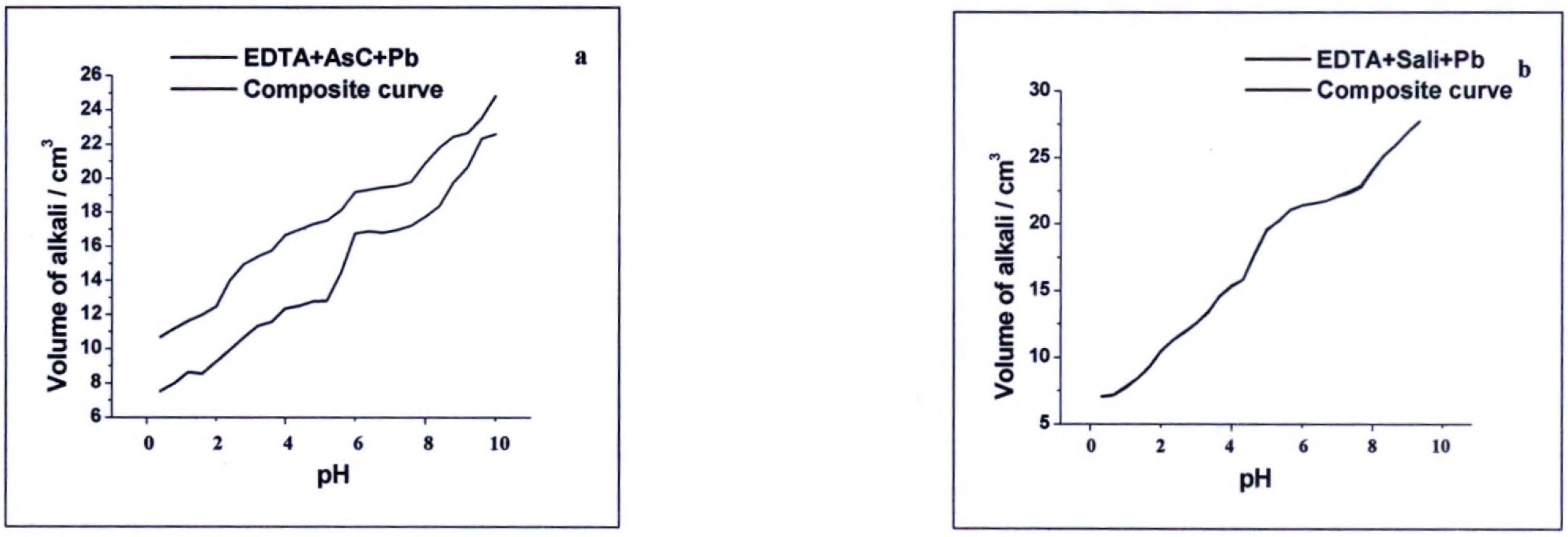


Figure 3: Composite curve and titration curve of a ternary system in which **a)** formed **b)** did not form a mixed complex.

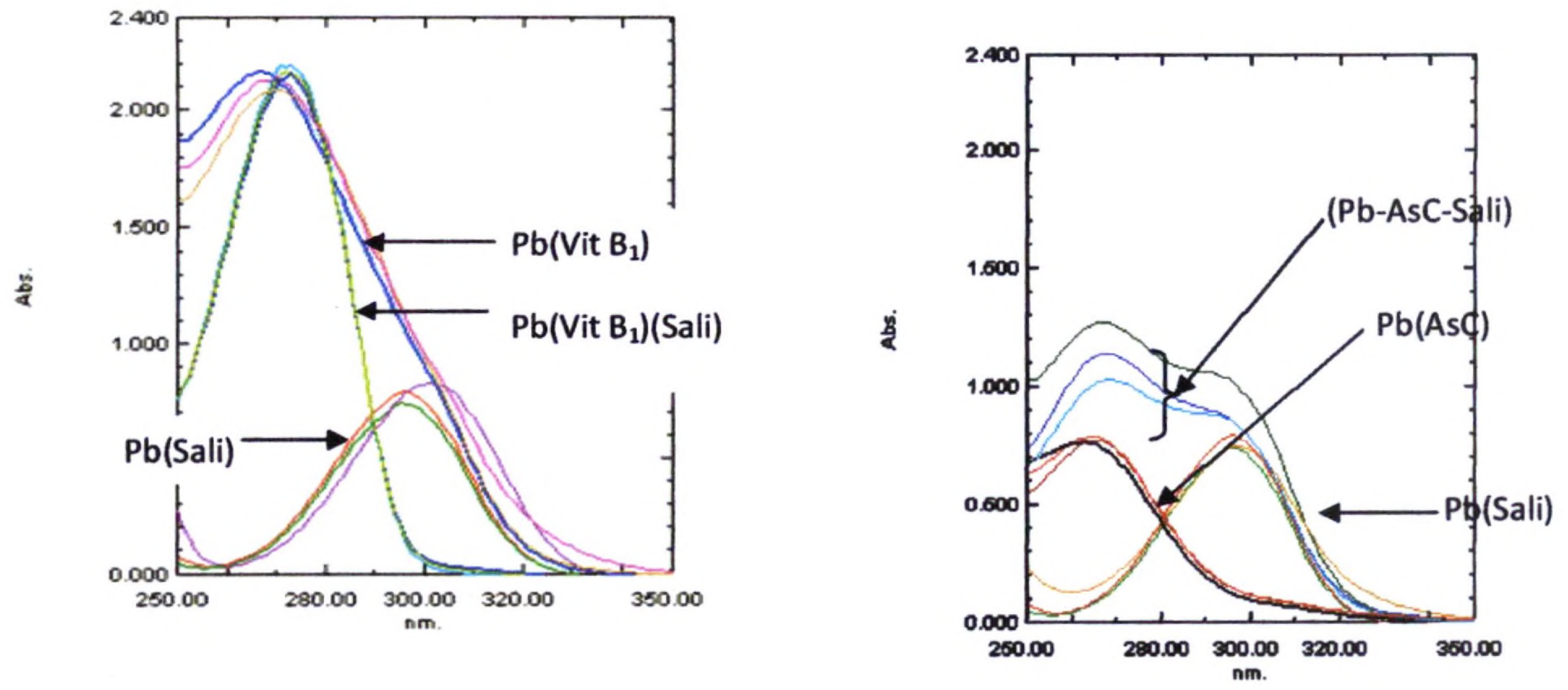
Further evidence for the formation of ternary complexes was obtained by plotting composites curves. All the systems that formed ternary complexes have clearly separated composite curves from their respective mixed ligand titration curves. Sample plots for two systems in which one shows curves for a system forming a mixed ligand complex and the other shows curves for a system not forming a mixed ligand complex are given in Figures 3**a** and 3**b** respectively.

UV experimental data

Ternary complex formation was confirmed with UV experimental data of complex systems as given in reported papers[49]. Spectra for two representative systems, in which one forming and the other not forming mixed complexes, are given in Figure 4**a** and 4**b** respectively.

According to the Figure 4a, mixed complex system has a λ_{max} value between λ_{max} values of two binary systems. This indicates that mixed system contained a species different from either of binary complexes. Thus, the new species must be the mixed complex of two ligands with lead. Rest of the systems which formed mixed complexes was similarly studied.

In figure 4b, a new peak did not appear; instead a broader peak area with two maximum regions appeared. This gave a clue for a system consisting of two different binary complexes. Thus, such a system cannot be expected to form any mixed complex under the experimental constitutions.



(a) (b)

Figure 4(**a**) UV absorption spectra for complexes; Pb(Vit B_1), Pb(Sali) and Pb(Vit B_1)(Sali) at three different pH values : (**b**) UV absorption spectra for complexes; Pb(AsC), Pb(Sali) and ternary system Pb-AsC-Sali at three different pH values

Comparative analysis of the Stabilities of single and mixed ligand complexes (Δlog K)

Δlog K values obtained for ternary complex systems formed are given in Table 10.

Table 10: Calculated Δlog K values for lead ternary complex systems

Ternary complex	**Δlog K**
EDTA-Pb-AsC	8.36
EDTA-Pb-Vi B_1	9.11
EDTA-Pb-Cit	3.92
Vit B_1-Pb-AsC	0.03
Vit B_1-Pb-Sali	0.58
Vit B_1-Pb-Ura	0.67
Vit B_1-Pb-Cit	2.78
Vit B_1-Pb-Vit B_3	0.78
AsC-Pb-Para	1.45
AsP-Pb-Ura	1.49
AsP-Pb-Para	2.58
Sali-Pb-Ura	6.10
Sali-Pb-Cit	3.81
Sali-Pb-Para	1.20
Ura-Pb-Para	3.19
Ura-Pb-Caf	1.38
Vit B_3-Pb-Para	1.25

Many of monomeric lead complexes reported so far are either regular or distorted octahedral in geometry[57,58] even though, few examples for other geometries are available in polymeric compounds[58]. Thus, limiting value for Δlog K was considered as -0.9. According to the results given in the Table 10, all ternary systems formed were more stable than their corresponding binary complexes. Hence, the equilibrium reaction given in equation 13 is more towards its right side resulting more percentage of ternary complexes in the solution.

(13)

Complexes of cadmium

Titration data for cadmium single ligand systems

Stability constants of binary complexes of cadmium studied at the concentration of 0.01 mol dm^{-3} with metal to ligand ratios, 1:1 and 1:2. obtained are listed in Tables 11 and 12 respectively and results for 1:1 binary at the concentration of 0.001 mol dm^{-3} are in Table 13.

Table 11: Stability constants for cadmium binary complex systems (1:1 ratio) with the pH at which permanent turbidity appeared

Binary system	**pH at permanent turbidity**	**Log K_1^f**
AsC	8.27	3.74
Cit	9.01	3.93
AsP	7.93	2.51
Caf	5.17	-
Ura	7.75	2.06
Sali	8.37	3.79
Vit B_1	9.28	4.04
Para	8.05	2.84
Vit B_3	8.10	3.18
Na_2EDTA	No turbidity	13.94

Table 12: Stability constants for cadmium binary complex systems (1:2 ratio) with the pH at which permanent turbidity appeared

Binary system	**pH at permanent turbidity**	**Log K_1^f**	**Log K_2^f**
AsC	8.56	3.37	1.95
Cit	No turbidity	4.02	-
AsP	8.46	3.27	-
Caf	5.74	-	-
Ura	8.63	2.21	-
Sali	8.71	3.86	-
Vit B_1	No turbidity	3.95	-
Para	8.33	3.32	-
Vit B_3	8.58	3.30	-
Na_2EDTA	No turbidity	13.68	-

According to the tables 11 and 12, caffeine has not formed complexes with cadmium at both ratios under the physiological conditions used. Ascorbic acid formed ML and ML_2 type complexes with cadmium. All the other ligands formed only ML type complexes in both ratios.

Table 13: Stability constants for cadmium binary complex systems (1:1 ratio) with the pH at which permanent turbidity appeared

Binary system	**pH at permanent turbidity**	**Log K_1^f**	**Log K_2^f**
AsC	No turbidity	6.28	4.17
Sali	9.03	4.60	-
Para	9.46	5.22	-
Cit	No turbidity	5.57	-
Ura	9.00	3.76	-

Vit B_1	9.14	4.82	-
Vit B_3	5.81	-	-
Asp	7.68	3.04	-
Caf	4.89	-	-
Na_2EDTA	No turbidity	13.75	-

UV experimental results

M: L ratios of the cadmium binary complexes were spectrophotometrically estimated using the similar method used in lead binary complexes. The percentage chelation values obtained are given in Table 14.

Table 14: Percentage chelation with cadmium at different pH values

Binary system	**pH**	**% chelation at different M:L ratios**					
		1:0.5	**1:1**	**1:1.5**	**1:2**	**1:2.5**	**1:3**
AsC-Cd	8.00	58.96	**76.64**	**76.85**	**76.45**	**76.38**	**76.46**
Sali-Cd	8.25	45.85	**80.49**	**80.56**	**80.18**	**80.60**	**80.50**
Para-Cd	8.00	64.04	**72.32**	**72.29**	**72.26**	**72.09**	**72.12**
Caf-Cd	5.00	0.63	0.98	1.62	2.00	2.16	-
Ura-Cd	7.50	46.52	**64.38**	**64.34**	**64.37**	**64.57**	-
Vit B_1-Cd	9.00	71.33	**86.02**	**85.95**	**86.02**	**86.12**	-
Vit B_3-Cd	8.00	62.00	**74.61**	**74.30**	**74.67**	**74.38**	-
Aspirin-Cd	7.90	54.37	**68.67**	**68.50**	**68.76**	**68.78**	-
AsC-Cd	8.50	68.63	70.73	72.93	**73.49**	**73.51**	**73.58**

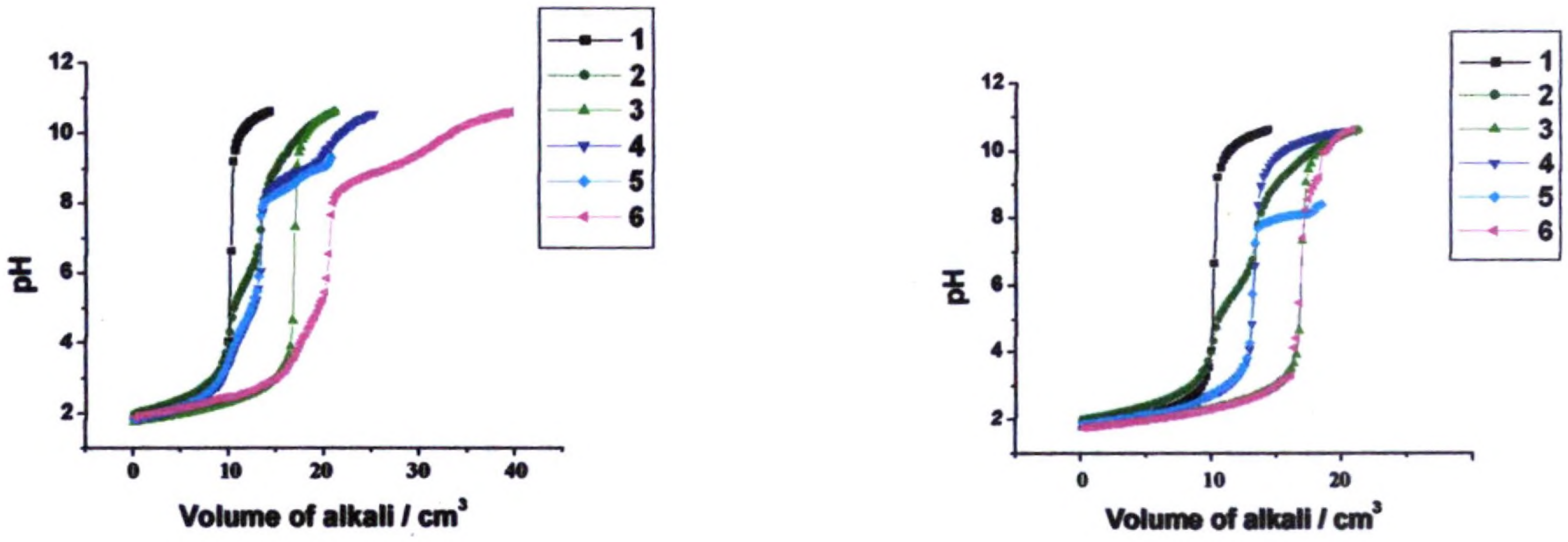
According to the UV data only ascorbic acid forms 1:2 binary complexes with Cd^{2+} at higher pH values. There are some literature evidences explaining 1:1 and 1:2, M:L complexes of cadmium with ascorbic acid[59]. There was no significant change in the absorbance values of caffeine spectra in the presence and absence of the cadmium.

According to the results obtained by both methods, it can be clearly noticed that percentage chelation was higher when the stability of the complex was higher.

Potentiometric studies for cadmium mixed ligand systems

Mixed ligand complex formations of each ligand pair was quantitatively studied with titration curves. Sample plots for two systems in which one shows curves for a system forming a mixed ligand complex and the other shows curves for a system not forming a mixed ligand complex are given in Figures 5**a** and 5**b** respectively.

In Figure 5a, mixed ligand titration curve and titration curve of EDTA binary complex system are different from each other showing the formation of Cd(EDTA)(vit B_1) complex. However, titration curve for Cd(EDTA)(Sali) mixture given in Figure 5b is much similar to the titration curve of Cd(EDTA) binary complex system implying the absence of mixed complex formation in the reaction mixture.



(a) (b)

Figure 5a: Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of Cd^{2+} with Na_2EDTA and Vitamin B_1 **1.** HNO_3; **2.** HNO_3+Na_2EDTA; **3.** HNO_3+Na_2EDTA+ $Cd(NO_3)_2$; **4.** HNO_3 + Vitamin B_1; **5.** HNO_3 + Vitamin B_1 + $Cd(NO_3)_2$; **6.** HNO_3+Na_2EDTA+Vitamin B_1+ $Cd(NO_3)_2$ **Figure 5b**: Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of Cd^{2+} with Na_2EDTA and Salicylic acid **1.** HNO_3; **2.** HNO_3+Na_2EDTA; **3.** HNO_3+Na_2EDTA+ $Cd(NO_3)_2$; **4.** HNO_3 + Salicylic acid; **5.** HNO_3 + Salicylic acid + $Cd(NO_3)_2$; **6.** HNO_3+Na_2EDTA+Salicylic acid+ $Cd(NO_3)_2$

Formation constants calculated for the cadmium ternary systems are listed in Table 15 and 16 for concentrations of 0.01 mol dm^{-3} and 0.001 mol dm^{-3} respectively. Out of 45 ligand combinations considered, only 11 and seven combinations formed ternary complexes with Cd^{2+} at two concentrations respectively.

Table 15: Stability constants for cadmium ternary complex systems together with the pH at which permanent turbidity appeared

L(primary ligand)	X(secondary ligand)	pH at permanent turbidity	log β_1	log β_2
EDTA	B_1	No turbidity	13.94	29.25
EDTA	Cit	No turbidity	13.94	20.37
B_1	Cit	9.40	9.28	-
EDTA	Sali	No turbidity	13.94	-
B_1	Sali	8.27	9.28	-
Cit	Sali	9.66	3.93	-
EDTA	AsC	No turbidity	13.94	25.42
B_1	AsC	8.39	9.28	-
Cit	AsC	No turbidity	3.93	9.83
Sali	AsC	No turbidity	3.79	12.06
EDTA	B_3	No turbidity	13.94	-
B_1	B_3	8.14	9.28	-
Cit	B_3	9.53	3.93	-
Sali	B_3	8.19	3.79	-
AsC	B_3	8.04	3.74	-
EDTA	Para	No turbidity	13.94	-
B_1	Para	8.45	9.28	-
Cit	Para	No turbidity	3.93	9.11

Sali	Para	8.15	3.79	-
AsC	Para	No turbidity	3.74	9.19
B_3	Para	8.16	3.18	-
EDTA	AsP	No turbidity	13.94	-
B_1	AsP	8.58	9.28	-
Cit	AsP	9.29	3.93	-
Sali	AsP	8.40	3.79	-
AsC	AsP	8.45	3.74	-
B_3	AsP	8.23	3.18	-
Para	AsP	8.97	2.84	5.45
EDTA	Ura	No turbidity	13.94	-
B_1	Ura	8.65	9.28	-
Cit	Ura	9.22	3.93	-
Sali	Ura	8.56	3.79	-
AsC	Ura	8.19	3.74	-
B_3	Ura	9.04	3.18	5.46
Para	Ura	8.12	2.84	-
AsP	Ura	9.28	2.51	6.37
EDTA	Caf	No turbidity	13.94	-
B_1	Caf	8.34	9.28	-
Cit	Caf	9.35	3.93	-
Sali	Caf	8.49	3.79	-
AsC	Caf	8.32	3.74	-
B_3	Caf	8.28	3.18	-
Para	Caf	9.02	2.84	5.67
AsP	Caf	8.24	2.51	-
Ura	Caf	7.80	2.06	-

Table 16: Stability constants for cadmium ternary complex systems together with the pH at which permanent turbidity appeared. (0.001 mol dm^{-3})

L	**X**	**pH at permanent turbidity**	**log β_1**	**log β_2**
EDTA	AsC	No turbidity	13.75	25.07
EDTA	Cit	No turbidity	13.75	18.75
AsC	Cit	No turbidity	6.28	13.28
EDTA	Para	No turbidity	13.75	-
AsC	Para	No turbidity	6.28	16.03
Cit	Para	9.97	5.57	-
EDTA	B_1	No turbidity	13.75	23.98
AsC	B_1	No turbidity	6.28	10.85
Cit	B_1	No turbidity	5.57	-
Para	B_1	9.75	5.22	-
EDTA	Sali	No turbidity	13.75	-
AsC	Sali	8.93	6.28	-
Cit	Sali	10.06	5.57	-
Para	Sali	9.84	5.22	-
B_1	Sali	9.20	4.82	-
EDTA	Ura	No turbidity	13.75	-
AsC	Ura	No turbidity	6.28	-
Cit	Ura	9.71	5.57	-
Para	Ura	9.47	5.22	-
B_1	Ura	8.82	4.82	-

Sali	Ura	9.22	4.60	-
EDTA	AsP	No turbidity	13.75	-
AsC	AsP	No turbidity	6.28	11.57
Cit	AsP	10.16	5.57	-
Para	AsP	8.60	5.22	-
B_1	AsP	8.70	4.82	-
Sali	AsP	9.15	4.60	-
Ura	AsP	8.62	3.76	-
EDTA	B_3	No turbidity	13.75	-
AsC	B_3	9.09	6.28	-
Cit	B_3	No turbidity	5.57	-
Para	B_3	8.76	5.22	-
B_1	B_3	9.08	4.82	-
Sali	B_3	9.47	4.60	-
Ura	B_3	9.20	3.76	-
AsP	B_3	7.87	3.04	-
EDTA	Caf	No turbidity	13.75	-
AsC	Caf	6.88	6.28	-
Cit	Caf	No turbidity	5.57	-
Para	Caf	8.80	5.22	-
B_1	Caf	9.00	4.82	-
Sali	Caf	9.40	4.60	-
Ura	Caf	9.00	3.76	-
AsP	Caf	7.91	3.04	-
B_3	Caf	5.95	-	-

Composite curves drawn for two mixed ligand mixtures are given in Figure 6**a** and 6**b**

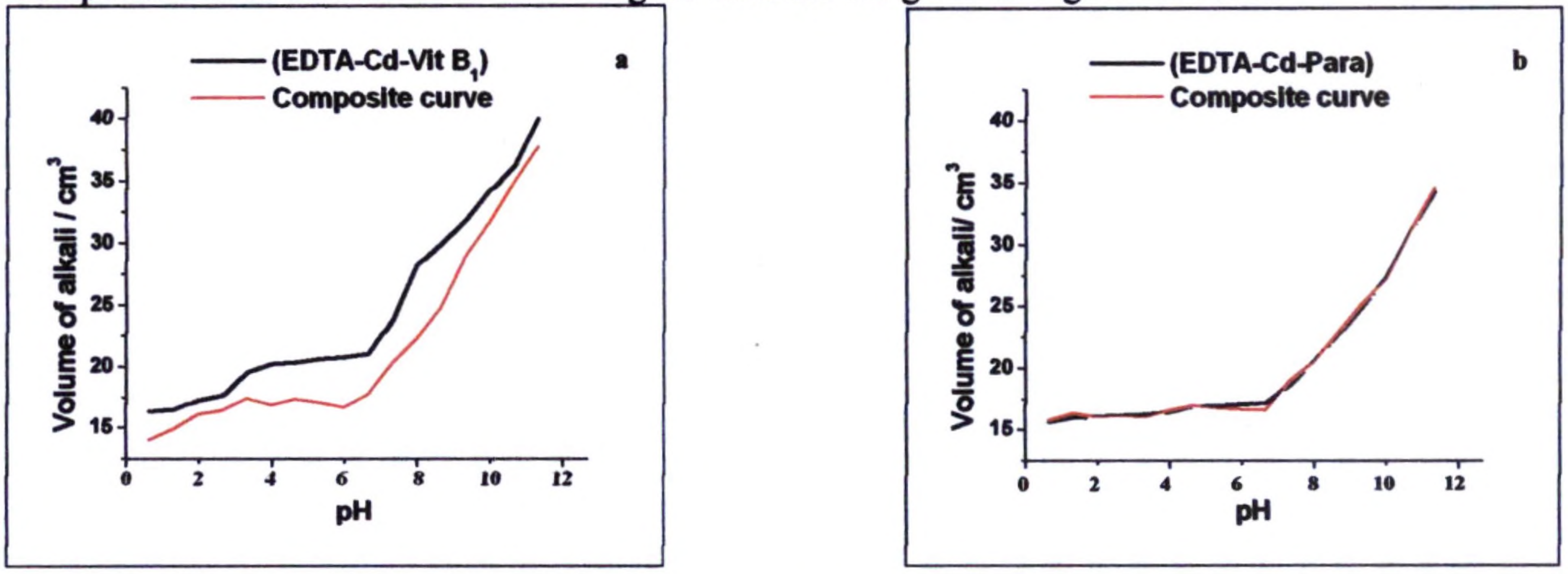
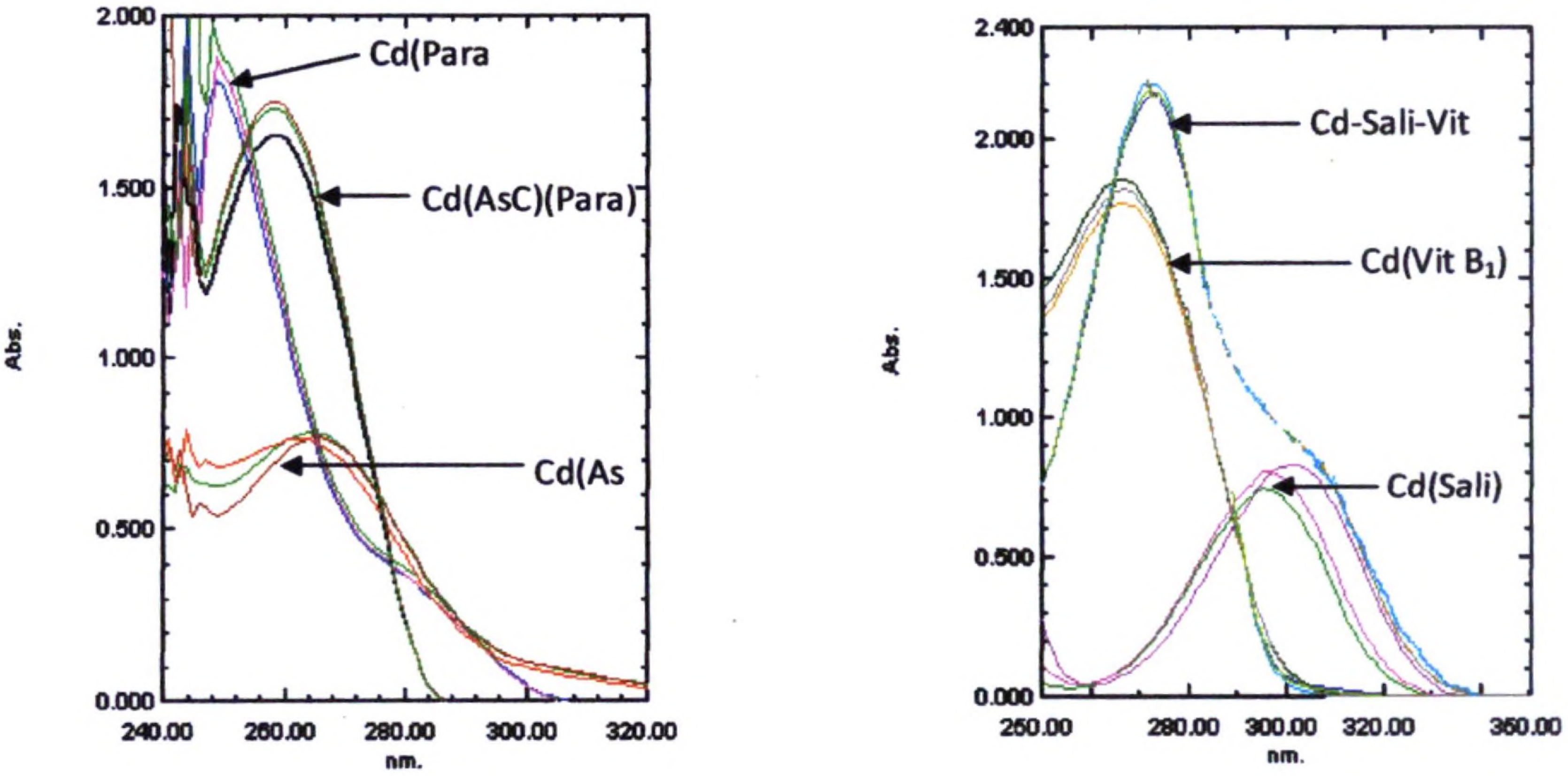


Figure 6: Composite curve and titration curve of a ternary system **a)** formed **b)** did not form a mixed ligand complex.

Only for the system that did not form a mixed complex, the two curves are superimposible by proving the inability of forming a mixed complex among those two ligands and Cd^{2+}.

UV experimental results

A representative set of spectra for a particular ternary system are given in Figure 7a. As shown in the diagram, a new peak appeared at a different position from the either peaks of binary complexes by indicating the formation of a mixed complex. Figure 7b illustrates a representative set of UV spectra for a ternary system which did not form a mixed ligand complex. There are two binary complexes in different amounts according to the spectral behaviour rather than a mixed ligand complex.



(a) (b)

Figure 7 : (a) UV absorption spectra for complexes; Cd(AsC), Cd(Para) and Cd(AsC)(Para) at three different pH values : (b) UV absorption spectra for complexes; Cd(Sali), Cd(Vit B_1) and ternary system Cd-Sali-Vit B_1 at three different pH values

Comparison of the Stabilities of single and mixed ligand complexes (Δlog K)

Stabilities of complexes formed were compared by considering the logarithmic values of their stepwise formation constants. Values obtained are listed in the Table 17.

Table 17: Calculated Δlog K values for cadmium ternary complex systems

Ternary complex	**Δlog K**
EDTA-Cd-AsC	**7.74**
EDTA-Cd-Vi B_1	**11.27**
EDTA-Cd-Cit	**2.50**
Cit-Cd-AsC	**2.16**
Sali- Cd -AsC	**4.53**
Cit- Cd -Para	**2.34**
AsC- Cd -Para	**2.61**
Para- Cd -AsP	**0.10**
Vit B_3- Cd -Ura	**0.22**
AsP- Cd -Ura	**1.80**
Para- Cd -Caf	**2.83**

According to the Δlog K values, all cadmium ternary complexes are more stable than their corresponding binary systems. It is very interesting to observe that in some ternary systems, Δlog K values are higher than K_1^f of both ligands.

Complexes of aluminium (Al^{3+})

pH titration studies of aluminium single ligand systems

Formation constants calculated for 1 : 1 and 1: 2 M : L systems are given in the Tables 18 and 19 respectively.

Table 18: Stability constants of aluminium binary complex systems (1:1 ratio) together with the pH at which permanent turbidity observed

Binary system	**pH at permanent turbidity**	**Log K_1^f**	**Log K_2^f**
AsC	5.82	3.86	4.13
Cit	8.42	8.81	3.57
AsP	5.27	2.41	-
Caf	4.58	-	-
Ura	No turbidity	9.24	7.37
Sali	5.49	3.30	3.55
Vit B_1	8.27	8.68	5.12
Para	No turbidity	9.12	7.62
Vit B_3	4.96	-	-
EDTA	No turbidity	14.17	-

Table 19: Stability constants for aluminium binary complex systems (1:2 ratio) reported together with the pH at which permanent turbidity observed

Binary system	**pH at permanent turbidity**	**Log K1f**	**Log K2f**
AsC	8.67	3.73	4.29
Cit	No turbidity	8.39	8.56
AsP	6.23	3.22	-
Caf	4.74	-	-
Ura	No turbidity	9.04	7.40
Sali	8.11	3.43	-
Vit B1	No turbidity	12.77	4.08
Para	No turbidity	9.08	7.79
Vit B3	6.06	-	-
EDTA	No turbidity	13.69	-

According to the results given in Tables 18 and 19, vitamin B_3 and caffeine have not formed any complex with aluminium in both M: L ratios under the experimental conditions used. However, other ligands have formed either ML or ML_2 type of complexes with aluminium. Specially, ligands with more basic properties, like paracetamol and uracil have formed very stable complexes.

Table 20: Stability constants for aluminium binary complex systems (1:1 ratio) reported together with the pH at which permanent turbidity observed (0.001 mol dm^{-3})

Binary system	**pH at permanent turbidity**	**Log K_1^f**	**Log K_2^f**
Asc	9.12	6.91	4.81
Sali	8.88	4.87	-
Para	9.35	8.85	-
Cit	9.25	7.83	4.97

Ura	10.12	9.37	-
Vit B_1	9.48	9.12	7.33
Vit B_3	5.16	-	-
Asp	6.82	-	-
Caf	4.45	-	-
Na_2EDTA	No turbidity	14.10	-

UV Experimental results for aluminium single ligand systems

Percentage chelation calculated for binary systems are given in the Table 21

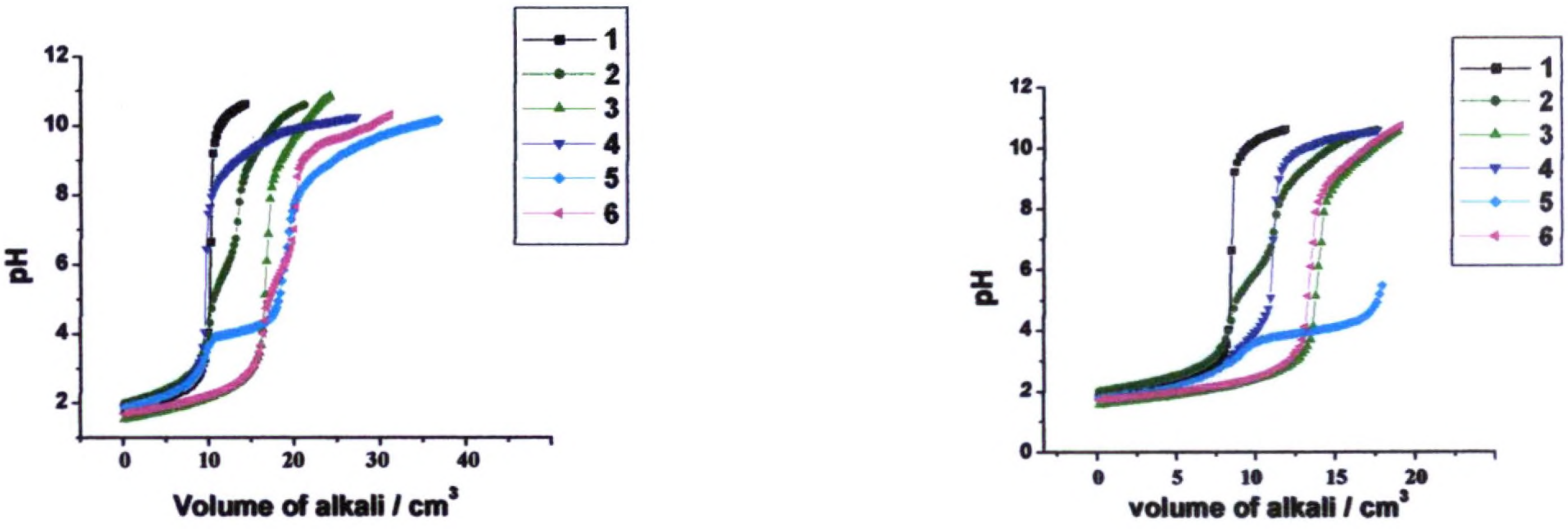
Table 21: Percentage chelation of each aluminium binary complex at relevant pH values

Binary system	**pH**	**% chelation at different M:L ratios**					
		1:0.5	**1:1**	**1:1.5**	**1:2**	**1:2.5**	**1:3**
AsC-Al	8.50	73.72	74.98	75.12	**82.48**	**82.46**	**82.36**
Sali- Al	8.00	45.85	55.55	59.44	**64.16**	**64.28**	**64.80**
Para- Al	10.00	76.71	81.38	81.46	**84.77**	**84.59**	**84.58**
Ura- Al	10.00	-	60.04	79.00	**85.13**	**85.61**	**85.76**
Vit B_1- Al	10.00	-	74.16	80.59	**83.35**	**83.35**	**83.41**
Aspirin- Al	5.00	54.37	**61.38**	**61.40**	**61.58**	**61.59**	-
Caf-Al	4.00	1.22	1.39	2.20	2.25	2.16	-
Vit B_3-Al	4.50	2.86	2.90	2.91	2.96	3.09	-

According to the data in Table 21, in each system, the percentage chelation with aluminium became a constant after a particular L: M ratio. That ratio was exactly similar to the ratio obtained from each potentiometric titration data and therefore, it confirmed further, the potentiometric results of each binary system. Percentage chelation of some ligands such as paracetamol, uracil and vitamin B_1 forming very stable binary complexes at higher pH values with Al^{3+} showed more than 80% chelation at higher stoicheometric ratios.

Potentiometric studies for aluminium mixed ligand systems

Figure 8a illustrates titration curves for EDTA-Ura-Al mixed system that formed a mixed complex in the solution. It can be clearly seen in the plot that mixed ligand system titration curve is different from the two binary system titration curves (curves 3 and 5 in figure 8a). Sample titration curves for a system which did not form a mixed ligand complex are given in Figure 8b.



(a) (b)

Figure 8 (a) Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of Al^{3+} with Na_2EDTA and uracil **1.** HNO_3; **2.** HNO_3+Na_2EDTA; **3.** HNO_3+Na_2EDTA+ $Al(NO_3)_3$; **4.** HNO_3 + uracil; **5.** HNO_3 + uracil + $Al(NO_3)_3$; **6.** HNO_3+Na_2EDTA+uracil+ $Al(NO_3)_3$ **(b)** Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of Al^{3+} with Na_2EDTA and ascorbic acid **1.** HNO_3; **2.** HNO_3+Na_2EDTA; **3.** HNO_3+Na_2EDTA+ $Al(NO_3)_3$; **4.** HNO_3 + ascorbic acid; **5.** HNO_3 + ascorbic acid + $Al(NO_3)_3$; **6.** HNO_3+Na_2EDTA+ascorbic acid+ $Al(NO_3)_3$

Formation constants of ternary systems calculated using a modified version of Irving and Rossotti method are given in the Table 22 and 23 for two concentrations used.

Table 22: Stability constants for aluminium ternary complex systems together with the pH at which permanent turbidity was appeared(0.01 mol dm^{-3})

L(primary ligand)	**X(secondary ligand)**	**pH at permanent turbidity**	**log β_1**	**log β_2**
EDTA	Ura	No turbidity	14.17	23.01
EDTA	Para	No turbidity	14.17	24.36
Ura	Para	8.40	9.24	-
EDTA	Cit	No turbidity	14.17	20.65
Ura	Cit	No turbidity	9.24	15.15
Para	Cit	No turbidity	9.21	14.51
EDTA	Vit B_1	No turbidity	14.17	29.36
Ura	Vit B_1	8.05	9.24	-
Para	Vit B_1	8.15	9.21	-
Cit	Vit B_1	No turbidity	8.81	22.41
EDTA	AsC	No turbidity	14.17	-
Ura	AsC	5.08	9.24	-
Para	AsC	4.71	9.21	-
Cit	AsC	No turbidity	8.81	17.50
Vit B_1	AsC	5.12	8.68	-
EDTA	Sali	No turbidity	14.17	-
Ura	Sali	8.79	9.24	-
Para	Sali	5.02	9.21	-
Cit	Sali	8.51	8.81	-
Vit B_1	Sali	9.38	8.68	12.93

AsC	Sali	5.22	3.86	-
EDTA	AsP	No turbidity	14.17	-
Ura	AsP	9.33	9.24	-
Para	AsP	8.11	9.21	-
Cit	AsP	8.44	8.81	-
Vit B_1	AsP	8.04	8.68	-
AsC	AsP	4.93	3.86	-
Sali	AsP	6.46	3.30	-
EDTA	Vit B_3	No turbidity	14.17	-
Ura	Vit B_3	7.43	9.24	-
Para	Vit B_3	7.28	9.21	-
Cit	Vit B_3	8.56	8.81	-
Vit B_1	Vit B_3	8.09	8.68	-
AsC	Vit B_3	5.03	3.86	-
Sali	Vit B_3	5.49	3.30	-
AsP	Vit B_3	5.15	2.41	-
EDTA	Caf	No turbidity	14.17	-
Ura	Caf	8.35	9.24	-
Para	Caf	8.91	9.21	-
Cit	Caf	8.34	8.81	-
Vit B_1	Caf	7.14	8.68	-
AsC	Caf	3.37	3.86	-
Sali	Caf	5.59	3.30	-
AsP	Caf	5.12	2.41	-
Vit B_3	Caf	5.01	-	-

The results given in Table 22 reveal that mixed complex formation of aluminium with considered ligand combinations is very poor.

Table 23: Stability constants for aluminium ternary complex systems together with the pH at which permanent turbidity was appeared(0.001 mol dm^{-3})

L	**X**	**pH at permanent turbidity**	**log β_1**	**log β_2**
EDTA	Ura	No turbidity	14.10	23.82
EDTA	B_1	No turbidity	14.10	28.83
Ura	B_1	No turbidity	9.37	14.45
EDTA	Para	No turbidity	14.10	23.94
Ura	Para	8.58	9.37	-
B_1	Para	8.44	9.12	-
EDTA	Cit	No turbidity	14.10	-
Ura	Cit	No turbidity	9.37	15.31
B_1	Cit	No turbidity	9.12	15.13
Para	Cit	No turbidity	8.85	15.54
EDTA	AsC	No turbidity	14.10	-
Ura	AsC	No turbidity	9.37	19.58
B_1	AsC	8.50	9.12	-
Para	AsC	No turbidity	8.85	18.78
Cit	AsC	9.57	7.83	-
EDTA	Sali	No turbidity	14.10	-
Ura	Sali	9.85	9.37	-
B_1	Sali	9.95	9.12	-

Para	Sali	9.38	8.85	-
Cit	Sali	9.37	7.83	-
AsC	Sali	8.13	6.91	-
EDTA	AsP	No turbidity	14.10	-
Ura	AsP	6.95	9.37	-
B_1	AsP	7.04	9.12	-
Para	AsP	8.56	8.85	-
Cit	AsP	No turbidity	7.83	11.15
AsC	AsP	8.00	6.91	-
Sali	AsP	8.26	4.87	-
EDTA	B_3	No turbidity	14.10	-
Ura	B_3	7.18	9.37	-
B_1	B_3	7.30	9.12	-
Para	B_3	8.46	8.85	-
Cit	B_3	9.68	7.83	-
AsC	B_3	7.58	6.91	-
Sali	B_3	8.28	4.87	-
AsP	B_3	7.00	-	-
EDTA	Caf	No turbidity	14.10	-
Ura	Caf	6.86	9.37	-
B_1	Caf	7.10	9.12	-
Para	Caf	7.70	8.85	-
Cit	Caf	9.52	7.83	-
AsC	Caf	8.10	6.91	-
Sali	Caf	8.22	4.87	-
AsP	Caf	7.12	-	-
B_3	Caf	5.13	-	-

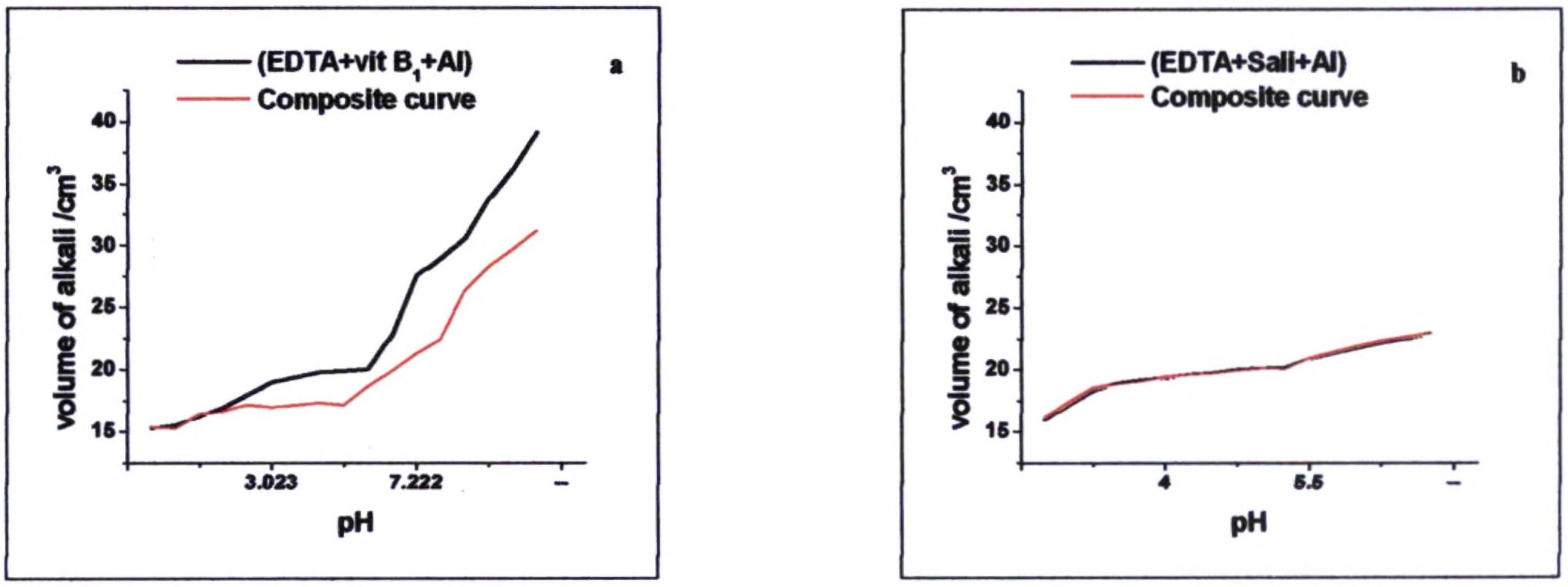
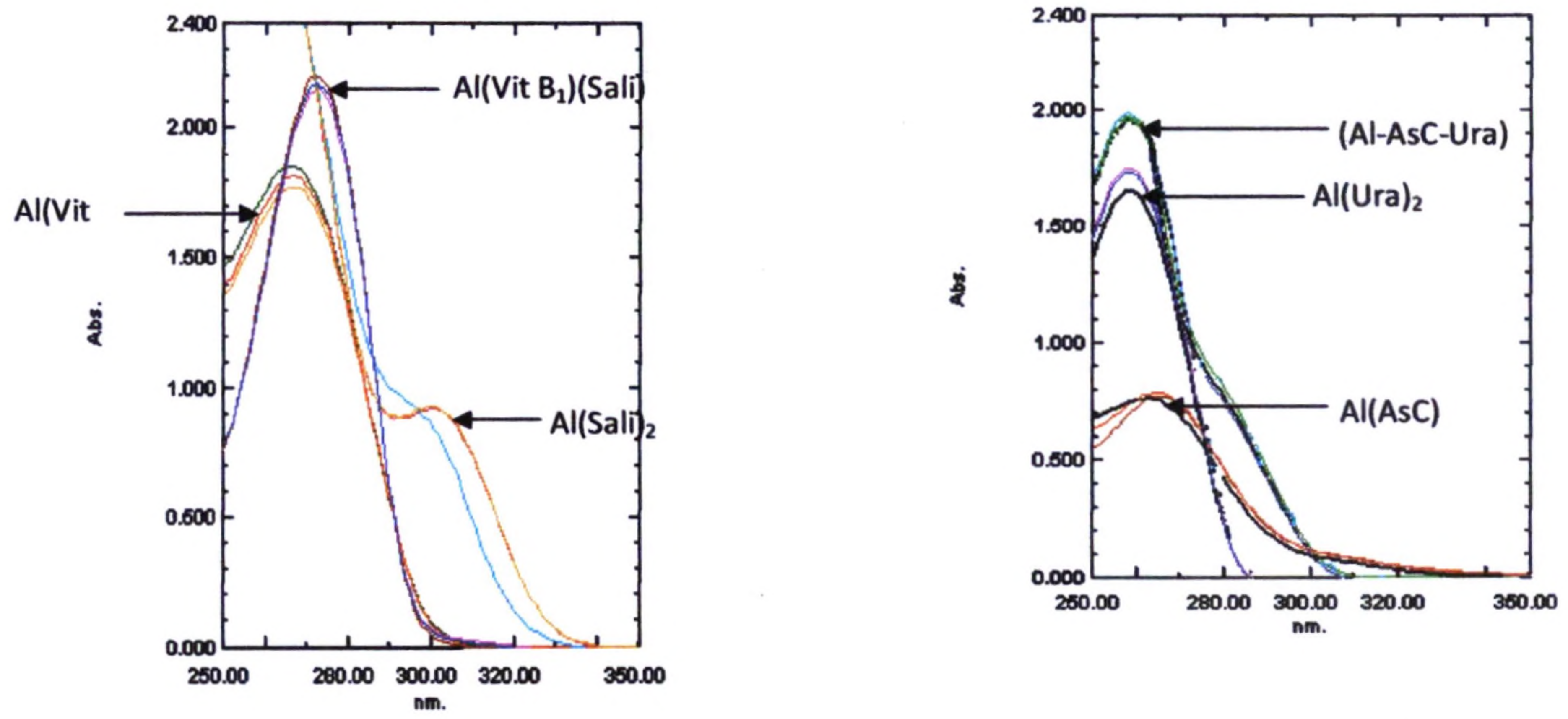


Figure 9: Composite curve and titration curve of a ternary system which **a.** formed **b.** did not form a mixed complex with aluminium.

Composite curves plotted further to confirm mixed complexation are given in Figure 9a) and 9b) for two systems. It is well evidence from the Figure 9a that, composite curve and ternary system titration curve has deviated much from each other when a strong interaction between ligand mixture and metal ion was present. Hence, systems which formed mixed complexes with aluminium showed this behaviour in their composite curves. Whereas, plots similar to one shown in Figure 9b were observed for all systems which did not form mixed complex with aluminium.

UV Experimental results for aluminum mixed ligand systems

Detailed spectra of Al(Vit B_1)(Sali) mixed complex system including spectra of Al(Vit B_1), Al(Sali) binary complexes are revealed in Figure 10a. The figure clearly shows a new peak for the new system which corresponds to the mixed ligand complex. Figure 10b shows UV absorbance spectra of a system that did not from a mixed ligand complex. Thus, for the mixed ligand system, there is no UV absorbance peak corresponds to a new mixed ligand complex.



(a) (b)

Figure 10 (a) UV absorption spectra for the complexes Al(Sali)$_2$, Al(Vit B_1)$_2$ and Al(Vit B_1)(Sali) at three different pH values. (b) UV absorption spectra for the complexes Al(AsC), Al(Ura)$_2$ and mixed ligand system Al-Ura-AsC at three different pH values.

Comparative analysis of the stabilities of single and mixed ligand complexes (Δlog K)

Stabilities of the mixed complexes formed over the binary complexes were analyzed by calculating the parameter Δlog K values and given in Table 24. The most frequently found coordination number of aluminium complexes is six and therefore, stability of octahedral complexes were considered[60].

Table 24: Calculated Δlog K values for aluminium ternary complex systems

Ternary complex	Δlog K
EDTA-Al-Ura	-0.40
EDTA-Al-Para	0.98
EDTA-Al-Cit	-2.33
EDTA-Al-Vit B_1	6.51
Ura- Al -Cit	-2.90

Para- Al -Cit	-3.51
Cit- Al –Vit B_1	4.92
Cit- Al -AsC	4.83
Vit B_1- Al -Sali	0.95

According to the values obtained for Δlog K, both stable and unstable ternary complex systems have been formed. The unstable nature can be explained considering their structures.

Complexes of copper

Even though, copper complexes are of different colors, the concentrations used in this study were not enough to give significant absorbance values in the visible region to be used in the analysis.

Formation constants computed using potentiometric titration data for binary systems with M : L ratios of 1: 1 and 1:2 are listed in Table 25 and Table 26 respectively and data for binary systems at 0.001 mol dm^{-3} concentration are in Table 27.

Table 25: Stability constants for Copper binary complex systems (1:1 ratio) together with the pH at which permanent turbidity appeared

Binary system	**pH at permanent turbidity**	**Log K_1^f**	**Log K_2^f**
AsC	No turbidity	7.56	6.33
Cit	No turbidity	4.48	-
AsP	6.33	2.96	-
Caf	5.30	-	-
Ura	5.69	2.03	-
Sali	9.90	3.56	2.91
B1	5.98	2.36	-
Para	5.56	1.54	-
B3	6.91	3.52	3.15
EDTA	No turbidity	13.18	-

Table 26: Stability constants for Copper binary complex systems (1:2 ratio) together with the pH at which permanent turbidity appeared

Binary system	**pH at permanent turbidity**	**Log K_1^f**	**Log K_2^f**
AsC	No turbidity	9.08	6.50
Cit	No turbidity	6.70	4.69
AsP	6.58	3.12	-
Caf	6.18	-	-
Ura	6.47	2.24	-
Sali	No turbidity	3.63	3.60
B1	6.57	1.88	-
Para	6.37	1.74	-
B3	7.31	3.55	3.53
EDTA	No turbidity	13.00	-

According to the results given in Tables 25 and 26, it is clear that both ML and ML_2 type complexes were formed by Cu^{2+} with considered ligand set in the aqueous medium.

Table 27: Stability constants for Copper binary complex systems (1:1 ratio) together with the pH at which permanent turbidity appeared(0.001 mol dm^{-3})

Binary system	pH at permanent turbidity	Log K_1^f	Log K_2^f
AsC	No turbidity	6.02	4.30
Sali	9.47	4.76	-
Para	9.19	3.42	-
Cit	No turbidity	6.07	4.16
Ura	9.37	4.49	-
Vit B_1	No turbidity	6.34	5.74
Vit B_3	9.66	4.92	-
Asp	6.18	3.34	-
Caf	5.99	-	-
Na_2EDTA	No turbidity	12.96	-

UV experimental results for copper single ligand systems

Percentage chelation calculated for binary complexes by changing M : L ratio in mixtures considering UV absorption peaks are given in Table 28.

Table 28: Percentage chelation of each copper binary complex at relevant pH values

Binary system	pH	% chelation at different M:L ratios					
		1:0.5	1:1	1:1.5	1:2	1:2.5	1:3
AsC-Cu	10.00	76.31	79.11	79.16	**84.22**	**84.21**	**84.44**
Sali- Cu	10.00	45.97	55.56	59.56	**65.14**	**65.04**	**65.14**
Para- Cu	6.30	22.24	**39.33**	**39.67**	**39.59**	**39.78**	**39.89**
Ura- Cu	6.50	48.21	**61.28**	**61.53**	**61.56**	**61.91**	-
Vit B_1- Cu	6.70	31.76	**62.66**	**62.97**	**62.81**	**62.86**	-
Vit B_3- Cu	6.75	-	48.96	60.23	**64.42**	**64.46**	**64.41**
Aspirin- Cu	6.30	56.36	**63.20**	**63.13**	**63.49**	**63.39**	-
Caf-Cu	5.00	1.34	1.68	1.68	1.97	2.14	-

The exact metal to ligand ratio was decided by considering the percentage chelation values given in Table 28. Strong chelators of Cu^{2+} like ascorbic acid, showed higher values for percentage chelation.

Potentiometric studies for copper mixed ligand systems

Calvin type pH titrations were carried out for all 45 ligand combinations as described previously. Sample titration curves for two mixed ligand systems are given in Figures 11**a** and 11**b**.

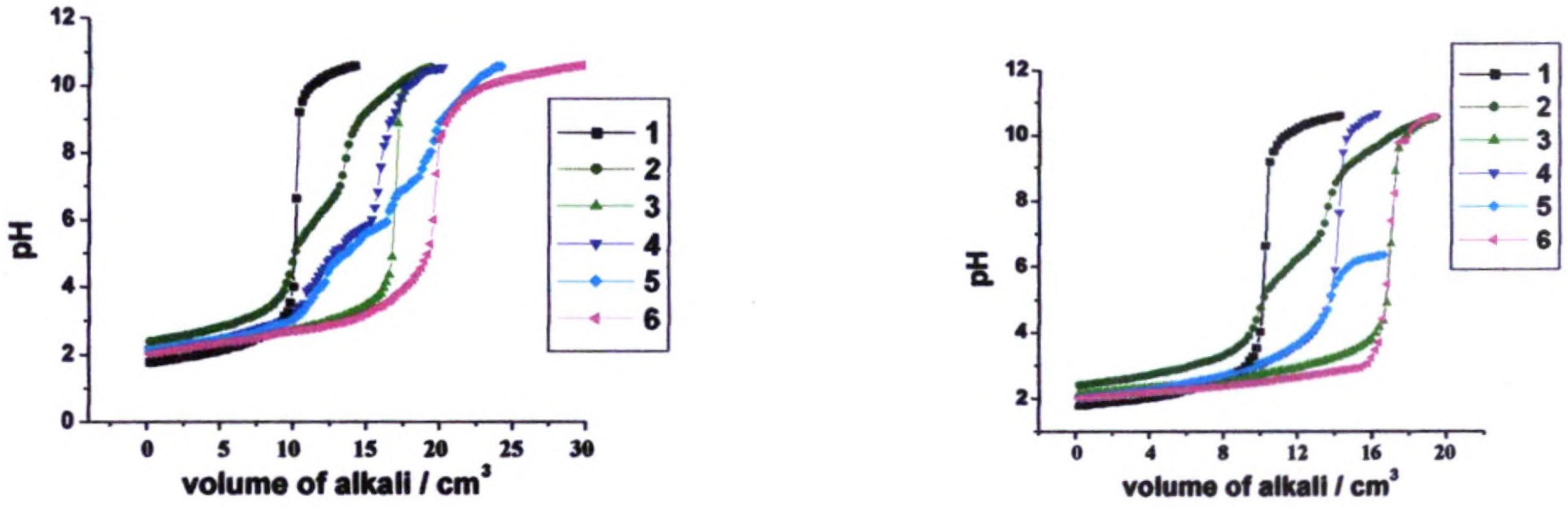


Figure 11 (a) Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of Cu^{2+} with Na_2EDTA and ascorbic acid **1.** HNO_3; **2.** HNO_3+Na_2EDTA; **3.** $HNO_3+Na_2EDTA+ Cu(NO_3)_2$; **4.** HNO_3 + ascorbic acid; **5.** HNO_3 + ascorbic acid + $Cu(NO_3)_2$; **6.** HNO_3+Na_2EDTA+ascorbic acid+ $Cu(NO_3)_2$ **(b)** Potentiometric titration curves of binary (1:1) and ternary (1:1:1) complexes of Cu^{2+} with Na_2EDTA and aspirin **1.** HNO_3; **2.** HNO_3+Na_2EDTA; **3.** $HNO_3+Na_2EDTA+ Cu(NO_3)_2$; **4.** HNO_3 + aspirin; **5.** HNO_3 + aspirin + $Cu(NO_3)_2$; **6.** HNO_3+Na_2EDTA+aspirin+ $Cu(NO_3)_2$

Figure 11a is for a system, in which a mixed ligand complex was formed between two ligand combinations and Cu^{2+} and its mixed ligand titration curve is away from curves of the two binary systems.Stability constants obtained using titration data for mixed ligand systems are given in Table 29 and 30 for two concentrations used.

Table 29: Stability constants for copper ternary complex systems together with the pH at which permanent turbidity appeared (0.01 mol dm^{-3})

L(primary ligand)	X(secondary ligand)	pH at permanent turbidity	log β_1	log β_2
EDTA	AsC	No turbidity	13.18	22.28
EDTA	Cit	No turbidity	13.18	20.48
AsC	Cit	No turbidity	7.56	-
EDTA	Sali	No turbidity	13.18	-
AsC	Sali	No turbidity	7.56	-
Cit	Sali	No turbidity	4.48	-
EDTA	Vit B_3	No turbidity	13.18	-
AsC	Vit B_3	No turbidity	7.56	-
Cit	Vit B_3	No turbidity	4.48	-
Sali	Vit B_3	6.42	3.56	-
EDTA	Asp	No turbidity	13.18	-
AsC	Asp	No turbidity	7.56	-
Cit	Asp	No turbidity	4.48	-
Sali	Asp	6.49	3.56	-
Vit B_3	Asp	6.37	3.52	-
EDTA	Vit B_1	No turbidity	13.18	-
AsC	Vit B_1	5.61	7.56	-
Cit	Vit B_1	No turbidity	4.48	-

Sali	Vit B_1	7.45	3.56	-
Vit B_3	Vit B_1	6.38	3.52	-
Asp	Vit B_1	6.34	2.96	-
EDTA	Ura	No turbidity	13.18	-
AsC	Ura	No turbidity	7.56	-
Cit	Ura	No turbidity	4.48	-
Sali	Ura	6.44	3.56	-
Vit B_3	Ura	6.61	3.52	-
Asp	Ura	6.65	2.96	-
Vit B_1	Ura	6.36	2.36	-
EDTA	Para	No turbidity	13.18	-
AsC	Para	No turbidity	7.56	12.47
Cit	Para	No turbidity	4.48	-
Sali	Para	6.63	3.56	-
Vit B_3	Para	5.91	3.52	-
Asp	Para	8.57	2.96	6.09
Vit B_1	Para	5.80	2.36	-
Ura	Para	8.28	2.03	6.45
EDTA	Caf	No turbidity	13.18	-
AsC	Caf	No turbidity	7.56	-
Cit	Caf	No turbidity	4.48	-
Sali	Caf	6.51	3.56	-
Vit B_3	Caf	6.54	3.52	-
Asp	Caf	6.32	2.96	-
Vit B_1	Caf	5.78	2.36	-
Ura	Caf	5.65	2.03	-
Para	Caf	5.08	1.54	-

Mixed complex formation of copper with considered ligand combinations was very much poor, as only five mixed complex were formed among the 45 systems considered.

Table 30: Stability constants for copper ternary complex systems together with the pH at which permanent turbidity appeared (0.001 mol dm^{-3})

L	**X**	**pH at permanent turbidity**	**log β_1**	**log β_2**
EDTA	B1	No turbidity	12.96	24.21
EDTA	Cit	No turbidity	12.96	16.88
B1	Cit	No turbidity	6.34	11.22
EDTA	AsC	No turbidity	12.96	22.89
B1	AsC	No turbidity	6.34	13.85
Cit	AsC	No turbidity	6.07	-
EDTA	B3	No turbidity	12.96	-
B1	B3	6.64	6.34	-
Cit	B3	No turbidity	6.07	-
AsC	B3	No turbidity	6.02	-
EDTA	Sali	No turbidity	12.96	-
B1	Sali	9.00	6.34	-
Cit	Sali	No turbidity	6.07	-
AsC	Sali	No turbidity	6.02	-
B3	Sali	8.7	4.92	-
EDTA	Ura	No turbidity	12.96	21.47
B1	Ura	9.1	6.34	-
Cit	Ura	No turbidity	6.07	-

AsC	Ura	No turbidity	6.02	-
B3	Ura	8.77	4.92	-
Sali	Ura	10.00	4.76	-
EDTA	Para	No turbidity	12.96	-
B1	Para	8.00	6.34	-
Cit	Para	No turbidity	6.07	-
AsC	Para	No turbidity	6.02	-
B3	Para	8.04	4.92	-
Sali	Para	No turbidity	4.76	-
Ura	Para	9.11	4.49	-
EDTA	AsP	No turbidity	12.96	-
B1	AsP	6.6	6.34	-
Cit	AsP	No turbidity	6.07	9.8
AsC	AsP	No turbidity	6.02	10.58
B3	AsP	6.52	4.92	-
Sali	AsP	8.68	4.76	-
Ura	AsP	8.72	4.49	-
Para	AsP	7.86	3.42	-
EDTA	Caf	No turbidity	12.96	-
B1	Caf	6.5	6.34	-
Cit	Caf	No turbidity	6.07	-
AsC	Caf	No turbidity	6.02	-
B3	Caf	6.5	4.92	-
Sali	Caf	8.65	4.76	-
Ura	Caf	8.78	4.49	-
Para	Caf	7.08	3.42	-
AsP	Caf	6.2	3.34	-

These results were further confirmed theoretically by plotting composite curves. Figure 12 a and 12b reveal composite and mixed ligand titration curves drawn for two systems consisting of Cu^{2+}, in which ligand combination has formed a mixed complex with Cu^{2+}, while the other systems did not, respectively.

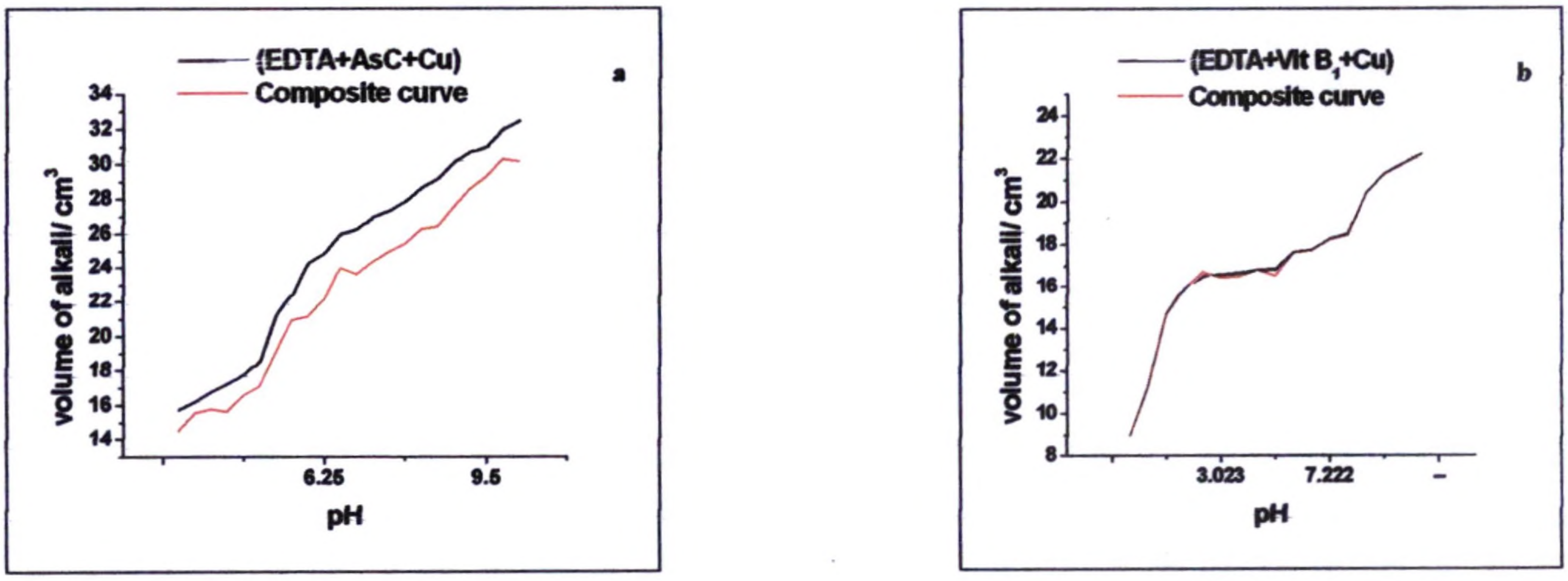
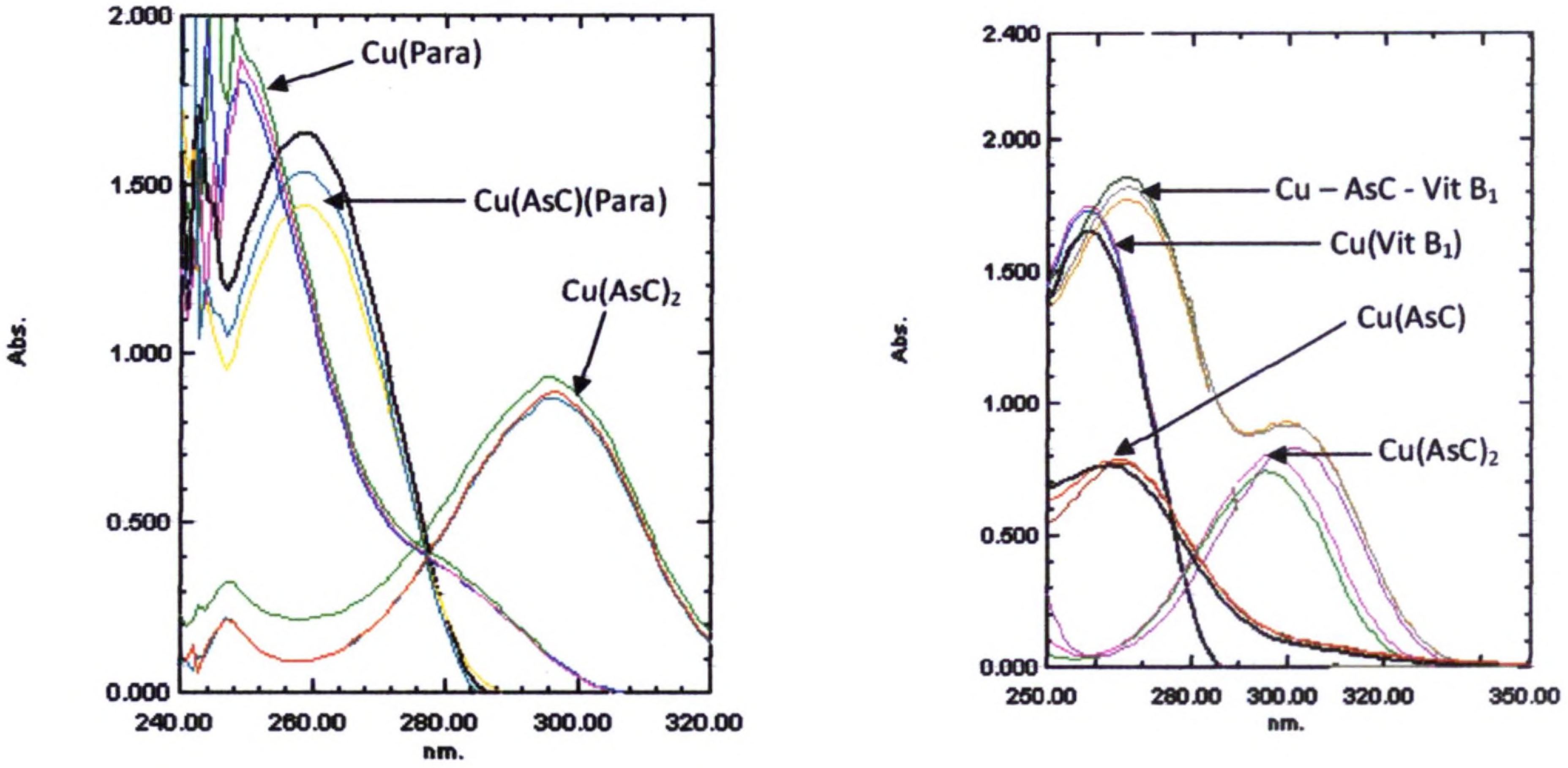


Figure 12: Composite curve and titration curve of a ternary system, in which **a.** formed **b.** did not form a mixed complex with copper.

In Figure 12a, titration curve of ternary system, EDTA-Cu-AsC has deviated from the composite curve calculated by confirming the formation of a mixed complex. However, this deviation was observed in composite curves of only five ternary systems of Cu^{2+}. All other composite curves and their respective titration curves were overlapped as same as in Figure 12b.

UV experimental results for copper mixed ligand systems

Figure 13a shows UV spectra for a system forming a mixed ligand complex with copper. In that figure a different λ_{max} value was obtained for the mixed complex. Figure 13b shows UV absorbance spectra for a ternary system not forming a mixed ligand complex with Cu^{2+}. Presence of two peaks in the spectra of mixed system corresponds to the two binary complexes formed in the reaction mixture.



(a) (b)

Figure 13 (a) UV absorption spectra for complexes; $Cu(AsC)_2$, Cu(Para) and Cu(AsC)(Para) complexes at three different pH values (b) UV absorption spectra for complexes; Cu(AsC), $Cu(AsC)_2$, Cu(Vit B_1) and mixed ligand system Cu-AsC-Vit B_1 at three different pH values

Comparative analysis of the Stabilities of single and mixed ligand complexes (Δ log K)

Stabilities of five Cu^{2+} mixed complexes formed compared to their respective binary complexes are listed in Table 31.

Table 31: Calculated Δlog K values for Copper ternary complex systems

Ternary complex	**Δlog K**
EDTA-Cu-AsC	1.54
EDTA-Cu-Cit	2.82
AsC-Cu-Para	3.37

AsP-Cu-Para	1.59
Ura- Cu -Para	2.88

With respect to Table 31 all Cu^{2+} ternary complexes formed are more stable than corresponding binary complexes as their Δlog K values are greater than one.

Discussion

The findings of this study indicate that most dietary ligands, analgesics and vitamin supplements frequently entered into the body can have strong interactions with these metal ions. Sometimes over individual molecules their mixtures have rather strong interactions with those metals resulting very stable mixed complexes. The most highly distributed metal; lead has shown much interesting results in this regards by forming larger number of mixed ligand complexes. In mild acidic, neutral or mild basic pH values lead formed stable binary complexes with ligands considered. According to the literature, lead is a borderline metal. Therefore, it acts in both ways either as a hard metal or as a soft one. Due to this reason, lead interacts with any ligand that carries at least one donor atom including N, O, S and P. When a ligand molecule carries several of these atoms, a chelated ring is observed[61]. However, the stability order observed for the binary complexes of lead can be different due to the functional groups attached to each considered ligand. Thus, the stability order for considered ligands is; EDTA > Vit B_1 > AsC > AsP > Sali > Ura > Cit > Vit B_3 > Para > Caf and that can also be well explained by taking the ligand structures. Vitamin B_3, paracetamol and Caffeine come at the end of the list as they do not form chelated rings with Pb^{2+}. Possible ligating sites of the molecule uracil are pyridine N atoms and carbonyl oxygen atoms. When NaOH is added, it can remove H^+ from –NH and make C=N before coordination[62] and that is a good molecular arrangement to chelate with metal ions. Due to the conjugation with ring double bond the donar capacity of the carbonyl oxygen of uracil is higher than a normal carbonyl group. Hence, the stability is higher in Ura-Pb(II) system than the other binary systems with caffeine and paracetamol, having carbonyl ligating sites. According to the structure of paracetamol, carbonyl or phenolic oxygen atoms are liable to coordinate with metal ions. However, coordination through both atoms cannot be possible at the same time since they are situated in para positions to each other specially in solutions when H_2O molecules are present in excess. Therefore, a single coordination bond through carbonyl carbon can be assumed with paracetamol. Thus, strong complex formation cannot be expected with lead in the solution.

As these ligands daily enter into the body via meals and drugs, such interactions are much significant for a person intoxicated with lead. Even though, stability values of single ligand complexes of lead with above ligands are much smaller than that of single ligand complex with EDTA, some ligands formed much stable mixed complexes with EDTA. Pb(EDTA)(AsC), Pb(EDTA)(Vit B_1) and Pb(EDTA)(Cit) are such complexes having very high stabilities. Moreover, some ligand mixtures of biologically important molecules also formed stable mixed complexes with lead. Thiamine plays an interesting role in this regard by forming five different mixed complexes with other ligands; ascorbic acid, salicylic acid, uracil and vitamin B_3. It is also very interesting to see that some ligand pairs containing a dietary ligand and an analgesic have strong interactions with lead at body temperature of 37^{0}C. Therefore, such ligand combinations like (ascorbic acid-Paracetamol), (salicylic acid-paracetamol) and (Vit B_3-paracetamol) may have some effects on the bioavailability of those individual ligands if they are entered together into a lead intoxicated person. However, caffeine can do a favour regarding the removal of lead from the body by the formation of stable Pb(Caf)(uracil) complex when taking several cups of tea per day, as uracil is available in the body as nucleic base.

A significant behaviour of Cd^{2+} with considered set of molecules was observed at higher pH values of around 8.50. After EDTA the best chelator of cadmium was vitamin B_1 as in the case of lead. Cadmium belongs to the category of soft metals[1]. Therefore, it prefers to bind with soft bases containing atoms in the order of S>N>O[63]. Vitamin B_1 is a special ligand in this regards, as it carries all these three atoms. In both ratios vitamin B_1+Cd binary complex has a stability constant ~ 10^4. In general, stability of cadmium complexes increases with increasing the order of coordinating sites in the ligand molecule[63]. Thus, strong complexes were found with EDTA, citric acid and vitamin B_1. On the other hand, due to the presence of aromatic rings, an extra stability has been observed in salicylic acid+Cd, Paracetamol+Cd and Vitamin B_3+Cd complexes although they are not mulidentate ligands. Though, caffeine alone did not show any strong interactions with cadmium, when it was mixed with paracetamol, formed a stable mixed complex revealing that for a person intoxicated with cadmium can have an advantage of removing cadmium by the use of this ligand combination. With cadmium also EDTA formed mixed complexes with Vitamin B_1, vitamin C, citric acid and salicylic acid, but with different stabilities. Results also revealed some considerable interactions of paracetamol with dietary ligands; ascorbic acid and citric acid in the presence of cadmium. Therefore, ligand combinations; (ascorbic acid-paracetamol) and (citric acid-paracetamol) may have some significant issues in the body, if they are administered together by a cadmium intoxicated person. On the other hand, he may need more paracetamol dosage than a normal person to get its real activity as a painkiller.

Findings of the work suggested totally a different complexation behaviour for Al^{3+} when compared with Pb^{2+} and Cd^{2+}. Depending on the ligands it formed complexes at any pH ranging from 2 to 11. ML_2 type aluminium complexes were common with the considered ligand set. Al^{3+} is a hard metal. Therefore, it prefers hard ligands/strong bases when complexes are formed. Among such bases, organic-oxygen and nitrogen containing compounds form complexes with higher stabilities[11]. Thus, it is expected to form stable complexes with variety of organic molecules including alcohols, ketones, aldehydes and ethers. Therefore, in this work, very stable complexes were observed with paracetamol, vit B_1 and uracil having basic oxygen and nitrogen atoms in their structures. It is also interesting to see that overall stabilities of these complexes were greater than the stability of Al(EDTA) complex. Aluminium did not have any interactions with caffeine and vitamin B_3. Ligand combinations among EDTA, Paracetamol, uracil and citric acid have formed highly stable mixed complexes with aluminium and their overall stability constant values were much higher than those of other three metals involved in this study. However, trend of aluminium mixed ligand complex formation towards considered ligand combinations was poor when lead or cadmium is considered. Besides, some mixed complexes were less stable than their corresponding binary complexes.

According to the results obtained, cu^{2+} preferred to have interactions with individual/single ligands of organic acids; ascorbic acid, salicylic acid and EDTA, consisting of more oxygen atoms in their structures. Theoretically, this observation is true because of being a borderline metal, Cu^{2+} has higher tendency towards the ligands having several oxygen and nitrogen atoms. Therefore, inside the body considerable interactions of these ligands can be expected with free copper ions. However, caffeine and vitamin B_3 did not form complexes with copper. Analgesics like aspirin and paracetamol also did have strong interactions with copper. Among the five mixed complexes found with copper three contained paracetamol as a ligand. Hence, it is clear that complexation ability of paracetamol has enhanced significantly when there was a second ligand in the system.

Structures proposed for the mixed ligand complexes of lead, cadmium, aluminium and copper are illustrated in Figures 14-17 respectively. However, the elucidation of more accurate structures of the complexes in the solution media is not so easy and that should be carried out with more advanced analytical techniques coupled with FT-IR and NMR spectroscopy.

Though the stability constant does not depends on the concentration of the starting compounds, in this study there was a significant difference in the stability constants calculated at two different concentrations, in which one was ten times lower. Specially, at lower concentration, the turbidity was observed at higher pH values, as the metal concentration was lower. In the calculations, pH values were taken up to the permanent turbidity. Thus, number of data points considered was greater in calculations at lower concentration. This also might affect the final value. However, at lower concentration, number of mixed ligand complexes formed was fewer especially in lead and cadmium.

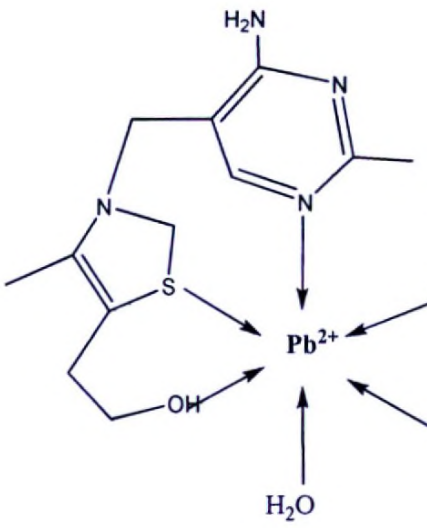
Mixed complex formation is dominated by aromatic ring stacking interactions[64-66] and hydrophobic interactions arising between the aliphatic side chains[67,68]. Hydrogen bonding interactions also can have the contribution to the stability of ternary complexes[69-72]. Another more important factor which enhances the stability of a mixed complex is electrostatic interactions arising between opposite charged moieties[73,74]. When two ligands together satisfy these stabilizing factors, a very strong mixed complex was observed in the presence of a metal ion. Majority of stable ternary complexes observed in this study contained acid-base ligand combinations (e.g. EDTA-B_1, AsC-B_1, B_1-Sali, Sali-Ura, B_1-Cit, AsC-Para, Sali-Para). Others mainly have electrostatic interactions and hydrogen boding interactions. Even though, many binary and ternary complexes were resulted, all of them may not give effective results in chelation therapy. Therefore, some more factors should be considered to decide the most suitable ligands/ligand mixtures for chelation therapy. Changing the pH of the medium can make some effects on the stability of complex. However, if a metal complex is stable over a range of pH and the formation constant is greater than 6.00 more suitable for used in chelation therapy[75.76]. Such complexes may not dissociate in the GUT (Gastro Intestinal Tract) or inside any organ and ultimately excreted from the body as metal complexes. Toxic metal ions present inside the body can be excreted by giving such individual or combinations of ligands. Moreover, it is very interesting to see that most mixed ligand complexes resulted in this study have stability values greater than six.

Conclusions

It was found that none of the dietary ligands, vitamins and analgesics considered is suitable for the chelation of lead and cadmium if they are used individually. However, solutions of citric acid, uracil, vitamin B_1 and paracetamol are well applicable in chelation therapy of aluminium through binary complex formation. Moreover, ascorbic acid is an effectual chelator of copper. However, as mixtures of (EDTA-Vit B_1), (EDTA-AsC) and (EDTA-Cit) showed the highest stability constants greater than M-EDTA by several hundred with all four metals, those will be the most effective mixtures for chelation therapy rather than using EDTA alone. Further, (AsC-Vit B_1), (Sali-Ura), (AsC-Para), (Ura-Para) will be very good chelating mixtures with harmless effects for lead and it has been clinically proven that (AsC-Vit B_1) can reduce the lead levels in liver of mice[77] and (AsC-Sali), (Cit-Para), (AsC-Para), (AsC-Cit) seem to be promising with chelation therapy of cadmium. As all mixed ligand complexes formed in this work had stability constants greater than 6 at the physiological pH and conditions, all will be very effective in chelation therapy, especially mixtures of vitamins, dietary ligands and uracil as they are non toxic to biological systems. As a nucleic base, uracil can show a good role in chelation therapy when it is taken with another suitable ligand.

On other hand, greater binding properties of paracetamol with four metals reflected that its therapeutic activity will be significantly lower for metal intoxicated person. On other hand bioavailability of vitamin

C, vitamin B_1 will be very low, if they are taken alone or together with other ligands like paracetamol and salicylic acid. However, caffeine and vitamin B_3 did not bind strongly with any of the four metals, thus it cannot make any influence in the body, if those toxic metals are present. Thus, findings of the study give some evidences for interesting behaviors of four metal ions with individual and mixtures of considered ligands at the biological conditions and it emphasizes an unrecognized property of those dietary ligands, vitamin supplements and analgesics.



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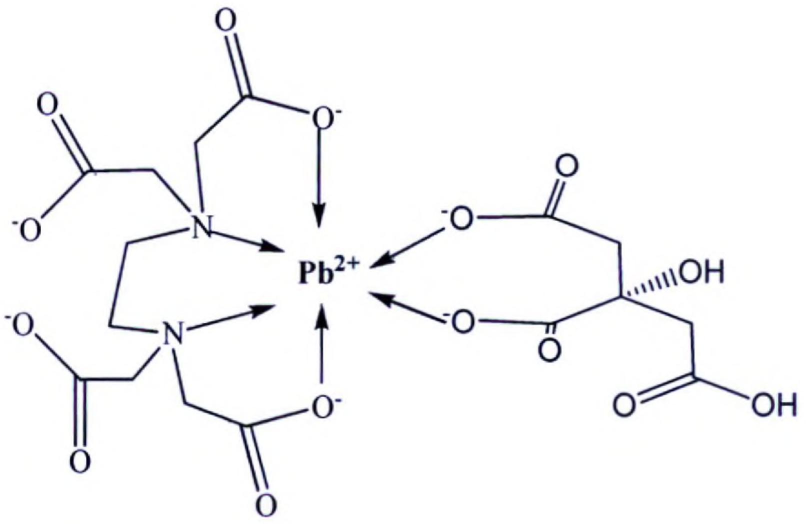

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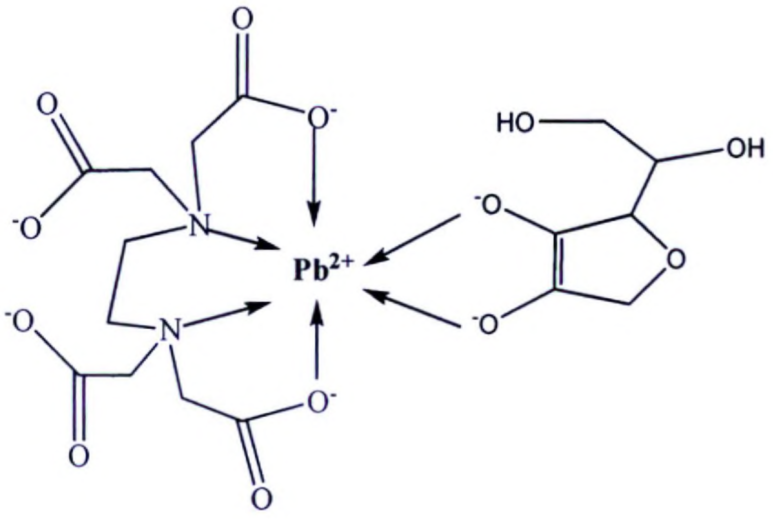

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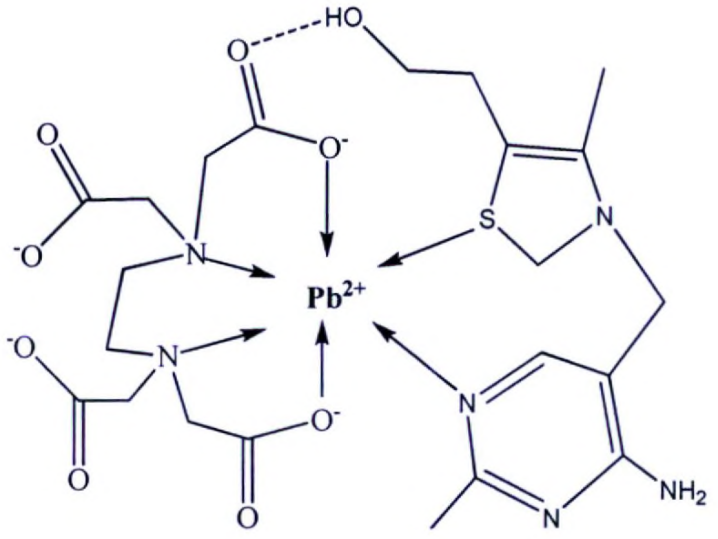

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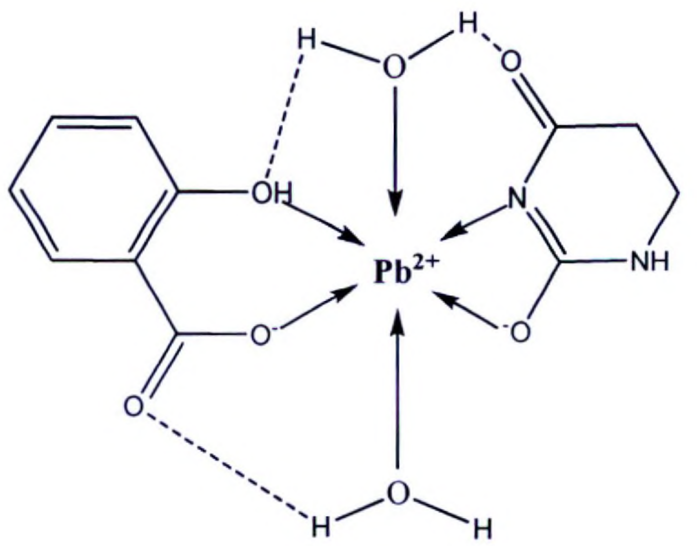

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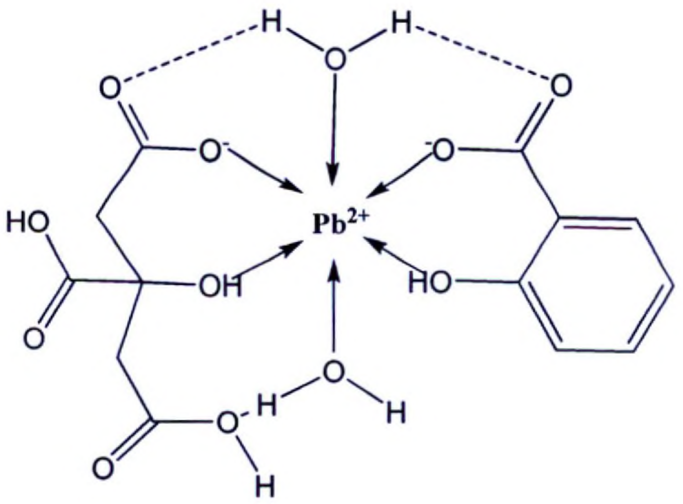

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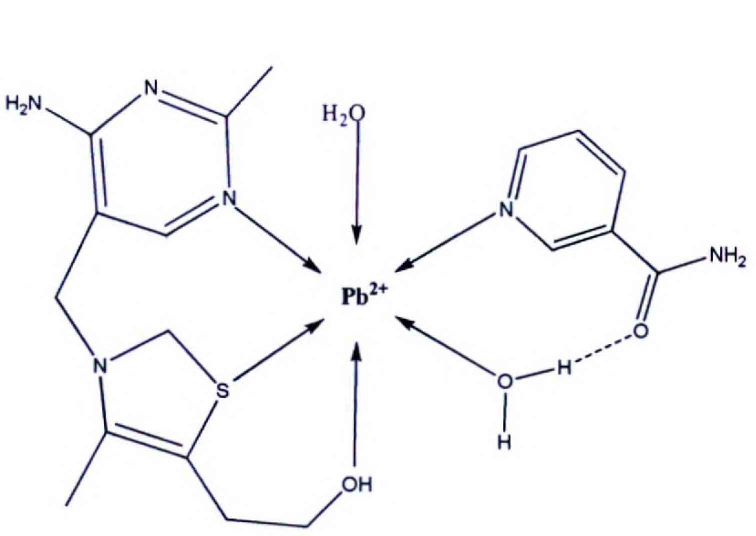

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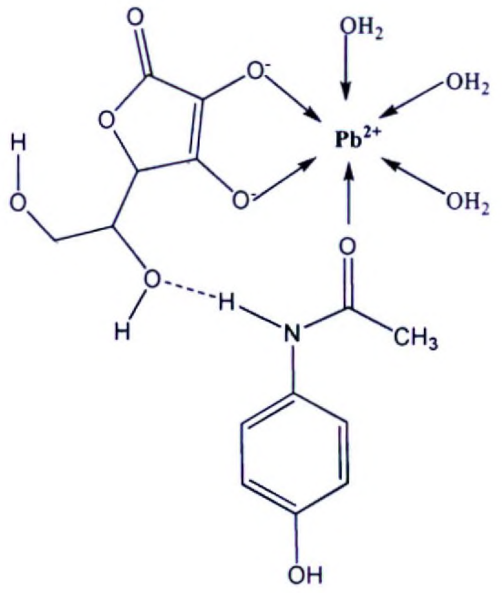

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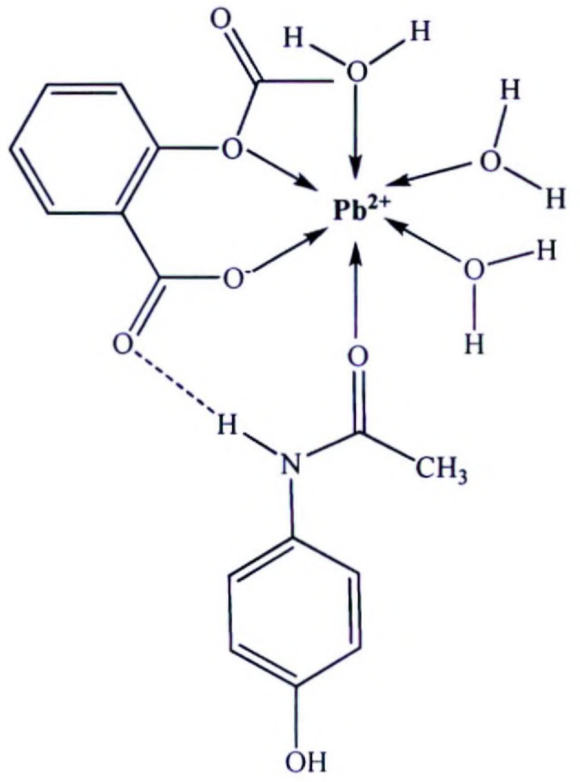

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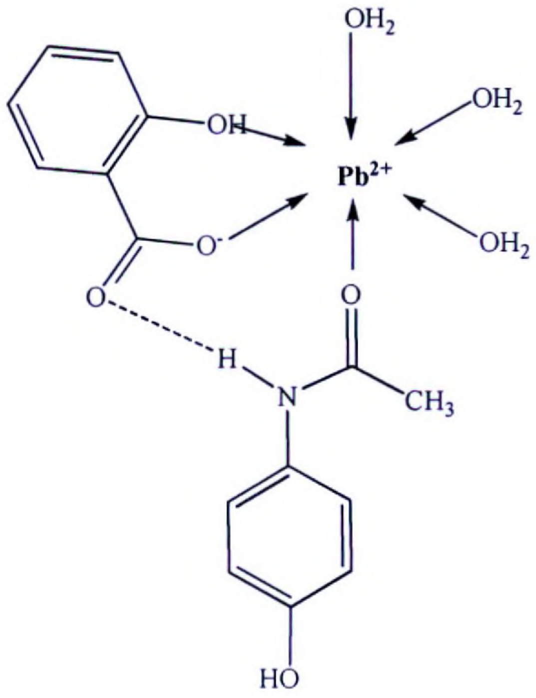

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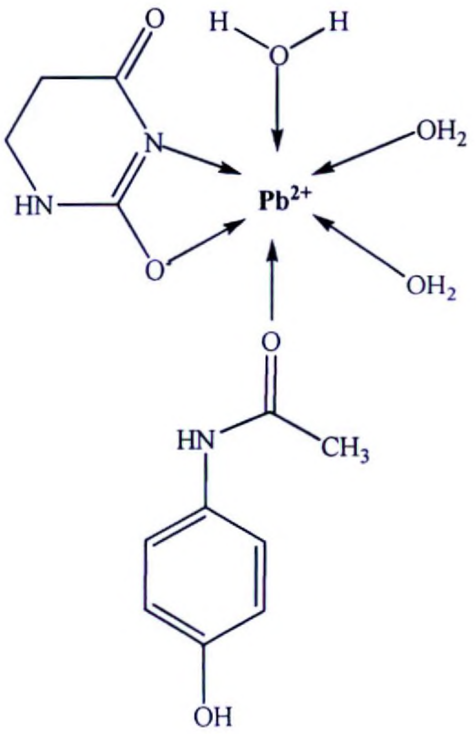

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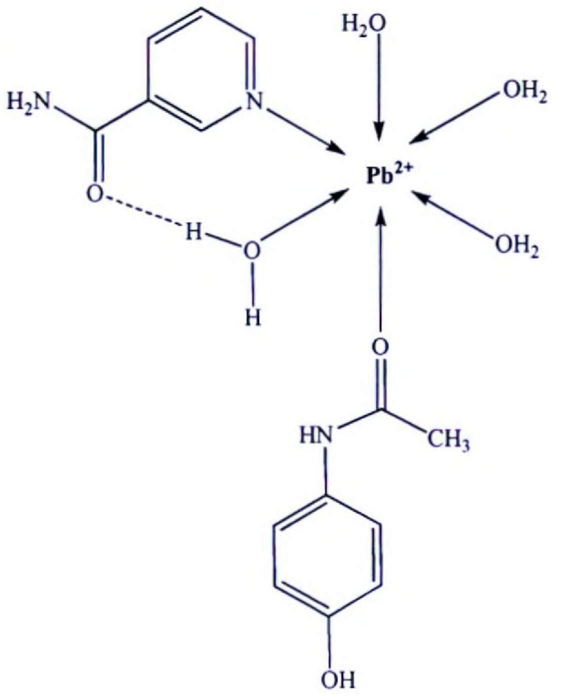

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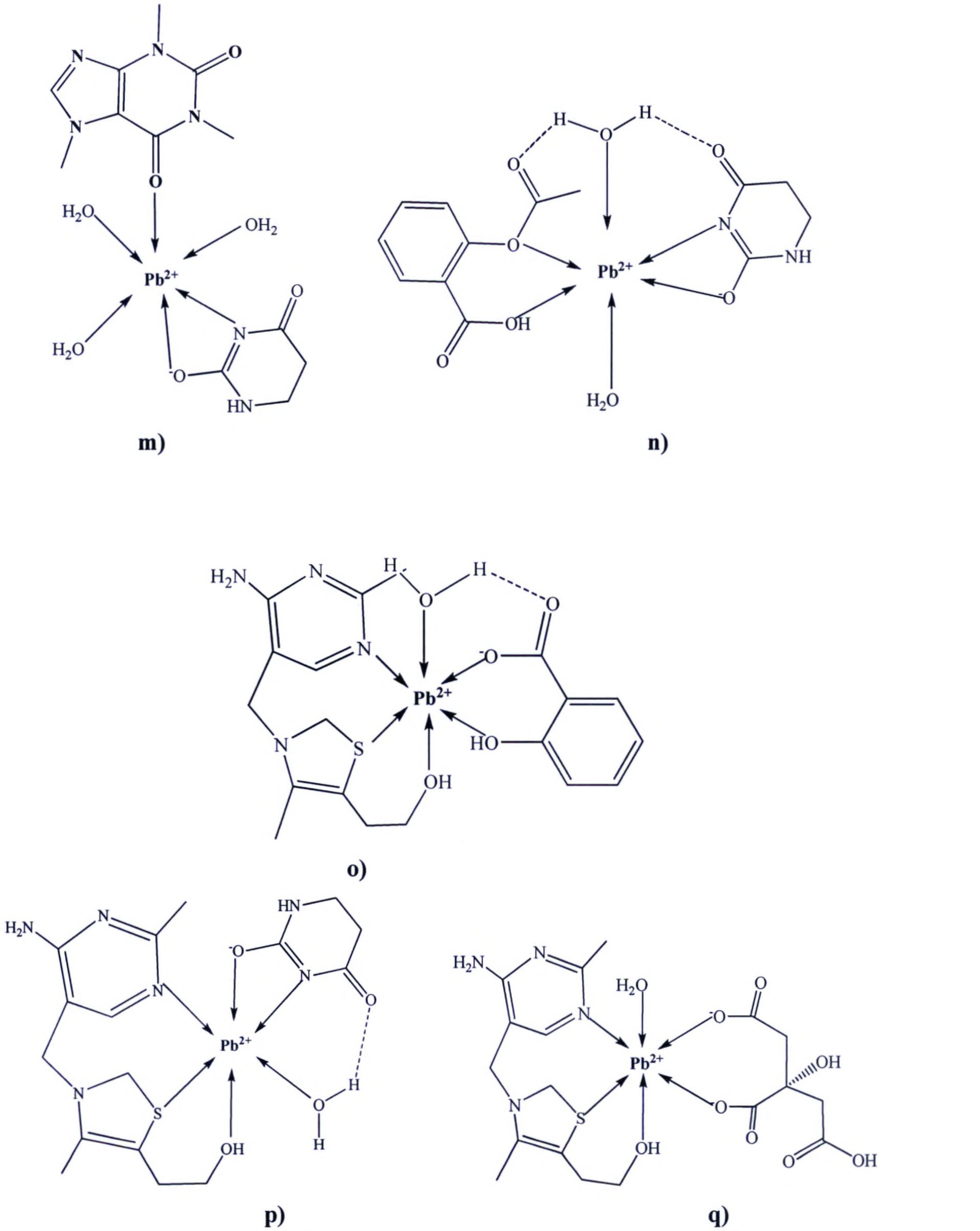
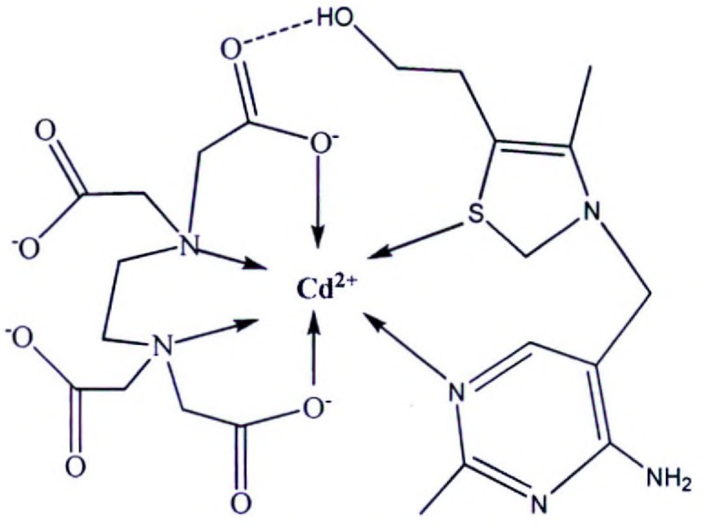


m) n) o) p) q)

Figure 14: Proposed structures for mixed ligand complexes of Pb^{2+} **a).**Pb(Vit B_1)(Asc) **b).**Pb(EDTA)(Cit) **c).**Pb(EDTA)(Asc) **d).**Pb(EDTA)(Vit B_1) **e).**Pb(Sali)(Ura) **f).**Pb(Sali)(Cit) **g).**Pb(Vit B_1)(Vit B_3) **h).**Pb(Asc)(Para) **i).**Pb(Asp)(Para) **j).**Pb(Sali)(Para) **k).**Pb(Ura)(Para) **l).**Pb(Vit B_3)(Para) **m).**Pb(Ura)(Caf) **n).**Pb(Asp)(Ura) **o).**Pb(Vit B_1)(Sali) **p).**Pb(Vit B_1)(ura) **q).**Pb(Vit B_1)(Cit)



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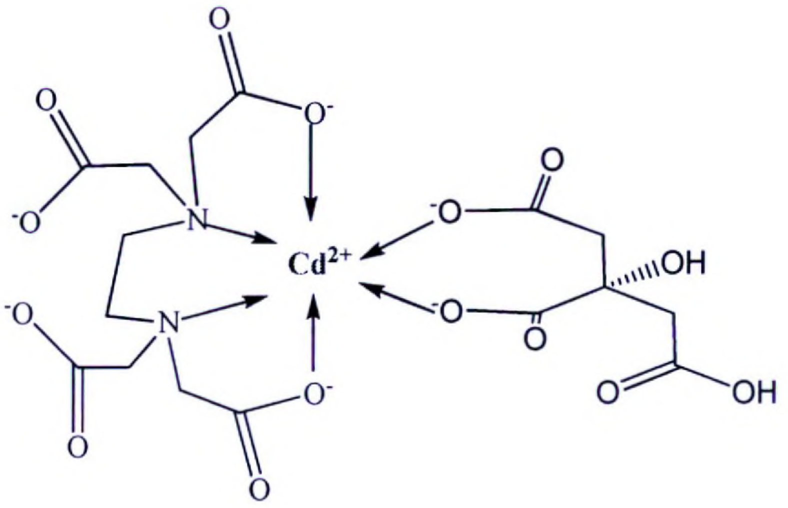

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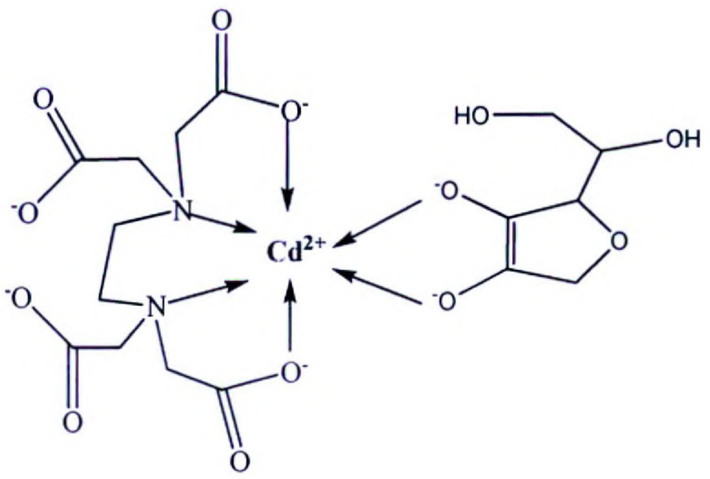

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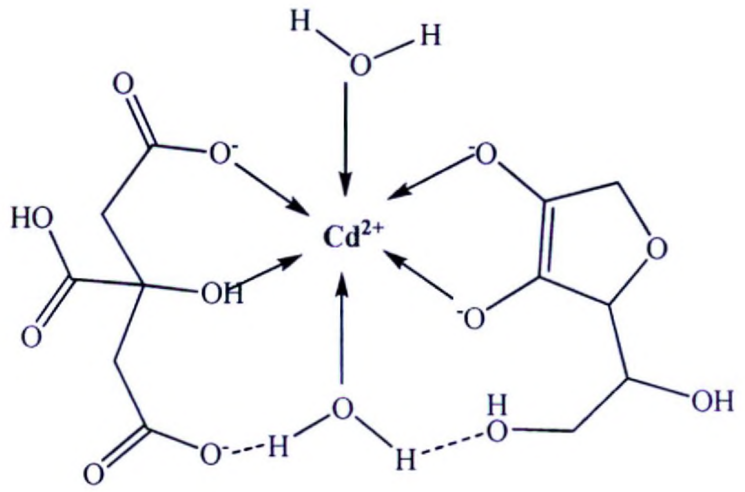

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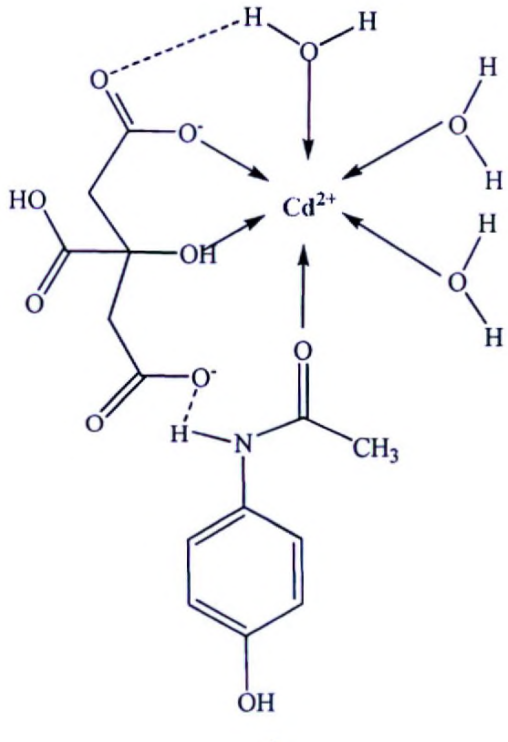

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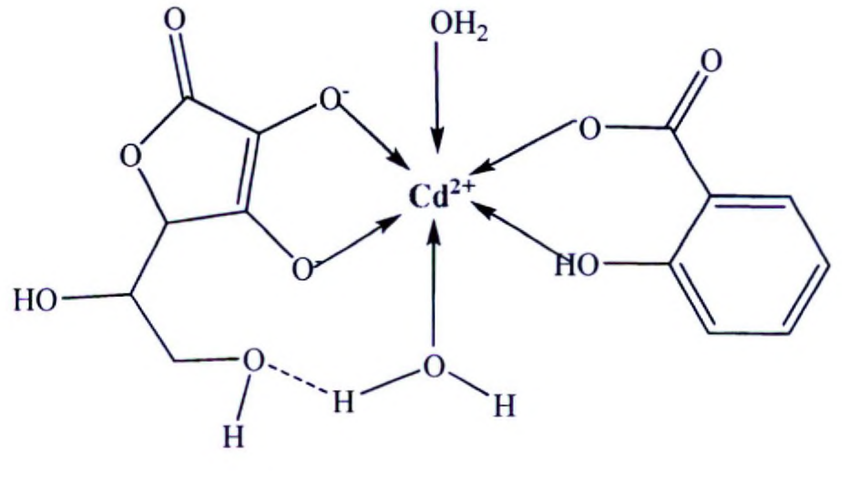
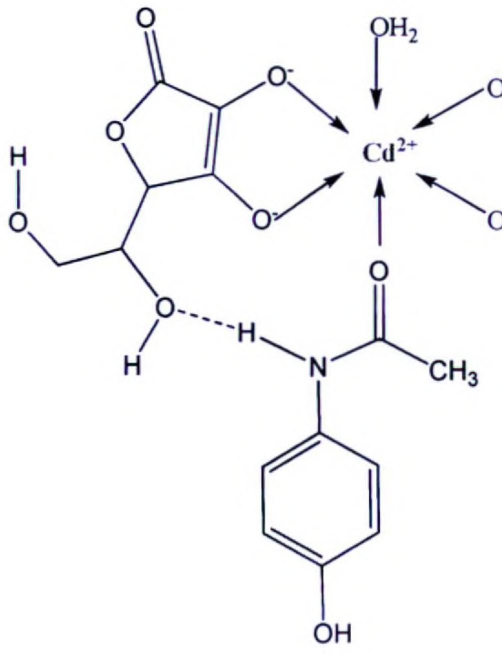
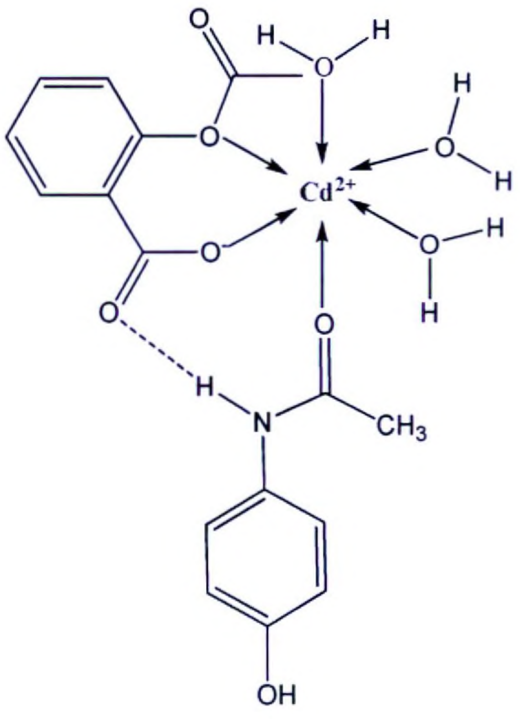


f)


O
O-
OH2
OH2
OH2
Cd2+
O
O-
H
O
O
H
H
O
N
CH3
OH

g)


O
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CH3
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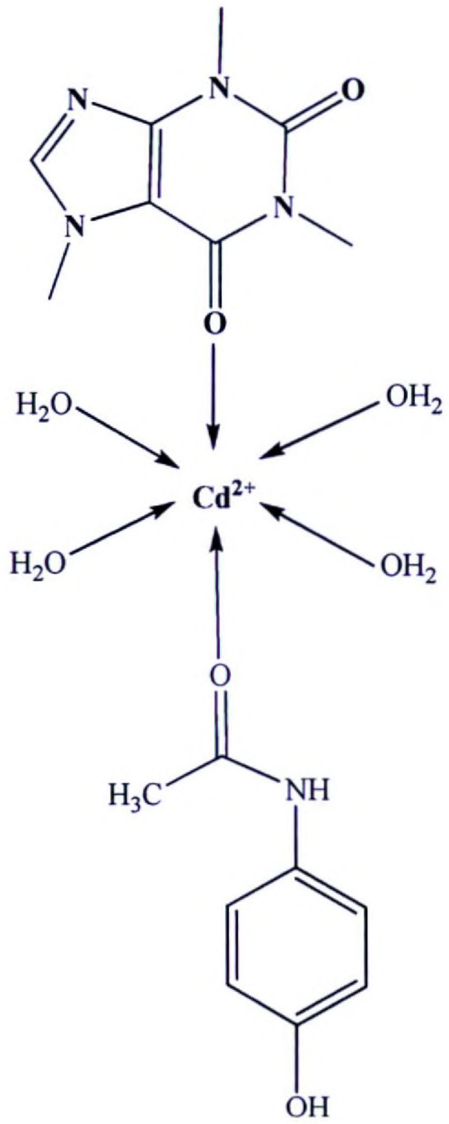
h)


N
N
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H2O
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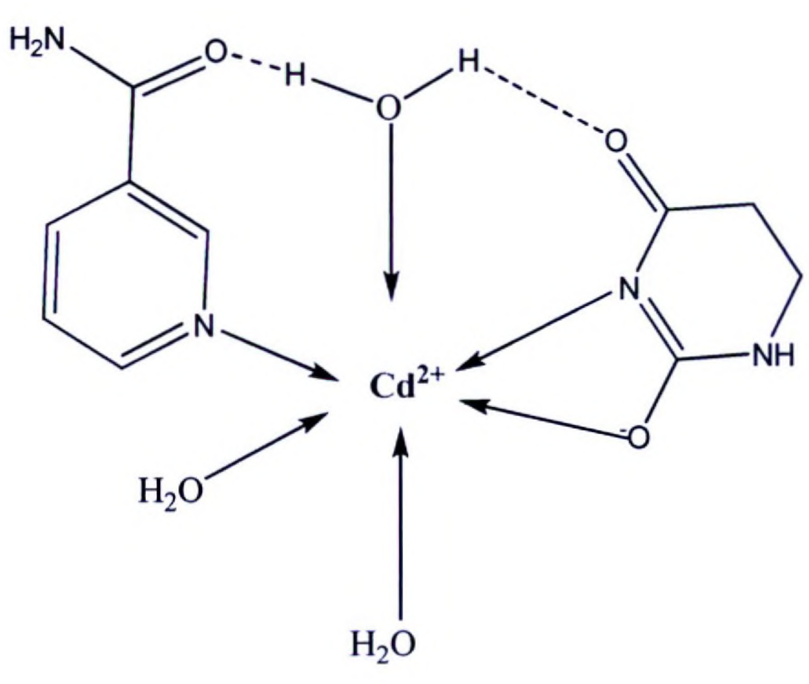
Cd2+
H2O
OH2
O
H3C
NH
OH

i)


H2N
O
H
O
H
O
N
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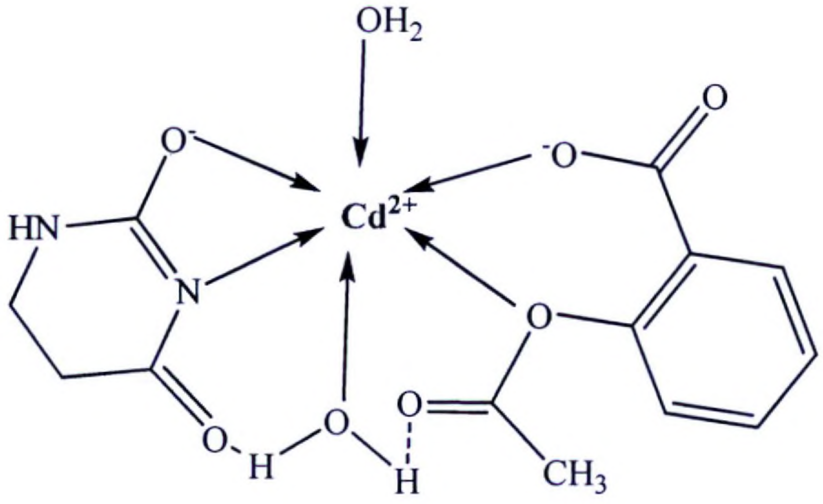
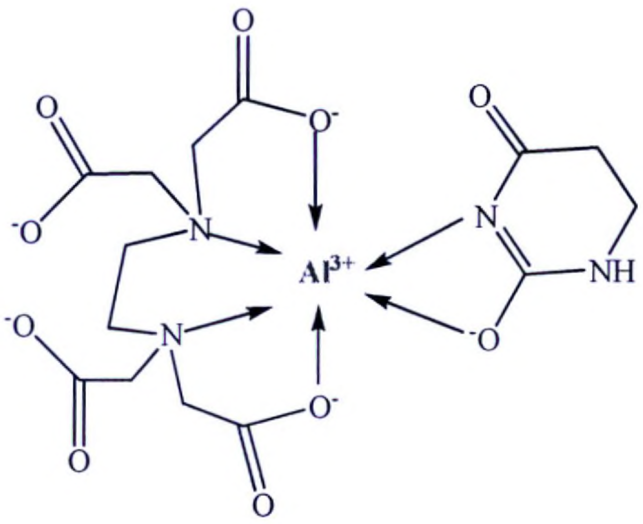
NH
Cd2+
O-
H2O
H2O

j)

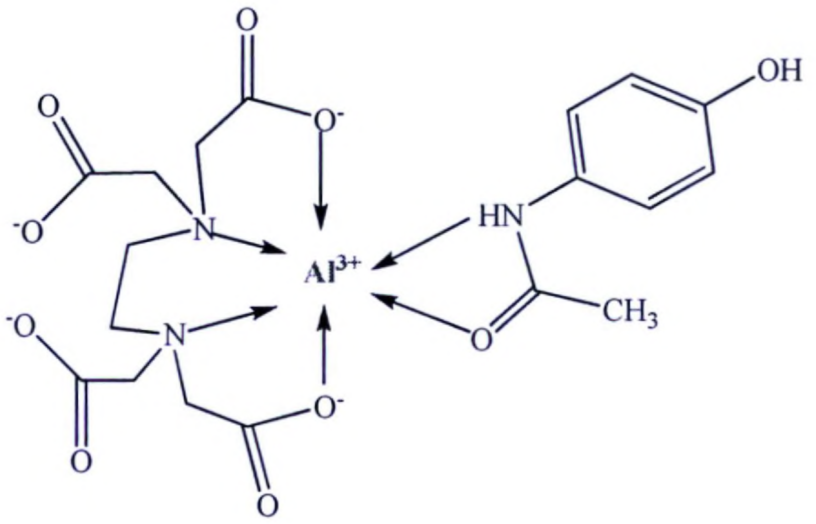


k)

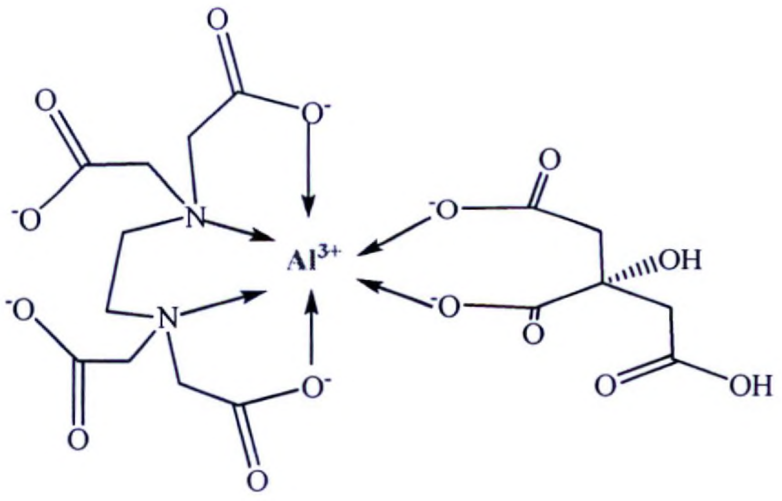
Figure 15: Proposed structures for mixed ligand complexes of Cd^{2+} **a)**.Cd(EDTA)(Vit B_1) **b)**.Cd(EDTA)(Cit) **c)**.Cd(EDTA)(Asc) **d)**.Cd(Asc)(Cit) **e)**.Cd(Cit)(Para) **f)**.Cd(Asc)(Sali) **g)**.Cd(Asc)(Para) **h)**.Cd(Asp)(Para) **i)**.Cd(Para)(Caf) **j)**.Cd(Vit B_3)(Ura) **k)**.Cd(Ura)(Asp)



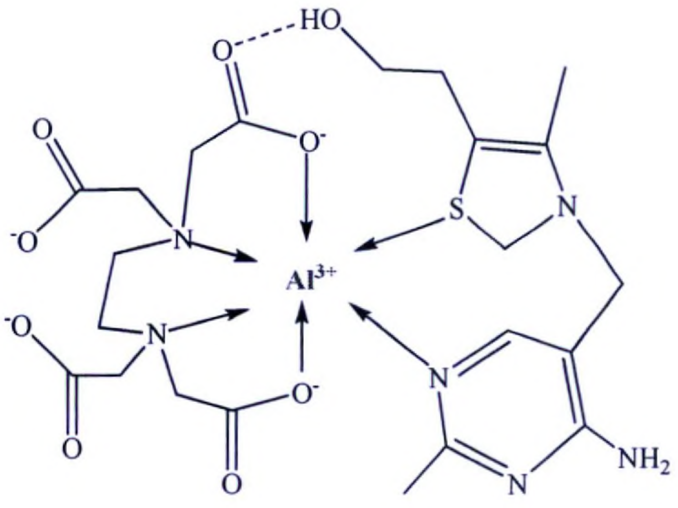
a)



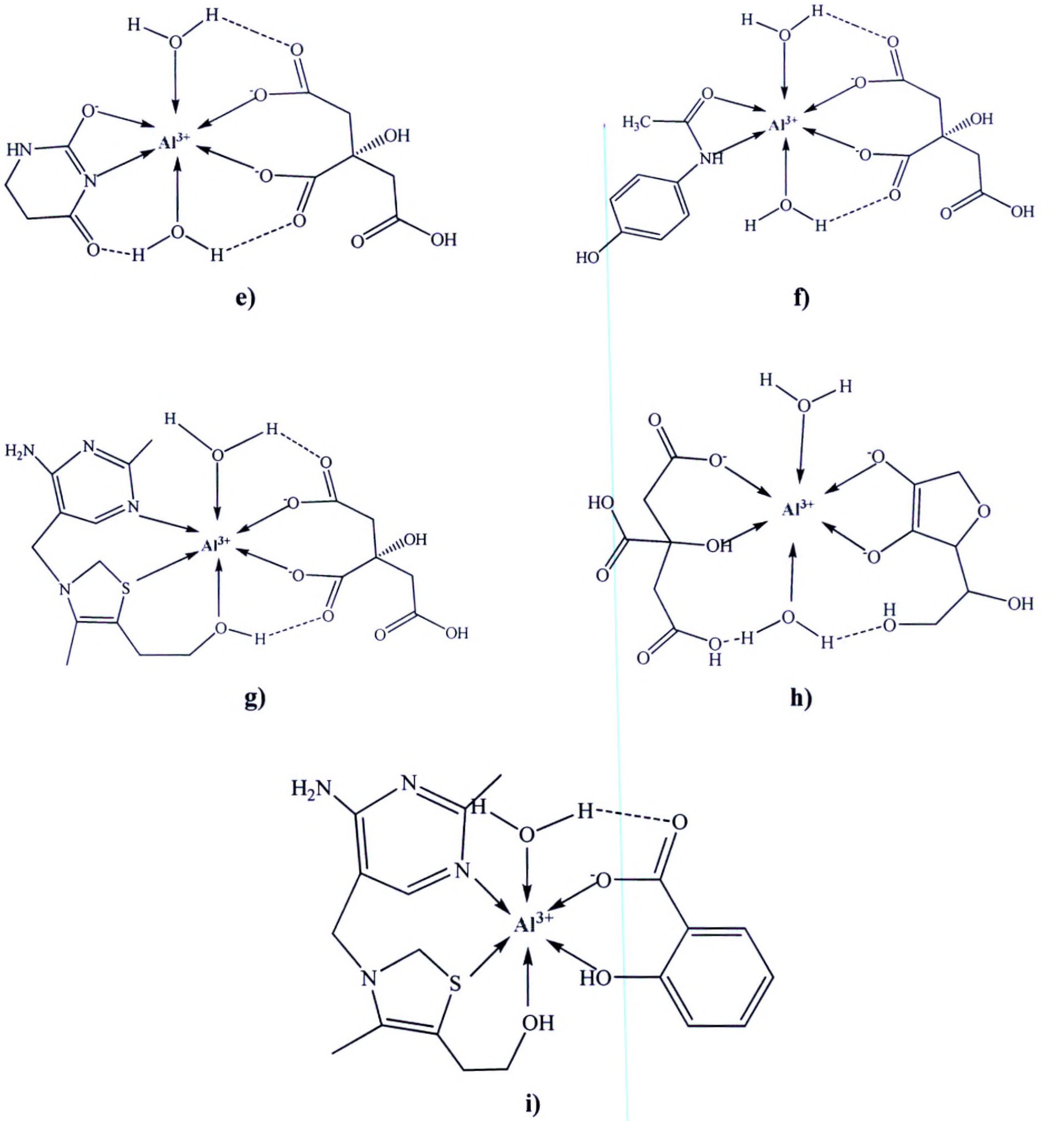
b)



c)

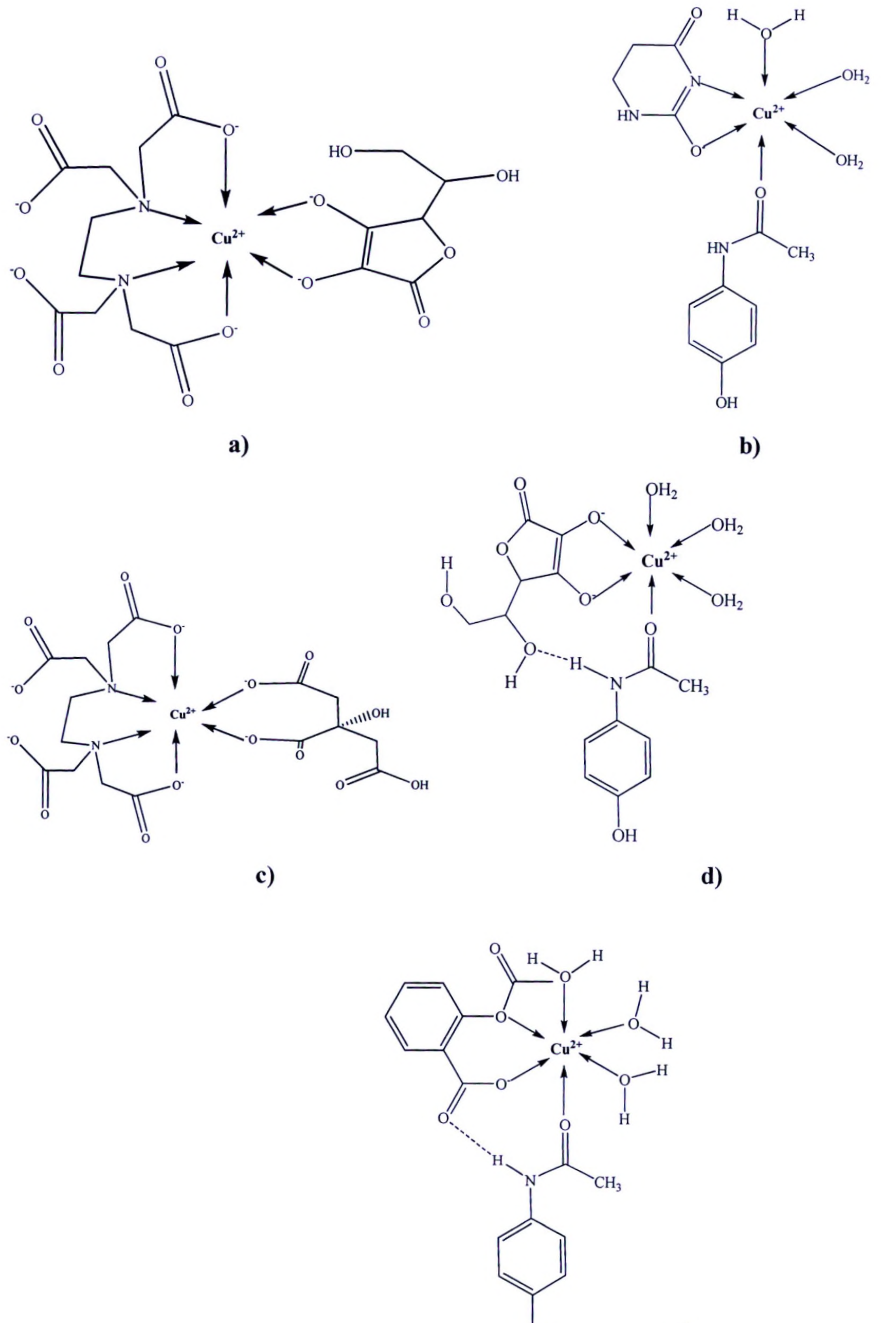


d)



e) f) g) h) i)

Figure 16: Proposed structures for mixed ligand complexes of Al^{3+} **a).**Al(EDTA)(Ura) **b).**Al(EDTA)(Para) **c).**Al(EDTA)(Cit) **d).**Al(EDTA)(Vit B_1) **e).**Al(Ura)(Cit) **f).**Al(Para)(Cit) **g).**Al(Vit B_1)(Cit) **h).**Al(Cit)(Asc) **i).**Al(Vit B_1)(Sali)



a) b) c) d) e)

Figure 17: Structures proposed for mixed ligand complexes of Cu^{2+} **a)**.Cu(EDTA)(AsC) **b)**.Cu(Ura)(Para) **c)**.Cu(EDTA)(Cit) **d)**.Cu(Asc)(Para) **e)**.Cu(Asp)(Para)

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Problems if any, encountered during the implementation of the project:

It took a long time to get the chemicals to our hands, as the purchasing of chemicals should be processed through a lengthy procedure that takes sometimes more than one year. In fact some of the chemicals ordered to purchase were not yet received, even after terminating the grant period.

During the grant period, there were technical problems with pH meter, thermostat, and UV-Visible spectrophotometer time to time and it took several weeks and months to repair them.

Though we had a plan to deduce the structures of major complexes formed using IR and NMR data, they were unable to work out as a result of technical faults, especially most of the time instruments were out of order.

Though it was supposed to calculate the data using a stability constant calculation software package, until the end of the project, it was unable to find out a reliable and good package free of charge to calculate the formation constants of especially mixed ligand systems. Thus, the calculation of constants manually using Microsoft excel took a long time. Moreover, until finding out the most reliable calculation method, formation constants of lead, cadmium and aluminium were calculated several times using different methods available in the literature.

Major findings and follow up activities.

Less harmful ligand mixtures for the chelation therapy of lead, cadmium, copper and aluminium were discovered as given in the conclusion. Besides, therapeutic ability of drugs and bioavailability of considered vitamins and natural ligands were found. Specifically, the interactions of paracetamol, the home drug for all aches and pains, with considered toxic metals were revealed.

Several manuscripts will be prepared within the next six months to send to national and international journals.

In the future interactions of these four metal ions with some more biologically important molecules and drugs used for common illness will be explored. Further, attempts are taken to recalculate the stability constants of binary and ternary systems of this project using a software, as an undergraduate project.

Section 4

Impact of research results:

Relevance of results achieved to scientific advancement

Results disclosed here will be very useful for scientist working on toxicology, bioinorganic chemistry, medicinal chemistry, environmental chemistry etc., as the project was mainly based on toxic metal-biologically and medicinally important ligands in the presence of each other in mixtures at the physiological conditions(*in vitro*).

Relevance of results achieved is useful not only to scientific field, but also to the people working at various industries based on raw materials of toxic metals, food chemistry, pesticides and all ordinary people. It is well known that most of the Sri Lankans have an inadequate knowledge on how the eating pattern, vitamin tablets and analgesics can affect the metal toxicity.

After publishing the data in peer reviewed journals, importance of results will be considered by other researchers, doctors and scientists in the world.

Relevance of results achieved to national/socio-economic development:

With the acceleration in industrialization, urbanization and excessive usage of pesticides and other toxic chemicals, the metal toxicity among Sri Lankans will be increased in the future. Hence it is necessary to keep them informed about the interactions between those metals and common drugs purchased over the counter and chelators in dietary substances to prevent the accumulation of toxic metals in the body. When, the people in a country is healthy, they can work harder. Therefore, the findings of the project will have a significant contribution to national development as well.

Dissemination/application of research output:

Four presentations were had at national research sessions. One manuscript was submitted to Indian Journal of chemistry-A and another 2/3 manuscript will be written within next 6 months based on the interesting results.

Section 5

Miscellaneous

List of major equipment acquired during the project and their functionality

Major Equipment	Functionality
Martini PH meter	O.K.

Thermostat	O.K.
Computer & a printer	O.K.

List of publications and communications arising from the project and or presentations made at seminars, workshops etc.

Chandrathilaka A. M. D. S, Hettiarachchi C.V and Ileperuma O. A., “Equilibrium study on binary and ternary complexes of Pb (II) with citric acid, uracil, caffeine, ascorbic acid, salicylic acid and paracetamol under physiological conditions, Sri Lanka Association for the Advancement of Science (SLAAS) , Annual research sessions, 2009

A. M. D. S . Chandrathilaka, C. V. Hettiarachchi and O. A. Ileperuma,Equlibrium study on binary and ternay complexes of Pb (II) withcommonly used drugs and Dietory ligands under physiological conditions”, Proceedings of the Peradeniya University Research Sessions, Sri Lanka, Vol. 14, 3rd December 2009

A. M. D. S . Chandrathilaka, C. V. Hettiarachchi and O. A. Ileperuma, “Complexation ability of Cd (II) with some organic ligands under physiological conditions”, Proceedings of the Peradeniya University Research Sessions, Sri Lanka, Vol. 15, 16th December 2010

A. M. D. S . Chandrathilaka, C. V. Hettiarachchi and O. A. Ileperuma, “Potentiometric study of Cd (II) and Al (III) binary and mixed systems with ascorbic acid, uracil and vitamin B_1 under physiological conditions, Sri Lanka Association for the Advancement of Science (SLAAS) , Annual research sessions, 2010

A.M.D.S. Chandrathilaka, C.V. Hettiarachchi* and O.A. Ileperuma, “Equilibrium Studies on Several Lead(II) Mixed Ligand Complexes Suitable for Chelation Therapy”, submitted to Indian Journal of Chemistry-A

Section 6

Summary statement of expenditure:

Personal: Rs. 877,067.00

Equipment: Rs. 254,009.40

Consumables: Rs. 103,199.01

Travel and subsistence: None

Miscellaneous : Rs. 149,556.00

Section 7

Grantee's signatures:

Comments of the Head of the Department/Signature:

Project successfully completed.

Head of the Institution's signature (PGIS director):

Head
Department of Chemistry
University of Peradeniya
Peradeniya, Sri Lanka.

Project completed successfully

29/2/2012

Prof. B. S. B. Karunaratne
Director
Postgraduate Institute of Science
University of Peradeniya, Peradeniya
SRI LANKA

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