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Equilibrium, Speciation and Bioactivity of Mg(II)/Ca(II)/Cd(II) with CTP and NA

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Abstract: *Biological activity and Equilibrium studies of solution have been taken at constant ionic strength I= 0.1 mol/l $NaNO_3$ and temperature $37+0.1^0C$ and the interaction of Mg (II), Ca (II) & Cd (II) divalent ions with Cytidine 5ˈ-triphosphate (CTP)&Nicotinic Acid (NA). The deep formation of 1:1:1 ternary and 1:1:1:1 quaternary complex was inferred from the potentiometric titration curve. Cytidine 5ˈ-triphosphate acts as a primary ligand in the ternary complexes involving the NA. The complexation model for systems of Cytidine 5ˈ-triphosphate (CTP) and the NA with the bio-metal ions have been established by the SCOGS Computer software from the potential metric data. The findings showed that nicotinic acid provides extra coordination sites, resulting in the development of stable mixed-ligand complexes, whereas CTP demonstrates a strong metal-binding affinity through its phosphate groups. Due to variations in ionic radius, charge density, and coordination preferences, the stability order of the complexes under investigation generally followed the trend Cd(II) > Mg(II) > Ca(II).*

Keywords: Divalent bio-metal ions, Cytidine 5ˈ-triphosphate (CTP), Nicotinic acid (NA), Potentiometer (pH -meter), ORIGIN 6.1 Software, SCOGS Computer programme.

I. INTRODUCTION

Mulberries have rich calcium sources, as 100 gm of it contains 39mg of calcium. Kiwi fruits come with more than 34mg of calcium every 100 gm. Other fruits high in calcium include blackberries these offer about 29 mg per 100 gm & milk cheese and other dairy foods, Green leafy vegetables such as curly kale, okra but not spinach (spinach does contain high level Calcium but the body cannot digest).

Human body needs calcium for building strong bone and teeth clotting blood, sending and receiving nerve signals and for muscles to move and for nerves to carry messages between your brain and every part of your body. Calcium also helps blood vessels move blood throughout your body and helps release hormones that affect many functions in your body.The food groups that contribute most of the dietary cadmium exposure are cereals and cereals products, vegetables, nut and pulses. Starchy roots are potatoes, meat and meat products. Due to their high consumption of cereals, nuts, oil seeds and pulses, vegetarian have a higher dietary exposure. Acute inhalation exposure to cadmium can result in flu-like symptoms like fever, muscle pain, etc. and can damage the lungs. Chronic exposure can result in kidney, bone and wing disease there is no clinical or experimental evidences that background environmental exposures to cadmium causes cancer. Cadmium toxicity primarily affects the lungs and kidney with secondary effects on the skeletal systems. There is no known disease associated with cadmium deficiency however since cadmium and zinc are closely related, Zinc deficiency can lead to cadmium toxicity. Women are more prone to cadmium toxicity than men.

Workers in industries producing or using cadmium have the greatest potential for cadmium exposure. Hobbyists such as jewellery makers and artists may also be at increased risk cigarette smoke can add to the body cadmium burden.

Cadmium has no known beneficial function in the human body. Cadmium is cumulative toxin. Cadmium is transported in the blood bound to metallothionein. The greatest cadmium concentration is found in the kidney and liver. Cadmium can enter the body from smoking tobacco, eating and drinking food and water containing cadmium and inhaling heat from the air. The skin does not easily absorb cadmium. The best screening and diagnostic test for chronic cadmium exposure is a 24-hour urinary cadmium label normalised to creatinine excretion. Globally, cadmium in white rice varied

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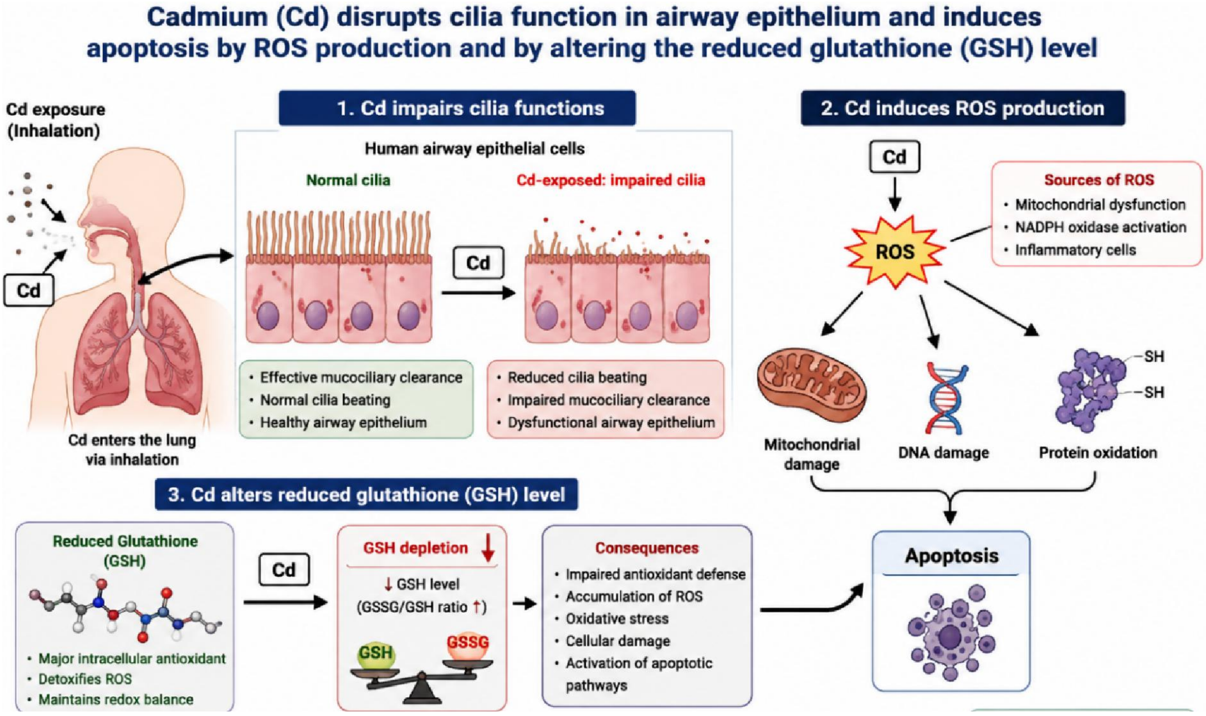
from less than 4.9 to 3712 microgram per kg with a global median of 19 microgram per kg. Cadmium (Cd) is a toxic heavy metal without known biological function in humans. It can be easily found in house dust and tobacco smoke [1]. As recently demonstrated in a human airway tissue model [2-5] Cd impairs cilia functions and enters the lung, where it can induce apoptosis by reactive oxygen species (ROS) production and by altering the reduced



Mechanism of ROS production and disruption of the redox balance

Nicotinic acid (niacin) is a water-soluble vitamin (B-3), which is used for the treatment of schizophrenia, hypercholesterolemia, diabetes, osteoarthritis, autoimmune diseases and pellagra [6]. Natural and synthetic derivatives of Nicotinic Acid are used in medicinal chemistry because of their physiological behaviour [7-9].

The effective centres of coordination with metal ions {Mg (II), Ca(II), Cd(II)} are the donor nitrogen atoms N(3) from pyrimidine bases and the oxygen atoms from the phosphate groups of the cytidine 5'-triphosphate (nucleotide). These centres are also the sites of non- covalent interactions with the other bio-ligands present in living organisms, such as small organic, polyamines, polycations or amino acids [10, 11].

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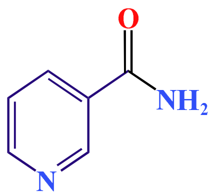
Natural Derivatives

Nicotinamide (Niacinamide)



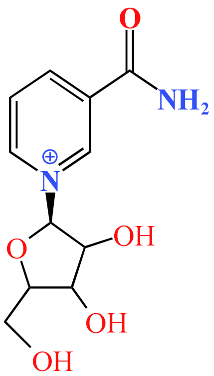
Found in:

Meat, Fish, Peanuts green vegetables

Physiological behaviour:

Precursor of NAD/NADP
DNA repair
Anti-inflammatory

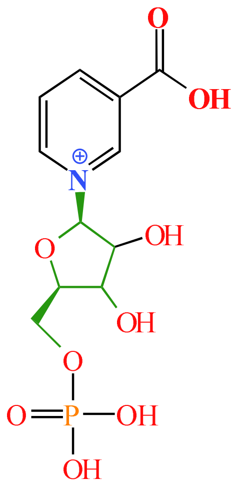
Nicotinamide Riboside



Milk, yeast, certain fruits, vegetables

+ Boost NAD^+ levels
MitochondrialFunction
Anti-aging effect

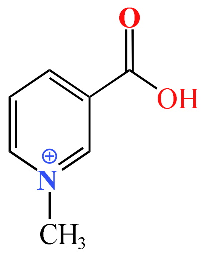
Nicotinic acid Mononucleotide



All living cells

NAD^+ biosynthesis
Support DNA repair

Trigonelline, Structurally, the N-methyl betaine of nicotinic acid (vitamin-B_3)

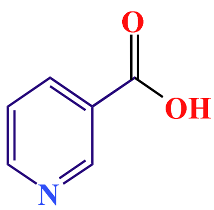


Coffee, Mushrooms

Antidiabetic
Antioxidant
Neuroprotective

Synthetic Derivatives

Niacin (Nicotinic acid)

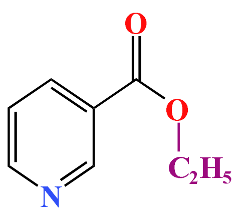


Uses:
Lipid lowering agent

Physiological behaviour:

Lovers LDL & VLDL
Raises HDL
Vasodilation

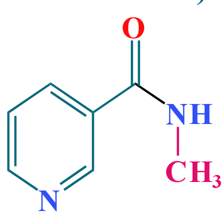
Nicotinate ester (Ethyl Nicotinate)



Vasodilator preparations

Improves circulation
Lowers blood presures
Anti-atherosclerotic

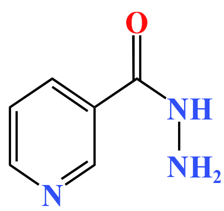
Nicotinamide derivative (N-Methyl Nicotinamide)



Investigational therapeutic agents

Neuroprotective
Anti-inflammatory
Anti-cancer potential

Nicotinic acid hydrazide



Anti-tubercular

Anti-microbial
Antitubercular
Enzyme-inhibition

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II. MATERIAL AND METHODS

Stock solutions of Nicotinic Acid and cytidine 5'- triphosphate were used as such Carbonate free sodium hydroxide solution which is prepared by standard method [12]. Metal nitrate solutions were prepared and standardized by EDTA titration. Solution of NaOH and HNO3 were prepared in double distilled water and standardized against standard oxalic acid as usual. The ionic strength of all mixture solutions was kept 0.01M NaNO3.The free acid concentration was kept 0.02M in each case. The following sets of solutions were prepared keeping the total volume 50 ml in each case. The molar ratio of binary ternary and quaternary system was taken in 1:1 ,1:1:1 and 1:1:1:1ratio respectively [13].

5ml NaNO3 (1.0M) + 5ml HNO3 (0.02M) + H2O
5ml NaNO3 (1.0M) + 5ml HNO3 (0.02M) + 5ml L1 (0.01M) + H2O
5ml NaNO3 (1.0M) + 5MLHNO3 (0.02M) + 5ml L2 (0.01M) + H2O
5ml NaNO3 (1.0M) + 5ml HNO3 (0.02M) + 5ml M (0.01M) + H2O
5ml NaNO3 (1.0M) + 5ml HNO3 (0.02M) + 5mlM (0.01M) + 5ml L1 (0.01M) + H2O
5ml NaNO3 (1.0M) + 5ml HNO3 (0.02M) + 5ml M (0.01M) + 5ml L2 (0.01M) + H2O
5ml NaNO3 (1.0M) + 5ml HNO3 (0.02M) + 5ml M (0.01M) + 5ml L1 (0.01M) + 5ml L2 (0.01M) + H2O
5ml NaNO3 (1.0M) + 5ml HNO3 (0.02M) + 5ml M1 (0.01M) +5ml M2 (0.01M) + 5ml L1 (0.01M) + 5ml L2 (0.01M) + H2O

Where M is {Cd(II), Mg (II) and Ca(II)} metal ions and L1, L2 are Nicotinic Acid and Cytidine 5'-triphosphate respectively. The titration curves of pH solution verses volume of NaOH for each set of solutions were plotted for potentiometric behaviour of solution at different pH. The species distribution curves were obtained by plotting % concentration vs. pH of the species through ORIGIN 6.1 software.

III. RESULTS AND DISCUSSION

3.1 Cd-CTP-NA System

Formation curve for Cd-CTP- NA system shown in fig. 1. The ternary complex species shows its remarkable presence at higher pH range. Protonated ligand species; HCTP, HNA both decreases with increasing pH, which is exist in higher concentration at different pH. The binary complex of Cd-CTP complex is the major binary complex species whose concentration 80% which is greater than Cd-NA at the same pH 3.8. It is clear from the

Table 1: Ternary complex (1:1:1) potentiometric titration of Cd-CTP-NA-system.

Volume of alkali (mL)	pH of solution			
	A	B	C	D
0.00	3.15	2.48	2.65	2.66
0.20	3.24	2.54	2.67	2.69
0.40	3.43	2.61	2.75	2.75
0.60	3.69	2.68	2.78	2.80
0.80	7.88	2.76	2.80	2.86
1.00	10.31	3.86	2.86	2.92
1.20	10.61	4.1	2.91	3.02
1.40	10.75	4.34	2.94	3.12
1.60	10.88	4.96	3.10	3.28
1.80	10.98	6.2	3.22	3.45
2.00	11.04	9.32	3.33	3.78

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2.20	11.11	9.48	3.59	4.24
2.40	11.16	11.52	3.98	4.69
2.60	11.20	11.98	6.06	5.14
2.80	11.25	12.48	7.06	5.72

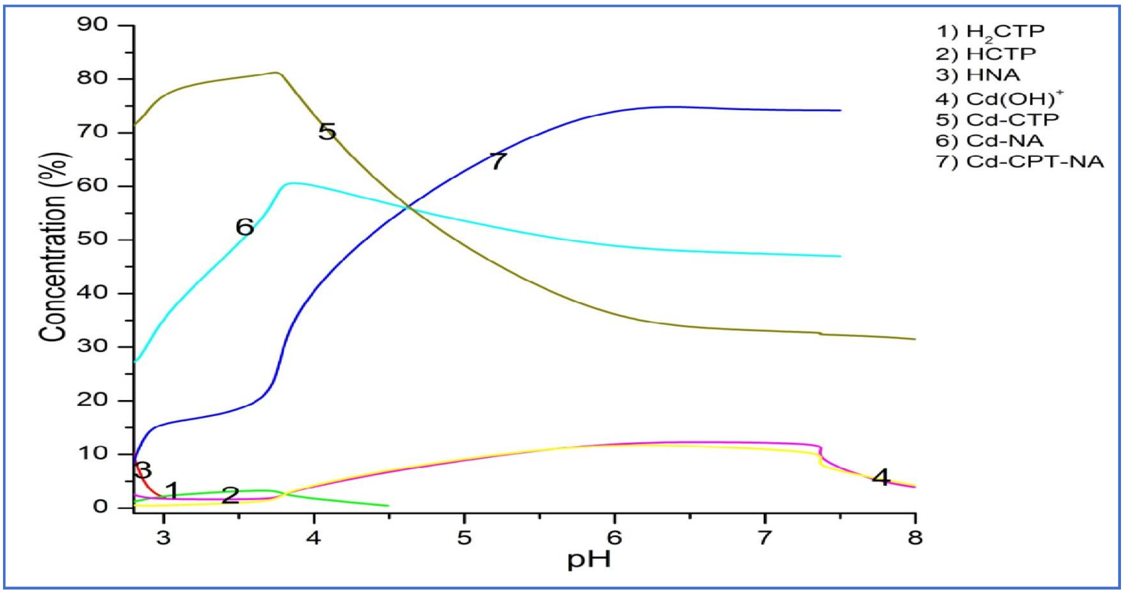

Fig. 1: Species formation curves of ternary (1:1:1) Cd-CTP-NA system.

speciation diagram that the Cd-NA complex is the minor binary complex species whose concentration is 60% at pH ~3.8. With further increase in pH, its concentration gradually decreases and the ternary species shows increase in concentration with further rise in pH ~ ⩾ 2.80. Therefore, a step wise formation of the binary and then ternary species is seen in the present systems [14]. The maximum concentration ternary complex is found to be ≈73.02% at pH ~6.7.

3.2 Ca-CTP-NA System

Speciation curve for Ca-CTP-NA system is represented by fig.2. Following species are assumed in the ternary system; Protonated ligand species viz. H2CTP, HCTP, HNA, binary species Ca-CTP and Ca -NA and ternary species Ca-CTP-NA. The tendency of complex formation in binary and ternary system of metal and ligand has represented their coordination behaviour in terms of stability [15]. This study, describes the relative stability constants and possible species concentration of metal complexes in binary and ternary system. The highest species percent concentration of binary complexes of Ca-CTP and Ca-NA systems was found to be ~45%, ~60% at different pH ranges from ~2.9, ~4.2 respectively, while (1:1:1) ternary system Ca-CTP-NA was found ~81% at (~3.5) pH [16,17].

Table 2; Ternary complex (1:1:1) potentiometric titration of Ca-CTP-NA-system.

Volume of alkali (mL)	pH of solution			
	A	B	C	D
0.00	2.42	2.48	2.75	2.67
0.20	2.5	2.54	2.8	2.75
0.40	2.56	2.61	2.88	2.87
0.60	3.6	2.68	3.04	3.01

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0.80	3.72	2.76	3.23	3.8
1.00	3.81	3.86	3.78	3.73
1.20	4.02	4.1	5.48	4.49
1.40	4.3	4.34	6.59	5.83
1.60	4.32	4.96	8.01	6.7
1.80	4.48	6.2	10.02	7.68
2.00	6.36	9.32	10.49	10.13
2.20	9.35	9.48	10.77	10.6
2.40	11.56	11.52	10.93	10.83
2.60	12.42	11.98	11.02	10.95
2.80	……	12.48	11.11	11.02
3.00	……	……	11.17	11.10
3.32	……	……	11.30	11.16

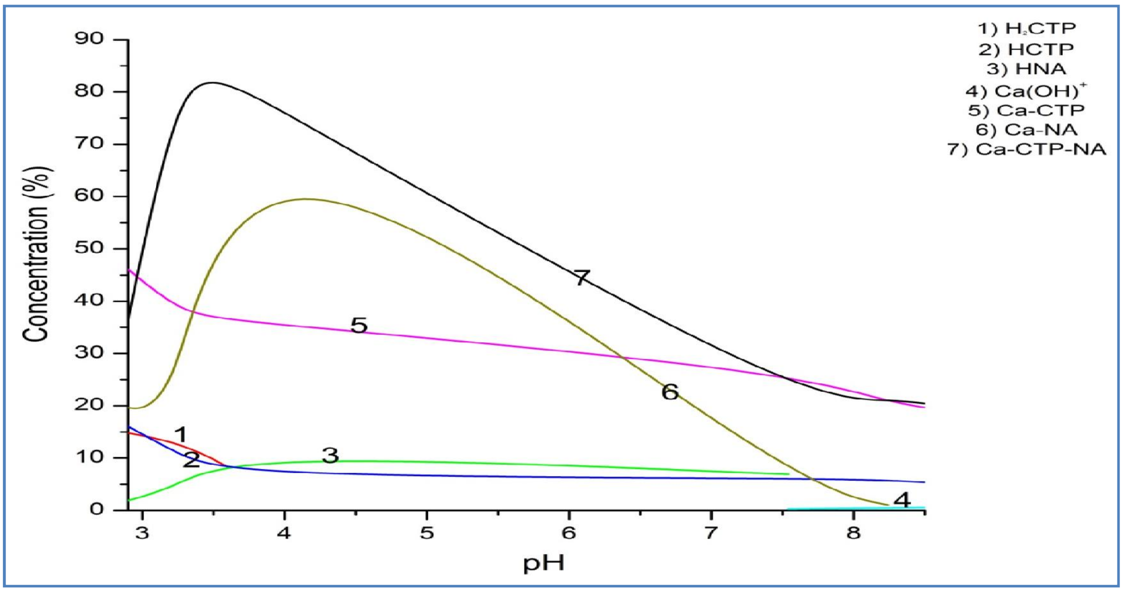

Fig. 2: Species formation curves of ternary (1:1:1) Ca-CTP-NA system.

3.3 Mg-CTP-NA System

The species distribution curves for ternary Mg-CTP-NA complex shown in fig.3. The tendency of complex formation in binary and ternary system of metal and ligand has also represented their coordination behavior in terms of stability. Protonated ligand species; H2CTP, HCTP, HNA all are formed low concentration range 0-20% at lower pH. This study, describes the relative stability constants and possible species concentration of metal complexes in binary and ternary system. The highest species percent concentration of (1:1) binary complexes of Mg-CTP, and Mg-NA systems were found to be 20%, and very low concentration at different pH range from ~ 6-6.7, and ~ 8-8.95 respectively, while (1:1:1) ternary system Mg-CTP-NA is 75.5% at pH =7. The similar pattern of complexes formation also reported by previous researchers [18].

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Table 3 Ternary (1:1:1) complex potentiometric titration of Mg-CTP-NA system.

Volume of alkali (mL)	pH of solution			
	A	B	C	D
0.00	2.42	2.48	2.73	2.75
0.20	2.5	2.54	2.78	2.79
0.40	2.56	2.61	2.88	2.83
0.60	3.6	2.68	3.03	2.91
0.80	3.72	2.76	3.2	2.99
1.00	3.81	3.86	3.77	3.10
1.20	4.02	4.1	4.91	3.22
1.40	4.3	4.34	6.78	3.45
1.60	4.32	4.96	10.53	3.88
1.80	4.48	6.2	10.76	4.35
2.00	6.36	9.32	11.00	4.99
2.20	9.35	9.48	11.09	5.56
2.40	11.56	11.52	11.11	6.21
2.60	12.42	11.98	11.17	7.06
2.80	……	12.48	11.22	7.80
3.00	…..	…….	11.27	8.13
3.32	…..	……	11.30	8.65

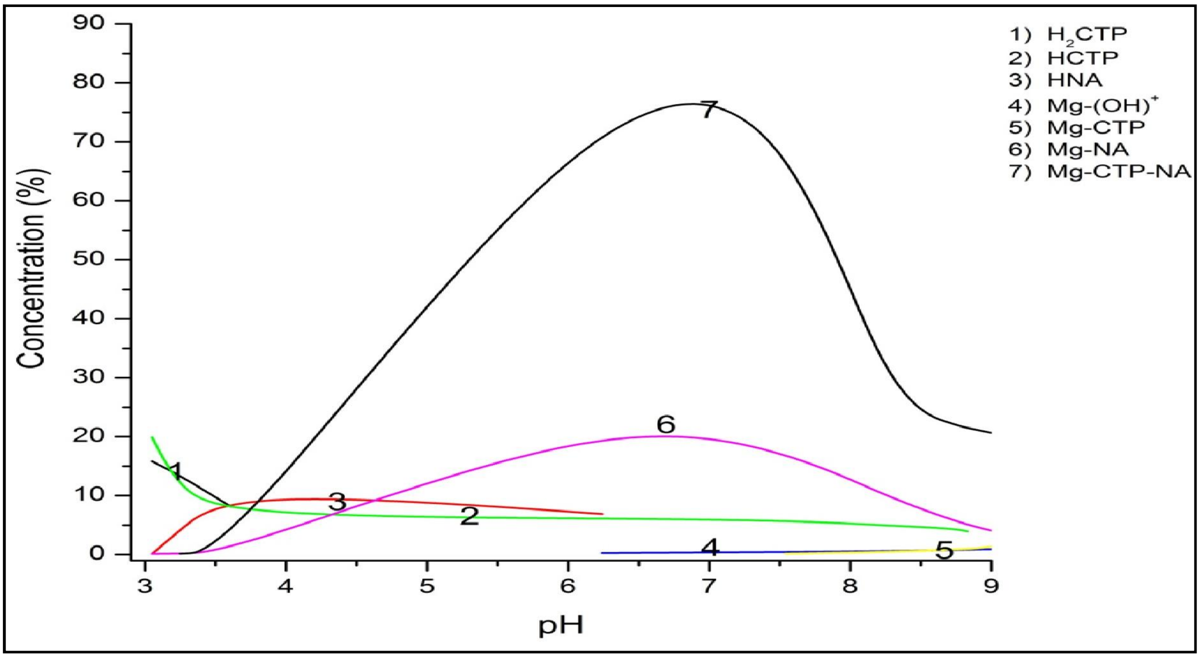

Fig. 3: Species formation curves of ternary (1:1:1) Mg-CTP-NA system.

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3.4 Mg-Ca-CTP-NA System

The Mg-Ca-CTP-NA species are depicted in Fig. 4 shows the formation of different species such as H3CTP, HCTP, HNA, Ca-CTP, Ca-NA, Mg-CTP, Mg-NA, Ca-CTP-NA, Mg-CTP-NA, Mg-Ca-CTP-NA. The protonated ligand species H3CTP, found at lower pH~3.04 is ~25% concentration. HCTP is very low concentration and HNA concentration reaches up to ~39% at low pH ~ 3.2. Species distribution curve of metal hydroxo species is not formed. The concentration of Mg-CTP, Mg-NA were found parallel concentration and reaches maximum concentration ~12% and ~7% at ~3.2 and ~3.5 pH, respectively [19]. The Ca-CTP, Ca-NA both were found in negligible amount. The ternary Mg-CTP-NA species were found to be lower concentration whereas the concentration of Ca-CTP-NA complex reaches maximum concentration ~ 62.2% at higher pH range ~ 6.4-9.0. The quaternary Mg-Ca-CTP-NA forms at the beginning of the titration and presents in the maximum concentration ~ 70.2% at lower pH ~ 3.2 and after that decreases with increasing pH

Table 4. Quaternary (1:1:1:1) complex potentiometric titration of Mg-Ca-CTP- NA.

Volume of alkali (mL)	pH of solution				
	A	B	C	D	E
0.00	2.42	2.48	2.75	2.67	2.75
0.20	2.5	2.54	2.79	2.75	2.81
0.40	2.56	2.61	2.83	2.87	2.92
0.60	3.6	2.68	2.91	3.01	3.05
0.80	3.72	2.76	2.99	3.8	3.30
1.00	3.81	3.86	3.10	3.73	3.67
1.20	4.02	4.1	3.22	4.49	4.64
1.40	4.3	4.34	3.45	5.83	5.49
1.60	4.32	4.96	3.88	6.7	6.77
1.80	4.48	6.2	4.35	7.68	8.86
2.00	6.36	9.32	4.99	10.13	10.12
2.20	9.35	9.48	5.56	10.6	10.54
2.40	11.56	11.52	6.21	10.83	10.80
2.60	12.42	11.98	7.06	10.95	10.92
2.80		12.48	7.80	11.02	11.03
3.00			8.13	11.10	11.10
3.20			8.65	11.16	11.13
3.40					11.18
3.60					11.31

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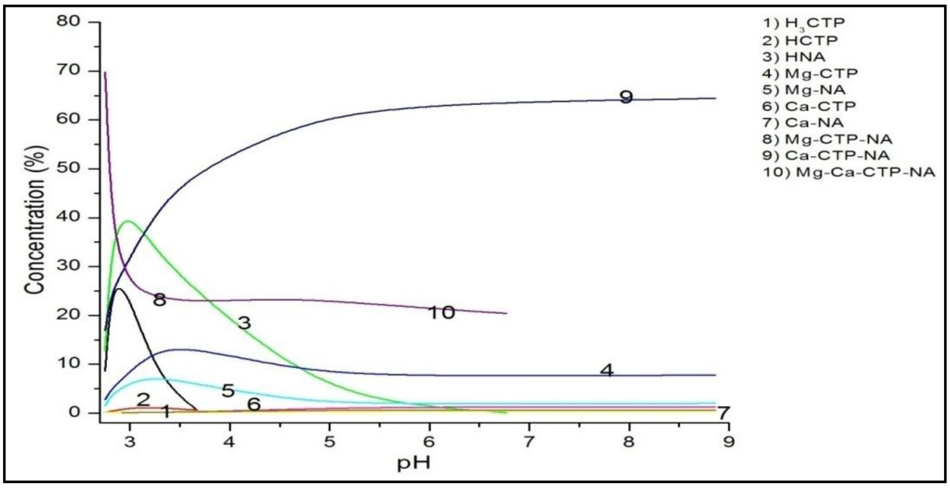

Fig. 4: Species formation curves of quaternary (1:1:1:1) Mg-Ca-CTP-NA system.

3.5 Cd-Ca-CTP-NA System

Fig. 5 of Cd-Ca-CTP-NA system shown the speciation curves of H3CTP, H2CTP, HNA, Ca-CTP, Ca-NA, Cd-CTP, Cd-NA, Ca-CTP-NA, Cd-CTP-NA, Cd-Ca-CTP-NA species The protonated ligand species H3CTP, H2CTP, HNA were found to be very low concentration.

Volume of alkali (mL)	pH of solution				
	A	B	C	D	E
0.00	2.42	2.48	2.66	2.67	2.77
0.20	2.5	2.54	2.69	2.75	2.86
0.40	2.56	2.61	2.75	2.87	2.95
0.60	3.6	2.68	2.80	3.01	3.13
0.80	3.72	2.76	2.86	3.8	3.34
1.00	3.81	3.86	2.92	3.73	3.90
1.20	4.02	4.1	3.02	4.49	4.55
1.40	4.3	4.34	3.12	5.83	5.37
1.60	4.32	4.96	3.28	6.7	6.26
1.80	4.48	6.2	3.45	7.68	6.78
2.00	6.36	9.32	3.78	10.13	8.26
2.20	9.35	9.48	4.24	10.6	9.93
2.40	11.56	11.52	4.69	10.83	10.50
2.60	12.42	11.98	5.14	10.95	10.70
2.80		12.48	5.72	11.02	10.88
3.00				11.10	11.04
3.20				11.16	11.12

Table 5: Quaternary complex 1:1:1:1 potentiometric titration of Cd-Ca-CTP- NA-system.

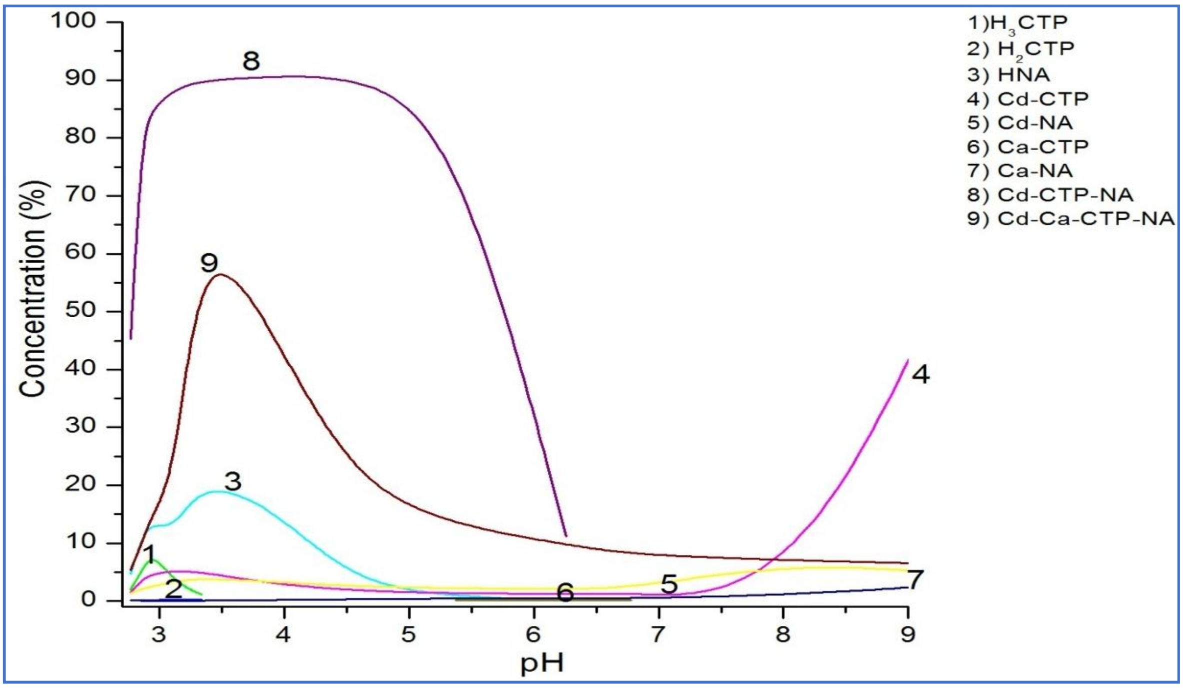

Fig.5: Species formation curves of quaternary (1:1:1:1) Cd-Ca-CTP-NA system.

The concentration of Cd-CTP and Cd-NA species were found to have constant concentration up to pH~ 2.7-7 after that pH concentration increases with increasing pH and cover maximum concentration ~ 40% and ~ 8% at same pH~ 9 respectively. The ternary Cd-CTP-NA and Ca-CTP-NA species firstly increases with increasing pH and obtained maximum concentration ~88.9% and 5.2% at same pH 2.8. The quaternary Cd-Ca-CTP-NA forms at the beginning of the titration and presents in the maximum concentration ~ 55.2% at lower pH ~ 3.47 and after that decreases with increasing pH.

IV. CONCLUSION

The ternary mixed ligand complexes of Cd, Mg and Ca with CTP and NA ligand systems were found to be 73.02 75.5 and 81% at different pH range from 6.7, 7.2 and 3.5-3.8, respectively. The formation of H2CTP and HCTP species in aqueous solution occurs at initial pH about 2.78-3.3 and observed that the complexes have concentration in range of 3.4-20.00%. The order of stability for complexes of the bivalent is given as under:

For ternary (1:1:1) M-CTP-NA types system Cd > Mg > Ca.

For quaternary (1:1:1:1) system Ca-Cd > Mg-Ca

The stability constants of complexes follow the Irving and Williams order [20].

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