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Implicatons of Metal Complexes in Biology and Medicine the System Cadmium (II)/ Iron (II)/ Zinc (II)-Hydroxyproline

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Abstract

The importance of metal ions to essential functions of living systems and for the well being of living organisms is known. Metal ions are fundamental elements for the maintenance of the life spans of the human, animals and plants. The stability constants of Cd^{2+}, Fe^{2+} and Zn^{2+} complexes with hydroxyproline were determined by Paper Electrophoretic Technique (PET). This method is based on the movement of a spot of metal ion in an electric field at various pH of background electrolyte. A graph of pH against mobility gives information about the formation of binary complexes and permit to calculate their stability constants. The stability constant of the ML and ML_2 complexes of Cd (II) – hydroxyproline, Fe (II) – hydroxyproline and Zn (II) – hydroxyproline, have been found to be (4.41 ± 0.01; 2.95 ± 0.06) (4.11 ± 0.01; 2.81 ± 0.11 and (4.83 ± 0.02; 3.28 ± 0.07) (logarithm stability constant values), respectively at ionic strength 0.1 mole L^{-1} (per chloric acid as background electrolyte) and a temperature of 35 ^{0}C, The first and second stability constants of metal complexes follow the order Zn (II) > Cd (II) > Fe (II). Metal complexes can offer their action such as anti-inflammatory, antibiotic, anti-thyroid and anticancer compounds. Metal based drugs bioactivity can be increased by metal chelation, which in turn increase their absorbance and stability. Recent advances in inorganic chemistry have made possible formation of a number of metal complexes with organic ligands of interest which can be use as therapeutic agents.

Keywords: Paper electrophoretic technique, cadmium (II) complexes, iron (II) complexes and zinc (II) complexes, overall mobility, stability constant.

Introduction

Stability constant is well known tool for solution chemist, biochemist and chemist. In general, to help for determining the properties of metal - ligand reactions in water and biological system (Wadekar et al., 2014). Transition metals exhibit different oxidation states and can interest with a few negatively charred molecules. These properties of transition metals led to development of metal-based drugs with promising pharmacological application and unique therapeutic opportunities (Sodhi & Paul, 2019). Beside several limitations and side effects, transition metal complexes are still the most widely used chemotherapeutics agents and make a large contribution to medicinal therapeutics (Wara, 2011). Metal complexes of amino acids have higher absorption, transport and metabolism efficiency in body than simple inorganic form (Ngu et al., 2013). Cadmium is a toxic metal for human, organisms, and for all ecosystems. The long biological half-life of cadmium makes it a cumulative toxin, chronic exposure and causes harmful effects from the metal stored in the organs (Peana et al., 2022). Zinc fingers (ZFs) are representative DNA –binding motifs found in many transcription factors. ZFs are known to involved in various biological functions such as developmental differentiation and suppression of tumors (Negi et al., 2023). Iron has many oxidation states ranging from 2^- to 6^+ but 2^+ (d^6) and 3^+ (d^5) oxidation states ranging are of greatest importance in biological systems and exquisitely sensitive to both pH and the nature of the ligating functionality (Naseem Akhtar & Ehsan, 2019). Polaprezinc a chelated for of zinc and L-carnosine is a new generation gastric mucosal protective agent that has been used in clinical for more than 20 years in Japan (Li et al., 2021). Synthesis of cadmium complexes using N (4)-phenyl-2- formylpyridine thiosemicarbazone (L_1) and 5-aminotetrazole (L_2) as organic ligands and evolution of their anti –cancer and nephrotoxic potential in vitro is reported by Abyar et al. (2019). In vitro antibacterial and antitumor potential of iron based on Schiff base is described by Aly et al. (2023). 4-Hydroxyproline are beneficial for preventing and treating obesity, cardiovascular dysfunction, neurological disorder, improving skin and bone health and promoting wellbeing in infants, children and adults (Wu, 2020).

Cadmium is one of the most toxic heavy metals. The major sources of cadmium release into the environment by waste streams are electroplating, smelting, alloy manufacturing, pigments, plastic, battery, mining and refining processes. Cadmium accumulated readily in living systems and has been reported to cause renal disturbances, lungs insufficiency, bone lesions, cancer and hypertension in humans (Benguella & Benaissa, 2002). Most organism require iron as an essential element in a variety of metabolic and informational cellular pathways. More than 100 enzymes acting in primary and secondary metabolism possess iron – containing cofactors such as iron sulfur clusturs or heme groups. The reversible Fe (II) / Fe (III) redox pair is best suited to catalyze a broad spectrum of redox reactions and to mediate electron chain transfer (Miethke & Marahid, 2007). Zinc (II) ion is known to be one of the important essential trace elements found in biological systems and also in many metalloproteins and metalloenzymes which exist in living organisms (Yoshikawa et al., 2001). Zinc complexes have attracted increasing interest in the field of synthetic and biological chemistry. Zinc complexes have attracted increasing interest in the field of synthetic and biological chemistry particularly due to the key role they play in biological systems. Binuclear zinc coordination moieties are found in various zinc enzymes such as phosphatase and metallo – ß – lactamases (Liu et al., 2003). The studies in complexation reactions of bivalent cadmium, iron and zinc is of interest because of their nutrient properties and toxicity (De Rosa & Crutchley, 2002; Lincoln, 2004; Grattan & Freake, 2012; Burgos et al., 2012; Vir et al., 2007; Fuch et al., 2003; Lieu et al., 2001; Purohit et al., 2007; Kini et al., 2009). Hydroxyproline is produced by hydroxylation of amino acid proline by the enzyme prolyl hydroxylose following protein synthesis. The enzyme catalyzed reaction takes place in the lumen of the endoplasmic reticulum. Hydroxyproline comprises roughly 4% of amino acids found in animal tissue. Hydroxyproline is found in few proteins other than collagen. For this reason, hydroxyproline content has been used as an indicator to determine collagen and or gelatin amount. Increased serum and urine levels of hydroxyproline have also been demonstrated in Paget's disease. Hydroxyproline has several significant roles in biological system (Weis et al., 2010; Han et al., 2007; Kaplon et al., 2010). Publications (Tewari, 2008; Tewari, 2008; Tewari, 2010) from our laboratory describe a new method for the study of binary and ternary complexes. The usual drawbacks of paper electrophoretic technique like variation in the temperature, during the electrophoresis, and adsorption affecting the mobility of charged moieties (Mc Donald, 1955).

A search of the literature indicated that very few reports available on cadmium (II), iron (II) and zinc (II) binary complexes with hydroxyproline. Hence, attempt was made to establish the optimum conditions for metal (II) – hydroxyproline complex formation. In addition, the present work describes an ionophoretic method for the determination of the stability constants of the cadmium (II) / iron (II) / zinc (II) – hydroxyproline binary complexes.

Experimental

Instruments: Systronics (Naroda, India) paper electrophoresis equipment horizontal-cum-vertical type, model 604, has been used. The apparatus consisted of a PVC moulded double tank vessel. In our laboratory significant change in the instrument has been made. Two hollow rectangular plates covered with thin polythene sheets have been used through which thermostated water is run for controlling the temperature. The tanks were closed with a transparent PVC molded lid. The whole assembly is tight, which prevents moisture changes that may upset the equilibria in a paper strip. This design thus keeps to a minimum the disturbing effects of evaporation from the unwanted liquid flow in the paper. Each electrolyte tank contains a separate electrode chamber. Whitman No. 1 filter papers for chromatography were used for the purpose of electrophoresis. Electrophoresis cell showing sandwiched paper strips is shown in Figure 1.

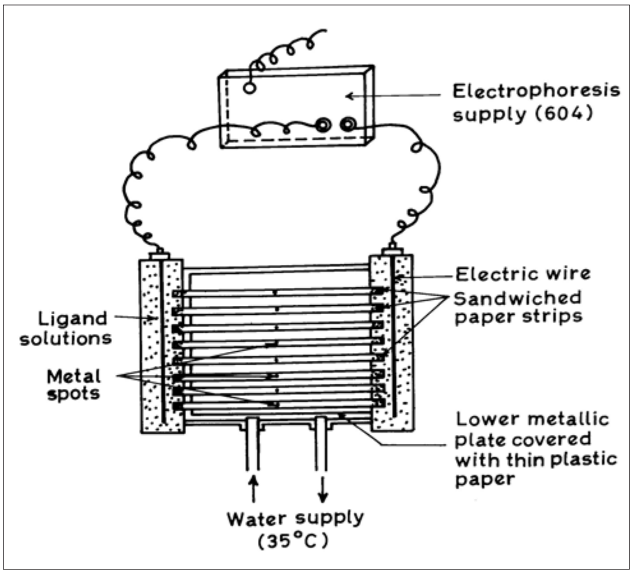


Figure 1: Electrophoresis cell showing sandwiched paper strips.

Elico (Hyderabad, India) Model L_{1-10} pH meter using a glass and calomel electrodes assembly working on 220 V/50 Hz, a. c. main was used for the pH measurements. pH meter was calibrated with buffer solution of pH 7.0.

Chemicals: Cadmium (II), iron (II) and zinc (II) perchlorate solutions were prepared by the precipitation of metal carbonate from a 1.0 M solution of Cd (II), Fe (II) and Zn (II) nitrates with the solution of sodium carbonate (chemically pure grade, BDH, Poole, UK). The precipitates were washed with boiling water and treated with calculates amounts of 1% perchloric acid. These reaction mixtures were boiled on water bath and filtered. The metal content of the filtrates was determined and final concentration kept at 5.0×10^{-3} mol L^{-1} (Mc Donald & Belcher, 1957). Metal spots were detected on the paper using dithizone in carbon tetrachloride for Zn (II).

A 0.1% (w/v) solution of 1 – (2 – pridylazo) – 2 – naphthol (PAN) (Merck, Darmstadt. Germany) in ethanol was used for detecting Cd^{2+} and Fe^{2+} metal ions. 0.005 M glucose (BDH, AnalaR) solution were prepared in water and used as an electro-osmotic indicator for the correction due to electro-osmosis. A saturated aqueous solution (0.9 ml) of silver nitrate was diluted with acetone to 20 ml. Glucose was detected by spraying with this silver nitrate solution and then with 2% ethanoic solution of hydroxide, when a black spot was formed. Paper strips showing the position of metal ion spots after electrophoresis is shown in Figure 2.

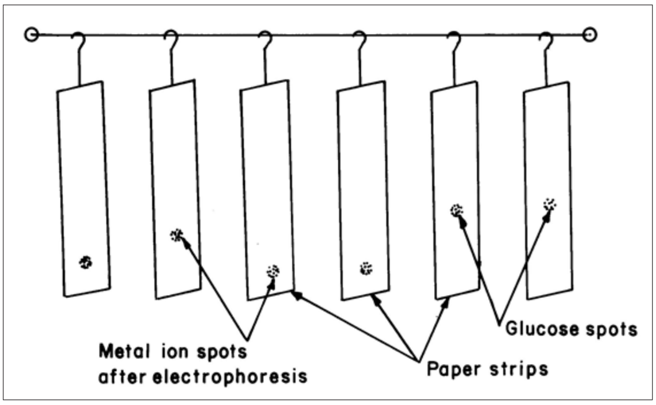


Figure 2: Paper strips showing position of metal ion spots after electrophoresis

Background Electrolytes

The background electrolytes used in the study of binary complexes were 0.1 M perchloric acid and 0.01 M hydroxyproline. Stock solutions of 5.0 M perchloric acid (SDS, Anala R), 2.0 M sodium and 0.5 M hydroxyproline (BDH, Poole, UK) were prepared.

Procedure

For recording the observation of particular metal ions, two paper strips were spotted with metal ion solutions along with additional two spotted with glucose using a 1.0 µL pipette and then mounted on insulate plate. Each of the two electrolyte vessels were filled with 150 m L of background electrolyte solutions containing 0.1 M perchloric acid and 0.01 M hydroxyproline. The paper become moistened with solution due to diffusion. The second insulated plate was placed on paper strips and then thermostatic water at 350C was circulated into plates to keep the temperature constants. A direct 240 V potential was applied between electrodes. Electrophoresis was carried out for 60 minutes. Paper strips were removed from the tank with glass rod. Dried and detected by specific reagents. Electrophoretic observation of metal ions was recorded at various pH values of back ground electrolytes. The mobility's were calculated by dividing the distance by the potential gradient and are expressed in cm2 V-1 min-1. The speed of metal ions /complex ions are reported with pH values. A plot of mobility against pH curve for M(II) – hydroxyproline is shown in Figure -3.

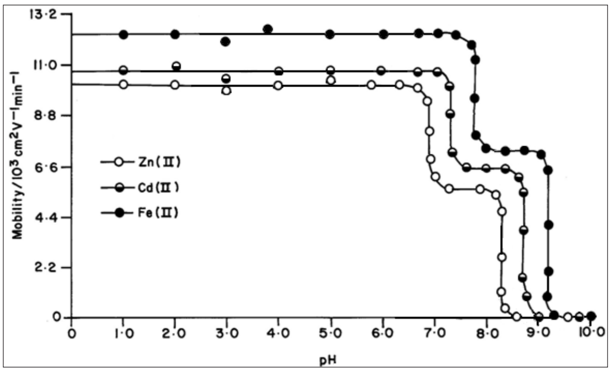


Figure 3: Mobility curves for the metal (II) – hydroxyproline complexes.

—o— = Zinc (II) – hydroxyproline;
—⊖— = Cadmium (II) – hydroxyproline;
—●— = Iron (II) – hydroxyproline.

Background electrolytes: 0.1 Mol L^{-1} perchloric acid and 0.01 Mol L^{-1} hydroxyproline. Concentration of
Cd (II), Fe (II) and Zn (II) = 0.005 Mol L^{-1}. Variation in the pH was made by the addition of sodium hydroxide.

Results

The electrophoretic mobility of the metal spot against pH gives a curve with numbers of plateaus is shown in Figure 3. A constant speed over a range of pH is possible only when a particular complex species is over whelmingly formed. Thus, every plateau is indicative of formation of a certain complex species. The first plateau in the beginning corresponds to a region in which metal ions are uncomplexed. In this region of low pH, concentration of unprotonated species of Hydroxyproline [CH_2 CH (OH) CH_2 CH (NH_2+) COOH] is maximum and this species is non complexing, beyond this range, metal ions spots have progressively decreasing mobility, complexation of metal ions should be taking place with anionic species of hydroxyproline, whose concentration increase progressively with increase of pH. Figure 3 shows three plateaus in Cd (II), Fe (II) and Zn (II) metal ions form two complexes with hydroxyproline anion.

Figure 3 discloses that Cd (II), Fe (II) and Zn (II) metal ions form their first complex movements toward negative electrode. Hence one anionic species of hydroxyproline [CH_2 CH (OH) CH_2 CH (NH) COO-], must have combined Cd (II), Fe (II) and Zn (II) metal ions to give 1:1, [Cd {CH_2 CH (OH) CH_2 CH (NH) COO}] + [Fe {CH_2 CH (OH) CH_2 CH (NH) COO}]+ and [Zn {CH_2 CH (OH) CH_2 CH (NH) COO}]+ complex cations, respectively. The third plateau in each case is due to 1:2 metal – ligand complex, hence, two anionic species of hydroxyproline [CH_2 CH (OH) CH_2 CH (NH) COO^-], must have combined Cd (II), Fe (II) and Zn (II) to give 1:2 [Cd {CH_2 CH (OH) CH_2 CH (NH) $COO\}_2$], [Fe{CH_2 CH (OH) CH2 CH (NH) $COO\}_2$] and [Zn{CH_2 CH (OH) CH_2 CH (NH) $COO\}_2$], neutral metal complexes, respectively. Further increase of pH has no effect on the mobility of metal ions, which indicates no further interactions between metal ions and ligand. In general, the complexation of metal ions with hydroxyproline anion may be represented as:

$$M^{2+} + L^- \overset{K_1}{\rightleftarrows} ML^+ \quad (1)$$

$$ML^+ + L^- \overset{K_2}{\rightleftarrows} ML_2 \quad (2)$$

Wherein $M^{2+} = Cd^{2+}$, Fe^{2+} and Zn^{2+} metal ions
$[L^-]$ = hydroxyproline anions; K_1 and K_2 are the first and second stability constants, respectively.

The metal spot on the paper is thus a combination of uncomplexed metal – ions, 1:1 and 1:2 metal complexes. The spot is moving under the influence of electric field and the overall mobility is given by equation of Jokl (1964).

$$U = \frac{U_{xp}\, ß_{xp} [\,HpL]^x}{ß_{xp} [\,HpL]^x} \quad (3)$$

Wherein $[HpL]^x$ = concentration of general complex species; $ß_{xp}$ = overall mobility constant of complex; U_{xp} = speed of general complex $[M(HpL)^x]$ present in the conglomeration. On taking into consideration different equilibria, the above equation is transformed into the following form

$$U = \frac{U_o + U_1 K_1 [L^-] + U_2 K_1 K_2 [L^-]^2}{1 + K_1 [L^-] + k_1 k_2 [L^-]^2} \quad (4)$$

Wherein U_0, U_1 and U_2 are mobilities of uncomplexed metal ion, 1:1 metal complex and 1:2 metal complex, respectively.

The protonation constants of pure hydroxyproline [$pka_1 = 1.80$; $pka_2 = 9.46$] were determined by the same electrophoretic technique. The mode of deprotonation of pure hydroxyproline can be represented as

$[CH_2\, CH\, (OH)\, CH_2\, CH\, (NH_2^+)\, COOH]$

$-H^+ \quad \downarrow\uparrow \quad pka_1$

$[CH_2\, CH\, (OH)\, CH_2\, CH\, (NH_2^+)\, COO^-]$

$-H^+ \quad \downarrow\uparrow \quad pka_2$

$[CH_2\, CH\, (OH)\, CH_2\, CH\, (NH)\, COO^-]$

Using protonation constants of pure hydroxyproline anion $[L^-]$ is determined for the pH value(s) of interest, from which K_1, can be calculated. The concentration of complexing hydroxyproline anion $[L^-]$ is calculated with the help of equation.

$$[L^-] = \frac{[L_T]}{1 + \frac{[H]}{pka_2} + \frac{[H]^2}{pka_1 . pka_2}} \quad (5)$$

Wherein $[L_T]$ = Total concentration of ligand hydroxyproline [0.001 mol L^{-1}], pka_1 and pka_2 = first and second protonation constants of pure hydroxyproline, respectively.

For calculating first stability constant, K_1, the region between first and second plateau is pertinent. The overall mobility will be equal to the arithmetic mean of the mobility of uncomplexed metal ion, U_0, and that of first complex, U_1, at a pH where $K = 1/[L^-]$. The second stability constant, K_2 of 1:2 complex can be calculated by taking into consideration, the region between second and third plateau of mobility curve, these calculated value of K_1 and K_2 are given in Table 1.

Metal Ions	Complexes	Stability constants	Logarithm stability constant values
Cadmium (II)	ML^+	K_1	4.41 ± 0.01
	ML_2	K_2	2.95 ±0.06
Iron (II)	ML^+	K_1	4.11 ± 0.0
	ML_2	K_2	2.81 ± 0.11
Zinc (II)	ML^+		4.83 ± 0.02
			5.03 ± [39]
	ML_2	K_2	3.28 ± 0.07
			4. 35 ± [39]

Ionic strength = 0.1 Mol L^{-1}; temperature = 35^O C; M= metal cations (Cd^{2+}, Fe^{2+} and Zn^{2+}); L= ligand (hydroxyproline); hydroxyproline anion = $[CH_2\, CH\, (OH)\, CH_2\, CH\, (NH)\, COO^-]$.
* = Literature values are given in the bracket.

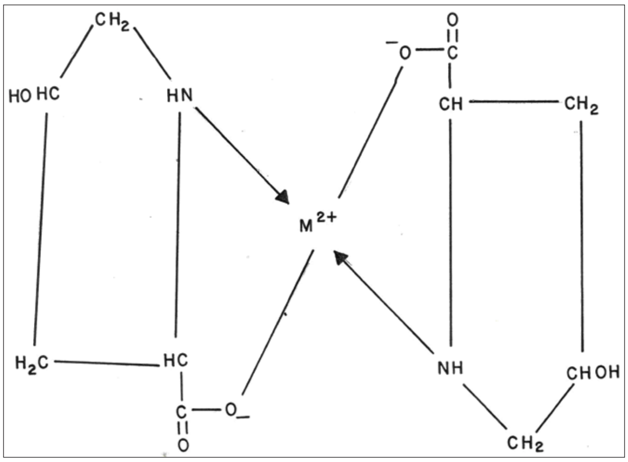
Table 1: Stability constants of the binary complexes of cadmium (II), iron (II) and zinc (II) – hydroxyproline.

Discussion

It is clear from Table 1 that order of stability constants viz: Zinc (II) > Cadmium (II) > iron (II) is same for ML^+ and ML_2 binary complexes. The stability constant of ML^+ and ML_2 complexes follow the order: $\log K_1 > \log K_2$. The corresponding second stability constant values are found to be lower for binary complexes. It is therefore inferred that coordinating tendency of a ligand decreases with higher state of aggregation. High stability constant values of zinc (II) – hydroxyproline complexes indicate strong bonding between zinc (II) cation and hydroxyproline anion. Whilst low stability constant value between iron (II) – hydroxyproline complexes indicate weak bonding between iron (II) cation and hydroxyproline anion. The precision of the method is limited to that of paper electrophoresis and uncertainly in the result is ± 5%, hence, it cannot replace the most reliable methods, even though it is new approach deserving further development. The stability constants of metal complexes, can be very easily calculated by this technique, therefore the present method is advantageous even other methods (viz: polarography, potentiometric, solubility etc.) reported in chemical literature.

The parallel studies on the metal complexes in biology and medicine and their stability constants determination are reported in chemical literature. A novel 5-nitro -8- hydroxyquinoline – proline hybrid and its rhodium (n^5 C^5 Me^5) and ruthenium (n^6 –p- cymene) complexes with excellent aqueous solubility were developed, characterized against sensitive and multiple drug resistance cells (Pivarcsik et al., 2023). A complex of zinc and carnosine called zinc –L- carnosine has been extensively used in tumor adjuvant therapy (Tang et al., 2022). Physiological and pharmacological applications of antioxidant activity of ferrozine-iron-amino acid complexes is reported by Berlette et al. (2001). Critical evaluation of stability constants of metal amino acid complexes in solution was investigated by Yamauchi and Omani (1996). Singh et al. (2019) has described various factors affecting the stability constants of metal complexes in solution.

The proposed structure for metal (II) – hydroxyproline. ML2 binary complexes may be given as follow



Proposed structure for metal (II) – hydroxyproline ML_2 binary complexes.

Conclusions

The following conclusions can be shown from the present study

1. Cadmium (II) / iron (II) / zinc (II) are significant but since they are toxic, hydroxyproline may be used to reduce the level of these cations in biological systems.
2. Zinc (II) – hydroxyproline and iron (II) – hydroxyproline metal complexes are found to have high and low stability constants value, respectively.
3. ML_2 complexes are found to have low stability constant value and less stable in comparison to ML^+ complexes.
4. The present paper ionophoretic technique is very helpful in determining whether a complex system is formed or not, and if formed its stability constants can also be determined.
5. Stability constants of metal complexes can be very easily calculated by this technique, so the present paper electrophoretic technique has significant advantages over the other physiochemical methods reported in chemical literature for the determination of stability constants of metal complexes.
6. The future work is to prepare Cd (II), Fe (II) and Zn (II) binary complexes with hydroxyproline at an optimum conditions mentioned in this paper, characterize them and study their possible medical potential as anti-biotic, anti-inflammatory and anti-cancer agent.

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