ISSN: 2754-4907

Journal of Drugs Addiction & Therapeutics

SCIENTIFIC Research and Community

Research Article

Open Access

Metal Complexes in Biology and Medicine: The System Aluminum (III)/Chromium (III)/Iron (III) – Norvaline

Ashish Kumar Tiwari[1] and Brij Bhushan Tewari[2]*

[1]Advanced Center for Material Science, Indian Institute of Technology Kanpur, Kanpur- 201085, Uttar Pradesh, India

[2]Department of Chemistry, Faculty of Natural Sciences, University of Guyana, P.O. Box 101110 Georgetown, Guyana

ABSTRACT

In coordination compounds studies, knowledge of the stability constants of complexes is necessary for preliminary quantitative treatment. Metal complexes can offer their action such as anti – inflammatory, antimicrobial, 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 number of transition metal complexes with organic ligands of interest which can be used as therapeutic agents. Aluminum prefers oxygen donor groups for complexation. The stability of complexes in biological systems depends on pH, which in blood plasma is 7.4. Chromium is a very adaptable metal, and it can form copious species with variable oxidation numbers from (- VI) to (+VI). Iron is a component of heme and chlorophyll and serves as micronutrients of plants and animals. Ferric ion from Industrial effluent has the potential to poison animals and plants. The present technique involving the use of paper ionophoresis is described for the study of equilibria in binary complex systems in solutions. The method is based on the movement of a spot of a metal ion in an electric field at various pH's of background electrolyte. A graph of pH versus mobility was used to obtain information in the binary complexes and to calculate its stability constants. Using this method, the stability constants of binary complexes metal (III) – norvaline have been determined to be (8.73 ± 0.03, 7.20 ± 0.04); (9.00 ± 0.01, 7.41± 0.07) and ((9.43 ± 0.01, 7.66 ± 0.11) (logarithm stability constant values) for aluminum (III) chromium (III) and iron (III) complexes, respectively, at ionic strength 0.1 Mol/L and a temperature of 35°C. The first and second stability constants of metal complexes follow the order Fe (III) > Cr (III) > Al (III).

***Corresponding author**

Brij Bhushan Tewari, Department of Chemistry, Faculty of Natural Sciences, University of Guyana, P.O. Box 101110 Georgetown, Guyana.

Received: March 06, 2025; **Accepted:** March 11, 2025; **Published:** March 20, 2025

Keywords: Paper Electrophoretic Technique, Overall Mobility, Metal Complexes, Stability Constants

Introduction

For the general case of complex MLn, the stepwise formation or stability constant (Kn) are:

$$M + L \leftrightharpoons ML,\ K_1 = \frac{[ML]}{[M]\,[L]}$$

$$ML + L \leftrightharpoons ML_2,\ K_2 = \frac{[ML_2]}{[ML]\,[L]}$$

$$ML_2 + L \leftrightharpoons ML_3,\ K_3 = \frac{[ML_3]}{[ML_2]\,[L]}$$

$$M_{n-1} + L \leftrightharpoons ML_n\ K_n = \frac{[ML_n]}{[ML_{n-1}]\,[L]}$$

Where M and L are metal cation and ligand anion, respectively.

For the calculation of total concentration of final complex product (ML_n), the overall formation constant is used.

$$\beta_n = \frac{[ML_n]}{[ML_{n-1}]\,[L]^n}$$

The overall formation constant is the product of stepwise formation constants

$$\beta_n = K_1.\ K_2.\ K_3.\ \ldots\ldots\ K_n$$

The inverse of formation constant, the dissociation constant k_d is also sometimes useful

$$ML \leftrightharpoons M + L,\ K_d = \frac{[M]\,[L]}{[ML]}$$

kd has the same form as k_a for acids, which facilities comparisons between metal complexes and Bronsted acids. Out of all techniques adopted for the study of metal ligand equilibria, we have opted for paper electrophoresis. Not much work, however, on record on the application of paper electrophoresis for examining complex reactions. Metal complexes play an important role in various biological systems; hence the formation, stability, and reactivity

of their complexes have been an active field of research [1].

Iron metal without which life cannot sustain. Aluminum is an indifferent metal, toxic at high level. Chromium is a is an essential beneficial metal which is helpful for a healthy life. The aluminum, chromium and iron elements in human diet (mg / day) at normal and toxic levels are (2 – 15, 5000), (0.01 – 1.2, 200) and (6 – 10, 200), respectively. Harmful effects of Al^{3+} include constipation and neurotoxicity. Increased concentration of Al^{3+} in the brain has been associated with Alzheimer's disease a malady that brings on senile dementia and even in death mostly elderly people. Essential and beneficial metals are also toxic at high concentration and their deficiency leads to disease conditions [2].

Mujika et al. has described a detailed account of the interaction of aluminum with proteic sidechains and how aluminum can exert oxidative stress by stabilizing superoxide radicals either as mononuclear aluminum or clustered in boehmite [3]. Speciation of aluminum in biological systems has been presented by Harris et al. [4]. Zhao et al. ported several examples where aluminum has been shown to irreversibly perturb and / or stabilize the natural conformation of molecules known to be important in energy metabolism, gene expression, cellular homeostasis and pathological signaling in neurological disease [4,5].

Mezencev and Gibbons has described a complex world of chromium species and their reactivity with DNA and other biologically relevant molecule in vitro to inform a more complete understanding of Cr (VI) – mediated toxicity [6].

Chromium is widely accepted as highly toxic and carcinogenic with no nutrional value. Wise and wise sr. has indicated that chromium causes genomic instability and also has no role in promoting genomic stability [7]. Lewicki et al. has aimed to present current knowledge about chromium, its organism and possible mechanisms of its action also in metabolic disorders such as diabetes or obesity [8]. Dwarzariski et al. has determined how a high – fat diet supplemental with various forms of chromium affects hematological and immune parameters of the blood of rats [9].

A review on chromium uptake, translocation and accumulation in plants discussed by Abdullah et al. [10]. It also provides a model to unravel the complexities of the Cr – plant interaction utilizing system biology and integrated OMICS approach. Rolic et al. has presented a review on iron metabolism and discussed role of Ca, Mg and trace elements (Cu, Zn, Pb, Hg, Ni, Cd, etc.) in absorption and transport of iron [11].

A review on the physiological significance of iron homeostasis in the body, the potential contribution of ferroptosis to the etiology and development of human diseases, along with the evidence supporting ferroptosis as a therapeutic approach, has been reported by Ru et al. [12]. Kulaszynska et al. has reported a review to systematize the available data on the role of iron in the function of the nervous system, especially in the brain [13]. This review summary's recent view on iron metabolism and its regulatory mechanism in human, including the essential action of hepcidin. Kontoghairghes has discussed iron load toxicity in medicine from molecular and cellular aspects to clinical implications [14].

The synthetic methods and pharmacological potentials of iron – imine complexes having in vitro activity to significant clinical performance from 2016 to present has been described by Anane et al. [15]. Aluminum (III), chromium (III) and iron (III) are well known for its biomedical applications and toxicity [16-20].

Norvaline is an amino acid $C_5 H_{11} NO_2$, isomeric with valine and usually made synthetically. It is not found in proteins. Norvaline may have been more abundant protein components during early stages of cell evolution. Norvaline is a drug used to treat vaginal inflammation with betamethasone and tyrothricin. Norvaline has several significant applications in biological systems [21-25]. Kiso has done comprehensive study on paper ionophoretic migration of metal complexes [26].

The paper ionophoretic technique usually suffers from several defects, electro – osmosis, temperature during electrophoresis, capillary flow on paper and adsorption affects the mobility of charged moieties [27]. The present technique is almost free from these destroying factors and very convenient in use. It gives results in fair agreement with accepted literature values. Communications from our laboratory have described a new method for the study of metal complexes [28-32]. A search of literature indicated coordination of amino acids with metal ions but no reports are available on binary complexes as aluminum (III), chromium (III) and iron (III) with norvaline [33,34]. In view of this, an attempt was made to establish the optimum conditions for metal (III) – norvaline complex formation. In addition, the present paper describes a paper electrophoretic method for the determination of the stability constants of aluminum (III), chromium (III) and iron (III) - norvaline binary complexes.

Experimental

Apparatus

A Systronic (Naroda, India) Model 604 electrophoretic system was used. The apparatus consisted of a (poly vinyl chloride) PVC molded double tank vessel. In our laboratory, significant change in the instrument has been made. Two hollow rectangular iron plates each weighing one kg. and covered with thin polythene sheets have been used through which thermostat water circulated for controlling the temperature. The tanks are closed with a transparent PVC molded lid. The whole assembly is tight to prevent moisture changes, which might upset the equilibria in the paper strip. The assembly design thus keeps to a minimum the disturbing effects of evaporation from unwanted liquid flow in the paper strip. Each electrolyte tank contains a separate electrode chamber in which Pt-wire anode and cathode are placed, respectively. Applied voltage was from a stabilized source. Paper electrophoresis equipment model 604 and electrophoresis assembly covered by thin polythene sheet is shown in Figure 1.

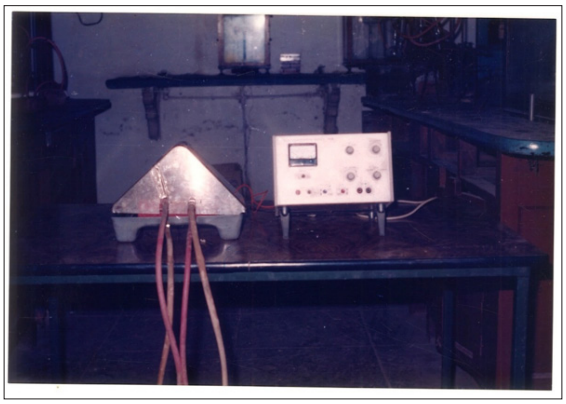


Figure 1: Paper Electrophoresis Equipment Model 604 and Electrophoresis Assembly Covered by Thin Polythene Sheet

Paper electrophoresis model 604 and lower metallic plate showing sandwiched paper strips is shown in Figure 2.

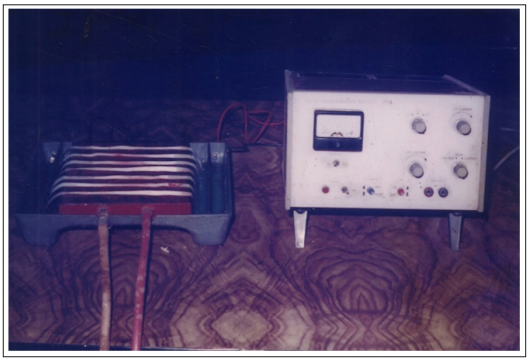


Figure 2: Paper Electrophoresis Model 604 and Lower Metallic Plate Showing Sandwiched Paper Strips

Whatman No. 1 filter paper for chromatography was used for the purpose of electrophoresis. Elico (Hyderabad, India), Model $L_{1\text{-}10}$, pH meter using a glass and calomel electrodes assembly working on 220 V/50 Hz established a. c. mains, was used for the pH measurement. pH meter was calibrated with buffer solution of pH 7.0. The thermostat 35 ^{0}C water supply in upper and lower metallic plates is shown in Figure 3.

Figure 3: The Thermostat 35 °C Water Supply in Upper and Lower Metallic Plates

The scheme for paper electrophoresis set-up is shown in Figure 4

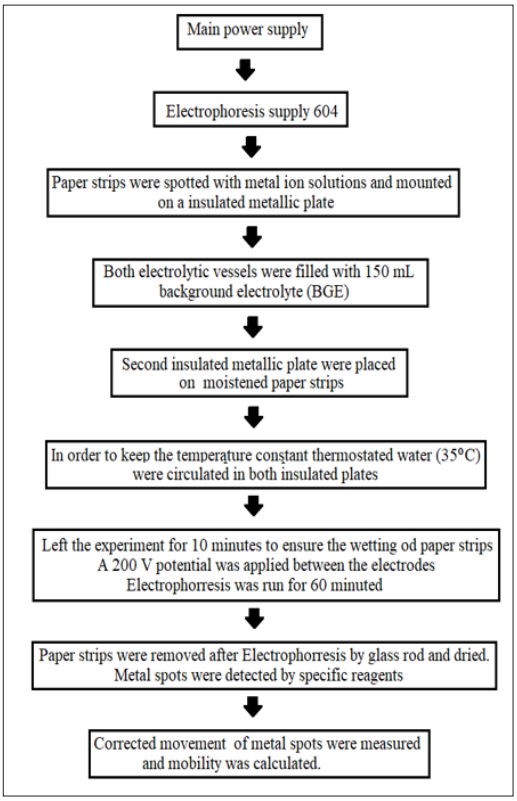


Figure 4: The Scheme for Paper Electrophoresis Set-up

Chemicals

Preparation of Metal Solution

Aluminum (III), chromium (III) and iron (III) metal perchlorate solutions were prepared by preliminary precipitation of metal carbonates from 0.1 Mol L^{-1} solution of sulfates of aluminum (III), chromium (III) and iron (III) with the solution of sodium carbonate (chemically pure grade BDH, Poole, UK). The precipitation was washed with boiling water and treated with measured amounts of 1 % perchloric acid. The metal contents of the filtrates were determined, and the final concentration was kept at 0.005 Mol L^{-1} [35,36].

Sodium Hydroxide Solution

Carbon dioxide free sodium hydroxide solution was prepared by dissolving 500 gms of sodium hydroxide in 500 mL of water in a flask. The flask was left overnight. The clear supernatant liquid was filtered rapidly using a high vacuum pump. A suitable volume of the filtrate was diluted, and the concentration determined by titrating against a standard oxalic acid solution. A solution (2.0 Mol L^{-1}) was obtained by suitable dilution. The concentration of stock solution was checked from time to time.

Detecting Reagents for Metal Ions and Glucose

Metal spots were detected on the paper using a aluminon solution (BDH, England) for Al^{3+}, 0.5 % solution of potassium ferrocyanide (BDH) for Fe^{3+} and 0.1 %, solution of 1 – (2 – pyridylazo) – 2 – napthol (PAN) (Merck, Darmstadt, Germany) in ethanol for Cr^{3+}. 0.005 Mol L^{-1} glucose (BDH, Analytical Reagent grade) solutions were prepared in water and used as an electron - 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% ethanolic sodium hydroxide, when a black spot was formed.

Background Electrolyte

The background electrolytes used in the study of binary complexes were 0.1 Mol L^{-1} perchloric acid and 0.01 Mol L^{-1} norvaline. The system was maintained at various pH by the addition of sodium hydroxide. Stock solutions of 5.0 Mol L^{-3} perchloric acid (SDS, Analytical Reagent grade), 2.0 Mol L^{-1} sodium hydroxide (Analytical Reagent grade) and 0.5 Mol L^{-1} norvaline were prepared. Each solution was standardized using the appropriate method.

Procedure

Whatman No.1 filter paper chromatography was used for the purpose of electrophoresis. For recording observation of metal ion, two strips were spotted with the metal ion solution along with additional two spotted with glucose using 1.0 µL pipette and mounted on insulated plate. Each of the two-electrolyte vessels were filled with 1.50 mL of background electrolyte containing 0.1 Mol L^{-1} perchloric acid and 0.01 Mol L^{-1} norvaline. The paper became moistened with the background electrolyte solutions due to diffusion. The second insulated plate was placed on paper strips and then thermostated water (35º C) was circulated in the plates to keep the temperature constant. The lid was then placed on the instrument to make it airtight. It was left for 10 minutes to insure wetting the strips. Subsequently a direct 200 Volts potential was applied between the electrodes. Electrophoresis was carried for 60 minutes after which these strips were removed from the tank and dried. The metal ion and glucose spots were detected by specific reagents. The leading and tailing edge were measured from the marked centre point and the mean were taken. The distance moved by glucose as subtracted (in case of migration toward anode) to obtain correct path length. Migration towards anode and cathode were designated by negative and positive signs, respectively.

Electrophoretic observations on metal ions were recorded at various pH values of the background electrolyte obtained by adding sodium hydroxide solution. The ionic strength being maintained at 0.1 Mol L^{-1}. The observed mobility of mobility of migrants was calculated by using the formula.

$$U = \frac{d}{x \cdot t}$$

after applying the correction factor the observed mobility is given as

$$U = \frac{d \pm d_G}{x \cdot t}$$

where U = mobility of metal ion / complex ion, d = mean of duplicate distance travelled by metal ion / complex ion; d_G = mean duplicate distance travelled by glucose spot; x = field strength; t = time for electrophoresis. The mobility of metal / complex ion spots on the paper strips were thus calculated and are reported with different pH values (Figure 5).

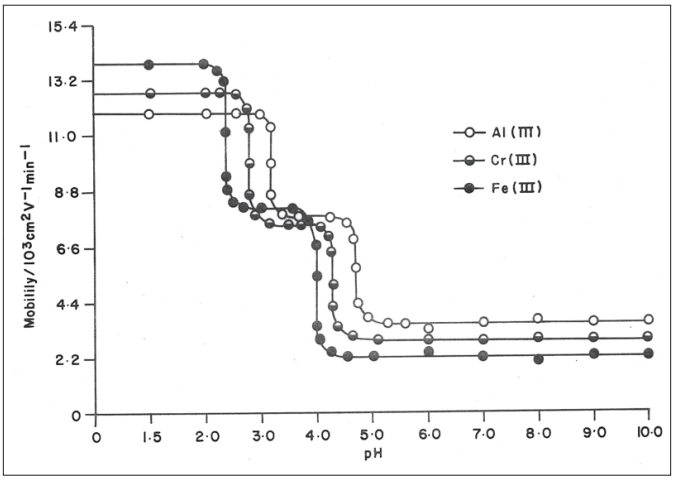


Figure 5: Mobility Curve for Metal (III) – Norvaline Systems. —○— Al (III) – Norvaline; —●— Cr (III) Norvaline; —●— Fe (III) – Norvaline. Background Electrolytes: 0.1M Perchloric Acid and 0.01M Norvaline pH's were Maintained by Addition of Sodium Hydroxide. The Paper Strip was Spotted with 0.1 µL of Sample Solutions and Glucose (for making osmotic corrections)

The protonation constants of pure norvaline were determined by the same paper electrophoresis technique. The two paper strips were spotted with norvaline along with glucose using 0.1 Mol L^{-1} perchloric acid only in a background electrolyte. The electrophoresis was carried out for 60 minutes as for metal ion. The electrophoresis speed was calculated. The speed of norvaline spots are reported with pH values. The individual speeds of duplicate spots were found to be equal.

Results

The plot of overall electrophoretic mobility of metal spot against pH is shown in Figure 5. A constant speed over a range of pH is possible only when a particular complex species is overwhelmingly formed. Thus, every plateau is indicative of the formation of a certain complex species. The first one in the beginning corresponds to a region in which metal ions uncomplexed. In this region of low pH, concentration of unprotonated species of norvaline [CH_3 CH_2 CH_2 CH (NH^+_3) COOH] is maximum and this species is non – complexing. Figure 5 reveals that aluminum (III), chromium

(III) and iron (III) ions form their first complex movement toward negative electrode, hence, one norvaline anionic [CH_3 CH_2 CH_2 CH (NH_2) COO^-] species must have combined with aluminum (III), chromium (III) and iron (III) metal ions to give 1:1, [Al {CH_3 CH_2 CH_2 CH (NH_2) COO}]$^{2+}$, [Cr {CH_3 CH_2 CH_2 CH (NH_2) COO}]$^{2+}$ and [Fe {CH_3 CH_2 CH_2 CH (NH_2) COO}]$^{2+}$ complex cations, respectively.

The third plateau in each case is still in a positive region of mobility curve showing cationic nature of metal - ligand complex. Hence, two anionic species of norvaline [CH_3 CH_2 CH_2 CH (NH_2) COO-] must have combined with Al (III), Cr (III) and Fe (III) metal ions to give 1:2 [Al {CH_3 CH_2 CH_2 CH (NH_2) COO1}$_2$}] $^+$; [Cr {CH_3 CH_2 CH_2 CH (NH_2) COO-}$_2$] $^+$ and [Fe {CH_3 CH_2 CH_2 CH (NH_2) COO^-}$_2$] $^+$ complex cations, respectively. Further increase of pH has no effect on the mobility of metal ions, which indicates no further interaction between metal ions and ligands.

Chemical literature also assigns prominent chelating properties of the unprotonated anionic species of norvaline ruling out any such property to zwitterions [37]. In view of the above observations, the complexation of metal ions with norvaline anion [L-] may be represented as

$$M^{3+} + L^- \overset{K_1}{\rightleftharpoons} ML^{2+} \qquad (1)$$

$$ML^{2+} + L^- \overset{K_2}{\rightleftharpoons} ML_2^{+} \qquad (2)$$

Where M^{3+} is Al^{3+}, Cr^{3+} and Fe^{3+} metal cations; [L^-] = norvaline ligand; ML^{2+}, ML_2^+ are 1:1 and 1:2 metal complexes; K_1 and K_2 are first and second stability constants, respectively.

The metal spot on the paper is thus a combination of the uncomplexed metal ions, 1:1 complex, and 1:2 complexes. The spot moving under the influence of electric field, and the overall mobility is given by equation of Jokl [38].

$$U = \frac{\Sigma u_{xp} \cdot \beta_{xp} [HpL]^X}{\Sigma \beta_{xp} [HpL]^x} \qquad (3)$$

Where $[HpL]^x$ is the concentration of general complex species; β_{xp} is the overall mobility constant of the complex; u_{xp} is the speed of the general combination. On taking into consideration different equilibria, the above equation is transformed into the following form:

$$U = \frac{u_0 + u_1 K_1 [L^-] + u_2 K_1 K_2 [L^-]^2}{1 + K_1 [L^-] + K_1 K_2 [L^-]^2} \qquad (4)$$

Wherein u_0, u_1 and u_2 are the mobilities of metal ions, 1:1 and 1:2 metal complexes, respectively. Equation (4) was used for the determination of the stability constant of metal complexes with norvaline.

The protonation constant of pure norvaline (pka_1 = 2.31; pka_2 = 9.65) were determined by same paper ionophoretic technique. The mode of deprotonation of pure norvaline can be represented as:

$$[CH_3\ CH_2\ CH_2\ (NH_3^+)\ COOH]$$
$$-H^+ \Updownarrow\ pka_1$$
$$[CH_3\ CH_2\ CH_2\ (NH_3^+)\ COO^-]$$
$$-H^+ \Updownarrow\ pka_2$$
$$[CH_3\ CH_2\ CH_2\ (NH_2)\ COO^-]$$

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

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

where [L_T] is the total concentration of ligand norvaline [0.001 / Mol L^{-1}], pka_1 and pka_2 are first and second dissociation constants of pure norvaline, respectively. For calculating first stability constant K_1, the region between first and secund plateau is pertinent. The overall mobility will be equal to the arithmetic mean of the mobility of uncomplex, u_0, and that of first complex, u_1 at a pH value where K_1 = 1 / [CH_3 CH_2 CH_2 (NH_2) COO^-].

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. The (se) calculated values of K_1 and K_2 are given in Table 1.

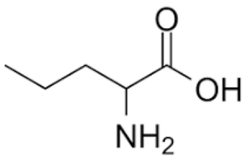
Table 1: Stability constants of binary complexes of aluminum (III) / chromium (III) /iron (III) – norvaline

Metal ions	Complexes	Stability constants	Logarithm stability constant values
Aluminum (III)	ML^{2+}	K_1	8.73 ± 0.03
	ML_2^+	K_2	7.20 ± 0.04
Chromium (III)	ML^{2+}	K_1	9.00 ± 0.01
	ML_2^+	K_2	7.41 ± 0.07
Iron (III)	ML^{2+}	K_1	9.43 ± 0.01
	ML_2^+	K_2	7.66 ± 0.11

Ionic strength = 0.1 mol L^{-1}; temperature 35º C; M = metal cations (Al^{3+}, Cr^{3+}, Fe^{3+}); L = Ligand (norvaline); norvaline anion = [CH_3 CH_2 CH_2 CH (NH_2) COO^-].

Discussion

It is clear from Table 1 that first and second stability constants of aluminum (III), chromium (III), and iron (III) – norvaline complexes follow the order Log K_1 > Log K_2, the corresponding second stability constant values are found to be lower for all complexes. It is therefore inferred that coordinating tendency of a ligand decreases with the higher state of aggregation. In other words, the metal progressive lessens its tendency of linkage with a ligand on progressive filling of vacant orbitals. This conclusion is of universal validity as evident in chemical literature [39-41]. The molecular structure of norvaline is given as



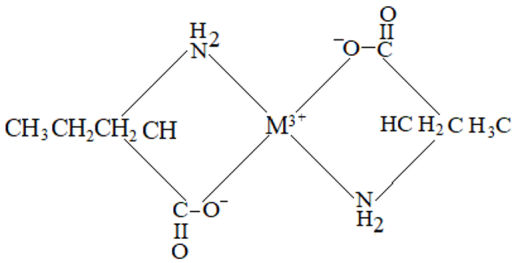
It is also clear from Table1 that stability constants of metal (III) – norvaline complexes follow the order Iron (III) > chromium (III) > aluminum (III). The high stability constant values of iron (III) – norvaline complex indicate strong bonding between iron (III) – norvaline complex may be due greater affinity to iron (III) cation for the oxygen donor ligands.

The stability constants of metal complexes may be very easily calculated by this technique; therefore, the present method has significant advantages over other methods (viz: polarographic, potentiometric, solubility etc.) reported in chemical literature for the determination of stability constants of metal complexes.

According to standard deviation (statistics) the precision of the method is limited to that of paper electrophoresis, and uncertainty in the result is ± 5%. Hence, it cannot immediately replace the most reliable methods, even though it is a new approach deserving further development.

The high stability constant values of iron (III) - norvaline complexes indicating strong bonding between iron (III) cation and norvaline anion, whilst low stability constant value of aluminum (III) – norvaline complexes indicate weak bonding between aluminum (III) cation and norvaline anion. The higher stability of iron (III) complexes may be due to its greater affinity for oxygen donor ligands.

The proposed structure for metal (III) – norvaline ML_2^+ binary complexes may be given as follow



In general calculation of first (K_1) and second (K_2) stepwise stability constants of binary metal complexes can be explained in following steps.

$$M + L \underset{}{\overset{K_1}{\rightleftharpoons}} ML$$

$$K_1 = \frac{[ML]}{[M][L]}$$

$$[ML] = K_1 [M] [L]$$

$$ML + L \overset{K_2}{\rightleftharpoons} ML_2$$

$$K_2 = \frac{[ML_2]}{[ML][L]}$$

$$[ML_2] = K_2 [ML] [L]$$

Where M = metal cation; L = Ligand anionic species; K_1 and K_2 = first and second stability constants, respectively.

$$U = \sum u_i f_i$$

Where U = overall mobility; u = mobility of particular species; f = mole fraction of particular species.

$$U = u_M f_M + u_{ML} f_{ML} + u_{ML2} f_{ML2}$$

$$\text{Mole fraction} = \frac{\text{Number of moles}}{\text{Total mole}}$$

$$\text{Total mole} = [M] + [ML] + [ML_2]$$

$$= [M] + K_1 [M] [L] + K_2 [ML] [L]$$

$$= [M] + K_1 [M] [L] + K_1 K_2 [M] [L]^2$$

$$= [M] [1 + K_1 [L] + K_1 K_2 [L]^2]$$

On considering $[1 + K_1 [L] + K_1 K_2 [L]^2] = J$

$$= MJ$$

$$f_M = \frac{[M]}{[M] J} = \frac{1}{J}$$

$$f_{ML} = \frac{K_1 [M][L]}{[M] \cdot J} = \frac{K_1 [L]}{J}$$

$$f_{ML2} = \frac{K_1 K_2 [M] [L]^2}{[M] J} = \frac{K_1 K_2 [L]^2}{J}$$

$$U = u_M \frac{1}{J} + u_{ML} \frac{K_1 [L]}{J} + u_{ML2} \frac{K_1 K_2 [L]^2}{J}$$

$$U = \frac{u_M + u_{ML} K_1 [L] + u_{ML2} K_1 K_2 [L]^2}{J}$$

On putting the value of J in above equation

$$U = \frac{U_M + u_{ML} K_1 [L] + u_{ML2} K_1 K_2 [L]^2}{1 + K_1 [L] + K_1 K_2 [L]^2}$$

For the calculation of first stability constant (K_1), first and second plateau are calculated then

$$U = \frac{U_M + u_{ML2} K_1 [L]}{1 + K_1 [L]}$$

when $K_1 [L] = 1$

then $K_1 = \frac{1}{[L]}$

$$U = \frac{u_M + u_{ML}}{2} \quad \text{(half mobility)}$$

Therefore, first stability constant (K_1) = 1/ [L]. [L] is concentration of ligating species at half of the mobility of first and second plateaus of mobility curve.

For the calculation of second stability constant, (K_2), second and third plateau are considered then

$$U = \frac{U_{ML} K_1 [L] + u_{ML2} K_1 K_2 [L]^2}{K_1 [L] + K_1 K_2 [L]^2}$$

$$U = \frac{K_1 [L] [u_{ML} + u_{ML2} K_2 [L]}{K_1 [L] [1 + K_2 [L]]}$$

when $K_2 [L] = 1$

then $K_2 = \frac{1}{[L]}$

$$U = \left\{ \frac{u_{ML} + u_{ML2}}{2} \right\} \quad \text{(half mobility)}$$

Therefore, second stability constant K_2 = 1/ [L]. [L] is the concentration of ligating species at half of the mobilities of second and third plateaus of mobility curve.

Conclusion

The following conclusions can be drawn from the present studies

- Aluminum (III), chromium (III) and iron (III) are significant for biological systems but as such they are toxic, the norvaline may be used to reduce the level of these metal ions in the biological systems.
- ML_2^+ complexes are found to have low stability constant values and are less stable in comparison to ML^{2+} complexes.
- The stability of metal (III) – norvaline complexes follow the order Fe (III) > Cr (III) > Al (III).
- Iron (III) metal ion have shown greater affinity with oxygen doner ligands in comparison to chromium (III) and aluminum (III) metal ions.
- Stability constants of metal complexes can be very easily calculated by the technique, so the present paper electrophoretic technique has significant advantages over the other physicochemical methods reported in chemical literature for the determination of stability constants of metal complexes.
- Future work is to prepare aluminum (III), chromium (III) and iron (III) binary complexes with norvaline at an optimum condition mentioned in this paper, characterize them and study their possible medical potential as a therapeutic agent.

References

1. Sherman SE, Lippard SJ (1987) Structural aspects of platinum anticancer drug interaction with DNA. Chem Rev 87: 1153-1181.
2. Banerjea D (1995) Some aspects on the role of metal ions in biological systems. Everyman's Sci 29: 176-184
3. Mujika JI, Rezabal E, Mercero JM, Ruiperez F, Costa D, et al. (2014) Aluminum in biological environments: A Computational Approach, Computational and Structural. Biotechnology Journal 9: 1-13.
4. Harris WR, Berthan G, Day JP, Exley C, Flaten TP, et al. (1996) Speciation of aluminum in biological systems. Journal of Toxicology and Environmental Health 48: 543-568.
5. Zhao H, Pogue AL, Alexandrov PN, Butler LG, Li W, et al. (2022) Alteration by aluminum – implications for protein misfolding disease. Molecules 27: 1-11.
6. Mezencer R, Gibbons C (2023) Interactions between chromium species and DNA in vitro and their potential role in the toxicity of hexavalent chromium. Metallonics 15: 1-12.
7. Wise SS, Wise Sr JP (2012) Chromium and genomic stability, NIH. Mutal Res 737: 78-82.
8. Lewick S, Zdanowski K, Krzyzowska M, Goniewicz M (2014) The role of chromium (III) in the organism and its possible use in diabetes and obesity treatment. Annals Agricultural and Environmental Medicine 27: 331-335.
9. Dworzariski W, Semfratowicz I, Cholewiriska E, Tutiz K., Fotchki B, et al. (2021) Effects of different chromium compounds on hematology and inflammatory cytokines in rate fed high – fat diet. Frontiers in Immunology 12: 1-14.
10. Abdullah, KI, Wani M, Naeem PK, Jha UC, Jha T, Aftab T, Persaud PVV (2024) Systems biology of chromium – plant interaction: Insights from omics approaches. Front Plant Sci 14: 1-11.
11. Rolic T, Yazdani M, Mandic S, Distante S (2024) Iron metabolism, calcium, magnesium and trace elements: A review. Biological Trace Element Research Springer 1-3.
12. Ru O, Li Y, Chen L, Wu Y, Min J, et al. (2024) Iron homeostasis and ferroptosis in human diseases: Mechanism and therapeutic prospects. Signal Transduction and Targeted Therapy 9: 1-64.
13. Kulaszyhbka M, Kwiatkowski S, Skonieczna – Zydecka K (2024) The iron metabolism with a specific focus on the functioning of the nervous systems. Biomedicines 12: 1-12.
14. Kontoghiorghes GJ (2023) Iron load toxicity in medicine: From molecular and cellular aspects to clinical implications. Int J Mol Sci 24: 1-28.
15. Anane J, Owusu E, Rievra G, Bandyopadhyay D (2024) Iron – imine cocktail in drug development: A contemporary update. Int J Mol Sci 25: 1-40.
16. Ogunbiyi EO, Kupa E, Adanma UM, Solomon NO (2024) Comprehensive review of metal complexes and nano –

composites: Synthesis, characterization and multifaceted biological applications. Eng Sci & Technol J 5: 1935-1951.
17. Andres CMC, De La Lastra JMP, Munguira EB, Juan CA, Perez – Leberia E (2024) Anticancer activity of metalodrugs and metallizing host defence peptides – current developments in structure – activity relationship. Int J Mol Sci 25: 1-45.
18. Adhikari S, Nath P, Das A, Datta A, Baildya N, et al. (2024) A review on metal complexes and its anti – cancer activities: Recent updates from in vivo studies. Biomedicine and Pharmacotherapy 171: 1-64.
19. Jimenez Perez A, Martinez Alonso M, Garcia Tozal J (2024) Hybrid hydroxyapatite – metal complex materials derived from amino acids and nucleobases. Molecules 29: 1-39.
20. Mohammed HS, Tripathi VD (2020) Medicinal applications of coordination complexes. J Phys Conf Ser IOP Publishing 1664: 1-12.
21. Chen J, Cui L, Lu S, Xu S (2024) Amino acid metabolism in tumor biology and therapy, Springer Nature. Cell Death and Disease 15: 1-18.
22. Li M, Wu Y, Ye L (2022) The role of amino acids in endothelial biology and function. Cells 11: 1-27.
23. Alvarez Carreno C, Becerra A, Lazcano A (2013) Norvaline and Norleucine may have been more abundant protein components during early stages of cell evolution. Orig. Life Evol. Biosph 63: 363-375.
24. Soini J, Falschlehner C, Liedert C, Bernhardt J, Vuaristo J, et al. (2008) Norvaline is accumulated after a down – shift of oxygen in Escherichia coli W3110. Microbiol Cell Factories 7: 1-14.
25. Gilinsky MA, Polityko YK, Markel AL, Latysheva TV, Samson AO, et al. (2020) Naumeanka, Norvaline reduces blood pressure and induces diuresis in rats with inherited stress – induced arterial hypertension. Hindwi Biomed. Research International 1-10.
26. Kiso Y (1972) Zone Electropheresis. New Attempts of Ionics Nankado.
27. Mc Donald HJ (1975) Ionography Electropheresis in Stabilized Media. Yearbook Publications Chicago.
28. Tewari BB (2023) Metal complexes in Biology and Medicine (Part – Mercury (II) / Nickel (II) / Lead (II) – Isoleucine). Novel Aspects in Chemistry and Biochemistry BP International London CH 9: 111-127.
29. Tewari BB (2014) Studies on biologically important cadmium (II) / iron (II) / zinc (II) – homoserine binary complexes in solution. Bolivian J Chem 7: 47-53.
30. Tewari BB (2005) Determination of stability constants of copper (II) / cobalt (II) / - methionine – cysteine (binary & mixed) complexes by Paper Electrophoretic Technique, Polish. Journal of Chemistry 79: 1853-1860.
31. Tewari BB (1995) Ionophoretic studi4es of lead (II) and uranyl (II) binary complexes with sulphur containing organic ligands (M – cysteine and M – methionine binary system), Trans. SAEST 30: 100-103.
32. Tewari BB, Singh RKP, Chander R, Yadava KL (1991) Electrophoretic Technique in determination of stability constants of mixed complexes, (M – nitrilotriacetate – cysteine system). Jour Chem Soc Pak 3: 224-248.
33. Micklitsch CM, Yu Q, Schneider JP (2006) Unnatural multidentate metal ligating alpha – amino acids. Teteahedron Lett 47: 6277-6280.
34. Remo M, Rode BM (2006) Effects of metal ions (Li+, Na+, K+, Mg2+, Ca2+ Ni2+, Cu2+ Zu2+) and water coordination on the structure of glycine and, Zwitterionic glycine. J Phys Chem A 110: 1960-1967.
35. Kolthoff M, Belcher R (1957) Volumetric Analysis. Intersciences Publisher Inc New York.
36. Vogel I (1978) Textbook of Quantitative Inorganic Analysis.4th Edition 1978, Longman London.
37. Blackburn R, Jones MM (1973) Stereoselective in the metal complex catalyzed hydrolysis of amino acid esters – III Distribution equilibria. J Inorg Nucl Chem 35: 1605-1620.
38. Jokl V (1964) Studies on complexation in solutions with paper electrophoresis. J Chromatogr 6: 432-439.
39. Pathak YZ, Joshi GB (1980) Stepwise stability constants and thermodynamic functions of Mn (II), Zn (II), and Cd (II) complexes with 2 – hydroxy – 5 – chlorobenzophenone – O – tolil (HCBOT). J Indian Chem Soc 38: 334-336.
40. Joshi D (1982) Mixed ligand complexes of zinc (II) and cadmium (II) with nitrilotriacetate acid as the primary ligand and amino acids as secondary ligands. Indian J Chem 21A: 446.
41. Bhattachanya PK (1981) Structure and stability of mixed ligand complexes. J Sci Ind Res 40: 382-384.