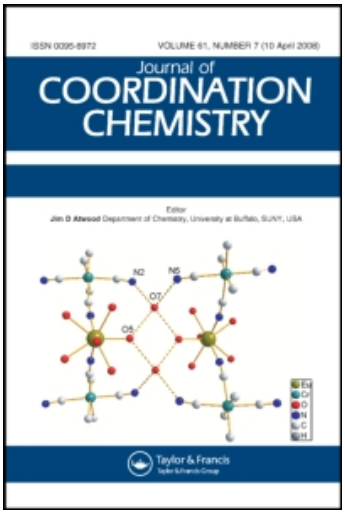


Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

Interaction of L-3,4-dihydroxyphenylalanin (L-DOPA) as a coordinating ligand with a series of metal ions; reaction of L-DOPA

Yahia Z. Hamada[a]; Cassietta Rogers[a]
[a] LeMoyne-Owen College, Memphis, TN, USA

First published on: 28 June 2007

To cite this Article Hamada, Yahia Z. and Rogers, Cassietta(2007) 'Interaction of L-3,4-dihydroxyphenylalanin (L-DOPA) as a coordinating ligand with a series of metal ions; reaction of L-DOPA', Journal of Coordination Chemistry, 60: 20, 2149 — 2163, First published on: 28 June 2007 (iFirst)
To link to this Article: DOI: 10.1080/00958970701256634
URL: http://dx.doi.org/10.1080/00958970701256634

Journal of Coordination Chemistry
Vol. 60, No. 20, 15 October 2007, 2149–2163

Taylor & Francis
Taylor & Francis Group

Interaction of L-3,4-dihydroxyphenylalanin (L-DOPA) as a coordinating ligand with a series of metal ions; reaction of L-DOPA

YAHIA Z. HAMADA* and CASSIETTA ROGERS

LeMoyne-Owen College, 807 Walker Avenue, Memphis, TN 38126 USA

(*Received 18 April 2006; in final form 20 September 2006*)

L-DOPA is an important neurotransmitter that is found in the brain and as a hormone in the circulatory system. We report in this article the similarities and differences in behaviour of this important neurotransmitter as a chelating agent among some divalent and trivalent metal ions using potentiometric titration in aqueous solutions at $25.0 \pm 1.0^{\circ}C$. The careful and detailed potentiometric titrations of L-DOPA with Al^{3+}, Cr^{3+}, Fe^{3+}, Cu^{2+}, and Zn^{2+} are discussed and compared. UV-Vis-spectroscopy is utilized for both the free L-DOPA and for the Fe^{3+}/L-DOPA system. The characteristic peak due to the $\pi \rightarrow \pi^*$ transition of the free L-DOPA at ~280 nm ($\varepsilon_{280\,nm} = 1927 \pm 65\,M^{-1}\,cm^{-1}$ between pH values of 2.0 to 3.0) disappeared when the iron solution was added to the L-DOPA sample in the same pH range. For the Fe^{3+}/L-DOPA system we have observed a new peak at 470 nm with $\varepsilon_{470} = 800 \pm 50\,M^{-1}\,cm^{-1}$. These comparison studies of the similarities and differences among these di- and tri-valent metal ions shed light on these systems in aqueous solutions. The appropriate metal simulation and speciation diagrams were constructed using the model that fit the titration data points.

Keywords: Aqueous solutions; L-DOPA; Potentiometric titrations; Protonated metal complexes; Speciation diagrams; UV-Vis spectra

1. Introduction

1.1. *L-DOPA*

In humans, L-DOPA is an important neurotransmitter. It also behaves as a hormone in the circulatory system. The initial enzymatic reaction in the biosynthesis of brain catecholamines involves the formation of L-DOPA from L-tyrosine [1], by the hydroxylation of tyrosine under the action of tyrosine hydroxylase [2, 3]. L-DOPA was first isolated from the seedlings of *Vicia faba*, which is a staple crop in Asia and the Mediterranean [4] and constitutes 6–9% of the dry seed weight of Mucuna species [5]. It is toxic to seed-eating beetle larvae at this 6–9% concentration [6]. L-DOPA is the

*Corresponding author. Tel.: (901) 435-1392. Fax: (901) 435-1424. Email: Yahia_hamada@loc.edu

Journal of Coordination Chemistry
ISSN 0095-8972 print/ISSN 1029-0389 online © 2007 Taylor & Francis
DOI: 10.1080/00958970701256634

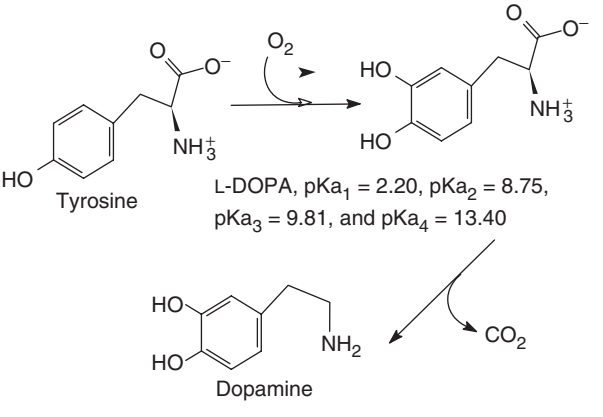
drug of choice for Parkinson's disease [7, 8]. Scheme 1 shows the structure of this biologically important ligand along with its synthetic route in humans along with its pKa values [9]. To our surprise, L-DOPA was also extracted and quantified from squid ink and it was found to be present with a concentration of 1.15×10^{-3} M which was significantly higher than the dopamine concentration of 0.19×10^{-3} M [10].

Aluminum, considered to be a neurotoxic metal ion, [11–16] and may play a role in the activation of δ-amino leuvlenate dehydrase which is involved in porphyrin synthesis, is blamed for many syndromes of dialysis such as dialysis encephalopathy, anemia, and osteomalacia [17–19]. There is evidence that Al plays a role in the pathology of Alzheimer's disease [20, 21]. Cr^{3+} is an essential trace metal necessary for the formation of the so-called 'Low Molecular Weight Chromium Complex' (LMWCr) but the site and mechanism of intestinal chromium absorption in humans have not been determined [22, 23]. Prion protein, responsible for Bovine Spongiform Encephalopathy (BSE) or 'mad cow disease' is a copper binding protein. The prion is remarkably selective for Cu^{2+} at which the metal ion is binding to two glycines following the histidine through the displacement of two amide protons [24, 25]. The concentration of zinc inside the biological cell is controlled by a complex system of transport and storage such that the free or loosely bound cytocolyic zinc is less than 1.0 femto-molar [26]. In contrast, the free or loosely bound zinc in the mammalian hippocampus is in the milli-molar range [27]. We are conducting titration studies at milli-molar concentrations in order to mimic some of the conditions for the chemistry of this important neurotransmitter L-DOPA with the zinc ion.

2. Experimental section

2.1. *Materials*

All solutions were prepared using reagent grade chromium(III) nitrate nonahydrate, $Cr(NO_3)_3 \cdot 9H_2O$, formula weight = 400.15 g mol^{-1}, iron(III) nitrate nonahydrate, $Fe(NO_3)_3 \cdot 9H_2O$, formula weight = 404.00 g mol^{-1}, aluminum nitrate nonahydrate, $Al(NO_3)_3 \cdot 9H_2O$, formula weight = 375.13 g mol^{-1}, (copper



Scheme 1. Synthetic routes for both L-DOPA and dopamine from the amino acid tyrosine in the biological milieu along with the pKa values of L-DOPA.

nitrate hemipentahydrate, $Cu(NO_3)_2 \cdot 2.5H_2O$, formula weight = 232.59 g mol^{-1}, or copper sulfate pentahydrate $Cu(SO_4) \cdot 5H_2O$, formula weight = 249.70 g mol^{-1} that was purchased from Sigma Chemical Company), and zinc nitrate hexahydrate, $Zn(NO_3)_2 \cdot 6H_2O$, formula weight = 297.49 g mol^{-1} using de-ionized (DI) water. L-DOPA was purchased from Spectrum Chemical Mfg. Corporation, Gardena, CA, $C_9H_{11}NO_4$, Formula weight = 197.19 g mol^{-1} which was used as received in the Zwitterion form. The pH values of all solutions were adjusted using standardized 0.1000 M sodium hydroxide, NaOH, solution. The pH values were measured using an advanced ISE/pH/mV/ORP Orion 720A+ meter connected to a combination Orion Gel-epoxy electrode. The pH values were measured accurately to the thousandths pH units. Some of the duplicated copper titration systems were conducted using the Orion model 250A pH meter that was connected to a combination Orion Gel-epoxy electrode. In this case, the pH values were measured accurately to the hundredths.

2.2. *Potentiometric titrations*

The total concentration of the metal ions used for the potentiometric protocol was in the range of 0.5–2.0 $\times 10^{-3}$ M. Before each titration, the titration solution mixtures were allowed to stir for 20–25 min to allow the titration system to attain complete equilibrium. All of the titrations were conducted at 25 ± 1.0°C.

2.3. *UV-Vis spectroscopy*

All UV-Vis spectra were measured using the T60 high performance spectrophotometer in connection with UVWIN software version 5.0 both purchased from Advanced ChemTech., Louisville, KY. Samples were prepared in D.I. water at 25 ± 1.0°C. The entire UV-Vis spectrum was scanned from 200 to 1100 nm using a quartz cuvette with an optical path length of 1.0 cm. Reference cuvettes filled with DI water were used with all measurements. The UV-Vis spectrum for the free L-DOPA was obtained at a concentration of 1.01 $\times 10^{-3}$ M at pH values of 3.01, 4.71, 9.10, and 10.61. The pH values were changed by the addition of a minimum amount of strong base to eliminate the dilution effect. We have repeated this experiment with another batch of free L-DOPA at a concentration of 5.07 $\times 10^{-4}$ M at pH values of 3.00, 4.70, 7.00, and 11.10 reproducing the spectra with minor differences at the higher pH values. The UV-Vis spectrum for the Fe^{3+} : L-DOPA in a 1 : 1 molar ratio was conducted with the concentration of 5.00 $\times 10^{-4}$ M at pH values of 2.36, 2.51, and 2.92.

3. Results and discussion

3.1. *Solubility of L-DOPA*

Researchers have published many reports regarding the aqueous solution chemistry of L-DOPA without reporting its limited solubility in aqueous solutions [28–32]. The Merck Index reports the solubility of L-DOPA in water in the range of 66 mg in 40 mL

of water [33] (8.4×10^{-3} M). We were able to prepare stock solutions of L-DOPA for the potentiometric titrations in the range of $\sim 10 \times 10^{-3}$ M quite easily but never succeeded at higher concentrations. Typical stock solution preparation of L-DOPA involved the following: exactly 1.0020 g powder L-DOPA was weighed and mixed with 100.0 mL DI water under stirring in a 600 mL beaker for 12 h. The white L-DOPA solid did not dissolve completely. Subsequent batches of 100.0 mL of DI water were added at a time with continuous stirring until all of the solid L-DOPA dissolved forming a homogenous solution. It took 500.0 mL of water in total and final volume, which gave the stock solution the concentration of 0.01014 M. This stock solution was kept in an amber bottle in a dark cabinet to minimize exposure to light. Aliquots of this stock solution were pipetted to prepare the potentiometric solutions described in the experimental section.

3.2. *Ligand pKa values*

L-DOPA has a total of four protonation sites, three are titratable within the normal range of potentiometric titration (1.50 to 11.50). These three groups are the carboxylate, one of the phenol groups (the first phenol group), and the amine group (see table 1). We have assigned $pKa_1 = 2.20$ to the carboxylate group, $pKa_2 = 8.75$ to the first phenol group, and $pKa_3 = 9.81$ to the amine group. Figure 1 shows the speciation diagram for the free L-DOPA under the following conditions: 0.20×10^{-3} moles total L-DOPA, pKw = 13.781 taken from Sweeton, Mesmer and Baes [34], and the titrant concentration = 0.10 M. This speciation diagram (figure 1) and all speciation diagrams presented in this study have been generated using the software program Hyss© [35]. It is clear from figure 1 that the cross points between the plots are the exact pKa values for this ligand. The major species present in solution in the acidic region is the tri-protonated ligand H_3dopa while the major species present in the alkaline solutions are the di-protonated and the mono-protonated ligand H_2dopa and Hdopa, respectively.

3.3. *Al^{3+}--L-DOPA titration system*

Figure 2 shows the family of potentiometric titration curves for the free L-DOPA and the Al^{3+}–L-DOPA complexes in the 1 : 1, 1 : 2, and 1 : 3 titration systems. The concentration of the Al^{3+} was set to 2.02×10^{-3} M. The pH values of the titration

Table 1. L-DOPA pKa values in aqueous solutions at 25 ± 1.0°C.

pKa values	L-DOPA pKa values[a]	Group assignment	Remarks
pKa_1	2.20	–COOH	Within the normal carboxylate pKa values[c]
pKa_2	8.75	–OH	Within the normal phenolate pKa values
pKa_3	9.81	$-NH_3^+$	Within the normal amine pKa values[c]
pKa_4	(13.40)[b]	(–OH) This pKa is too high to be measured potentiometrically[a]	Outside the normal titration range

[a]Martell and Smith reference number 9. [b]The second phenolic group of L-DOPA is outside the normal range to be measured potentiometrically. [c]See references 2, 3 and 9.

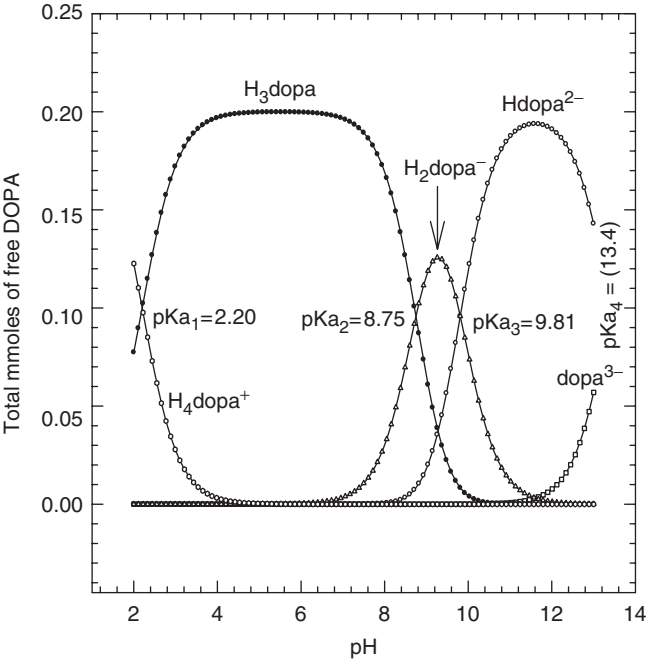


Figure 1. Speciation diagram of free L-DOPA using program Hyss [35] under the following conditions: total L-DOPA = 0.2×10^{-3} moles, initial pX = 2, final pX = 13, and pKw = 13.781 from reference [34].

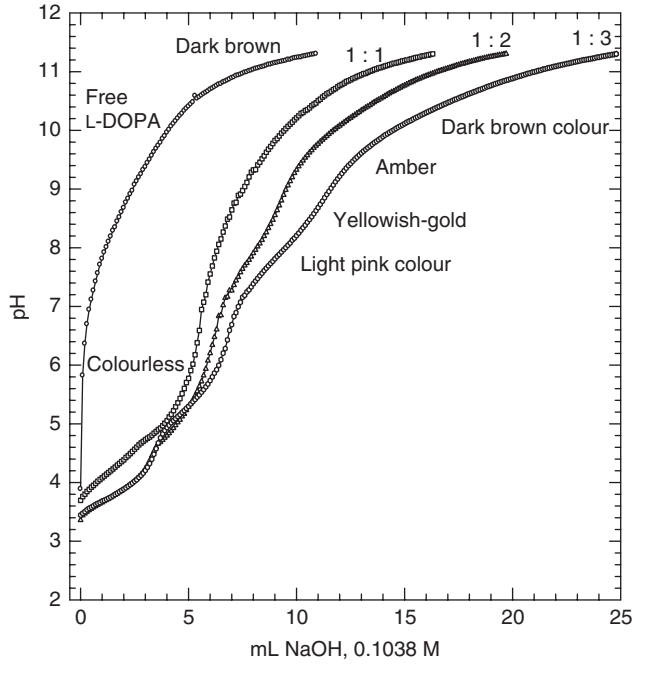


Figure 2. Potentiometric titration of Al^{3+}/L-DOPA in 1 : 1, 1 : 2, and 1 : 3 molar ratios. The inflection points appeared at 3.07 eq., 3.33 eq., and 3.73 eq., respectively. The equivalent (eq.) is defined as moles of NaOH/moles of Al^{3+}, $[Al^{3+}] = 2.02 \times 10^{-3}$ M.

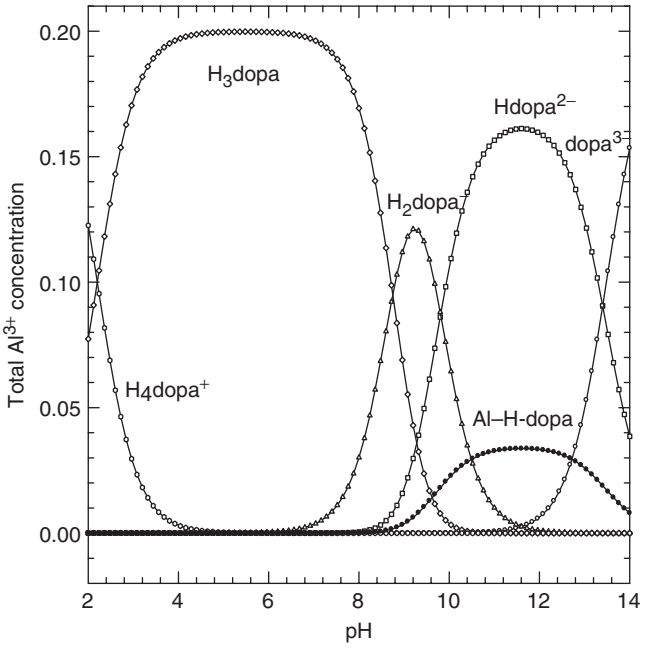


Figure 3. Speciation diagram of equimolar Al : L-DOPA using program Hyss. The mono-protonated species Al–H-dopa built-up to about 20%.

solutions were increased using 0.1038 M NaOH as the titrant. The free L-DOPA curve showed a sharp curve at the beginning of the titration starting at pH 4.0, indicating that L-DOPA has already lost its carboxylate hydrogen before addition of the first aliquot of the titrant. See figure 4 below for further confirmation. The free L-DOPA curve extended with a basic buffer region representing the release of the protons of the phenol and amine with pKa values of 8.75 and 9.81, respectively.

For the 1 : 1, 1 : 2, and 1 : 3 titration systems the titrations started within the acidic pH range of 3.50–3.70, which indicated that L-DOPA in the Al^{3+}–L-DOPA complex lost its carboxylate hydrogen before addition of the first 100 μL aliquot of the titrant. Release of protons per aluminum will be associated with the phenol groups and/or the amine group and/or the hydrolysis of the aqua ligand associated with the Al^{3+} ion. The following equivalents have been observed at the inflection points at pH 6.50: The 1 : 1 titration system showed an inflection at 3.07 equivalents per Al^{3+} ion, the 1 : 2 titration system showed an inflection at 3.33 equivalents per Al^{3+} ion, and 1 : 3 titration system showed an inflection at 3.74 equivalents per Al^{3+} ion.

Figure 3 shows the speciation diagram and distribution of total aluminum among the various species formed for Al^{3+} : L-DOPA in 1 : 1 ratio at 0.20×10^{-3} moles total Al^{3+}, $pKw = 13.781 \pm 0.006$ [34], and the titrant concentration = 0.10 M. In this speciation diagram it appears that only the protonated Al^{3+}–H-DOPA monomer built-up to about 20%.

There were some colour changes as the pH increased for the aluminum–L-DOPA titration system: colourless or clear titration mixture between pH 3.0 to 5.5, followed by light cloudy pink solutions between pH 5.5 to 6.5 which cleared above pH 6.5, followed by a light yellow colour between pH 7.5 to 9.0, followed by a darker yellow solution

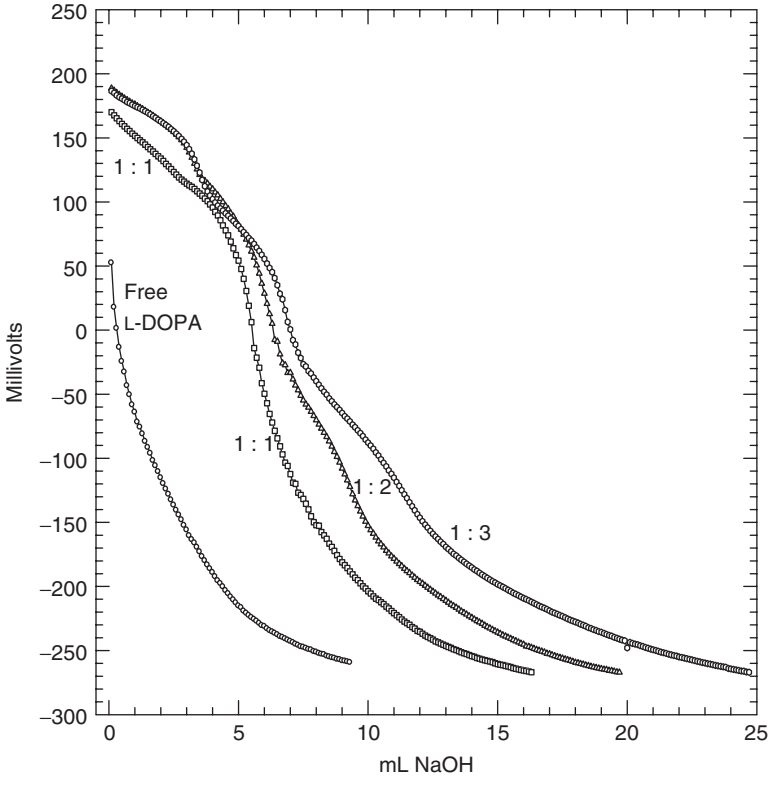


Figure 4. Correlation of added mL of titrant *vs.* millivolts for the free L-DOPA, Al^{3+} : L-DOPA in 1 : 1, 1 : 2, and 1 : 3 molar ratios. $[Al^{3+}] = 2.02 \times 10^{-3}$ M. Inflection points are right on the zero potential mark.

between pH values of 9.0 to 10.5, and purple or almost black solution at pH 11.0 to 11.5. These changes in colour were consistent and reproducible for the 1 : 1, 1 : 2, and 1 : 3 titration systems, repeated three times. It was somewhat surprising to see the development of bright colours in aqueous aluminum solutions [11–13].

To confirm whether the species formed are neutral, cationic, or anionic, we have generated millivolt response plots for free L-DOPA and the aluminum–L-DOPA complexes against the added volume of titrant. Figure 4 shows the correlation and the change in the values of mV *versus* mL of added titrant for the free L-DOPA titration system. At the beginning L-DOPA existed in the Zwitterionic form as purchased, but for the remainder of the titration curve the ligand existed mainly in the anionic state. Figure 4 also shows the potential response of the 1 : 1, 1 : 2, and 1 : 3 titration systems. The potential differences extend from ~(+200) to (−250 mV) in which there are a mixture of cationic and anionic species present above and below the zero potential value.

3.4. *Cr^{3+}--L-DOPA titration system*

Figure 5 shows the potentiometric titration curves for free L-DOPA and those for the Cr^{3+} : L-DOPA in 1 : 1, 1 : 2, and 1 : 3 molar ratios. It is clear from these curves that the 1 : 1, 1 : 2, and 1 : 3 titration graphs overlap. Because Cr^{3+} has slow ligand exchange

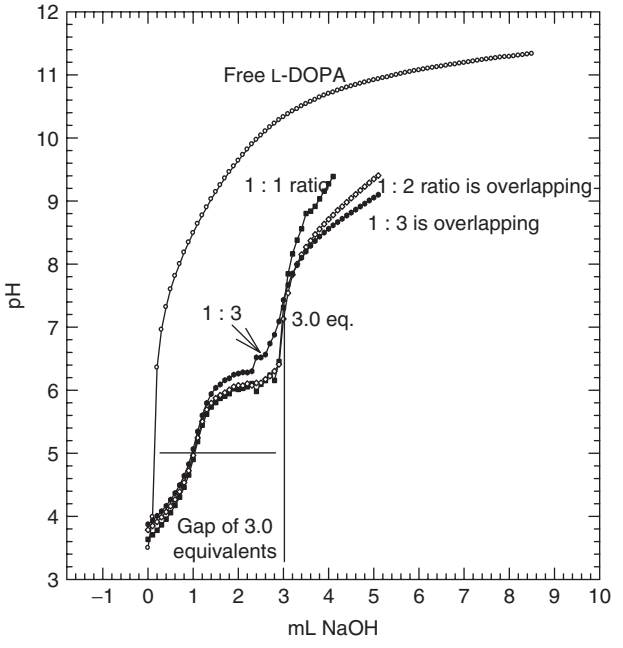


Figure 5. Titration of free L-DOPA along with titrations of Cr^{3+} : L-DOPA in 1 : 1, 1 : 2 and 1 : 3 ratios. It seems that the same kind of species are formed for all titration ratios because allcurves are overlapping. $[Cr^{3+}] = 1.0 \times 10^{-3}$ M.

in aqueous solutions (the rate constant for the exchange of water molecules in the hexa-aqua Cr^{3+} ion $k_{exch} = \sim 10^{-5}\,s^{-1}$) [12], the Cr^{3+} titration system was left standing for 24 h before starting the titration. Figure 5 shows two inflection points, an initial inflection at a pH of 4.5 and a major inflection at pH 7.5. The inflection at 7.5 appeared at $3.0 \pm 1.0 \times 10^{-3}$ equivalent protons.

Figure 6(a) shows the correlation of the mV values *versus* mL of added titrant for the free L-DOPA along with that of the Cr^{3+} : L-DOPA titration system in a 1 : 2 molar ratio. The potential response of this titration system is similar to that of the Al^{3+} : L-DOPA and the Fe^{3+} : L-DOPA systems (see figure 4 and figure 7b for comparison). Figure 6b shows the correlation of mV *versus* pH for the Cr^{3+} : L-DOPA in a 1 : 2 molar ratio. The mV values extend from ~(+150) to (−150 mV).

3.5. *Fe^{3+}–L-DOPA titration system*

Figure 7(a) shows the potentiometric titration curves for free L-DOPA and Fe^{3+}–L-DOPA in 1 : 1, 1 : 2, and 1 : 3 molar ratios. Figure 7(b) shows the potential response of the 1 : 3 titration system from ~(+200) to (−250 mV). Table 2 is the summary of the potentiometric titrations for the Al^{3+} : L-DOPA, Cr^{3+} : L-DOPA, and Fe^{3+} : L-DOPA in various molar ratios with at least three equivalents released per metal ion; the amine group (one proton) and the catecholate group (two protons). When iron was added to the L-DOPA solution, a deep blue colour was generated immediately that changed to green then deep purple colour upon addition of NaOH. These solutions have been studied using visible spectroscopy.

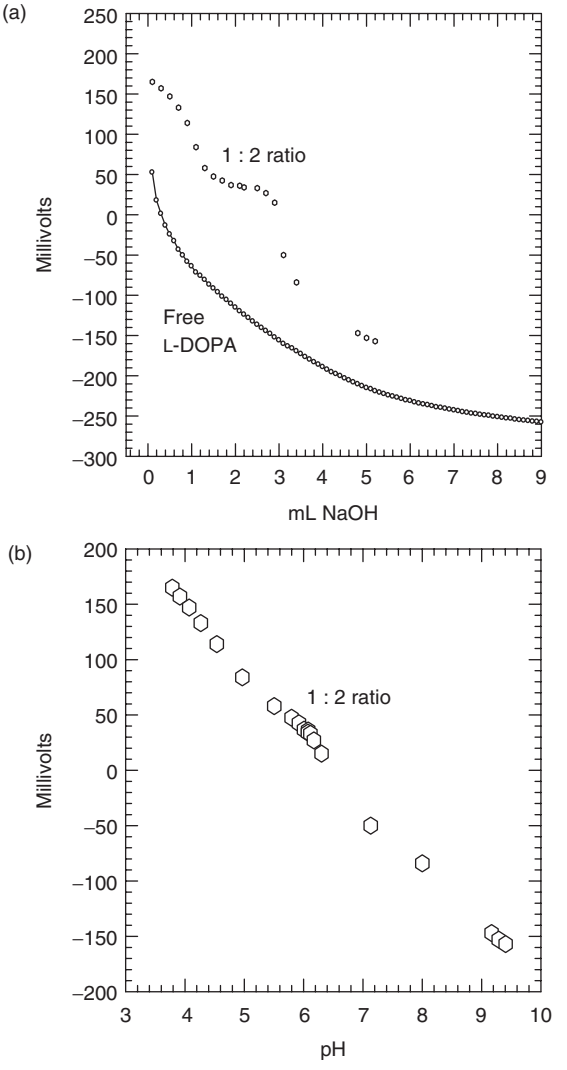


Figure 6. (a) Corrleation of mL NaOH *vs.* millivolts for the free L-DOPA and that of Cr^{3+} : L-DOPA in 1 : 2 ratio, (b) Correlation of millivolts *vs.* pH. Total Cr^{3+} concentration was set to $[Cr^{3+}] = 1.0 \times 10^{-3}$ M.

Figure 8 is the speciation diagram and distribution of total iron among the various complexes formed for the iron : L-DOPA system in the 1 : 1 ratio as a function of pH under the following conditions: 0.20×10^{-3} moles total Fe^{3+}, pKw = 13.781 ± 0.006 [34], and the titrant concentration = 0.10 M. It appeared that Fe^{3+}–L-DOPA monomer built up to about 75%.

3.6. *UV-Vis spectroscopy*

The UV-Vis spectrum of free L-DOPA at 1.01×10^{-3} M at pH 3.01 gave a sharp and distinct absorption peak with a maximum wavelength of ~280 nm with

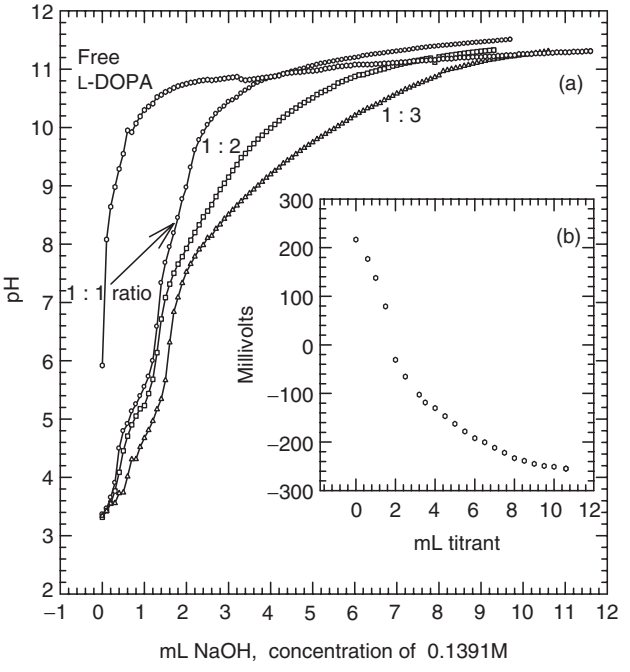


Figure 7. (a) Potentiometric titration for free L-DOPA, Fe^{3+}–L-DOPA titration systems in 1 : 1, 1 : 2, and 1 : 3 ratios. $[Fe^{3+}] = 0.50 \times 10^{-3}$ M. (b) Correlation of millivolts *vs.* mL of added titrant for the 1 : 3 ratio, most of the graph is in the negative region.

Table 2. Summary of the potentiometric titrations for the Al^{3+} : L–DOPA, Cr^{3+} : L-DOPA, and Fe^{3+} : L-DOPA in various molar ratios.

Metal ion	Number of equivalents per metal ion[a]			
Metal : DOPA ratio	1 : 1	1 : 2	1 : 3	σ[b]
Al^{3+}	3.07 eq.	3.33 eq.	3.74 eq.	0.010
Cr^{3+}	3.00 eq.	3.00 eq.	3.00 eq.	0.001
Fe^{3+}	3.63 eq.	3.90 eq.	4.74 eq.	0.010

[a]The equivalent of titrant is defined as the number of mol of titrant per number of mol of L-DOPA unless the metal ion is present. [b]σ is the standard deviation of typically 3–5 titration replicas.

$\varepsilon_{280} = 1881\ M^{-1}\,cm^{-1}$. When this experiment was repeated at 5.07×10^{-4} M at the same pH value the same sharp and distinct peak was reproduced with the same maximum absorption peak at the same wavelength. The molar extinction coefficient was averaged to $\varepsilon_{280} = 1927 \pm 65\ M^{-1}\,cm^{-1}$. We attribute this peak to the $\pi \rightarrow \pi^*$ transition of the aromatic ring. When the pH values increased from 3.0 to 4.7, then to 9.0, and then to 11.0; new bands with shoulders between 390 and 470 nm were observed, in good agreement with the literature for free de-protonated L-DOPA [28, 36, 37].

We recorded the UV-Vis spectra for the Fe^{3+} : L-DOPA in 1 : 1 molar ratio at 5.00×10^{-4} M at the pH values of 2.36, 2.51, and 2.92. The sharp and distinct peak at 280 nm disappeared from the Fe^{3+}–L-DOPA complex mixture while another peak appeared with shoulders, due to internal electron transfer from L-DOPA to the ferric ion as reported in the literature [37]. The disappearance of the $\pi \rightarrow \pi^*$ peak and the

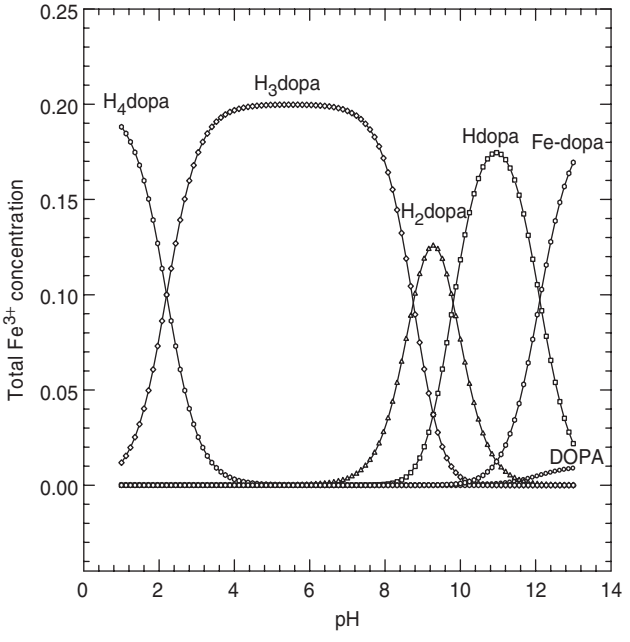


Figure 8. Speciation diagram of Fe^{3+} : L-DOPA in equimolar ratio, $0.20 \times 10^{-}3$ moles Fe^{3+} solution using program Hyss, and pKw = 13.781 taken from Sweeton, Mesmer and Baes [34]. The Fe–DOPA complex built up to a maximum of 75%.

appearance of the new peak with shoulders at low pH values (i.e. 2.36, 2.51, and 2.92) indicated that the iron is coordinated to the catecholate. There was a new peak at 470 nm with $\varepsilon = 800 \pm 50\,M^{-1}\,cm^{-1}$. This is the first report of a molar extinction coefficient for the Fe^{3+}–L-DOPA system. This represents an internal electron transfer in which L-DOPA is converted to dopaquinone [37] and the Fe^{3+} was reduced to Fe^{2+} Linert *et al*. We are in the process of measuring the oxidation–reduction potential using cyclic voltammetry for this important titration system.

3.7. *Cu^{2+}–L-DOPA titration system*

Figure 9 shows the potentiometric titration curves for the free L-DOPA, the free hexa-aqua copper solution and the Cu^{2+}–L–DOPA titration systems in the 1 : 1, 1 : 2, 1 : 3, 1 : 4, and 1 : 5 molar ratios; the titration system is well equilibrated and uniform. The free hexa-aqua copper solution showed an inflection point at exactly two equivalents with precipitation beginning around pH 7.30, indicating release of two net protons from the two axial aqua ligands. Equation (1) shows formation of copper hydroxide precipitate within the titration of the hexa-aqua copper solution. These two proton equivalents (2.04 ± 0.1) show the accuracy of the titration systems. If systems are not well equilibrated the free hexa-aqua copper will show an inflection point at a value other than two.

$$Cu^{2+} + 2OH^{-} \rightarrow Cu(OH)_2 \downarrow \text{ppt} \quad (1)$$

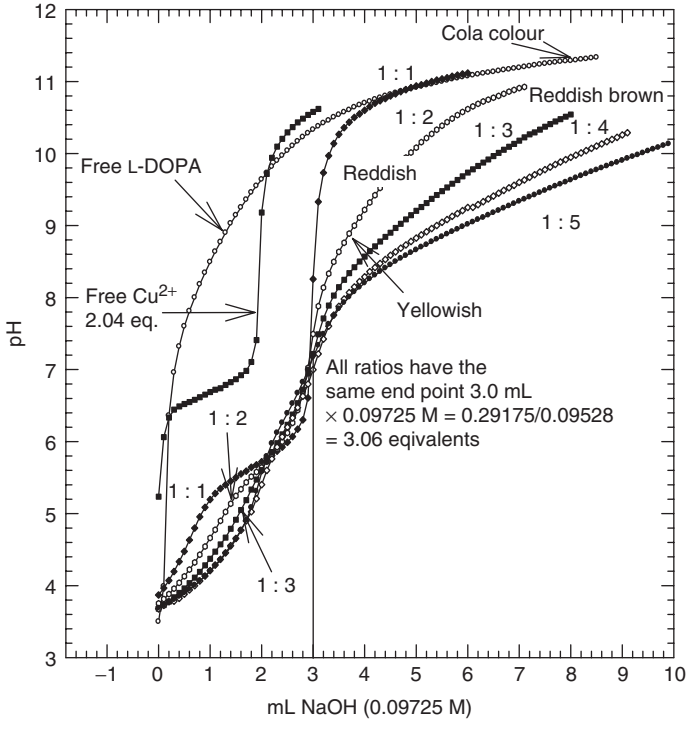


Figure 9. Potentiometric titrations of free L-DOPA, free Cu^{2+}, Cu^{2+} : L-DOPA in 1 : 1, 1 : 2, 1 : 3, 1 : 4, and 1 : 5 molar ratios. $[Cu^{2+}] = 1.014 \times 10^{-3}$ M. All ratios showed the same equivalence point that appeared at 3.06 equivalents of titrant per copper ion.

L-DOPA as a chelating ligand solubilized the copper ion at the physiological pH value. The Cu^{2+}–L-DOPA system produces the same species regardless of the metal to ligand ratio. This is evident from the appearance of the inflection points at 3.0 equivalents for the 1 : 1, 1 : 2, 1 : 3, 1 : 4, and the 1 : 5 titration ratios. These experiments have been repeated seven times with the following average and standard deviation (3.00 ± 0.10, $n = 7$ trials). There is no precipitation at any pH value for any of the Cu^{2+}–L-DOPA titration systems and three protons released each time the copper ion binds with L-DOPA. Because of the high pKa value of the second catecholate (pKa = 13.40), we suspect that one of the aqua ligands releases a proton via metal hydrolysis. It is not clear whether the second catecholate group has been de-protonated or not. We have launched detailed spectroscopic and kinetic studies to account for the type of species produced by these titration systems.

3.8. *Zn^{2+}–L-DOPA titration system*

Figure 10 shows the potentiometric titration curves for free L-DOPA, hexa-aqua zinc and the Zn^{2+}–L-DOPA systems in 1 : 1, 1 : 2, and 1 : 3 molar ratios. L-DOPA as a chelating ligand solubilized the zinc ion throughout the pH range and particularly the physiological pH value. Although the zinc titration system looks completely different from the copper titration system, there is a similarity between the two titration systems. The Zn^{2+} : L-DOPA titration system showed inflections at about three equivalents,

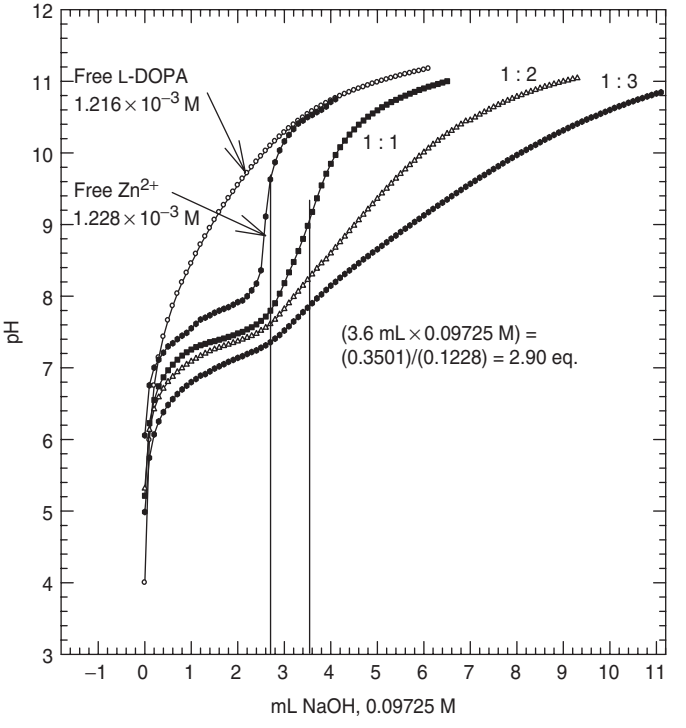


Figure 10. Potentiometric titrations of free 1.216×10^{-3} M free L-DOPA. Overlaying 1.228×10^{-3} M free Zn^{2+} solution along with these of Zn^{2+} : L-DOPA in 1 : 1, 1 : 2, and 1 : 3 molar ratios. $[Zn^{2+}] = 1.228 \times 10^{-3}$ M.

similar to the copper titration system. On the other hand the 1 : 1, the 1 : 2, and the 1 : 3 Zn^{2+} : L-DOPA titration systems did not superimpose as was the case with the copper titration system.

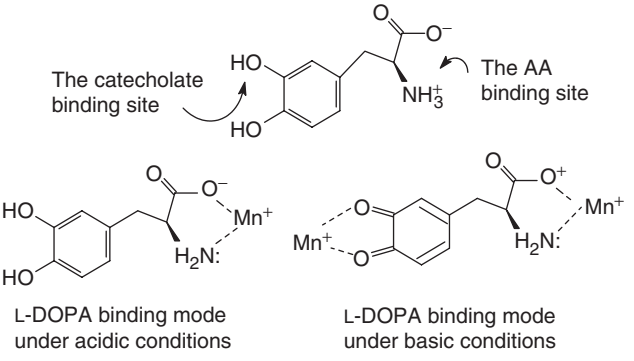
Titration of the free hexaaqua zinc solution showed an inflection at (2.06 ± 0.03) proton equivalents, proof that the potentiometric titration is accurate in measuring the number of total protons released per metal ion. Equation (2) shows the formation of the zinc hydroxide precipitate within the titration of the free hexa-aqua zinc solution around pH 8.4.

$$Zn^{2+} + 2OH^- \rightarrow Zn(OH)_2 \downarrow \text{ppt} \tag{2}$$

The Zn^{2+} : L-DOPA 1 : 1, 1 : 2, and 1 : 3 titration systems showed a well-buffered system between pH 6.1 and 7.5 in addition to the basic buffer region for the 1 : 2 and the 1 : 3 titration systems. When there is excess ligand present the ligand has a greater buffer capacity [2, 3].

4. Conclusion

Potentiometric titrations provide data more reliable than from titrations that use chemical indicators. They are particularly useful with coloured solutions and for detecting the presence of unexpected species [38]. The potentiometric titrations become even more powerful tools when coupled with UV-Vis-spectroscopy in determining the



Scheme 2. Proposed binding modes of L-DOPA as a chelating ligand of metal ions in aqueous solutions under acidic and basic conditions. The scheme shows the amino acid (AA) binding site and the (catecholate) binding site. Under acidic conditions the two catecholate protons will be intact. Under basic conditions the catecholate protons will be removed and eventually transformed into oxo-ligands. The oxo-ligand also has a very strong metal binding capacity.

identity of the coloured species. The potentiometric titrations of L-DOPA with various metal ions presented in this study are novel in their accuracy and the elucidation of the number of protons released within each system. L-DOPA as a chelating or coordinating ligand solubilized a variety of di- and tri-valent metal ions, Al^{3+}, Cr^{3+}, Fe^{3+}, Cu^{2+}, and Zn^{2+}. For the Fe^{3+}/L-DOPA system, the electronic transition at 470 nm with $\varepsilon = 800 \pm 50\,M^{-1}\,cm^{-1}$ was observed in the literature at 458 nm.

It is known from the literature that L-DOPA undergoes oxidation to form dopasemiquinone and even further oxidation to form the stable dopaquinone. Scheme 2 shows the proposed binding modes of L-DOPA as a chelating ligand of metal ions in aqueous solutions under acidic and basic conditions. The potentiometric titrations presented indicate that at least three protons are released from the interaction of L-DOPA with each metal ion. In these potentiometric titrations, it makes no difference whether the L-DOPA is in the original un-oxidized or in its oxidized, dopaquinone, state from the metal chelation point of view because both have the oxygen/nitrogen with the proper chelating capacity to chelate the metal ion under consideration. Synthesis of the various L-DOPA complexes that appeared in the speciation diagrams with the metal ions is being attempted in our laboratory.

Acknowledgments

This work was supported in part by NSF HBCU-UP under grant # HRD-0411493. We also would like to thank the Division of Natural and Mathematical Sciences for financial support. Special thanks are for Professor D. Harris and Mr. H. Holeyfield for reading the manuscript.

References

[1] J.P. Chalmer, R.J. Baldessarini, R.J. Wurtman. *Proc. Nat. Acad. Sci. US*, **68**, 662 (1971).
[2] Biochemistry, Instructor third edition by Garrett and Grisham. Thomson, Brooks/Cole, Belmont, CA (2005).

[3] A.L. Lehninger. In *Principles of Biochemistry*, D.L. Nelson and M.M. Cox (Eds), 3rd Edn, WORTH, New York, NY (2000).
[4] Antiparkinsonian, Special issue of C&EN News, June 20 2005.
[5] E.A. Bell. *J. Agric. Food Chem.*, **51**, 2854 (2003).
[6] D.H. Janzen, H.B. Juster, E.A. Bell. *Phytochemistry*, **16**, 223 (1977).
[7] F. Garcia-Garcia, S. Ponce, R. Brown, V. Cussen, J. Kruger. *Brian Res.*, **1042**, 160 (2005).
[8] H. Heikkinen, J.G. Nutt, P.A. LeWitt, W.C. Koller, A. Gordin. *Clinical Neuropharmacol.*, **24**, 150 (2001).
[9] A.E. Martell, R.M. Smith. *Critical selected Stability Constants of Metal Complexes Database, Version 6.0 for windows*, National Institute of Standards and Technology, Gaithersburg, MD, April (2001).
[10] M.T. Lucero, H. Farrington, W. Gilly. *Biol. Bull.*, **187**, 55 (1994).
[11] J. Huheey, E. Keiter, R. Keiter. *Inorganic Chemistry Principals of Structure and Reactivity*, 4th Edn, Harper Collins College Publishers, New York (1993), and references therein.
[12] B. Douglas, D. MacDaneil, J. Alexander. *Concepts and Models of Inorganic Chemistry*, 3rd Edn, John Wiley & Sons INC., New York (1994).
[13] D. Shriver, P. Atkins. *Inorganic Chemistry*, 3rd Edn, W.H. Freeman and Company, New York (1999).
[14] M. Venturini, G. Berthon. *J. Inorg. Biochem.*, **37**, 69 (1989).
[15] D.C. Ackley, R.A. Yokel. *Toxicology*, **120**, 89 (1997).
[16] R.A. Yokel, M. Wilson, R.W. Harris, A.P. Halestrap. *Brain Res.*, **930**, 101 (2002).
[17] I.M. Parkinson, K. Ward, D.N.S. Kerr. *J. Clin. Pathol.*, **34**, 1258 (1981).
[18] M.R. Wills, J. Savory. *Lancet*, **2**, 29 (1983).
[19] A.C. Alfrey. *New Eng. J. Med.*, **310**, 1113 (1984).
[20] R.J.P. Williams. *J. Inorg. Biochem.*, **76**, 81 (1999).
[21] C.J. Exley. *J. Inorg. Biochem.*, **76**, 133 (1999).
[22] John B. Vincent. *Polyhedron*, **20**, 1 (2001), and references therein.
[23] Carl A. Burtis and Edward R. Ashwood. In *Tietz Textbook of Clinical Chemistry*, Carl A. Burtis and Edward R. Ashwood (Eds), 2nd Edn, Saunders, Philadelphia, PA (1994).
[24] E. Frieden. *Sci. Am.*, **227**, 54 (1972).
[25] G.L. Millhauser. *Acc. Chem. Res.*, **37**, 79 (2004).
[26] C.E. Outten, T.V. O'Halloran. *Science*, **292**, 2488 (2001).
[27] C.C. Woodroofe, A.C. Won, S.J. Lippard. *Inorg. Chem.*, **44**, 3112 (2005), and references therein.
[28] F. Zhang, S. Bi, J. Liu, X. Wang, X. Yang, L. Yang, Q. Yu, J. Hu, Z. Bai. *Anal. Lett.*, **35**, 135 (2002).
[29] I.F. Sedeh, S. Sjöberg, L. Öhman. *J. Inorg. Biochem.*, **50**, 119 (1993).
[30] T. Kiss, I. Sóvágó, B.R. Martin. *J. Am. Chem. Soc.*, **111**, 3611 (1989).
[31] K. Prasad, K. Rao, M.S. Mohan. *J. Coord. Chem.*, **16**, 251 (1987).
[32] V.K. Patel, P.K. Bhattacharya. *Inorg. Biochem.*, **21**, 169 (1984).
[33] E. Rytting, K.A. Lentz, X.-Q. Chen, F. Qian, S. Venkatesh, *The Merck Index*, 10th Edn, pp. 784–785, Merck & Co. Inc., Rahway, NJ (1983).
[34] F.-H. Sweeton, R.-E. Mesmer, C.F. Baes Jr. *J. Sol. Chem.*, **3**, 191 (1974).
[35] L. Alderighi, P. Gans, A. Ienco, D. Perters, A. Sabatini, A. Vacca. *Coord. Chem. Rev.*, **184**, 311 (1999).
[36] B.A. Hasan, K.D. Khalaf, De La Guardia. *Talanta*, **42**, 627 (1995).
[37] W. Linert, R.F. Jameson, E. Herlinger. *Inorgan. Chim. Acta*, **187**, 239 (1991).
[38] D.A. Skoog, D.M. West, F.J. Holler, S.R. Crouch. *Fundamentals of Analytical Chemistry*, 8th Edn, Brooks-Cole/Thomson, Belmont, CA (2004).