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Iron(III), Chromium(III), and Copper(II) Complexes of L-Norvaline and Ferulic Acid

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Supporting Information

ABSTRACT: The complexation equilibria of L-norvaline (Nva) and ferulic acid (FA) were studied in aqueous solutions at room temperature (298 K) and in fixed ionic strength (0.15 $mol \cdot dm^{-3}$ $NaNO_3$) by means of potentiometry and spectrophotometry techniques. The ferric (Fe^{3+}), chromium (Cr^{3+}), and cupric (Cu^{2+}) complexing capacities of Nva and FA and their overall stability constants in aqueous solutions were obtained by the HYPERQUAD 2008 program from the potentiometric data. The concentration distributions of the various complex specia in solution were evaluated and discussed. The spectroscopic UV−visible measurements are carried out to give qualitative information about the confirmation of the complexes formed in these solutions.

INTRODUCTION

It is known that nonprotein amino acids, which are common in fodder plants, consumed animal feeds, and human foods, are a threat to human and animal health.[1] They possess a wide array of chemical structures and often contain functional groups not commonly present in natural products.[1,2] Some act as agonists or antagonists in the neurotransmission of higher animals.[1,2] Others influence the growth of fungi and bacteria.[1,2] Norvaline ($C_5H_{11}NO_2$) is an unusual nonproteinogenic branched-chain amino acid which has been of interest particularly in early enzymological research work.[3] Recently, it has been proved that L-norvaline (Nva) has antifungal activity as it inhibits arginase thus increasing arginine concentrations.[4] More specifically, Nva has been shown to inhibit homoserine dehydrogenase.[5] Moreover, some biological studies have indicated that Nva has antitubercular and antifungal actitvities.[6,7] Also, a lot of research has been focused on the role of antioxidant ferulic acid (FA) in several biological processes.[8]

Taking into account the natural occurrence, biosynthesis, metabolism, and functions of nonprotein amino acids and phenolic acids, the study of their metal complexes gains much attention and is attracting more attention in equilibrium and biological chemistry. Hence, a study of metal−Nva−FA complexes may lead to better elucidation of the behavior of such compounds in biological systems and metal−Nva−FA interactions might be of therapeutic interest. A search in the literature shows that there has been quite a number of studies reported on the interaction between metal ions and Nva.[9−27] Most of these research works investigated the complexation equilibria of the copper(II), nickel(II), cobalt(II), manganese(II), uranyl(II), terbium(III), lanthanum(III), praseodymium(III), neodymium(III), and trimethyltin(IV) metal ions with Nva using different electroanalytical techniques. However, there is no study conducted on the iron(III) or chromium(III) metal ion binary complexes of Nva, and any metal ion−norvaline mixed ligand complexes.

Our recent publications describe the protonation equilibrium phenomena of essential amino acids,[28] hydroxamic acids,[29] phenolic compounds,[30] and nonprotein Nva,[31] L-canavanine[32] amino acids in aqueous and in aqueous−organic solvent solutions. The present work reports potentiometric and spectrophotometric results on the binary and mixed ligand complexes involving transition metal ions (Fe^{3+}, Cr^{3+}, Cu^{2+}), with Nva and FA. The objective of this study was to gain insight into the complexation properties of Nva and FA. The quantification of metal-chelating activity was measured by the potentiometric technique with a glass electrode, and the experimental data were analyzed using the TINET,[33] GLEE,[34] and HYPERQUAD 2008[35] computer programs. A detailed quantitative examination of the complexation species formed in the metal ion−Nva−FA systems is presented. The graphic representation of the complex species concentration curves is given by the distribution diagrams performed by means of the HySS modeling program,[36] which furnishes a variety of data presentations, including tables of concentrations of all species present in solution in the selected pH ranges. Spectroscopic analyses of different complex species were characterized by means of UV−visible spectroscopy.

EXPERIMENTAL SECTION

Materials and Solutions. Nva ($C_5H_{11}NO_2$) of analytical grade (Merck, Germany) was used without further purification. The Nva obtained was assayed in triplicate by titration with a carbonate-free solution of standard sodium hydroxide (Acros Organics, USA). This assay showed that the mass fraction purity of the Nva was (0.99 ± 0.05). A carbonate-free sodium hydroxide solution was prepared by dissolving Analar pellets in ultra pure water, and the solution was standardized potentiometrically with potassium hydrogen phthalate (Sigma-Aldrich, USA).[37] A nitric acid (Pancreac, Spain) solution (≈ 0.04 $mol \cdot dm^{-3}$) was prepared and used after being standardized. Ferric nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$), sodium hydroxide and sodium nitrate were supplied by Acros Organics, USA. *trans*-Ferulic acid, copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), and chromium

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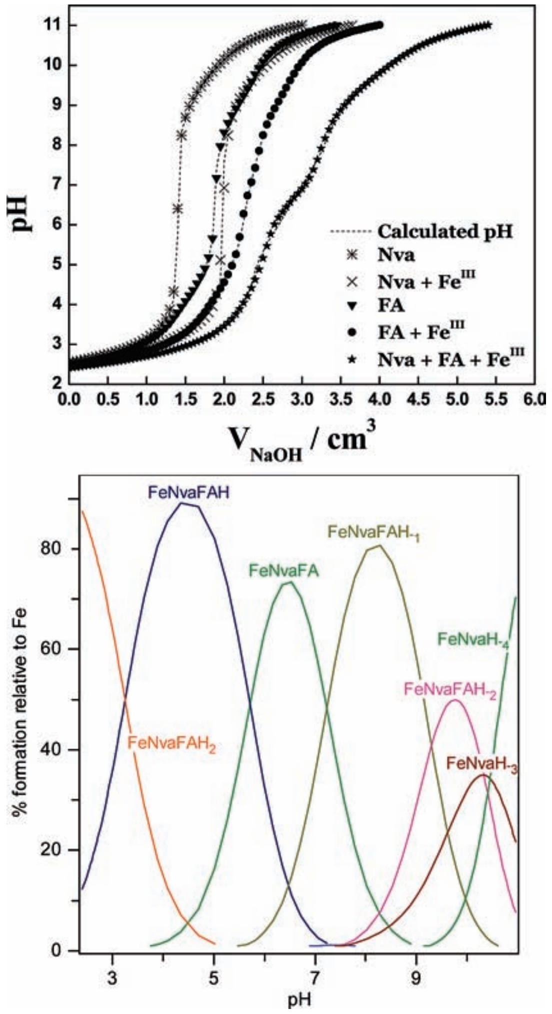


Figure 1. Potentiometric titration and species distribution curves for the Fe(III) + L-norvaline (Nva) + ferulic acid (FA) system at T = 298.15 K and I = 0.15 mol·dm^{-3} NaNO$_3$. Percentages are calculated with respect to the analytical concentration of the metal ion.

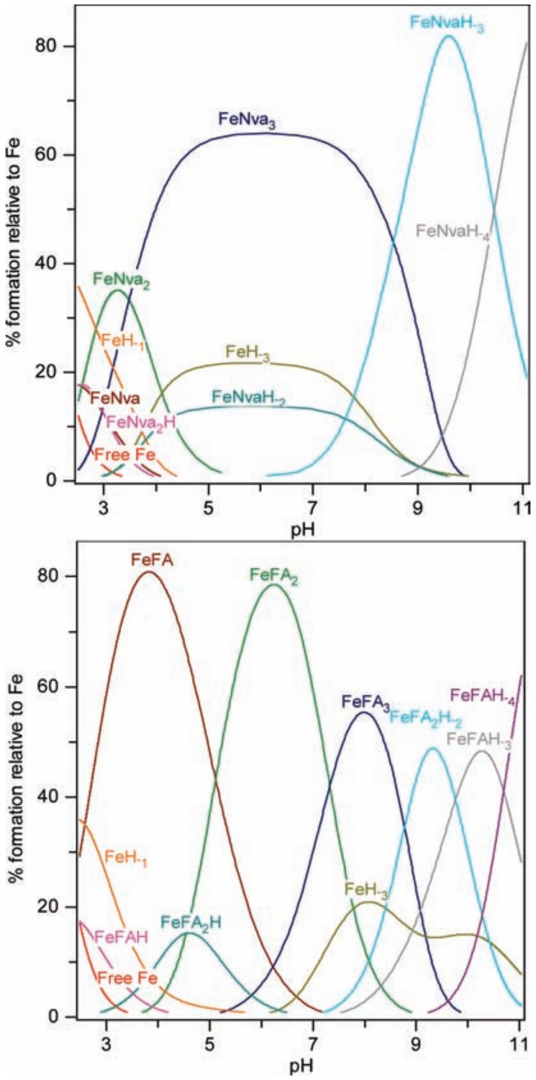


Figure 2. Species distribution diagrams for (top) Fe(III) + Nva and (bottom) Fe(III) + FA binary systems at T = 298.15 K and I = 0.15 mol·dm^{-3} NaNO$_3$. Percentages are calculated with respect to the analytical concentration of the metal ion.

chloride hexahydrate (CrCl$_3$·6H$_2$O) were obtained from Sigma-Aldrich, USA. All solutions used throughout the experiments were prepared freshly in ultra pure water obtained from a NANO pure-Ultrapure water system that was distilled and deionized with a resistance of 18.3 MΩ·cm^{-1}.

Apparatus and Equilibrium Titration Procedures. The pH-potentiometric titrations were performed using a Metrohm 702 SM titroprocessor with a 664 dosimate, a 728 magnetic stirrer, coupled with a dosino buret model 683. The electrode response can be read to the third decimal place in terms of pH units with a precision of ± 0.001 and the potential with a precision of ± 0.1 mV. The titroprocessor was coupled to a personal computer, and the titration software TINET version 2.4 was used to control the titration and data acquisition. The pH titrations were carried out in a 150 cm^3 commercial glass vessel. The ionic strength of the solutions was maintained at a constant level by using the desired concentration of NaNO$_3$ solution as supporting electrolyte. The pH meter was calibrated with standard buffer solutions (pH 4.0 and 7.0) before and after each series of pH measurements. The results of strong acid versus alkali titrations were analyzed using a computer program (GLEE, glass electrode evaluation)[36,37] that has been used for the calibration of the glass electrode by means of a strong acid−strong base titration. The program GLEE has been developed as part of the HYPERQUAD suite of programs for stability constant determination.[35] This program provides an estimate of the carbonate concentration of the base, the pseudo-Nernstian standard potential, and slope of the electrode and optionally the concentration of the base and the values of pK_w (pK_w = 13.77 at ∼298 K). For the protonation constants estimation of Nva and FA, the following solutions were prepared (total volume 50 cm^3) and titrated potentiometrically against a standard carbonate-free NaOH (0.1 mol·dm^{-3}) solution:

(a) 0.003 mol·dm^{-3} HNO$_3$ + 0.1 mol·dm^{-3} NaNO$_3$.
(b) Solution (a) + 0.001 mol·dm^{-3} Nva.
(c) Solution (a) + 0.001 mol·dm^{-3} FA.

For the determination of the binary metal complexes, solutions containing Nva or FA and metal ions were titrated at 1:1, 1:2, and 1:3 metal ion to ligand mole ratios to fulfill the maximum coordination number of the metal ion, and for ternary systems, a 1:1:1 metal ion/Nva/FA ratio was used. The concentration of metal ion and ligand solutions in the titrated samples were always

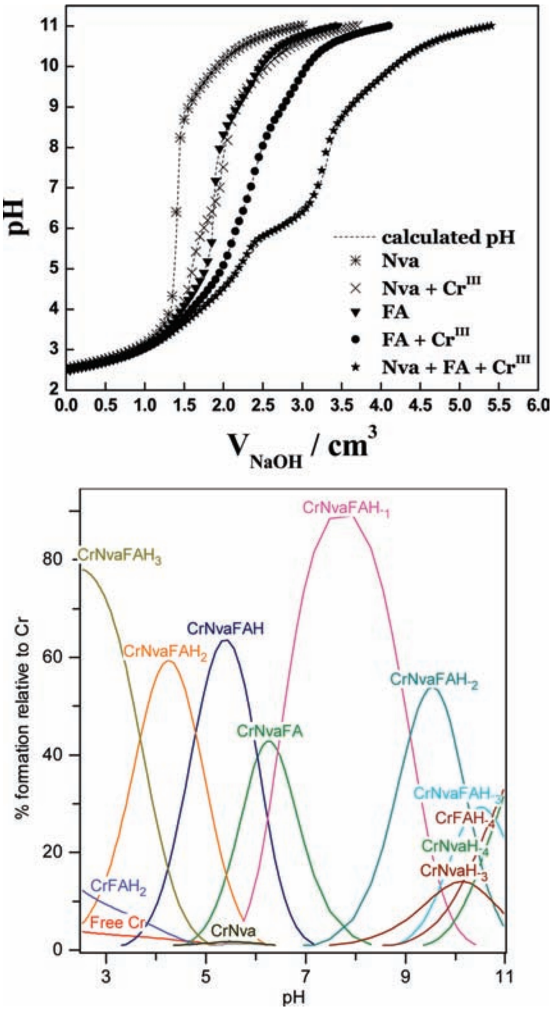


Figure 3. Potentiometric titration and species distribution curves for Cr(III) + Nva + FA system at T = 298.15 K and I = 0.15 mol·dm^{-3} NaNO$_3$. Percentages are calculated with respect to the analytical concentration of the metal ion.

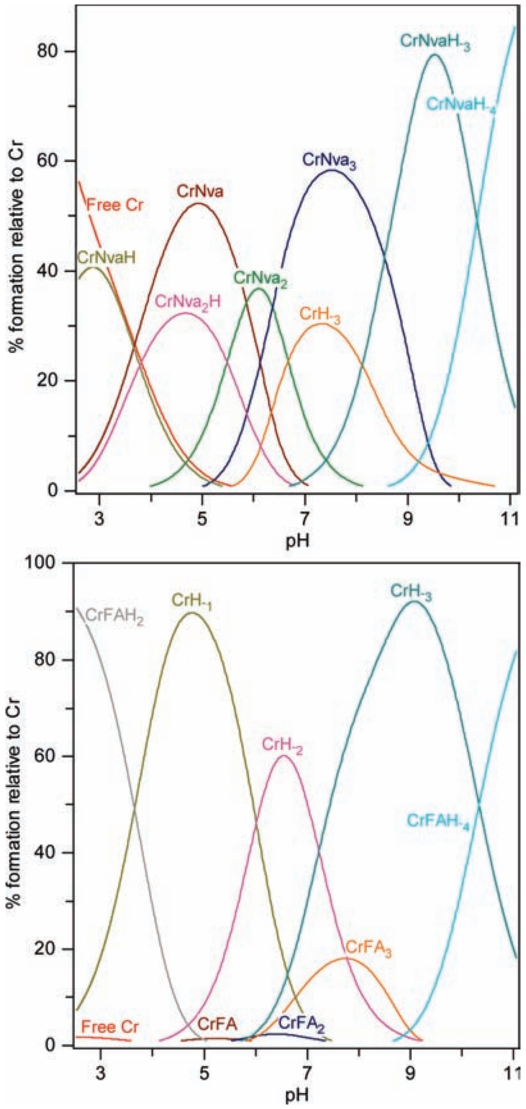


Figure 4. Species distribution diagrams for (top) Cr(III) + Nva and (bottom) Cr(III) + FA binary systems at T = 298.15 K and I = 0.15 mol·dm^{-3} NaNO$_3$. Percentages are calculated with respect to the analytical concentration of the metal ion.

the same and varied in the range (0.00004 to 0.0001) mol·dm^{-3}. Each solution was thermostatted at 298.15 K, where the solutions were left to stand for several minutes before titration. A magnetic stirrer was used during all titrations. Each titration was repeated at least three times under carefully controlled experimental conditions. Typically, more than 80 pH readings (points of potentiometric measurements) were collected and taken into account for each titration.

Calculations. For computing the protonation constants of the ligands (Nva and FA) and their metal complexes from pH titration data, the best equilibrium model was sought by systematically testing *pqr* conditions using an advanced software program HYPERQUAD 2008.[35] This software facilitates visual interpretations of refinement which in turn helps greatly in obtaining the best fit. To compute stability constants from potentiometric and spectrophotometric data, many software programs such as BEST, LETAGROP, MINIQUAD, PKAS, SUPERQUAD, and HYPERQUAD have been used to date. All of these programs use the least-squares approach. Among them, HYPERQUAD 2008 is one of the most recent versions of the HYPERQUAD program to determine stability constants from potentiometric and spectrometric data.[35]

Spectrophotometric Measurements. Absorption spectra were obtained using UV—vis spectra (Perkin-Elmer; model Lambda 25).

RESULTS AND DISCUSSION

By fitting the experimental data at the titration curves (Figures 1 to 6) of both ligands, Nva and FA using HYPERQUAD 2008program, the protonation constants (log β_1 and log β_2) were evaluated for Nva and FA are presented in Table 1. As is known, certain functional groups found in biological molecules, in particular carboxylic acids or amino groups can gain or lose H$^+$ depending on the availability of hydrogen ions (or protons) in the solution. In computer programs such as HYPERQUAD the protonation equilibira of the ligand are defined as overall association constants. This means that for Nva or FA two equilibrium constants are expressed as

$$H^+ + A^{2-} \rightleftharpoons HA^-; \quad [HA^-] = \beta_1[H^+][A^{2-}]$$

$$2H^+ + A^{2-} \rightleftharpoons H_2A; \quad [H_2A] = \beta_2[H^+]^2[A^{2-}]$$

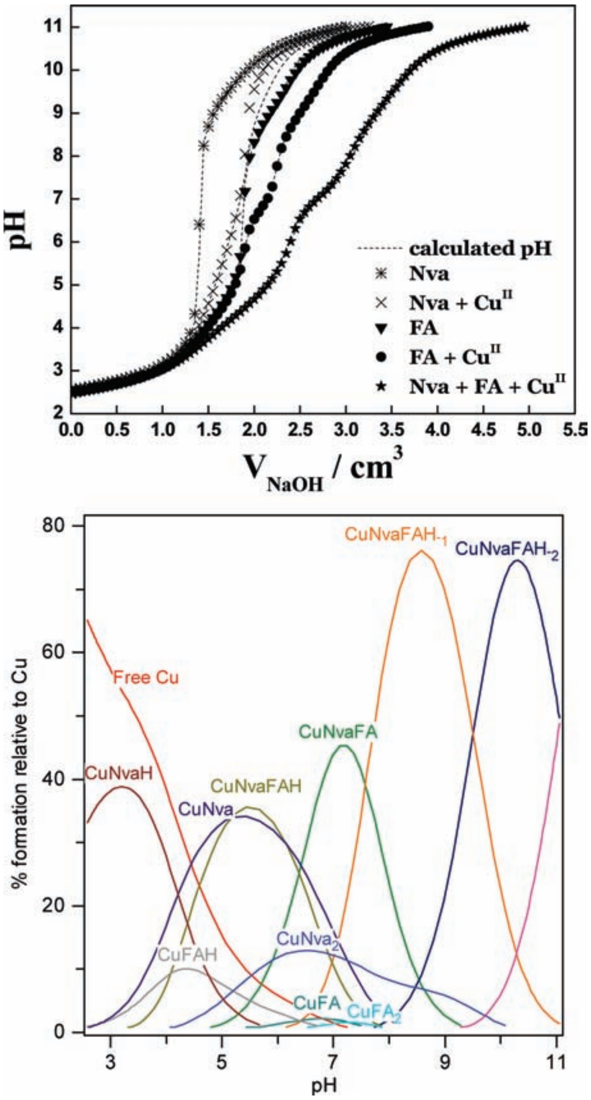


Figure 5. Potentiometric titration and species distribution curves for Cu(II) + Nva + FA system at $T = 298.15$ K and $I = 0.15$ mol·dm^{-3} NaNO$_3$. Percentages are calculated with respect to the analytical concentration of the metal ion.

The stepwise acid dissociation constants of Nva or FA can be defined as

$$H_2A \rightleftharpoons H^+ + HA^-; \quad [H_2A] = K_{a1}[H^+][HA^-]$$

$$HA^- \rightleftharpoons H^+ + A^{2-}; \quad [HA^-] = K_{a2}[H^+][A^{2-}]$$

It is worth mentioning that the pK_{a1} (2.61) and pK_{a2} (9.29) values of Nva investigated can be associated with the carboxylic acid and amino functions, respectively, since log β_1 = pK_{a2} and log β_2 = pK_{a1} + pK_{a2} As for FA, the protonation constants pK_{a1} (4.46) and pK_{a2} (8.77) were found for the carboxylic acid and the hydroxyl group at C(4) in the *meta*-position, respectively. From the chemical structures shown in Scheme 1 and the protonation equilibria of Nva and FA, one can conclude that protonation equilibrium constants are controlled by the electronic effects of substituent groups, aromatic nuclei, and the space between the functional groups.

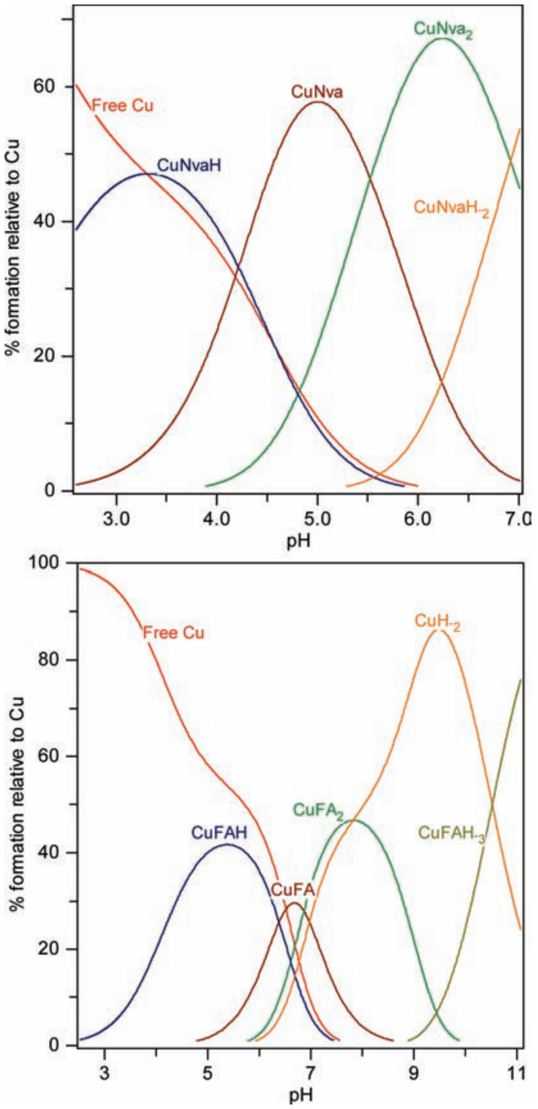


Figure 6. Species distribution diagrams for (top) Cu(II) + Nva and (bottom) Cu(II) + FA binary systems at $T = 298.15$ K and $I = 0.15$ mol·dm^{-3} NaNO$_3$. Percentages are calculated with respect to the analytical concentration of the metal ion.

From the experimental data shown in Table 1, it was noted that the protonation constants of Nva and FA obtained in this work were consistent with those reported in the literature within a very reasonable range, after allowing for the variation in experimental conditions such as ionic strength and background medium as well as the methods of calculation.[38,39]

The formation constants (log β_{pqrs}) for the binary and mixed ligand systems involving transition metal ions (Fe^{3+}, Cr^{3+}, and Cu^{2+}) with Nva and FA as well as the pH intervals in which the data were collected are presented in Tables 2 to 4. The complexation between metal ion (M) and Nva and FA can be described by the general equilibrium:

$$pM + qNva + rFA + sH \rightleftharpoons M_pNva_qFA_rH_s$$

$$\beta_{pqrs} = \frac{[M_pNva_qFA_rH_s]}{[M]^p[Nva]^q[FA]^r[H]^s}$$

where p, q, r, and s are the coefficients that indicate the stoichiometry associated with the possible equilibria in solution;

p, q, r, and s are the coefficients for metal ion, ligand Nva, ligand FA, and H-atoms, respectively. The stoichiometries and stability constants of the complexes formed were determined by trying various possible composition models. The selected model was the one that gave the best statistical fit and seemed chemically sensible and consistent with the titration data, without giving any systematic drifts in the magnitudes of the various residuals.

Figures 1 to 5 show the pH-metric titration curves of metal ion—Nva—FA binary and ternary systems. Experimental and calculated titration curves are represented by symbols and dashed lines. Good fitting between these curves confirms the validity of the obtained complexation models. In those figures, we can see a good overlap of experimental and calculated values of pH. Refinements using the entire pH range were successful for all complexes except for binary Cu(II) complexes with Nva which were carried out over the pH range 2.60 to 7.01. In all titrations, the experimental pH titration curves recorded for the binary and ternary metal ion—Nva—FA systems, when compared with the pH titration curves of Nva and FA ligands alone as shown in Figures 1 to 5, proves that complex formation between the metal ions studied (Fe^{3+}, Cr^{3+}, and Cu^{2+}) and the two ligands (Nva and FA) did occur. The ternary complex formation may proceed either through a stepwise or simultaneous mechanism depending on the chelating potentials of Nva and FA.

The color of the solution was light purple for the Fe^{3+}—Nva—FA system at pH < 6 and changed to yellow at pH ~ 6—8 and orange at pH > 8. The solution was colorless initially and changed to greenish blue for the Cr^{3+}—Nva—FA system at 4.04 < pH < 6.18 and then clear blue at pH > 6.18. For the Cu^{2+}—Nva—FA system, its

Table 1. Logarithms of the Protonation Constants (β_{pqrH}) of Nva and FA in Water at Ionic Strength (I = 0.15 mol·dm^{-3} $NaNO_3$) and Temperature (T = 298 K)[a]

species	p	q	r	s	log β_{pqrs}	pH range	SD
					Nva		
$NvaH^-$	0	1	0	1	9.29 (9.68)[13]	2.62—11.12	0.0496
$NvaH_2$	0	1	0	2	11.90 (11.99)[13]	2.62—11.12	0.1332
					FA		
FAH^+	0	1	1	1	8.77 (8.94)[38]	2.53—11.10	0.0274
FAH_2	0	1	1	2	13.23 (13.42)[38]	2.53—11.10	0.0412

[a] All of the values were calculated with the program Hyperquad 2008 from potentiometric investigations at 298 K and I = 0.15 mol·dm^{-3} $NaNO_3$. The symbols p, q, r, and s are used in the programs to indicate the stoichiometric coefficients associated with the possible equilibria in solution.

Table 2. Logarithms of the Overall Stability Constants (β_{pqrH}) of Binary and Ternary Ferric (Fe(III)) Complexes with Nva and FA in Water at Ionic Strength (I = 0.15 mol·dm^{-3} $NaNO_3$) and Temperature (T = 298 K)[a]

complex series	p	q	r	s	log β_{pqrs}	pH range	SD
				Nva Complexes			
$FeNva^+$	1	1	0	0	10.47	2.52—11.08	0.0663
$FeNva_2^-$	1	2	0	0	20.70	2.52—11.08	0.0338
$FeNva_3^{3-}$	1	3	0	0	30.15	2.52—11.08	0.0833
$FeNva_2H$	1	2	0	1	23.30	2.52—11.08	0.0399
$FeNvaH_{-2}^-$	1	1	0	−2	3.35	2.52—11.08	0.0501
$FeNvaH_{-3}^{2-}$	1	1	0	−3	−4.26	2.52—11.08	0.0635
$FeNvaH_{-4}^{3-}$	1	1	0	−4	−14.72	2.52—11.08	0.1213
$Fe(OH)^{2+}$	1	0	0	−1	−2.05	2.52—11.08	0.0853
$Fe(OH)_3$	1	0	0	−3	−9.52	2.52—11.08	0.0459
				FA Complexes			
$FeFA^+$	1	0	1	0	11.61	2.47—11.03	0.0929
$FeFA_2^-$	1	0	2	0	18.78	2.47—11.03	0.0301
$FeFA_3^{3-}$	1	0	3	0	24.10	2.47—11.03	0.0650
$FeFAH^{2+}$	1	0	1	1	13.86	2.47—11.03	0.0933
$FeFA_2H$	1	0	2	1	23.32	2.47—11.03	0.1056
$FeFA_2H_{-2}^{3-}$	1	0	2	−2	2.63	2.47—11.03	0.0404
$FeFAH_{-3}^{2-}$	1	0	1	−3	−10.51	2.47—11.03	0.0554
$FeFAH_{-4}^{3-}$	1	0	1	−4	−21.20	2.47—11.03	0.0689
$Fe(OH)^{2+}$	1	0	0	−1	−2.16	2.47—5.03	0.0713
$Fe(OH)_2^+$	1	0	0	−2	−5.04	2.49—5.74	0.1332
$Fe(OH)_3$	1	0	0	−3	−14.31	2.47—11.03	0.0780
				Mixed Ligand Complexes			
$FeNvaFA^{1-}$	1	1	1	0	25.86	2.39—10.96	0.0516
FeNvaFAH	1	1	1	1	31.58	2.39—10.96	0.0547
$FeNvaFAH_2^+$	1	1	1	2	34.82	2.39—10.96	0.0675
$FeNvaFAH_{-1}^{2-}$	1	1	1	−1	18.63	2.39—10.96	0.0462
$FeNvaFAH_{-2}^{3-}$	1	1	1	−2	9.33	2.39—10.96	0.0622

[a] All of the values were calculated with the program Hyperquad 2008 from potentiometric investigations at 298 K and I = 0.15 mol·dm^{-3} $NaNO_3$. The symbols p, q, r, and s are used in the programs to indicate the stoichiometric coefficients associated with the possible equilibria in solution.

Scheme 1. Protonation Equilibria of Nva and FA

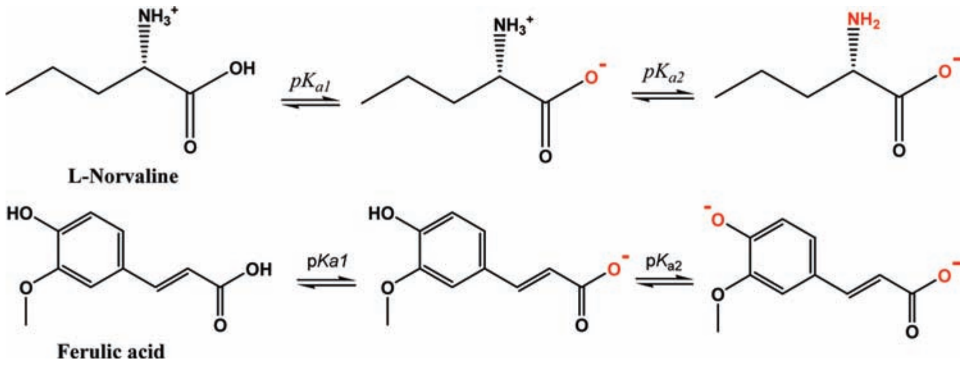


Table 3. Logarithms of the Overall Stability Constants (β_{pqrH}) of Binary and Ternary Chromium (Cr(III)) Complexes with Nva and FA in Water at Ionic Strength ($I = 0.15$ mol·dm^{-3} $NaNO_3$) and Temperature ($T = 298$ K)[a]

complex series	p	q	r	s	log β_{pqrs}	pH range	SD
			Nva Complexes				
$CrNva^+$	1	1	0	0	8.85	2.60−11.09	0.0656
$CrNva_2^-$	1	2	0	0	15.80	2.60−11.09	0.0466
$CrNva_3^{3-}$	1	3	0	0	22.36	2.60−11.09	0.103
$CrNvaH^{2+}$	1	1	0	1	12.52	2.60−11.09	0.1113
$CrNva_2H$	1	2	0	1	21.29	2.60−11.09	0.1007
$CrNvaH_{-3}^{2-}$	1	1	0	−3	−11.95	2.60−11.09	0.0576
$CrNvaH_{-4}^{3-}$	1	1	0	−4	−22.30	2.60−11.09	0.1043
$Cr(OH)_3$	1	0	0	−3	−16.76	2.60−11.09	0.0979
			FA Complexes				
$CrFA^+$	1	0	1	0	8.17	2.53−11.06	0.0182
$CrFA_2^-$	1	0	2	0	14.33	2.53−11.06	0.0216
$CrFA_3^{3-}$	1	0	3	0	20.10	2.53−11.06	0.0460
$CrFAH^{2+}$	1	0	1	1	12.49	2.53−11.06	0.0307
$CrFAH_2^{3+}$	1	0	1	2	18.12	2.53−11.06	0.0798
$CrFAH_{-4}^{3-}$	1	0	1	−4	−22.37	2.53−11.06	0.0871
$Cr(OH)^{2+}$	1	0	0	−1	−1.93	2.53−11.06	0.0727
$Cr(OH)_2^+$	1	0	0	−2	−7.49	2.53−11.06	0.0814
$Cr(OH)_3$	1	0	0	−3	−15.17	2.53−11.06	0.0848
			Mixed Ligand Complexes				
$CrNvaFA^{1-}$	1	1	1	0	17.83	2.54−10.94	0.0783
CrNvaFAH	1	1	1	1	23.87	2.54−10.94	0.0764
$CrNvaFAH_2^+$	1	1	1	2	28.66	2.54−10.94	0.0838
$CrNvaFAH_3^{2+}$	1	1	1	3	32.35	2.54−10.94	0.0992
$CrNvaFAH_{-1}^{2-}$	1	1	1	−1	11.39	2.54−10.94	0.0584
$CrNvaFAH_{-2}^{3-}$	1	1	1	−2	2.29	2.54−10.94	0.0671
$CrNvaFAH_{-3}^{4-}$	1	1	1	−3	−8.01	2.54−10.97	0.1108

[a] All of the values were calculated with the program Hyperquad 2008 from potentiometric investigations at 298 K and $I = 0.15$ mol·dm^{-3} $NaNO_3$. The symbols p, q, r, and s are used in the programs to indicate the stoichiometric coefficients associated with the possible equilibria in solution.

Table 4. Logarithms of the Overall Stability Constants (β_{pqrH}) of Binary and Ternary Cupric (Cu(II)) Complexes with Nva and FA in Water at Ionic Strength ($I = 0.15$ mol·dm^{-3} $NaNO_3$) and Temperature ($T = 298$ K)[a]

complex series	p	q	r	s	log β_{pqrs}	pH range	SD
			Nva Complexes				
CuNva	1	1	0	0	8.26 (8.11)[16]	2.60−7.01	0.0558
$CuNva_2^{2-}$	1	2	0	0	15.37 (15.14)[16]	2.60−7.01	0.0593
$CuNvaH^+$	1	1	0	1	12.47	2.60−7.01	0.0647
$CuNvaH_{-2}^{2-}$	1	1	0	−2	−4.21	2.60−7.01	0.037
			FA Complexes				
CuFA	1	0	1	0	5.41 (5.12)[39]	2.53−11.08	0.0671
$CuFA_2^{2-}$	1	0	2	0	10.52 (10.71)[39]	2.53−11.08	0.0584
$CuFAH^+$	1	0	1	1	11.80	2.53−11.08	0.0876
$CuFAH_{-3}^{3-}$	1	0	1	−3	−20.96	2.53−11.08	0.0767
$Cu(OH)^+$	1	0	0	1	−8.03	2.53−11.08	0.0681
$Cu(OH)_2$	1	0	0	−2	−13.55	2.53−11.08	0.0577
			Mixed Ligand Complexes				
$CuNvaFA^{2-}$	1	1	1	0	13.97	2.59−11.05	0.0449
$CuNvaFAH^{-1}$	1	1	1	1	20.35	2.59−11.05	0.063
$CuNvaFAH_{-1}^{3-}$	1	1	1	−1	6.39	2.59−11.05	0.0376
$CuNvaFAH_{-2}^{4-}$	1	1	1	−2	−3.11	2.59−11.05	0.0426
$CuNvaFAH_{-3}^{5-}$	1	1	1	−3	−14.17	2.59−11.05	0.0709

[a] All of the values were calculated with the program Hyperquad 2008 from potentiometric investigations at 298 K and $I = 0.15$ mol·dm^{-3} $NaNO_3$. The symbols p, q, r, and s are used in the programs to indicate the stoichiometric coefficients associated with the possible equilibria in solution.

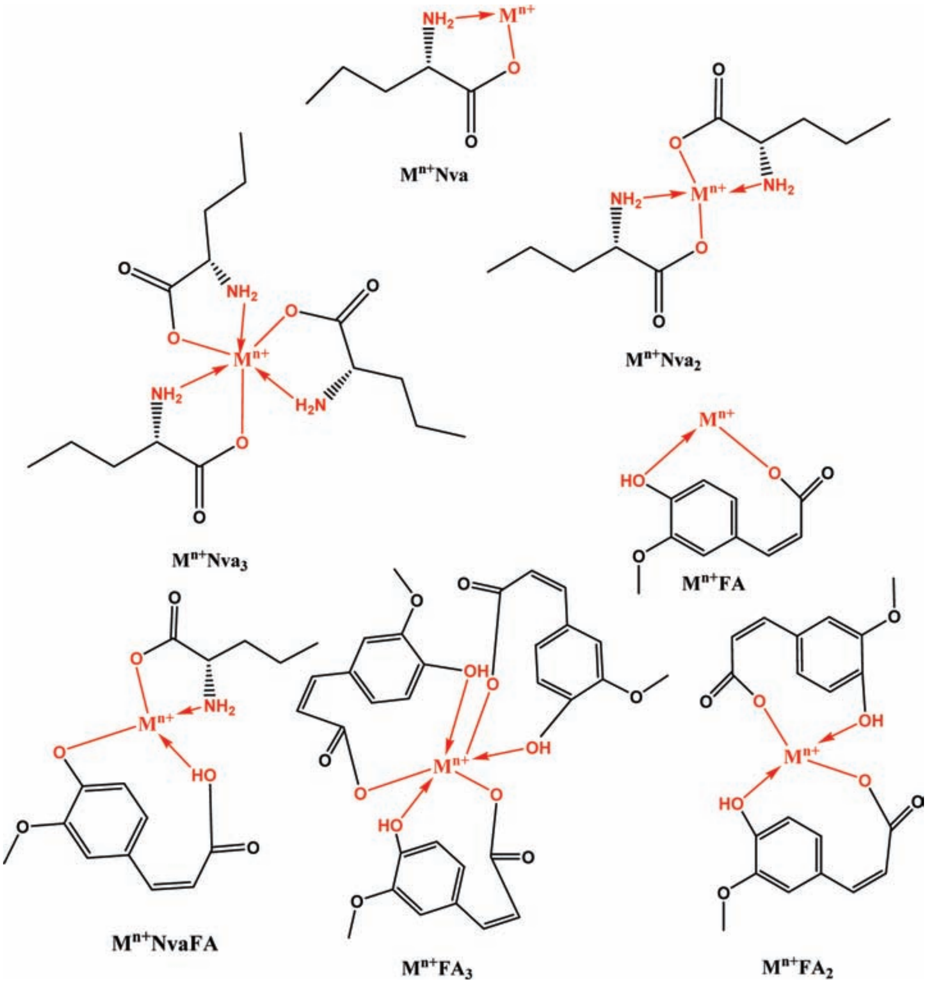
color changed from colorless initially to light blue at pH 4.5 then to greenish blue at pH 8.37. No precipitation occurred in all solutions during the titrations. However, slight turbidity was observed in the case of the Cr^{3+}−Nva−FA system at pH ∼ 6.18−8.37.

The bioavailability of metals and their physiological and toxicological effects depend on the actual species present. The equilibrium distributions of various complex species formed in water solutions are shown as functions of pH (Figures 1 to 5). The graphical distribution diagrams in Figures 1 to 6 show that the protonated binary and mixed ligand complex species which include $FeNva_2H$, $FeFAH^{2+}$, $FeFA_2H$, FeNvaFAH, $FeNVaFAH_2^+$, $CrNvaH^{2+}$, $CrNva_2H$, $CrFAH^{2+}$, $CrFAH_2^{3+}$, CrNvaFAH, $CrNvaFAH_2^+$, $CrNvaFAH_3^{2+}$, $CuNvaH^+$, $CuFAH^+$, and $CuNvaFAH^{1-}$ started to form at very low pH. The deprotonated binary and mixed ligand complexes such as $FeNva^+$, $FeNva_2^-$, $FeNva_3^{3-}$, $FeFA^+$, $FeFA_2^-$, $FeFA_3^{3-}$, $FeNvaFA^{-1}$, $CrNva^+$, $CrNva_2^-$, $CrNva_3^{3-}$, $CrFA^+$, $CrFA_2^-$, $CrFA_3^{3-}$, $CrNvaFA^{1-}$, CuNva, $CuNva_2^{2-}$, CuFA, $CuFA_2^{2-}$, and $CuNvaFA^{1-}$ were formed in acidic conditions. On the other hand, the species $FeNvaH_{-2}^-$, $FeNvaH_{-3}^{2-}$, $FeNvaH_{-4}^{3-}$, $FeFA_2H_{-2}^{3-}$, $FeFAH_{-3}^{2-}$, $FeFAH_{-4}^{3-}$, $FeNvaFAH_{-1}^{2-}$, $FeNvaFAH_{-2}^{3-}$, $CrNvaH_{-3}^{2-}$, $CrNvaH_{-4}^{3-}$, $CrFAH_{-4}^{3-}$, $CrNvaFAH_{-1}^{2-}$, $CrNvaFAH_{-2}^{3-}$, $CrNvaFAH_{-3}^{4-}$, $CuNvaH_{-2}^{2-}$, $CuFAH_{-3}^{3-}$, $CuNvaFAH_{-1}^{3-}$, $CuNvaFAH_{-2}^{4-}$, and $CuNvaFAH_{-3}^{5-}$ started to form at neutral conditions. The metal hydroxyl complexes such as $Fe(OH)^{2+}$, $Fe(OH)_2^+$, $Fe(OH)_3$, $Cr(OH)^{2+}$, $Cr(OH)_2^+$, $Cr(OH)_3$, $Cu(OH)^+$, and $Cu(OH)_2$ were formed in alkaline conditions.

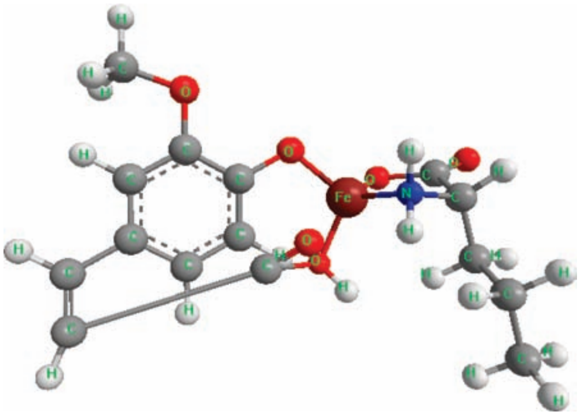
In coordination chemistry, HSAB theory classifies acceptors (metal ions, proton) and donors (ligands: Nva, FA) as hard or soft based on their thermodynamic behavior (or coordination affinities) in aqueous solutions. The simple rule of this useful theory is "hard acceptors prefer hard donors and soft acceptors prefer soft donors".[40] Both ferric(III) and chromium(III) are considered as hard acceptors, while copper(II) could be considered as a borderline ion. In other words, metals that tend to bond covalently preferentially form complexes with ligands that tend to bond covalently, and similarly, metals that tend to bond electrostatically preferentially form complexes with ligands that tend to bond electrostatically. On the other hand, Nva possesses two different donor atoms: N (of amino group) and O (of carboxyl group), while FA possesses one donor atom O (of phenolic group) and another O (of carboxylic group). Hence, during complexation of Fe^{3+}, Cr^{3+}, and Cu^{2+} with Nva or FA, these donor atoms were involved, as shown in the proposed molecular structures of some of these complexes in Scheme 2, and in the molecular modeling of the Fe(III)−Nva−FA ternary complex (Scheme 3).

The logarithms of the overall stability constants (log β_{pqrs}) of the binary and mixed ligand complexes investigated are given in Tables 2 to 4. As can be seen from this data, it was observed that:

Scheme 2. Proposed Molecular Structure of Some Binary and Ternary Complexes: M^{n+} = Fe^{3+}, Cr^{3+}, and Cu^{2+}



Scheme 3. Proposed Molecular Structure of a Fe(III)−Nva−FA Ternary Complex



(i) The stability constants of Fe^{3+}-FA, Cr^{3+}-FA, Cu^{2+}-Nva, and Cu^{2+}-FA binary complexes agreed well with the data reported previously in the literature.[13,16,38,39]

(ii) The stability of ferric(III), chromium(III), and cupric(II) binary complexes of Nva are higher than those of FA.

(iii) For the Fe^{3+}−Nva−FA acid system, the overall stabilities of the ternary complex species formed decrease in the order $FeNVaFAH_2^{+}$ > FeNvaFAH > $FeNvaFA^{1-}$ > $FeNvaFAH_{-1}$ > $FeNvaFAH_{-2}$, while the stabilities of its binary complexes with Nva decrease in the order $FeNva_3^{3-}$ > $FeNva_2H$ > $FeNva_2^{-}$ > $FeNva^{+}$ > $FeNvaH_{-2}$ > $FeNvaH_{-3}$ > $FeNvaH_{-4}$. The stability of complex formation of ferric−FA complexes decrease in the order $FeFA_3^{3-}$ > $FeFA_2H$ > $FeFA_2^{-}$ > $FeFAH^{2+}$ > $FeFA^{+}$ > $FeFA_2H_{-2}$ > $FeFAH_{-2}^{3-}$ > $FeFAH_{-3}^{2-}$ > $FeFAH_{-4}^{3-}$.

(iv) In the case of the Cr^{3+}−Nva−FA system, it was found that the overall stabilities of binary Nva complexes decrease in the order $CrNva_3^{3-}$ > $CrNva_2H$ > $CrNva_2^{-}$ > $CrNvaH^{2+}$ > $CrNva^{+}$ > $CrNvaH_{-3}^{2-}$ > $CrNvaH_{-4}^{3-}$ and $CrFA_3^{3-}$ > $CrFAH_2^{3+}$ > $CrFA_2^{-}$ > $CrFAH^{2+}$ > $CrFA^{+}$ > $CrFAH_{-4}^{3-}$ in the case of binary FA complexes. For the mixed ligand complexes, the stability trend is as follows: $CrNvaFAH_3^{2+}$ > $CrNvaFAH_2^{+}$ > CrNvaFAH > $CrNvaFA^{1-}$ > $CrNvaFAH_{-1}^{2-}$ > $CrNvaFAH_{-2}^{3-}$ > $CrNvaFAH_{-3}^{4-}$.

(v) For the Cu^{2+}−Nva−FA system, it was found that the overall stability of the binary Nva complexes decrease in the order $CuNva_2^{2-}$ > $CuNvaH^{+}$ > CuNva > $CuNvaH_{-2}^{2-}$, and $CuFAH^{+}$ > $CuFA_2^{2-}$ > CuFA > $CuFAH_{-3}^{3-}$ in the

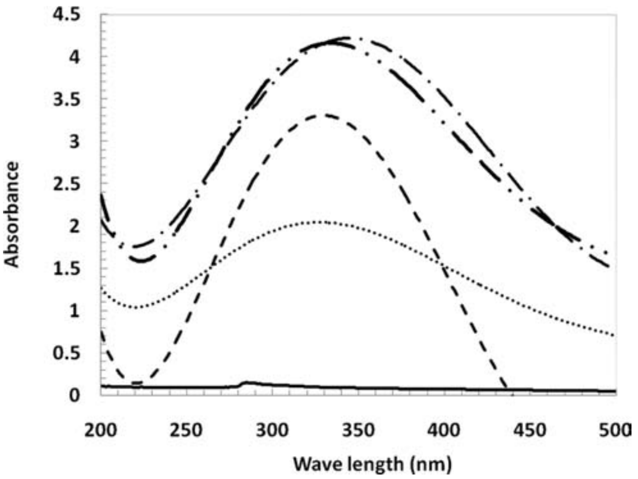


Figure 7. Absorption spectra for (—) Nva; (·····) FA; (− − −) binary system (0.001 mol·dm^{-3} Fe^{III} + 0.001 mol·dm^{-3} Nva); (— • —) binary system (0.001 mol·dm^{-3} Fe^{III} + 0.001 mol·dm^{-3} FA); (— • • —) ternary system (0.001 mol·dm^{-3} Fe^{III} + 0.001 mol·dm^{-3} Nva + 0.001 mol·dm^{-3} FA).

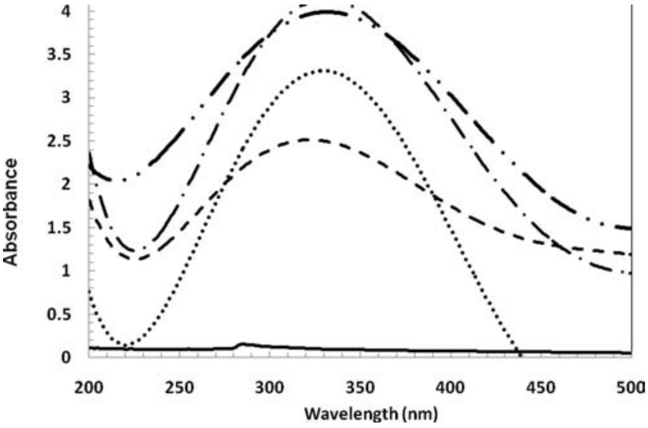


Figure 8. Absorption spectra for (—) Nva; (·····) FA; (− − −) binary system (0.001 mol·dm^{-3} Cr^{III} + 0.001 mol·dm^{-3} Nva); (— • —) binary system (0.001 mol·dm^{-3} Cr^{III} + 0.001 mol·dm^{-3} FA); (— • • —) ternary system (0.001 mol·dm^{-3} Cr^{III} + 0.001 mol·dm^{-3} Nva + 0.001 mol·dm^{-3} FA).

case of binary FA complexes. For mixed ligand complexes, the stability trend is CuNvaFAH^{-1} > CuNvaFA^{2-} > CuNvaFAH$_{-1}^{3-}$ > CuNvaFAH$_{-2}^{4-}$ > CuNvaFAH$_{-3}^{5-}$.

(vi) By comparing the stability constants of binary and mixed ligand complexes, it was demonstrated that ternary complexation is responsible for the stabilization of the mixed complexes.

UV−visible absorption spectra are known to be useful for proving the formation of Nva and FA binary and ternary systems of iron(III), chromium(III), and copper(II) metal ions. As shown in Figures 7 to 9, Nva shows almost no absorption signals, while FA shows a strong broad absorption band at 228 nm. The UV−visible spectroscopic measurements clearly show an obvious absorption trend for each binary and ternary complex species as shown in Figures 7 to 9. The spectra of the ternary systems are quite different from those of the binary systems, emphasizing the formation of the former in solution.

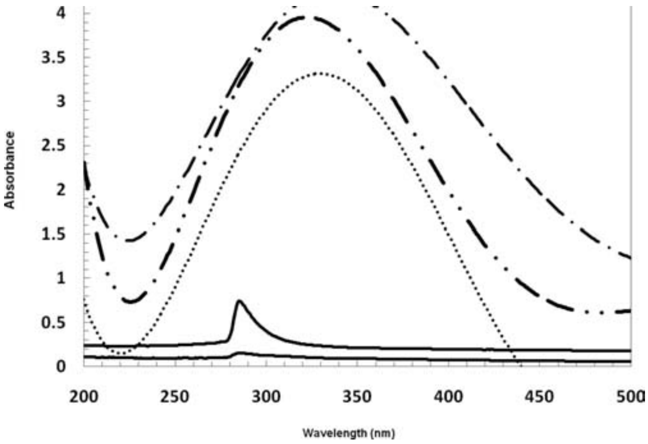


Figure 9. Absorption spectra for (—) Nva); (·····) FA; (− − −) binary system (0.001 mol·dm^{-3} Cu^{II} + 0.001 mol·dm^{-3} Nva); (— • —) binary system (0.001 mol·dm^{-3} Cu^{II} + 0.001 mol·dm^{-3} FA); (— • • —) ternary system (0.001 mol·dm^{-3} Cu^{II} + 0.001 mol·dm^{-3} Nva + 0.001 mol·dm^{-3} FA).

■ ASSOCIATED CONTENT

S Supporting Information. Potentiometric titration experimental data points for all systems associated with this article are listed in three tables and attached as a supplementary file and can be found in the online version. This material is available free of charge via the Internet at http://pubs.acs.org.

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