

# Nickel and Cobalt Complexes of Non-protein L-Norvaline and Antioxidant Ferulic Acid: Potentiometric and Spectrophotometric Studies

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**Abstract** Binary and mixed-ligand complexes of  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  involving L-norvaline (Nva) and ferulic acid (FA) have been investigated in aqueous solutions by pH potentiometry and UV–visible spectrophotometric techniques, at 298.15 K and fixed ionic strength ( $0.15 \text{ mol}\cdot\text{dm}^{-3}$ ,  $\text{NaNO}_3$ ). The overall stability constants of the  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  complexes with the ligands studied were obtained by the HYPERQUAD2008 program from the pH-potentiometric data. As a result of the numerical treatment, a model composed of seven species  $\text{NiNva}^+$ ,  $\text{NiNva}_2$ ,  $\text{NiNvaH}_{-1}$ ,  $\text{NiNva}_{-2}$ ,  $\text{NiFA}$ ,  $\text{NiFAH}_{-1}$  and  $\text{NiNvaFA}^-$  was obtained for the  $\text{Ni}^{2+} + \text{Nva} + \text{FA}$  system, whereas for the  $\text{Co}^{2+} + \text{Nva} + \text{FA}$  system the complexes  $\text{CoNva}^+$ ,  $\text{CoNva}_2$ ,  $\text{CoNvaH}_{-1}$ ,  $\text{CoNvaH}_{-2}$ ,  $\text{CoFA}$ ,  $\text{CoFAH}_{-1}$ , and  $\text{CoNvaFA}^-$  were obtained. The complex species distributions in certain pH ranges were calculated by the HySS2009 simulation program. Spectroscopic UV–visible measurements were carried out to give qualitative information about the complexes formed in these solutions.

**Keywords** L-norvaline · Ferulic acid · Complexation · Equilibria

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## 1 Introduction

Nickel and cobalt are essential trace elements for the human body. Nickel is a cofactor of many enzymes, whereas cobalt is a primary component of vitamin B12. This vitamin plays an important role in growth development, metabolic processes, and nervous system and brain functions. It is used in the treatment for anemia, since it helps red blood cell production [1–7]. However, exposure of the human body to these elements in excessive amounts causes various negative effects on health. The most commonly reported adverse health effect of excess nickel exposure is contact dermatitis, which is the result of an allergic reaction to nickel. Moreover, nickel is a respiratory toxicant that can cause acute lung inflammation, chronic bronchitis, emphysema, pulmonary fibrosis, and can induce lung or nasal cancer [8, 9]. Similar to nickel, cobalt exposure mainly affects the human respiratory system. Exposure of these elements also affects the gastrointestinal tract, kidney, cardiac, immunological and neurological systems [8–12].

Metal–ligand complexes have been used as metal-poisoning antidotes. Such an antidote circulates in the blood stream and reduces metal concentrations in the human body [13–15]. A number of ligands have been used as antidotes to combat metal poisoning, such as EDTA, amino acids, hydroxamic acids and phenolic acids. In a recent study [16] we investigated the stability constants of a number of complexes formed by L-norvaline (Nva) and *trans*-ferulic acid (FA) with transition metal ions ( $\text{Fe}^{3+}$ ,  $\text{Cr}^{3+}$  and  $\text{Cu}^{2+}$ ). Since Nva and FA show strong chelating ability [17–20], it is natural to extend the earlier work to complexation of these two ligands with  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$ . The objective of this study is to quantify the metal chelating activity of Nva and FA with  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$ . Besides, these compounds also show many beneficial effects for the human body, such as antioxidant, anti-inflammatory, and anticancer effects and have a therapeutic effect on tumors [21–32]. Monitoring of complex formation of ligands and metal ions was accomplished with a potentiometric technique with a glass electrode, and the experimental data were analyzed using HYPERQUAD2008 [33]. Graphic representations of the complex species formed in certain pH ranges, as distribution diagrams, were produced with the HySS2009 [34] modeling program. Also, UV–vis spectroscopic analyses were used to verify the formation of metal ligand complexes.

## 2 Experimental

### 2.1 Material and Solution

All chemicals were analytical grade and were used without further purification. L-Norvaline (Nva,  $\text{C}_5\text{H}_{11}\text{NO}_2$ , 99 %) was purchased from Merck (Germany) and *trans*-ferulic acid (FA, 99 %) was obtained from Aldrich (Germany). Carbonate-free sodium hydroxide solution was purchased from Acros Organics (USA) and the solution was standardized potentiometrically with potassium hydrogen phthalate (Sigma–Aldrich, USA).

Nickel chloride hexahydrate ( $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ), cobalt nitrate hexahydrate ( $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ), and sodium nitrate ( $\text{NaNO}_3$ ) were purchased from Acros Organics (USA). The nitric acid (Pancreac, Spain) solution used to acidify other solutions was prepared and standardized before use. All solutions in the experiments were prepared freshly before use, using ultra pure water obtained from a NANO Pure Ultrapure water system (distilled and deionized giving a resistance of  $18.3 \text{ M}\Omega \cdot \text{cm}^{-1}$ ).

## 2.2 pH-Potentiometric Measurements

### 2.2.1 Procedure

The pH-potentiometric titrations were performed using a Metrohm 702 SM Titroprocessor with a 664 Dosimat, a 728 magnetic stirrer, coupled with a Dosino burette model 683. The electrode response can be read to the third decimal place in terms of pH units with a precision of  $\pm 0.001$ . 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 meter was calibrated with standard buffer solutions (pH = 4.00, 7.00 and 9.21) before and after each series of pH measurements.

For the determination of binary and mixed ligand complexes, the following solutions were prepared (total volume  $50 \text{ cm}^3$ ) and titrated potentiometrically against a standard carbonate-free NaOH ( $0.1 \text{ mol}\cdot\text{dm}^{-3}$ ) solution:

- $0.003 \text{ mol}\cdot\text{dm}^{-3} \text{ HNO}_3 + 0.15 \text{ mol}\cdot\text{dm}^{-3} \text{ NaNO}_3$ ;
- solution (a) + Nva or FA;
- solution (a) + Nva or FA + metal ions ( $\text{Ni}^{2+}$  or  $\text{Co}^{2+}$ );
- solution (a) + Nva + FA + metal ions ( $\text{Ni}^{2+}$  or  $\text{Co}^{2+}$ ).

The protonation constants of Nva and FA were determined potentiometrically by titrating mixture (b). The stability constants of the binary complexes of  $\text{Ni}^{2+}$  or  $\text{Co}^{2+}$  with Nva and FA were determined by titrating mixture (c). The stability constants of the mixed-ligand complexes of Nva and FA with  $\text{Ni}^{2+}$  or  $\text{Co}^{2+}$  were calculated using potentiometric data obtained from mixture (d).

The ionic strength of the solutions was maintained at  $0.15 \text{ mol}\cdot\text{dm}^{-3}$  by using the desired concentration of  $\text{NaNO}_3$  as the supporting electrolyte. The ligand concentrations in the titrated samples were varied from  $0.0004$  to  $0.0012 \text{ mol}\cdot\text{dm}^{-3}$ . For binary complexes, potentiometric titrations were carried out using four molar ratios of metal-to-ligand (1:1, 1:2, 1:2.5 and 1:3) to fulfill the maximum possible coordination number of the metal ion, and the ratio 1:1:1 was used for the ternary systems. Each solution was thermostatted at  $25 \pm 0.1 \text{ }^\circ\text{C}$ , and the solution was 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.

### 2.2.2 Data Analysis

The determinations of the protonation constants of ligands and the overall stability constants of their metal complexes from the pH titration data were performed using the HYPER-QUAD2008 program [33]. Corresponding to previous work [16], the ionization constant of water ( $\text{p}K_w$ ) in  $0.15 \text{ mol}\cdot\text{dm}^{-3} \text{ NaNO}_3$  is 13.77 at 298 K. The stoichiometries and stability constants of the complexes formed were determined by trying various possible composition models (using titration results obtained before precipitation or opalescence was observed in the solution). 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.

## 2.3 Spectrophotometric Measurements

The absorption spectra were obtained using an UV-vis spectrophotometer (Varian Cary 50, USA). Spectra of solutions containing  $\text{Ni}^{2+} + \text{FA}$ ,  $\text{Co}^{2+} + \text{FA}$ ,  $\text{Ni}^{2+} + \text{Nva} + \text{FA}$  and

$\text{Co}^{2+} + \text{Nva} + \text{FA}$  were taken separately between  $\text{pH} = 4.5$  and 11 over the wavelength interval of 200 to 450 nm. The solutions were prepared with the same metal-to-ligand molar ratios as used in the pH-potentiometric titrations.

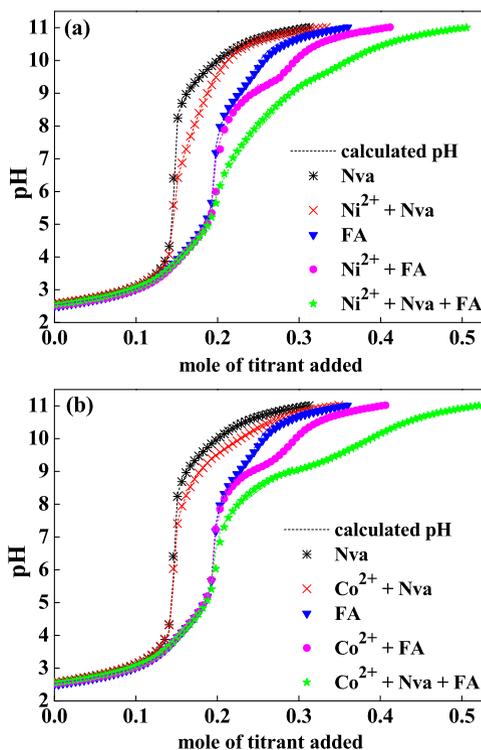
### 3 Results and Discussion

#### 3.1 Potentiometric Results

In our recent study [16], we reported the protonation constants of Nva and FA. Both of these ligands have two protonation sites. For Nva, associations of the protons occur at the amino group ( $-\text{NH}_2$ ) and the carboxylic acid group ( $-\text{COO}^-$ ), with overall proton association constant ( $\log_{10} \beta$ ) values of 9.29 and 11.90, respectively. In contrast, the overall proton association constants for ferulic acid are 8.77 and 13.23, and protonation occurs at the para phenolic hydroxyl ( $-\text{O}^-$ ) and at the carboxylate group, respectively. These protonation constants values were obtained using the HYPERQUAD2008 program, and were used as input data for the evaluation of the overall stability constants of metal complexes using the same program. These values are in good agreement with those reported in literatures [17, 18, 35, 36] and the small differences are caused by differences in experimental conditions or calculation procedures used.

From the (typical) titration curves shown in Fig. 1, it can be seen that at a fixed titrant volume there is a shift in pH from that for the ligand alone to lower pHs for ligand–metal ion complexes. These shifts indicate that complexes formed in the binary and ternary systems.

**Fig. 1** Potentiometric titration curves of (a) the  $\text{Ni}^{2+} + \text{Nva} + \text{FA}$  system and (b) the  $\text{Co}^{2+} + \text{Nva} + \text{FA}$  system, both at  $T = 298.15 \text{ K}$  and  $I = 0.15 \text{ mol}\cdot\text{dm}^{-3} \text{ NaNO}_3$ ;  $[\text{M}^{2+}] = 0.0004 \text{ mol}\cdot\text{dm}^{-3}$  and  $[\text{ligands}] = 0.001 \text{ mol}\cdot\text{dm}^{-3}$  for the binary systems;  $[\text{M}^{2+}]$  and  $[\text{ligands}] = 0.001 \text{ mol}\cdot\text{dm}^{-3}$  for the ternary system



**Table 1** Logarithms of the overall stability constants ( $\log_{10} \beta_{pqrs}$ )<sup>a</sup> of binary and ternary complexes of Ni<sup>2+</sup> with L-norvaline (Nva) and ferulic acid (FA) in aqueous solution at 298.15 K and  $I = 0.15 \text{ mol}\cdot\text{dm}^{-3} \text{ NaNO}_3$ 

Complex	<i>p</i>	<i>q</i>	<i>r</i>	<i>s</i>	$\log_{10} \beta_{pqrs}$	S.D.
NiNva <sup>+</sup>	1	1	0	0	5.49 (5.42) [18]	0.0102
NiNva <sub>2</sub>	1	2	0	0	9.73 (9.87)	0.0157
NiNvaH <sub>-1</sub>	1	1	0	-1	-3.54	0.0222
NiNvaH <sub>-2</sub>	1	1	0	-2	-14.74	0.0652
NiFA	1	0	1	0	3.70	0.0893
NaFAH <sub>-1</sub>	1	0	1	-1	-5.37	0.0626
NiNvaFA <sup>-</sup>	1	1	1	0	8.83	0.0740
$\Delta \log_{10} K$					-0.35	

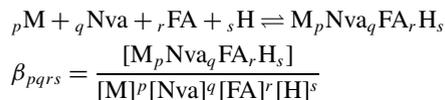
<sup>a</sup>All the values were calculated with the program HYPERQUAD2008 from potentiometric investigations at 298 K and  $I = 0.15 \text{ mol}\cdot\text{dm}^{-3} \text{ NaNO}_3$ . The symbols *p*, *q*, *r*, and *s* are used in the programs to indicate the stoichiometric coefficients associated with the possible equilibria in solutions

**Table 2** Logarithms of the overall stability constants ( $\log_{10} \beta_{pqrs}$ )<sup>a</sup> of binary and ternary complexes of Co<sup>2+</sup> with L-norvaline (Nva) and ferulic acid (FA) in aqueous solution at 298.15 K and  $I = 0.15 \text{ mol}\cdot\text{dm}^{-3} \text{ NaNO}_3$ 

Complex	<i>p</i>	<i>q</i>	<i>r</i>	<i>s</i>	$\log_{10} \beta_{pqrs}$	S.D.
CoNva <sup>+</sup>	1	1	0	0	4.29 (4.29) [18]	0.0100
CoNva <sub>2</sub>	1	2	0	0	7.26 (7.81)	0.0572
CoNvaH <sub>-1</sub>	1	1	0	-1	-5.72	0.0554
CoNvaH <sub>-2</sub>	1	1	0	-2	-15.06	0.0095
CoFA	1	0	1	0	3.06	0.0553
CoFAH <sub>-1</sub>	1	0	1	-1	-5.88	0.0745
CoNvaFA <sup>-</sup>	1	1	1	0	7.71	0.0775
$\Delta \log_{10} K$					0.36	

<sup>a</sup>All the values were calculated with the program HYPERQUAD2008 from potentiometric investigations at 298 K and  $I = 0.15 \text{ mol}\cdot\text{dm}^{-3} \text{ NaNO}_3$ . The symbols *p*, *q*, *r*, and *s* are used in the programs to indicate the stoichiometric coefficients associated with the possible equilibria in solutions

Stability constant determinations for the complexes formed were accomplished by fitting the experimental data corresponding to the titration curves of Ni<sup>2+</sup> and Co<sup>2+</sup> with Nva and FA (using HYPERQUAD2008 program). The complexation reactions between metal ion (M<sup>2+</sup>), Nva and FA can be described by the general equilibrium:



where *p*, *q*, *r* and *s* are the stoichiometric coefficients associated with the complexes formed from the metal ion, Nva, FA and proton, respectively.

The evaluated stability constants ( $\log_{10} \beta_{pqrs}$ ) for Nva and FA are presented in Tables 1 and 2. The stability constant values for the Nva system with both Ni<sup>2+</sup> and Co<sup>2+</sup> are quite

similar to those of the corresponding complexes with the  $\alpha$ -amino acid glycine; these results are reasonable since both systems have similar chelating sites ( $-\text{NH}_2$  and  $-\text{COO}^-$ ). The  $\log_{10} \beta$  values of the  $\text{M}^{2+}$  + glycine [37] system are 5.80 for  $\text{Ni}^{2+}$  and 4.66 for the  $\text{Co}^{2+}$  system, while for the  $\text{M}^{2+}$  + Nva system the overall stability constant values are 5.49 for  $\text{Ni}^{2+}$  and 4.29 for  $\text{Co}^{2+}$ , respectively. These results suggest that the metal ions binding to the Nva ligand occur in a glycine-like structure.

For binary system with ferulic acid as ligand, it is suggested that FA acts as bidentate ligand for both  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  since the obtained  $\log_{10} \beta_{\text{ML}}$  values for the  $\text{M}^{2+}$  + FA complex are greater than the  $\log_{10} \beta$  values of the individual chelating sites ( $-\text{COO}^-$  and  $-\text{O}^-$ ). The  $\log_{10} \beta$  value of a  $\text{Ni}^{2+}$  system is 0.67 at a carboxyl site, while in a  $\text{Co}^{2+}$  system the  $\log_{10} \beta$  value is 0.56 [38]. Unfortunately,  $\log_{10} \beta$  values for  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  systems could not be found in the literature at the hydroxyl site. However, these values are expected to be slightly higher than those at the corresponding carboxyl site.

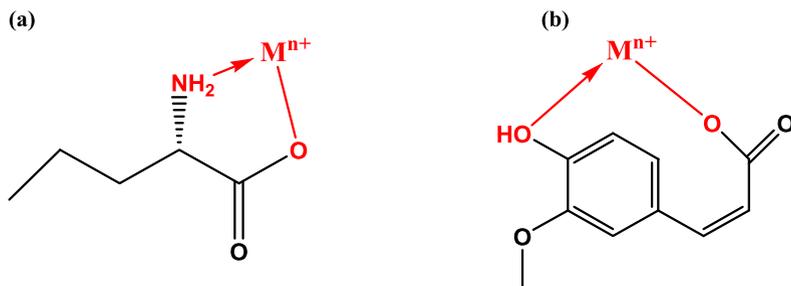
The stability constant of a metal–ligand complex in a ternary system is related to those of the corresponding binary systems by  $\Delta \log_{10} K$ , which compares the stability of the ternary system to the binary systems, by the following relation:

$$\Delta \log_{10} K = \log_{10} \beta_{\text{MNvaFA}}^{\text{MNva}} - \log_{10} \beta_{\text{MNva}}^{\text{M}} - \log_{10} \beta_{\text{MFA}}^{\text{M}}$$

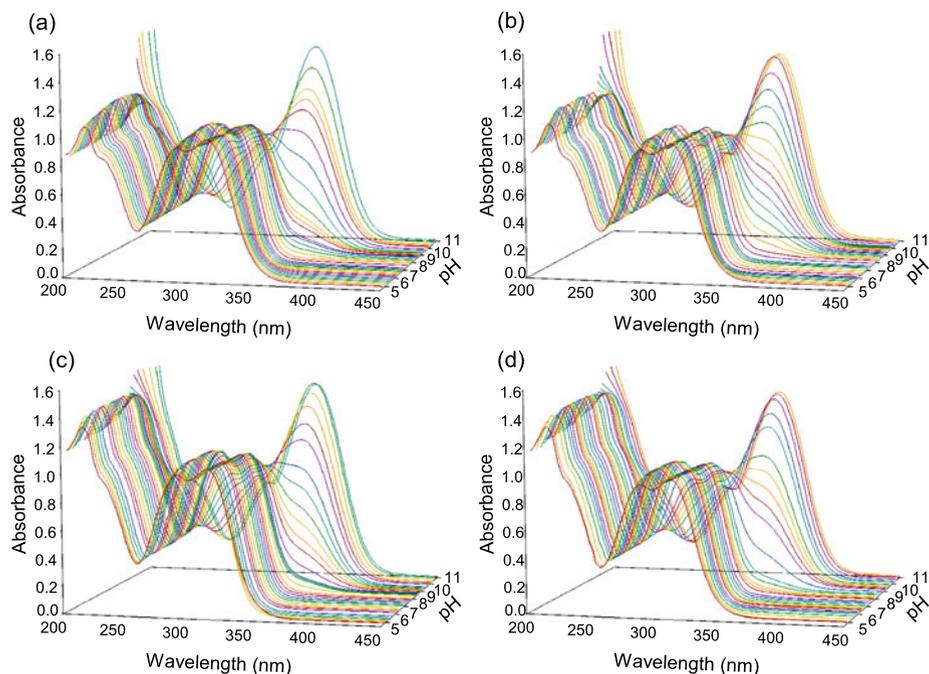
A positive value of  $\Delta \log_{10} K$  indicates that the ternary complex is more stable than the binary complexes, while the opposite is true if  $\Delta \log_{10} K$  is negative [39]. From the calculated  $\Delta \log_{10} K$  values of the  $\text{Ni}^{2+}$  system, it can be seen that the ternary system is less stable, because there is a steric effect when FA binds to the metal–Nva complex. In contrast, for  $\text{Co}^{2+}$  the ternary system is more stable since  $\text{Co}^{2+}$  forms less stable complexes in the binary systems, so it has less steric hindrance to the secondary ligand binding with the metal–Nva complex.

From the trend of stability constant values, it can be concluded that the binary  $\text{Ni}^{2+}$  complexes are more stable than those of the  $\text{Co}^{2+}$  binary systems. This trend follows the Irving–Williams series of stability [40]. By comparing the ligand chelating abilities, it can be seen that Nva complexes more strongly with metal ions than FA. This phenomenon happens as a result of the ligand structure (Fig. 2). Since FA produces bulkier a chelate ring when complexing with a metal ion, the complex formed tends to be less stable [41–43].

HySS provides an integrated environment for setting up a calculation, performing it and creating speciation diagrams, etc. There are no limits imposed on the numbers of reagents, complexes or partially soluble products that may be present. Three types of calculation can be performed: (i) simulation of a titration curve, (ii) calculation of species' concentrations for a range of conditions, and (iii) speciation for a single set of conditions (single data



**Fig. 2** Coordination models of (a) L-norvaline and (b) *trans*-ferulic acid



**Fig. 3** UV-vis spectra of (a)  $\text{Ni}^{2+} + \text{FA}$ , (b)  $\text{Ni}^{2+} + \text{Nva} + \text{FA}$ , (c)  $\text{Co}^{2+} + \text{FA}$ , and (d)  $\text{Co}^{2+} + \text{FA} + \text{Nva}$  systems at  $T = 298.15 \text{ K}$ ;  $[\text{M}^{2+}] = 0.0004 \text{ mol}\cdot\text{dm}^{-3}$  and  $[\text{ligands}] = 0.001 \text{ mol}\cdot\text{dm}^{-3}$  for binary systems;  $[\text{M}^{2+}]$  and  $[\text{ligands}] = 0.001 \text{ mol}\cdot\text{dm}^{-3}$  for ternary systems

points) [34]. From the species distribution diagrams, a simulation of the amounts of complex formed in binary and ternary system at a certain pH value can be performed. In the  $\text{Ni}^{2+} + \text{Nva}$  system, the  $\text{NiNva}$  complex starts to form at  $\text{pH} = 5$  and the maximum concentration (approximately 33 %) occurs at  $\text{pH} = 7.9$ , which is then followed by formation of the  $\text{NiNva}_2$  complex (ca. 28 %) at  $\text{pH} = 9.2$ . For the  $\text{Co}^{2+} + \text{Nva}$  system, the  $\text{CoNva}$  complex starts to form at  $\text{pH} = 6$  and the maximum concentration occurs at  $\text{pH} = 9$  (ca. 30 %).

For the binary complexes of a metal ion with FA, the  $\text{NiFA}$  complex starts to form at  $\text{pH} = 6$  and reaches a maximum concentration (ca. 20 %) at  $\text{pH} = 8.7$ , while the  $\text{CoFA}$  complex concentration at  $\text{pH} = 8.9$  is approximately 10 %. The mixed ligand species distribution diagrams show that the percent formation of ternary species overlaps with the binary complex of Nva and this result is consistent with the  $\Delta \log_{10} K$  values.

### 3.2 Spectrophotometric Results

The UV-vis spectra of  $\text{Ni}^{2+}/\text{Co}^{2+} + \text{Nva} + \text{FA}$  at  $\text{pH} = 4.5\text{--}11$  are shown in Fig. 3. Three absorbance peaks are found at 215 nm, 289 nm and 313 nm ( $\text{pH} = 4.5$ ). In the  $\text{Ni}^{2+} + \text{FA}$  system, as the pH is increased to 7.2, the 289 nm and 313 peaks are shifted hypsochromically to 286 nm and 310 nm, respectively, while the peak at 215 nm shifts hyperchromically to greater absorbance value. The peaks start to shift bathochromically with increasing pH and, at about  $\text{pH} = 9$ , the peak at 286 nm slowly subsides while the peak at 313 nm broadens and shifts bathochromically to 345 nm. The shifting of  $\text{Ni}^{2+} + \text{FA}$  peaks around  $\text{pH} = 7.2$  might be caused by the formation of the  $\text{NiFA}$  complex in solution.

For the  $\text{Ni}^{2+} + \text{Nva} + \text{FA}$  ternary system, the hypsochromic shift occurs at  $\text{pH} = 6$  (286 nm and 309 nm), then the peaks start to shift bathochromically, and at about  $\text{pH} = 9.2$  the peak at 286 nm slowly subsides while the peak at 313 nm broadens and shifts bathochromically to 345 nm. Similar results were obtained for the  $\text{Co}^{2+}$  system. Initially the peak shifts hyperchromically, then at about  $\text{pH} = 8-9$  the 313 nm peak broadens and shifts to 345 nm. This shifting in the spectra indicates that the ligand is bound to the metal and forms binary and mixed ligand complexes.

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