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Complex Stability and Molecular Structure Studies of Divalent Metal Ion with L-Norleucine and Vitamin B3

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ABSTRACT: The stability constants of L-norleucine (Nle) and nicotinic acid (NA) with divalent metal ion (Cu^{2+}, Ni^{2+}, and Co^{2+}) binary and mixed ligand systems at 298 K and ionic strength 0.15 $mol \cdot dm^{-3}$ $NaNO_3$ water solutions were determined using pH-potentiometry. The stability constants data were estimated using the HYPERQUAD 2008 program, and each complex species was shown graphically using the HySS program. UV−visible spectrophotometric measurements were performed to prove the complex species formation. In addition, the possible binding sites of the ligands Nle and NA to bind metal ions and the Gibbs free energy of some complex species were predicted and evaluated by using the Gaussian 09 program.

INTRODUCTION

Divalent metal ions such as copper, nickel, and cobalt metal ions are known essential metals in the human body for maintaining health. Although they have beneficial effects in humans, these ions can be toxic if over-accumulated in the human body.[1] To prevent certain diseases caused by metal poisoning and to reduce the concentration of metal ions in blood and urine in the body, the coordination chemistry of some chelating agents of such metals or recently known ligand therapy has been studied extensively.[2−4] Thus, we have studied recently in our laboratory the complex formation of some divalent and trivalent metal ions with (O, N) and (O, O) donor ligands such as L-norvaline, L-norleucine, ferulic acid, and gallic acid.[5,6] L-Norleucine (Nle) is a nonprotein amino acid that is formed by the deamination of L-lysine, and it is very powerful in preventing brain injury in maple syrup urine disease.[7] Also, Nle acts as a transdermal penetration enhancer drug,[8] supporting proper growth and bone development[9] and maintaining nitrogen balance in human body.[10,11] Moreover, Nle was found to have antifungal,[12] anti-inflammatory, and antioxidant activities.[13]

Nicotinic acid (NA) is one of the pyridinecarboxylic acids which belong to vitamin B groups and is known as vitamin B3. It plays an important role in the human body, animals, and biochemical processes.[14] It is used in food, medicines, and cosmetics as an indispensable nutriment, and it is also widely used in the synthesize of some medicines and dyes.[15] In the present paper, the determination of protonation constants of Nle and NA and their stability constants with divalent metal ions (Cu^{2+}, Ni^{2+}, and Co^{2+}) at 298 K and an ionic strength of 0.15 $mol \cdot dm^{-3}$ $NaNO_3$ water solutions were studied using pH-potentiometric titrations. The titration data were analyzed by the computer program HYPERQUAD 2008. The complex species distribution was represented graphically using HySS programs. The complex species formation was also spectrophotometrically studied. The complex species Gibbs free energy calculations were done using the Gaussian 09 program.

EXPERIMENTAL SECTION

Materials and Solutions. All of the chemical materials used are analytical reagent grade and used without further purification. Nle and NA were purchased from Sigma Aldrich (St. Louis, MO). A nitric acid (Pancreac, Spain) solution was prepared and used after being standardized. Carbonate-free sodium hydroxide (NaOH) from Acros Organics (Morris Plains, NJ) was standardized with potassium hydrogen phthalate (Sigma Aldrich, St. Louis, MO).

All metal salts (copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$) from Sigma Aldrich (St. Louis, MO), nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$) and cobalt nitrate hexahydrate ($Co(NO_3)_2 \cdot 6H_2O$) from Acros Organics (Morris Plains, NJ)) were weighed accurately before solution preparation. To maintain the ionic strength in the solution, sodium nitrate ($NaNO_3$) from Acros Organics (Morris Plains, NJ) was used.

All solutions used throughout the experiments were prepared freshly in ultrapure water obtained from a nanopure ultrapure water system in which water was distilled and deionized with a resistance of 18.3 $M\Omega \cdot cm^{-1}$.

Apparatus and Procedure. *pH-Potentiometric Titration.* All pH titrations were performed using a Metrohm 702 SM titrator 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 with a precision of ± 0.001. The titrator was coupled to a personal computer, and titration software TINET version 2.4 was used to control the titration and data acquisition. The titration was carried out in a 80 cm^3 commercial double-walled glass vessel. The pH meter was calibrated with standard buffer solutions (pH 4.01, 7.00, and 9.21) before and after each series of pH titration measurements.

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For the protonation constant determination of Nle and NA, an aqueous solution ($1 \cdot 10^{-3}$ $\text{mol} \cdot \text{dm}^{-3}$) of each ligand was titrated with 0.1 $\text{mol} \cdot \text{dm}^{-3}$ carbonate-free NaOH under nitrogen atmosphere, and the ionic strength of the solution was maintained at a constant level (0.15 $\text{mol} \cdot \text{dm}^{-3}$) by using $NaNO_3$ solution as the supporting electrolyte. For the stability constant determination of binary complexes, solutions containing Nle or NA and metal ions with molar ratios of 1:1; 1:2, and 1:3 were titrated. Meanwhile, for mixed ligand systems, a molar ratio of 1:1:1 (metal ions/Nle/NA) was used. The concentrations of metal ion and ligand solutions in the titrated samples were varied in the range (0.00004 to 0.0001) $\text{mol} \cdot \text{dm}^{-3}$.

The temperature of each solution was maintained at 298 K during the titration run by circulating thermostatted water using an oil thermostatted setup. A magnetic stirrer was used during all titrations. Each titration was repeated at least three times under carefully controlled experimental conditions.

UV-Spectrophotometric Measurement. Divalent metal ion complex species were spectrophotometrically studied in solutions at ca. 298 K using a double-beam Jasco V-670 spectrophotometer with standard 1 cm quartz cells. The UV—vis bandwidth, scan speed, and data interval used are 0.1 nm, 100 $\text{nm} \cdot \text{min}^{-1}$, and 1.0 nm, respectively.

These solutions were prepared with the same metal-to-ligand molar ratio as done previously in the pH-potentiometric titration and analyzed at a pH where the maximum amount of binary and mixed ligand complexes was formed according to the HySS results.

Computations. All of the titration data sets were added to the HYPERQUAD 2008 program. This program permits the determination of formation constants from potentiometric data especially when different equilibrium reactions take place in aqueous solution.[16] For this purpose, a fitting criterion based on the minimization of the nonlinear least-squares sum defined by the difference between the calculated and the experimental data of the titration curves was used

$$X^2 = \frac{\sum (E_{\text{cal}} - E_{\text{exp}})^2}{E_{\text{cal}}}$$

In HYPERQUAD 2008, stability constants are parameters that are refined. This program uses the convention that all equilibrium constants are overall association constants (β).

Besides the HYPERQUAD 2008 program, results from Gaussian09 simulation were also used to support our experimental results.[17] Gaussian 09 is a series of electronic structure programs to predict the energies, molecular structures, vibration frequencies, and molecular properties of molecules and reactions in chemical environments. Gaussian 09 is based on the basic laws of quantum mechanics to predict the energy and structure of a molecule. This computation has been performed using density functional theory (DFT) at the B3LYP level. The B3LYP functional consisting Becke's three-parameter (B3) hybrid exchange functional was combined with Lee—Yang—Parr correlation functional (LYP) with the standard 6-31+G(d) as a basis set in water solvation.[18,19] The density functional B3LYP can accurately and economically produce the heats of formation for compounds containing transition metals.[20,21] Previous research also indicated that B3LYP is one of the best choices for metal complexes.[22,23] The vibration frequencies were determined by evaluating analytically the second derivatives of the energy with respect to the nuclear coordinates at the same levels.

Scheme 1. Protonation Equilibria of L-Norleucine (Nle) and Nicotine Acid (NA)

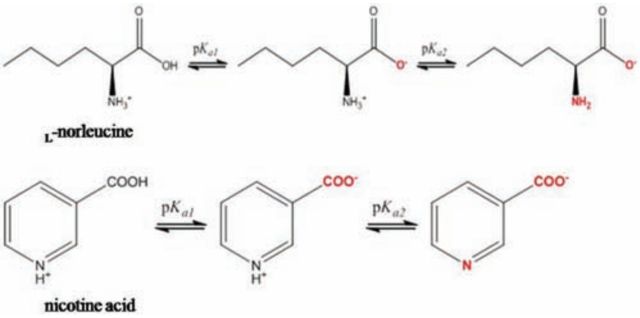

Table 1. Stability Constants of Binary and Mixed Ligand Copper (Cu^{2+}) Complexes with L-Norleucine (Nle) and Nicotinic Acid (NA) in Water (I = 0.15 $\text{mol} \cdot \text{dm}^{-3}$ $NaNO_3$, T = 298 K)[a]

complex species	p	q	r	s	log β_{pqrst}	SD
Nle Complexes						
$CuNle^+$	1	1	0	0	8.14 (8.18)[29]	0.0543
$CuNle_2$	1	2	0	0	15.41	0.0475
$CuNleH^{2+}$	1	1	0	1	12.52	0.0654
NA Complexes						
$CuNA^+$	1	0	1	0	3.34 (3.23)[30]	0.0242
CuNAOH	1	0	1	−1	−2.28	0.0225
Nle and NA Complexes						
CuNleNA	1	1	1	0	11.93	0.0772
$CuNleNAH^+$	1	1	1	1	16.99	0.0841
$CuNleNAH_2^{2+}$	1	1	1	2	21.25	0.0811

[a] The symbols p, q, r, and s are used in the HYPERQUAD 2008 program to indicate the stoichiometric coefficients associated with the possible equilibria in solution.

The geometries of the supposed structures of some of the investigated binary and ternary complexes were fully optimized by minimizing the energies with respect to all of the geometrical parameters without imposing any molecular symmetry constraints. The validity of the optimized structures was checked using normal-mode frequency analysis, in which the real minimum structure must indicate a positive value for all frequencies.

RESULTS AND DISCUSSION

The protonation constants of Nle and NA free ligands and their stability constants with divalent metal ions were evaluated by fitting the titration data of each system using HYPERQUAD 2008. The use of this software permits the determination of stability constants from potentiometric titration data especially when different equilibrium reactions take place in aqueous solution.[16] The results of stability constant refinement include information such as goodness of fit of the objective function, parameter values, standard deviation, calculated data point, residuals, and concentration of all species in the model at all data points. The calculations were not performed for pH regions in which experimental findings showed a continuous decrease in pH or formation of a precipitate or obvious turbidity.

Table 2. Stability Constants of Binary and Mixed Ligand Nickel (Ni^{2+}) Complexes with Nle and NA in Water ($I = 0.15$ mol·dm^{-3} $NaNO_3$, $T = 298$ K)[a]

complex species	p	q	r	s	$\log \beta_{pqrst}$	SD
			Nle Complexes			
NiNle	1	1	0	0	5.48 (5.4)[29]	0.0654
$NiNle_2^{2-}$	1	2	0	0	10.11 (10.01)[29]	0.0627
			NA Complexes			
$NiNA^+$	1	0	1	0	2.64 (2.66)[31]	0.1209
			Nle and NA Complexes			
NiNleNA	1	1	1	0	9.32	0.1162
$NiNleNAH_2^{2+}$	1	1	1	2	19.11	0.1194

[a] The symbols p, q, r, and s are used in the HYPERQUAD 2008 program to indicate the stoichiometric coefficients associated with the possible equilibria in solution.

Table 3. Stability Constants of Binary and Mixed Ligand Cobalt (Co^{2+}) Complexes with Nle in Water ($I = 0.15$ mol·dm^{-3} $NaNO_3$, $T = 298$ K)[a]

complex species	p	q	r	s	$\log \beta_{pqrst}$	SD
			Nle Complexes			
CoNle	1	1	0	0	4.25 (4.26)[29]	0.0666
$CoNle_2^{2-}$	1	2	0	0	7.77 (7.79)[29]	0.0588
			NA Complexes			
CoNA	1	0	1	0	NA	

[a] The symbols p, q, r, and s are used in the HYPERQUAD 2008 program to indicate the stoichiometric coefficients associated with the possible equilibria in solution.

In computer program such as HYPERQUAD 2008, the protonation constant of the ligand is expressed as the overall reaction, β_n:

$$H^+ + L^{2-} \leftrightarrow HL^- \qquad [HL^-] = \beta_1[H^+][L^{2-}]$$

$$2H^+ + L^{2-} \leftrightarrow H_2L \qquad [H_2L] = \beta_2[H^+]^2[L^{2-}]$$

where $\log \beta_1$ is equal to pK_{a2} and $\log \beta_2$ is equal to $pK_{a1} + pK_{a2}$.

The numerical protonation constant values of Nle and NA showed that Nle has two protonation constants: pK_{a1} 2.31 corresponding to the liberation of the carboxylic acid proton, and pK_{a2} 9.35 related to the liberation of the amino acid proton. Meanwhile, the protonation constants of NA were found to be pK_{a1} 2.22 and pK_{a2} 4.59, resulting from the liberation of the proton of the carboxylic acid and pyridine nitrogen groups, respectively. The plausible protonation equilibria processes of Nle and NA were presented as shown in Scheme 1.

The equilibrium of the complex species formation is represented by the overall stability constant, β_{pqrs}, as shown in the following relations:

$$pM + q\text{Nle} + r\text{NA} + s\text{H} \rightleftharpoons \text{M}p\text{Nle}q\text{NA}r\text{H}s$$

$$\beta_{pqrs} = [\text{M}p\text{Nle}q\text{NA}r\text{H}s]/[\text{M}]^p[\text{Nle}]^q[\text{NA}]^r[\text{H}]^s$$

where β_{pqrs} represents the stability constants of the complex species.

Table 4. Complex Gibbs Free Energy of Reaction Obtained by the Gaussian 09 Program

molecule	G (hartree/particle)[a]	Δ_rG (hartree/particle)
	Metal Ions	
Cu^{2+}	−1639.813789	
Ni^{2+}	−1507.725005	
Co^{2+}	−1382.251038	
	Ligands	
Nle	−441.110896	
NA	−436.375509	
	Nle Complexes	
$[CuNle]^+$	−2081.149815	−0.225130
$[NiNle]^+$	−1949.015974	−0.180073
$[CoNle]^+$	−1823.498972	−0.137038
	NA Complexes	
$[CuNA]^+$	−2076.365366	−0.176068
$[NiNA]^+$	−1944.205740	−0.105226
$[CoNA]^+$	−1818.684987	−0.058440
	NleNA Complexes	
[CuNleNA]	−2517.571059	−0.270865
[NiNleNA]	−2385.430267	−0.218857
[CoNleNA]	−2259.914210	−0.176767

[a] The calculation was done using a density functional theory (DFT)-B3LYP method combined with 6-31+G(d) as a basis set, 1 hartree/particle = 2.6255048·10^3 kJ·mol^{-1}.

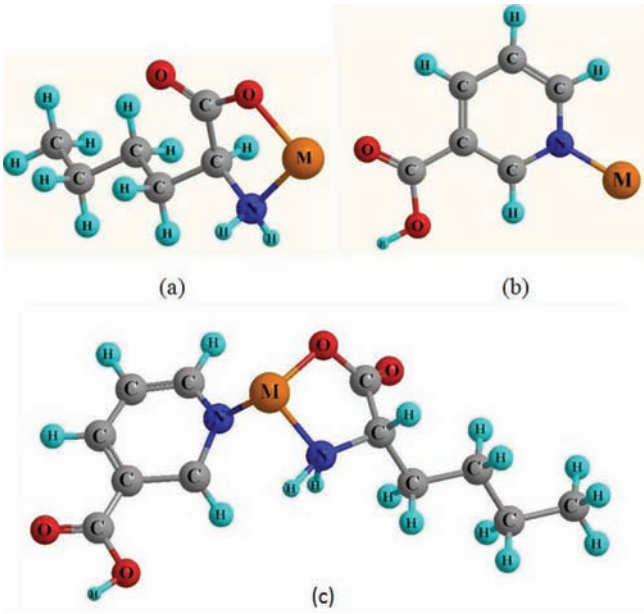

Figure 1. Modeling of possible binding sites using Gaussian 09 for (a) Nle−divalent metal ion (M) binary systems, (b) NA−divalent metal ion (M) binary systems, and (c) Nle−NA−divalent metal ion (M) mixed ligand systems.

The stability constant data ($\log \beta_{pqrs}$) for the binary and mixed ligand systems involving divalent metal ions (Cu^{2+}, Ni^{2+}, and Co^{2+}) and Nle and NA, which include several complex species with different stoichiometries, are presented in Tables 1 to 3.

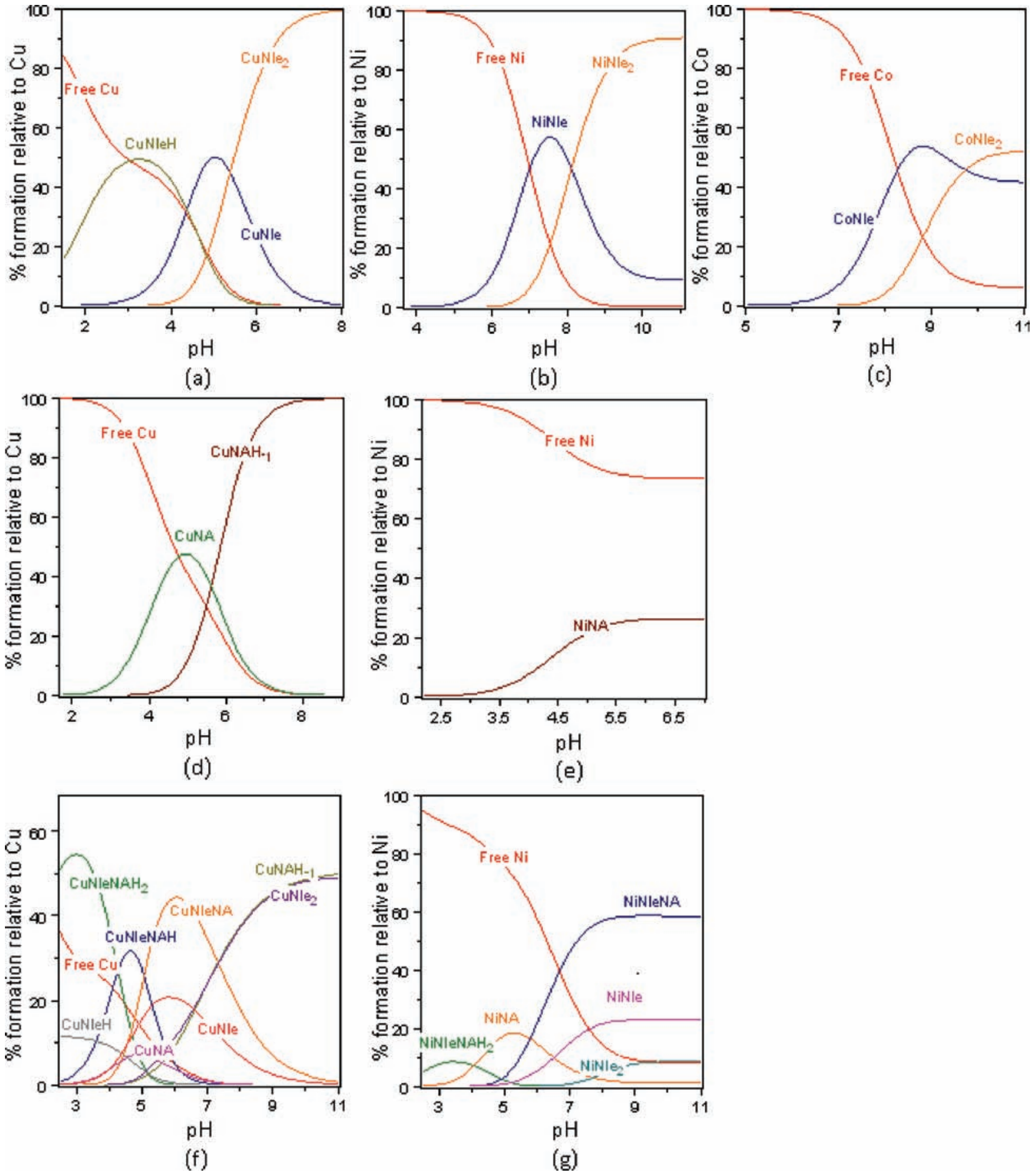

Figure 2. Species distribution curves for (a) Cu^{2+} + Nle, (b) Ni^{2+} + Nle, (c) Co^{2+} + Nle, (d) Cu^{2+} + NA, (e) Ni^{2+} + NA, (f) Cu^{2+} + Nle + NA, and (g) Ni^{2+} + Nle + NA systems at $T = 298$ K and $I = 0.15$ mol·dm^{-3} $NaNO_3$. Percentages are calculated with respect to the analytical concentration of the metal ion.

The stability constant data for both binary and mixed ligand systems was found to be decreased in the order $Cu^{2+} > Ni^{2+} > Co^{2+}$. This trend is in agreement with the Irving–Williams series where the complex of divalent metal ions follow the order $Cu^{2+} > Ni^{2+} > Co^{2+}$.[24]

The trend of stability constants of these divalent metal ions with Nle and NA were further supported by the optimization and frequency simulation using Gaussian 09. In this simulation, a number of assumptions are used as simplification, such as the usage of fully deprotonated ligands, only considering the complex formation of one metal ion and one ligand, not considering the usage of salt to maintain the solution's ionic strength and not considering the addition of acid/base during the potentiometric titration. From the thermochemistry result presented in Gaussian 09 output, the Gibbs free energy (G) of each molecule can be obtained, and thus the Gibbs free energy of reaction ($\Delta_r G$) can

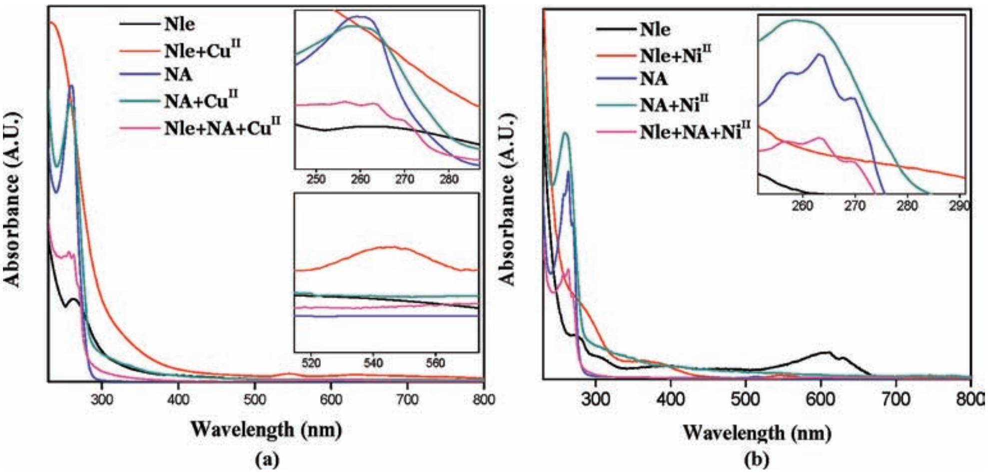

Figure 3. Absorption spectra of free Nle and NA ligands, binary system (0.001 mol·dm^{-3} metal + 0.001 mol·dm^{-3} Nle/NA), and mixed ligand systems (0.001 mol·dm^{-3} metal + 0.001 mol·dm^{-3} Nle + 0.001 mol·dm^{-3} NA) using (a) Cu^{2+} at ∼ pH 5, (b) Ni^{2+} at ∼ pH 10.

be calculated. The Gibbs free energy of reaction is related to its equilibrium constant by the following relations:

$$M^{2+} + L^- \rightleftharpoons [ML]^+$$

$$\Delta_r G = \sum G_{\text{product}} - \sum G_{\text{reactant}}$$

$$\Delta_r G = -RT \ln K_c$$

From the equation above, it is shown that, when the Gibbs free energy of reaction is more negative, the equilibrium constant will be larger which means that the complex formed is more stable. It is also well-known that all complexes always tend to possess minimal energy corresponding to the formation of the most stable state.[25] Hence a more negative energy means the formation of a more stable complex. Interestingly, we found in this work that the stability constants of the NA−Co^{2+} system cannot be determined by using pH-potentiometric titration. This is because the stability constant value in the NA−Co^{2+} system was found to be very low and the complex formed is unstable. This fact can be supported by the $\Delta_r G$ value of the NA−Co^{2+} system using Gaussian 09 calculations. By comparing all values of $\Delta_r G$ in Table 4, it can be seen that the NA−Co^{2+} system possesses the least negative values in all binary systems. The same is true with the formation of the CoNleNA complex in which the $\Delta_r G$ value also indicates it to be less stable than CuNleNA and NiNleNA systems. The $\Delta_r G$ value of NA−CO^{2+} can be calculated by the Gaussian 09 program but cannot be determined by the pH-potentiometric method apparently because of the simplifications implied during the Gaussian simulation.

The Gaussian 09 program also can predict the molecular structure of the complex (Figure 1). Figure 1a shows that divalent metal ion binds Nle at its two binding sites (carboxylic acid and amine groups), while the binding of NA and divalent metal ion (shown in Figure 1b) only occurs at the pyridine nitrogen ring. This can be explained according to HSAB (hard soft acid base) theory.[26]

In coordination chemistry, HSAB theory classifies acids (metal ions) and bases (ligands) as hard and soft based on their affinity binding in the solution. All divalent metal ions are classified as soft acids, and the pyridine nitrogen ring is categorized as a soft base, while the oxygen donor is classified as a hard base. Hence, during complexation, hard acids prefer to bind to hard bases, while soft acids prefer to bind to soft bases.[26]

The tendency of the mixed ligand complex formation can be obtained by subtracting the stability constant of the mixed ligand complex from those of the binary complexes and defined in terms of Δ log *K*.

$$\Delta \log K = \log \beta_{\text{MNleNA}} - \log K^{\text{M}}_{\text{MNle}} - \log K^{\text{M}}_{\text{MNA}}$$

The Δ log *K* values of Cu^{2+} and Ni^{2+} metal ion complexes were calculated to be 0.45 and 1.04, respectively. The positive values of Δ log *K* indicated that the mixed ligand system tends to form more stable complexes than the binary system due to ligand−ligand interaction.[27,28]

The color of solution was bluish for the CuNleNA system at pH ∼6 and turbid bluish at pH ∼8. For the NiNleNA system, the solution changed from clear to turbid at pH 10.

A species distribution diagram is a visualization tool to show the species that are present and their concentrations as functions of pH. Figure 2 shows the binary and mixed Nle and NA ligand species distributions based on the data showed in Tables 1 to 3. The tendency of mixed ligand systems to form more stable complex species than their correspondent binary ones can be seen in Figures 2f−g. These figures show that CuNleNA reaches a maximum around 50 % while the maxima are found to be only about ∼20 % and 7 % for CuNle and CuNA, respectively. On the other hand, the NiNleNA species attains about ∼58 % relative concentration, while NiNle and NiNA only reach about ∼23 % and 19 %, respectively.

To prove the formation of the binary and mixed Nle and NA systems in solutions, UV−visible spectrophotometric measurements were performed at (220 to 800) nm. From the speciation distribution diagram that performed by the HySS program, it can be known that the complex formation of Cu^{2+} and Ni^{2+} approximately occurs at pH 5 and 10, respectively. The UV−visible spectra results (Figure 3) show the maximum absorption wavelengths (nm) for Nle and NA at ∼ pH 5 are 263 and 259, respectively. While at higher pH (∼ pH 10) values, the maximum absorption wavelengths of Nle and NA were shifted and resulted in different shapes in absorption curves. By comparing the maximum absorption wavelengths, the shapes of absorption curve of each ligand, binary, and mixed ligand complexes, the shifting peaks and/or the new peaks that occur in those graphs; it shows that there are complex species formation was existed.

CONCLUSIONS

(1) The stability constant data of the studied binary and mixed ligand systems involving the nonprotein amino L-norleucine acid and vitamin B3 (nicotinic acid) was found to be decreased in the order $Cu^{2+} > Ni^{2+} > Co^{2+}$, in agreement with the Irving–Williams stability series.

(2) The cobalt(II)–vitamin B3 binary and mixed ligand complexes were found to be unstable; thus, their stability constants cannot be determined. This was supported by the least negative value of $\Delta_r G$ achieved by a Gaussian 09 simulation program.

(3) The calculated $\Delta \log K$ and minimum energy values of the divalent metal ions and both Nle and NA complex species formed in water solutions indicated that the mixed ligand systems were more stably formed than the binary complexes, as shown in the complex species distribution diagrams.

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