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Kinetic Studies of Fast Equilibrium by Means of High-performance Liquid Chromatography. V. Stepwise Complex Formation of the Nickel(II) Ion with 2,2′-Bipyridine and 1,10-Phenanthroline

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The stepwise complex formation of the nickel(II) ion (M^{2+}) with 2,2′-bipyridine(bpy) and 1,10-phenanthroline(phen) ($M_{n-1}^{2+}+L \rightleftharpoons ML_n^{2+}$ with $K_n=[ML_n^{2+}]/[ML_{n-1}^{2+}][L]$, where $n=1$—3), has been investigated by means of high-performance liquid chromatography. Peaks of the labile partially ligand substituted complexes, ML^{2+} and ML_2^{2+}, have appeared on chromatograms, along with peaks of the ML_3^{2+} and excess free ligands, on reverse phase ion-pair chromatography using sodium dodecyl sulfate in acidic media. Each labile species has been found to be eluted without any noticeable change, such as ligand addition, dissociation, or disproportionation, during chromatography. Thus, the chromatograms obtained have directly indicated the equilibrium concentrations of each species in the solutions prior to chromatography. The stepwise complex formation constants of nickel(II) with bpy or phen have been determined by means of high-performance liquid chromatography.

In this series of papers[1-4] it has been demonstrated that a considerably fast reaction can be traced by means of high-performance liquid chromatography(HPLC) based on conventional principles. The present report deals with the complex formation of the nickel(II) ion (M^{2+}) with 2,2′-bipyridine(bpy) and 1,10-phenanthroline(phen) in aqueous media.

The representative stepwise complex formation of M^{2+} with these *N,N*-coordinates chelating reagents(L) is expressed by the following equations:

$$M^{2+} + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ML^{2+} \quad (1)$$

$$ML^{2+} + L \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ML_2^{2+} \quad (2)$$

$$ML_2^{2+} + L \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} ML_3^{2+} \quad (3)$$

$$K_1 = [ML^{2+}]/[M^{2+}][L] \quad (4)$$

$$K_2 = [ML_2^{2+}]/[ML^{2+}][L] \quad (5)$$

$$K_3 = [ML_3^{2+}]/[ML_2^{2+}][L] \quad (6)$$

The rate constant k_1, which was determined by several authors by means of the stopped-flow method,[5-10] is a bimolecular rate constant of the order of 10^3 mol^{-1} dm^3 s^{-1}, suggesting that mono-complex formation proceeds promptly. In strongly acidic conditions, since bpy and phen exist as the protonated form HL^+, another path for complex formation has been proposed:[8]

$$L + H^+ \underset{k_-}{\overset{k}{\rightleftharpoons}} HL^+ \quad (7)$$

$$K = [HL^+]/[H^+][L] \quad (8)$$

$$M^{2+} + HL^+ \underset{k'_{-1}}{\overset{k'_1}{\rightleftharpoons}} ML^{2+} + H^+ \quad (9)$$

$$ML^{2+} + HL^+ \underset{k'_{-2}}{\overset{k'_2}{\rightleftharpoons}} ML_2^{2+} + H^+ \quad (10)$$

$$ML_2^{2+} + HL^+ \underset{k'_{-3}}{\overset{k'_3}{\rightleftharpoons}} ML_3^{2+} + H^+ \quad (11)$$

The rate constant k'_1 has been reported to be of the order of 10^0—10^1 mol^{-1} dm^3 s^{-1},[8-10] much smaller than k_1. When M^{2+} and L are mixed, the complex formation reaches equilibrium almost instantaneously, except in strong acidic conditions. If the equilibrated solution is supplied to HPLC, it seems that the equilibrium prior to HPLC is displaced rapidly and that each labile species undergoes some reactions in the column promptly. This, however, is not always true for the following reasons. Among the various equilibrium equations mentioned above, the acid-base equilibrium shown in Eq. 7 will reach the equilibrium fastest. Thus, the rate constants k and k_- will be large, and when L (or HL^+) is chromatographed, it will reach equilibrium ($L^+ + H^+ \rightleftharpoons HL^+$) in the eluent almost instantaneously. The progress of other reactions in the column is not so fast. Since the forward reaction of Eq. 1 is a bimolecular one, the progress of the reaction is retarded during chromatography because of the decrease in the probability of collision between M^{2+} and L. Furthermore, mono-complex formation will be suppressed in an acidic eluent because, in acidic media, almost all ligands exist as the protonated form HL^+, which reacts with M^{2+} only slowly. The backward reaction of Eq. 1 is also slow. The dissociation rate constant, k_{-1}, of the $[Ni(bpy)]^{2+}$ complex has been reported to be of the order of 10^{-4} s^{-1}.[11] Our previous report[4] demonstrated that the progress of a unimolecular reaction during chromatography could be suppressed when the rate constant was of the order of 10^{-4} s^{-1}.

A similar discussion might hold for the second and the third steps of complex formation described in Eqs. 2, 3, 10, and 11. Aside from the reactions mentioned above, another type of reaction of ML^{2+} and ML_2^{2+} should be taken into consideration.

$$2ML^{2+} \underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} ML_2^{2+} + M^{2+} \quad (12)$$

This type of "disproportionation" reaction is not always negligible because the "reactants" are not separated by HPLC. We ourselves have previously described the disproportionation reaction for the sys-

tem of nickel(II) dithiocarbamate chelates[1] and demonstrated that the disproportionation did not occur during chromatography. Similarly, it might safely be concluded that disproportionation can also be neglected in the present case.

When all the above premises are fulfilled, each labile species is eluted without any change in the column. In this case, since the concentrations of each species prior to HPLC can be determined from the peak height (or area) appearing on chromatograms, the equilibrium constants, K_1, K_2, and K_3 in Eqs. 4, 5, and 6 will be determined.

Experimental

Reagents. A standard solution of nickel(II) was prepared by dissolving $NiCl_2 \cdot 6H_2O$ in water. The chelating reagents (bpy and phen) were also dissolved in water.

Apparatus. The HPLC apparatus used in this study is similar to the one described previously.[1] Several reverse phase packings (Nucleosil 5C18 (5 μm), LiChrosorb RP 18 (5 μm), Hypersil ODS (5 μm), Nucleosil 5C8 (5 μm), and LiChrosorb RP 8 (5 μm)) were slurry packed into stainless steel columns. A digital pH meter (Model 225, Iwaki Glass Co., Ltd.) was used to measure the accurate pH of the equilibrated solutions prior to HPLC.

Results and Discussion

Optimum Conditions of Reverse Phase Ion-pair Chromatography. The separation of some heavy metal chelates of bpy[12] and phen[13,14] has been reported by several authors using a proper ion-pair reagent such as sodium 1-pentanesulfonate. We here chose sodium dodecyl sulfate as an ion-pair reagent. The ion-pair reagent was dissolved in the mixed solvent system of water-acetonitrile, and the pH of the solution was controlled by adding a suitable quantity of salt. A satisfactory separation was attained when the solvent system was strongly acidic. Among the several acids tested, tartaric acid gave best results, and so we here chose this solvent system. The elution behavior varied markedly when different packings were used. Among these, Nucleosil 5C18 gave satisfactory results. Other packings gave a poor resolution due to either serious tailing or the overlap of two peaks. When free bpy or phen was injected into the column, a peak corresponding to red-colored $[Fe(bpy)_3]^{2+}$ or $[Fe(phen)_3]^{2+}$ appeared on chromatograms, along with the peak of free bpy or phen because of the contamination of iron ion from the HPLC apparatus. In order to eliminate the contamination, a minute amount of ethylenediaminetetraacetic acid(EDTA,H_4edta) was added to the eluent.[15] Thus treated, the peak of $[Fe(bpy)_3]^{2+}$ or $[Fe(phen)_3]^{2+}$ disappeared and the enlarged peak of free bpy or phen alone appeared on chromatograms. This treatment, which permits the quantitative elution of free bpy (or phen), is not always preferred because there exists some possibility that coordinated bpy (or phen) might be substituted for edta during chromatography. As will be shown in the following section, this ligand exchange is negligible when the concentration of EDTA added is small. Therefore, the content of EDTA should be so chosen that free bpy (or phen) may be eluted quantitatively and the peak heights of ML^{2+}, ML_2^{2+}, and ML_3^{2+} may not be affected by EDTA addition.

The best conditions for HPLC were as follows: solvent system: acetonitrile: 0.05 mol dm^{-3} tartaric acid: 0.001 mol dm^{-3} EDTA: sodium dodecyl sulfate =80: 50:0.3:0.5 g; Column: Nucleosil 5C18 (4.6 mm×15 cm); Column temperature: 25 °C (for phen chelate) or 10 °C (for bpy chelate); Flow rate: 1.5 cm^3 min^{-1}; Detector: UV 254 nm. Hereafter, the experiments were carried out under these conditions.

HPLC of Ni^{2+}-bpy System. Two solutions of Ni^{2+} and bpy were mixed, and after the pH had been adjusted to 1.9—2.0 by $HClO_4$-$NaClO_4$, the solutions were made up to a definite volume. The concentrations of the total nickel(II) ion $[M_t]$ and ClO_4^- were chosen to be 1.0×10^{-3} mol dm^{-3} and 1.0×10^{-1} mol dm^{-3}. The ratio of the $[M_t]$ to the total bpy $[L_t]$ was changed from 0.25 to 3.5 consecutively in the molar ratio. Then, the aliquots of these samples was submitted to HPLC; the chromatograms are shown in Fig. 1. Several peaks appeared on the chromatograms. Peak 2, which was

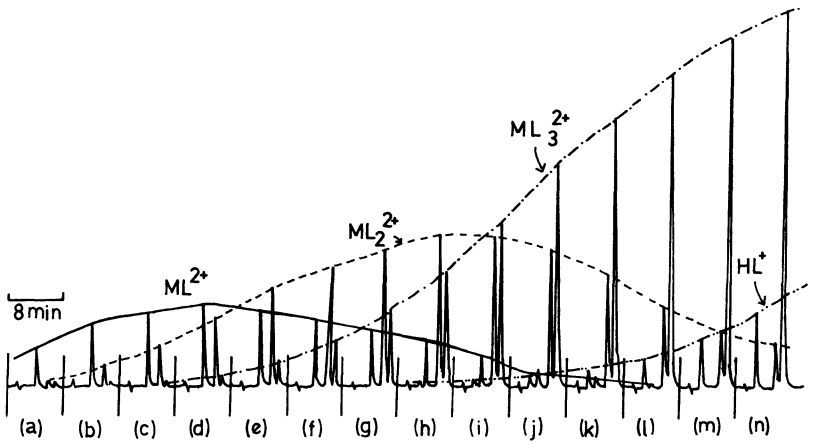

Fig. 1. Chromatograms of nickel(II)-bpy system in acidic media. For chromatographic conditions, see text.
$[M_t]=1.0$ mM, pH=1.93—1.99($HClO_4$-$NaClO_4$, $ClO_4^-=0.1$ M)
$[L_t]/[M_t]$ 0.25(a), 0.50(b), 0.75(c), 1.00(d), 1.25(e), 1.50(f), 1.75(g), 2.00(h), 2.25(i), 2.50(j), 2.75(k), 3.00(l), 3.25(m), 3.50(n).

relatively large when the $[L_t]/[M_t]$ ratio was small and which reached the maximum under the condition $[L_t]/[M_t]=1.0$, corresponds to the 1:1 complex ML^{2+}. Peak 3, which gave highest peak when $[L_t]/[M_t]$ was equal to 2.0, is the peak of the 1:2 complex ML_2^{2+}. The last peak (Peak 4), which increased gradually with the increase in $[L_t]$, corresponds to the 1:3 complex ML_3^{2+}. When $[L_t]/[M_t]$ exceeded 2.0, a new peak (Peak 5) appeared on chromatograms. This peak, which grew with the increase in $[L_t]/[M_t]$ is attributed to the peak of free bpy. Near the present HPLC conditions, free ligands should be chromatographed as the protonated form HL^+, because the eluent is strongly acidic. In Fig. 1, the peak of the aquanickel(II) ion M^{2+} did not appear on chromatograms because the aqua ion does not have any absorption in the UV range. When the portion corresponding to the solvent front was collected and sodium diethyldithiocarbamate was added to the portion, a brownish yellow color of nickel(II) diethyldithiocarbamate chelate appeared, suggesting that the aquanickel(II) ion was eluted without retention.

It seems that the elution sequence, M^{2+}, ML^{2+}, ML_2^{2+}, and ML_3^{2+}, is reasonable for the following reason. With an increase in the coordination number of the hydrophobic bulky bpy ligand, the hydrophobicity of the chelate increases. Thus, the 1:3 complex will form a very hydrophobic ion pair with the dodecyl sulfate ion, which has a strong affinity with the hydrophobic ODS stationary phase. Thus, the retention time of the 1:3 complex will be the longest, and the elution sequence described above will result. Since the aquanickel(II) ion is very hydrophilic, it is not retained on the stationary phase at all.

The addition of EDTA to the eluent was necessary for the quantitative elution of free ligands. In order to investigate the effect of EDTA on other peaks, HPLC was carried out in the presence and absence of EDTA. Each peak height was found to be indifferent to the addition of EDTA, with the exception of the peak of free bpy. This result suggests that the bpy ligand coordinated with ML^{2+}, ML_2^{2+}, and ML_3^{2+} is not replaced by edta during chromatography when the EDTA concentration is low.

Determination of the Stepwise Complex Formation Constants of the Ni^{2+}–bpy System. Two solutions of Ni^{2+} and bpy were mixed so that $[L_t]/[M_t]$ was equal to 2.0. Then the pH of the solution was changed from 1 to 8 by the use of $HClO_4$–$NaClO_4$ or $NaClO_4$–NaOH. The concentration of $[M_t]$ was chosen as 1.0×10^{-3} mol dm^{-3}. Figures 2(a) and (b) depict examples of chromatograms near acidic and almost neutral conditions. These two chromatograms suggest that some difference in chromatograms exists when the pH values of the sample solutions are different. This can be explained in terms of the "protonation reaction" of bpy. The concentration of ligands effective on complex formation is smaller in acidic media than in basic or neutral media, because in acidic conditions most of the bpy exists as the protonated form HL^+. Thus, the ratio of the 1:3 complex is smaller in acidic media than in neutral media because of the low effective concentration of bpy available for complex formation.

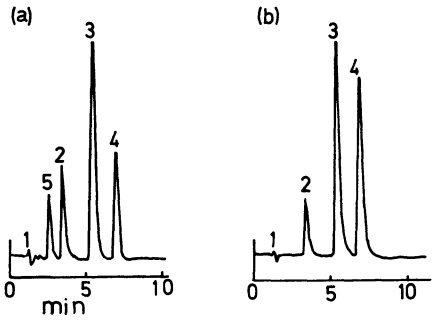

Fig. 2. Chromatograms of nickel(II)–bpy system at $[L_t]/[M_t]=2.0$. For chromatographic conditions, see text.
$[M_t]=1.0$ mM, $[L_t]=2.0$ mM, pH=1.2(a), 5.1(b) ($HClO_4$–$NaClO_4$, $ClO_4^-=0.1$ M)
1 M^{2+}(solvent front), 2 ML^{2+}, 3 ML_2^{2+}, 4 ML_3^{2+}, 5 HL^+.

Now peaks of HL^+, ML_3^{2+}, ML_2^{2+}, ML^{2+}, and possibly M^{2+} appeared on chromatograms in acidic conditions, as is shown in Fig. 2(a), the determination of the stepwise complex formation constants, K_1, K_2, and K_3, is, therefore, possible provided that the equilibrium concentrations of each species are known. For this purpose, the following facts are noteworthy: (1) The chromatograms shown in Fig. 2 should directly indicate the equilibrium concentration of each species prior to HPLC. The ion-pair formation of these metal chelates with the dodecyl sulfate ion might result in a displacement of the equilibrium. If this phenomenon really occurs, the reaction proceeds instantaneously in the column. The fast separation speed of HPLC and the moderate lability of these chelates suggest that this reaction is unlikely to occur. Furthermore, when the content of SDS in the eluent was chosen as 0.1 and 1.0 g instead of 0.5 g, the percentage of ML_3^{2+} in the sample shown in Fig. 2(a) was not changed. (2) Calibration graphes of each species are necessary, the procedure being carried out as follows: First, a linear calibration graph of bpy is obtained. Second, a calibration graph of ML_3^{2+} is obtained by mixing nickel(II) and bpy so that the $[L_t]/[M_t]$ ratio is 4.0 or higher near neutral pH conditions and by supplying the sample to HPLC. This sample gave only two peaks of ML_3^{2+} and excess free ligands, suggesting the complete 1:3 complex formation. The calibration graph of ML_3^{2+} was also linear. Calibration curves of other species could not be obtained directly because of either the lack of absorption(M^{2+}) or the lability of the chelates(ML^{2+} and ML_2^{2+}). It is noteworthy here that the measurements of each peak area in Fig. 2(a) do not give the concentrations of each species because of the difference in the absorption coefficients of the species. The absorption coefficients were found to decrease in this order: $ML_3^{2+}>ML_2^{2+}>ML^{2+}$, which was parallel to the coordination numbers of bpy. Calibration graphs of these labile species were obtained by the following procedures. Fractions corresponding to M^{2+}, ML^{2+}, and ML_2^{2+} were collected, and a slight excess of bpy was added. After the pH was adjusted to approximately neutral, each portion was diluted to a definite volume

Table 1. Stepwise complex formation constants of the nickel(II)-bpy system at 25 °C

$\log K_1$	$\log K_2$	$\log K_3$	Ref.
6.92±0.11	6.81±0.07	6.25±0.06	This work*
6.80	6.46	5.20	17
7.13	6.88	6.53	18
7.07	6.86	6.20	19
6.9	6.8	5.9	20
6.95	6.83	6.35	21

* Chromatograms are shown in Fig. 2(a).
The concentrations of each species are as follows: $[M]_t=1.00$ mM (1M=1 mol dm^{-3}); $[L]_t=2.00$ mM; $[M^{2+}]=(0.16\pm0.02)$ mM; $[ML^{2+}]=(0.29\pm0.01)$ mM; $[ML_2^{2+}]=(0.41\pm0.01)$ mM; $[ML_3^{2+}]=(0.16\pm0.005)$ mM; $[L]+[HL^+]\fallingdotseq[HL^+]=(0.35\pm0.03)$ mM; pH=1.21.
From the measurements of the peak height, the total concentration of $[M_t]$ was calculated to be (1.02±0.045) mM.

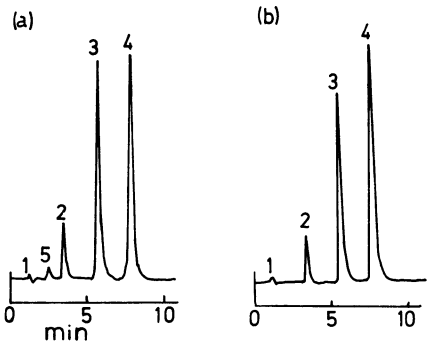

Fig. 3. Chromatograms of nickel(II)-phen system at $[L_t]/[M_t]=2.0$. For chromatographic conditions, see text.
$[M_t]=0.5$ mM, $[L_t]=1.0$ mM, pH=1.3(a), 6.2(b) (HCl-NaCl, $Cl^-=0.05$ M)
1 M^{2+} (solvent front), 2 ML^{2+}, 3 ML_2^{2+}, 4 ML_3^{3+}, 5 HL^+.

by the use of the HPLC solvent. Each sample solution was left to stand at least 2 h and then submitted to HPLC. From the peak heights (or areas) of ML_3^{2+} which then appeared on chromatograms, the initial concentrations of ML_2^{2+}, ML^{2+}, and M^{2+} were calculated. In order to confirm that each species was converted into the 1:3 complex thoroughly, the following experiments were carried out. The aquanickel(II) ion (M^{2+}) was dissolved in the HPLC solvent in the presence and absence of EDTA and tartaric acid. A slight excess of bpy was added to these solutions and pH was adjusted to be almost neutral. These two solutions were then diluted to a definite volume and were submitted to HPLC. The peak heights of ML_3^{2+} were similar for these two sample solutions, suggesting the complete 1:3 complex formation.

Now, the concentrations of M^{2+}, ML^{2+}, ML_2^{2+}, ML_3^{2+}, and HL^+ were determined by HPLC, K_1, K_2, and K_3 can be determined if the dissociation constant of bpy (K in Eq. 8) is known. Table 1 indicates these values by assuming log K=4.4.[16] The stepwise complex formation constants reported by other authors[17–21] are also included in this table. The present values agree very well with the results of other authors, suggesting the reliability of the present HPLC method.

Table 2. Stepwise complex formation constants of the nickel(II)-phen system at 25 °C

$\log K_1$	$\log K_2$	$\log K_3$	Ref.
8.97±0.35	8.82±0.16	8.40±0.15	This work*
8.60	8.10	7.55	22
8.80	8.30	7.70	23
8.8	8.3	7.7	18
8.0	8.0	7.9	19
8.65	8.43	7.83	24

* Chromatograms are shown in Fig. 3(a).
The concentrations of each species are as follows: $[M]_t=0.50$ mM; $[L]_t=1.00$ mM; $[M^{2+}]=(0.03\pm0.01)$ mM; $[ML^{2+}]=(0.10\pm0.01)$ mM; $[ML_2^{2+}]=(0.21\pm0.01)$ mM; $[ML_3^{2+}]=(0.17\pm0.01)$ mM; $[L]+[HL^+]\fallingdotseq[HL^+]=(0.015\pm0.003]$ mM; pH=1.33.
The concentration of $[M_t]$ was calculated to be (0.51±0.04) mM.

Ni^{2+}-phen System. Similar experiments were carried out for the Ni^{2+}-phen system. The chromatogram patterns obtained resembled those for the Ni^{2+}-bpy system. The results at $[L_t]/[M_t]=2.0$ are exemplified in Figs. 3(a) and (b) under acidic and almost neutral conditions. The equilibrium concentrations of M^{2+}, ML^{2+}, ML_2^{2+}, ML_3^{2+}, and HL^+ were determined by HPLC with similar procedures; thus, the stepwise complex formation constants were calculated. The results are tabulated in Table 2, assuming that log K=5.0.[16] The results reported by other authors[18,19,22–24] are also appended in this table. The present results agree fairly well with those of other authors, though the present data are slightly larger. A comparison of Table I and Table II indicates that the ratio of HL^+ and ML^{2+} is smaller in the Ni^{2+}-phen system than in the Ni^{2+}-bpy system. This may be attributed to the fact that the stepwise complex formation constants are larger for the Ni^{2+}-phen system than for the Ni^{2+}-bpy system.

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