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Equilibrium Analysis of Reactions of Metal-Pyridylazoresorcinolato Chelates with Quaternary Ammonium Ion, Nonionic Surfactant and Polyethylene Glycol in Aqueous Solution by Capillary Zone Electrophoresis

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The resolutions of metal-4-(2-pyridylazo)resorcinol chelates by capillary zone electrophoresis (CZE) were investigated in the presence of some interacting reagents; also, equilibrium reactions between the chelates and the interacting reagents were analyzed in an aqueous solution. Among nine metal chelates formed in aqueous solution, the chelates of V^{V}, Fe^{II}, Co^{III}, Ni^{II}, and Cu^{II} were resolved and detected by CZE, while other chelates were decomposed during electrophoretic migration. The electrophoretic mobility of the chelates of Fe^{II}, Ni^{II}, and Cu^{II} increased with increasing pH of the migrating solution; also, the acid-dissociation constants of these three chelates were determined by analyzing the mobility change. The ion-association constants of the five anionic chelates and pyridylazoresorcinolate ion with quaternary ammonium ions were also determined by analyzing the mobility change. The binding behavior of the ligand and its chelates with nonionic surfactant micelle, as well as with polyethylene glycol, were investigated, and their binding constants were determined through the mobility change. When Brij 35 was used as a nonionic surfactant interacting with the anionic chelates, the Fe^{II} chelate decomposed at Brij 35 concentrations over 6.67 mM. The equilibrium constants and the reactivity were compared with each other.

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Capillary zone electrophoresis (CZE) is a powerful tool for the separation and determination of ionic substances. Metal ions have been determined after derivatizing them with chelating reagents to convert anionic complexes. Ethylenediaminetetraacetic acid[1] and 8-quinolinol-5-sulfonic acid[2] were utilized as derivatizing reagents for the determination of alkaline earth metals by CZE analysis. Some pyridylazo compounds were also used for the derivatization and determination of transition metal ions.[3–5]

4-(2-Pyridylazo)resorcinol (par) has been used as a spectrophotometric reagent and a metallochromic indicator for metal determination, and equilibrium reactions of chelate formation have been investigated in aqueous solutions, as well as in liquid-liquid distribution systems, including ion-pair extraction.[6–10] The properties of the chelates were utilized for the separation of heavy metal ions by ion-pair reversed-phase high performance liquid chromatography,[11,12] and also for CZE separations.[13–16] Recently, sodium dodecyl sulfate has been used in micellar electrokinetic chromatography (MEKC), and in the separation of the par chelates in MEKC.[17] Cationic polymers have also been used as an interacting reagent for anionic chelates.[18] In the capillary electrophoretic separation of the par chelates, it was reported that some metal chelates decomposed during migration.[13,17]

The hydroxyl groups of the par chelates have been reported to dissociate in aqueous solutions. However, such interesting properties as acid dissociations, interactions with cations and surfactants, and their equilibrium reactions in aqueous solutions have not yet been clarified, because of the lack of an analysis method. The electrophoretic mobility of ions can provide useful information on the dissolved states of analytes, and equilibrium constants can be determined by analyzing the mobility change. In this study, the authors investigated several properties of the par chelates, including acid dissociation, ion association with quaternary ammonium ions, as well as binding reactions to nonionic surfactant micelle and polyethylene glycol. The utilization of CZE measurements has major advantages in that the analyte concentrations can be lowered to the 10^{-5} M level or less,[19] and that the migration time of the species of interest can be used even when they gradually decompose during migration.[20]

Experimental

Apparatus

A Hewlett Packard ^{3D}CE was used as a capillary electrophoresis system. A fused-silica capillary (Hewlett Packard) was attached to the system; the dimensions of the capillary were 56.5 cm in total length, 50 cm in effective length from the injection point to the detector, and 50 μm inner diameter. A Hewlett Packard ChemStation (Ver. A. 05.04) was used to record and analyze the electropherograms. A Corning Ion Analyzer M-250, calibrated daily with standard pH solutions, was used to measure the pH of the solutions.

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Reagent

4-(2-Pyridylazo)resorcinol was purchased from Dojindo Laboratories (Kumamoto, Japan), and used after being dissolved in slightly alkaline water. Standard metal solutions were prepared with reagent-grade metal chlorides or metal nitrates with 0.1 M HCl or 0.1 M HNO_3 solutions, except for vanadium; ammonium metavanadate(V) was dissolved in a 0.1 M HCl solution. The solutions were standardized by a complexometric titration. Quaternary ammonium salts of tetramethylammonium bromide (TMA^+Br^-) and tetrabutylammonium bromide (TBA^+Br^-) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and used without further purification. Polyoxyethylene (23) lauryl ether (Brij 35) and polyethylene glycol with an average molecular mass of 1000 (PEG#1000) were from Wako (Osaka, Japan). Sodium tetraborate (borax), KH_2PO_4, and Na_2HPO_4 were used as pH buffer components. The water used was deionized and distilled.

Procedure

Metal ions were derivatized with par before CZE separation. A solution of a metal ion with their final concentrations of 2×10^{-5} M containing 1×10^{-3} M par and phosphate buffer (pH 7.0; 10 mM) was allowed to stand for completing the complexation reaction. A mixture of metal-chelates was also prepared in a similar manner.

A solution containing the pH buffer and an interacting reagent was used as a migrating solution; the ionic strength of the solution was adjusted to 0.05 M by adding appropriate amounts of NaCl. A migrating solution thus prepared was filled both in a cathodic and an anodic reservoir, as well as into a capillary. A sample solution containing the metal chelates (2×10^{-5} M each) was introduced into the capillary by hydrodynamic injection from the anodic end for 5 s (250 mbar·s). After injection, a voltage of 15 kV was applied, and electrophoresis was started. The par chelates were photometrically detected at 500 nm. Throughout the experiments, the capillary, as well as the vials of the migrating solution and the sample solution, was thermostated at 25.0˚C. The electrophoretic mobility of the chelates was obtained in the usual manner.

Results and Discussion

Analysis of an acid-dissociation reaction

Prior to CZE separation of the par chelates, the complexation reaction was examined photometrically under the conditions of pH 7.0 and 9.2. Among the fourteen metal ions examined, V^V, Mn^{II}, Fe^{III} (Fe^{II} as chelate), Co^{II} (Co^{III} as chelate), Ni^{II}, Cu^{II}, Zn^{II}, Cd^{II}, and Pb^{II} formed par chelates, while Mg, Al, Ca, Sr, Ba did not form colored chelates at both pHs.

The electrophoretic separation of metal chelates was performed under several pH conditions. Typical electropherograms are shown in Figs. 1(a) and (b). Among nine par chelates formed in an aqueous solution, the peaks of V^V, Fe^{II}, Co^{III}, Ni^{II}, and Cu^{II} chelates were obtained. The oxidation number for iron was considered from the electrophoretic mobility and the absorption spectrum; it is attributed to be Fe^{II} chelate. Other par chelates (Mn^{II}, Zn^{II}, Cd^{II}, and Pb^{II}) should be decomposed during migration, as mentioned by Iki *et al.*;[13] the decomposition is attributed to the kinetic differentiation mode in the CZE separation between the chelates and par, which resulted in the dissociation of the chelates during migration. Overlapped peaks in the migration time ranging over 12 – 13 min (preceding to the par signal) in Fig. 1(b) can be attributed to the Mn, Zn, Cd, and Pb chelates.

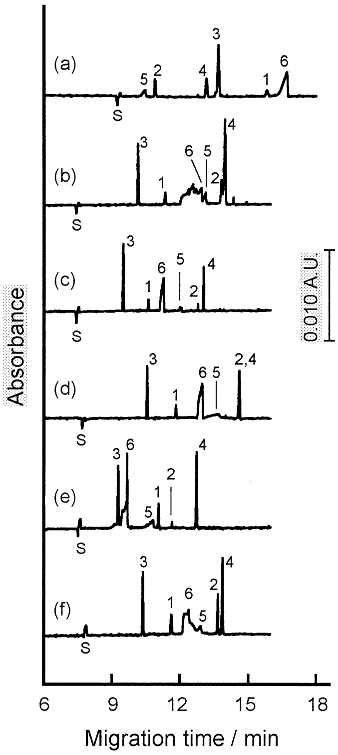

Fig. 1 Typical electropherograms for par and its metal chelates in the absence and presence of an interacting reagent. Interacting reagent and pH conditions: (a) none, pH 6.31; (b) none, pH 9.46; (c) 7.5 mM TBA^+Br^-, pH 9.5; (d) 7.5 mM TMA^+Br^-, pH 9.5; (e) 6.67 mM Brij 35, pH 9.5; (f) 10 mM PEG#1000, pH 9.5. CE conditions: applied voltage, 15 kV; detection wavelength, 500 nm; temperature, 25˚C; injection period, 5 s (250 mbar·s). Migrating solution: 10 mM pH buffer + interacting reagent + NaCl (I = 0.05). Sample solution: nine kinds of 2×10^{-5} M metal ions (VO_3^-, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}) + 1×10^{-3} M par + 10 mM phosphate buffer (pH 7.0). Signals: 1, V-par; 2, Fe-par; 3, Co-par; 4, Ni-par; 5, Cu-par; 6, par; S, EOF.

The order of migration and the electrophoretic mobility of the chelates can be changed by changing the pH of the migrating solution, as can be seen in Figs. 1(a) and (b); therefore, the pH conditions can be utilized for improving the resolution of the par chelates; further, we can analyze the acid-dissociation reactions of the chelates using the electrophoretic mobility change. The changes in the electrophoretic mobility of par and their chelates are shown in Fig. 2. It is noted from Fig. 2 that par chelates of Fe^{II}, Ni^{II} and Cu^{II} became more anionic under alkaline pH conditions, whereas the chelates of V^V and Co^{III} exist as identical species over the examined pH conditions. The par chelates of Fe^{II}, Ni^{II} and Cu^{II} can release the protons of the resorcinol moiety stepwise. The acid-dissociation reactions of the par chelates are represented as in Eq. (1) with equilibrium constants (2):

$$\begin{aligned} \mathrm{M(Hpar)_2} &\rightleftharpoons \mathrm{M(Hpar)(par)^-} + \mathrm{H^+}; \\ \mathrm{M(Hpar)(par)^-} &\rightleftharpoons \mathrm{M(par)_2^{2-}} + \mathrm{H^+}, \end{aligned} \tag{1}$$

$$K_{a1} = \frac{[\mathrm{H^+}][\mathrm{M(Hpar)(par)^-}]}{[\mathrm{M(Hpar)_2}]};\ K_{a2} = \frac{[\mathrm{H^+}][\mathrm{M(par)_2^{2-}}]}{[\mathrm{M(Hpar)(par)^-}]}, \tag{2}$$

where K_{a1} and K_{a2} are the acid-dissociation constants. The apparent electrophoretic mobility of a chelate, $-\mu_{ep}'$, is related to

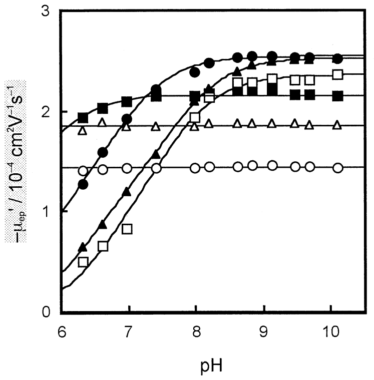

Fig. 2 Changes in the electrophoretic mobility of par and its metal chelates as a function of the pH. The CE conditions and the sample solution are the same in Fig. 1. Migrating solution: 10 mM pH buffer + NaCl (I = 0.05). △, V-par; ▲, Fe-par; ○, Co-par; ●, Ni-par; □, Cu-par; ■, par.

Table 1 Acid-dissociation constants of par chelates

Acid	pK_a	
	This study[a]	Reference
Fe^{II} (Hpar)$_2$	6.37 ± 0.10	6.47[b]
Fe^{II} (Hpar) (par)$^-$	7.69 ± 0.10	7.66[b]
Ni^{II} (Hpar)$_2$	5.68 ± 0.26	6.2[b]
Ni^{II} (Hpar) (par)$^-$	6.90 ± 0.13	7.1[b]
Cu^{II} (Hpar)$_2$	6.66 ± 0.57	—
Cu^{II} (Hpar) (par)$^-$	7.67 ± 0.59	8.2[c]

a. Error: 3σ.
b. Ref. 10.
c. Ref. 21.

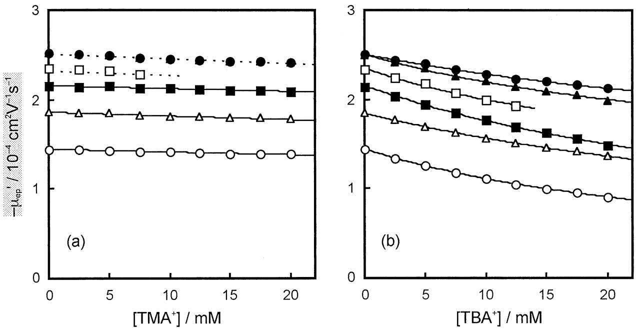

Fig. 3 Changes in the electrophoretic mobility of par and its metal chelates with increasing concentrations of quaternary ammonium salts (Q$^+$Br$^-$). The CE conditions and the sample solution are the same in Fig. 1. Migrating solution: 10 mM borate buffer (pH 9.5) + (0 - 20) mM Q$^+$Br$^-$ + NaCl (I = 0.05). Q$^+$Br$^-$: (a) TMA$^+$Br$^-$; (b) TBA$^+$Br$^-$. △, V-par; ▲, Fe-par; ○, Co-par; ●, Ni-par; □, Cu-par; ■, par.

the acid-dissociation constants, and is represented as in Eq. (3), where the activity of H$^+$ obtained by a pH measurement, a_{H^+}, is used instead of the concentration of H$^+$,

$$-\mu_{ep}' = \frac{(a_{H^+}^2/K_{a1}K_{a2})(-\mu_{ep,M(HL)2}) + (a_{H^+}/K_{a2})(-\mu_{ep,M(HL)(L)}) + (-\mu_{ep,ML2})}{(a_{H^+}^2/K_{a1}K_{a2}) + (a_{H^+}/K_{a2}) + 1}. \tag{3}$$

In Eq. (3), $(-\mu_{ep,M(HL)2})$, $(-\mu_{ep,M(HL)(L)})$, and $(-\mu_{ep,ML2})$ are the electrophoretic mobility of M(Hpar)$_2$, M(Hpar)(par)$^-$, and M(par)$_2^{2-}$, respectively. In the equilibrium analysis, M(Hpar)$_2$ is electrically neutral, an electrophoretic mobility of zero was used for $(-\mu_{ep,M(HL)2})$. Based on the charge and the molecular volume, the value for $(-\mu_{ep,M(HL)(L)})$ was estimated to be one half of that of $(-\mu_{ep,ML2})$. A series of pairs of a pH and a $(-\mu_{ep}')$ value were used as input variables, and the values of K_{a1}, K_{a2} and $(-\mu_{ep,ML2})$ were optimized using a non-linear least-squares analysis.[20] The determined acid-dissociation constants are summarized in Table 1. The acid-dissociation constants were already obtained by spectrophotometry;[10,21] their values are also summarized in Table 1 for a comparison. In the study,[21] the pK_a value for the Cu^{II} chelate was determined in 10%(v/v) aqueous acetone. In this study, however, we succeeded to determine the two steps of the pK_a values in an aqueous solution, although the deviations are relatively large. Although a slightly large difference is observed in Ni(Hpar)$_2$, the equilibrium constants obtained in this study agreed well with the reported values.

Ion-association reaction of the par chelates with quaternary ammonium ions

Some par chelates are present as anionic species, and can be extracted from an aqueous phase into an organic phase as an ion associate with a quaternary ammonium ion. The extraction constants of the anionic chelates have already been reported.[6-10] Generally, in the ion-association extraction, or ion-pair extraction, the extraction equilibrium was present as an extraction constant (K_{ex}), which includes both the ion-association reaction in an aqueous solution (K_{ass}) and the distribution reaction of the ion associate between aqueous and organic phases (K_D). The authors have succeeded to determine the ion-association constants in an aqueous solution, and have clarified the contribution of the ion-association reactions in an aqueous solution to the whole extraction process.[22,23] In this study, the ion-association reactions of the par chelates with quaternary ammonium ions in an aqueous solution were investigated at pH 9.5. At that pH, par and its chelates exist as stable species of Hpar$^-$, VO$_2$(par)$^-$, Fe(par)$_2^{2-}$, Co(par)$_2^-$, Ni(par)$_2^{2-}$, and Cu(par)$_2^{2-}$. Typical electropherograms using tetrabutylammonium ion (TBA$^+$) and tetramethylammonium ion (TMA$^+$) are shown in Figs. 1(c) and (d), respectively. The changes in the electrophoretic mobility of the chelates with changing the concentrations of the quaternary ammonium ions are shown in Fig. 3. At TMA$^+$ concentrations more than 7.5 mM or for TBA$^+$ concentrations of more than 12.5 mM, the peak for the Cu^{II} chelate diminished. The presence of the quaternary ammonium ion would accelerate the decomposition of the Cu(par)$_2^{2-}$ chelate, which should be attributed to a resolution improvement between Hpar$^-$ and Cu(par)$_2^{2-}$.

The ion-association reactions of the chelates were analyzed using the mobility change. The ion-association reaction between a quaternary ammonium ion (Q$^+$) and a chelates (M-par^{n-}) is represented as in Eq. (4) with an equilibrium constant (5):

$$Q^+ + \text{M-par}^{n-} \rightleftharpoons Q^+\cdot\text{M-par}^{n-}, \tag{4}$$

$$K_{ass} = \frac{[Q^+\cdot\text{M-par}^{n-}]}{[Q^+][\text{M-par}^{n-}]}, \tag{5}$$

where K_{ass} is an ion-association constant. The ion-association constants were also determined using the decreases in the electrophoretic mobility in the same manner as in our previous

Table 2 Ion-association constants and binding constants of par and its metal chelates

Equilibrium constant[a]	Interacting reagent	Par chelates					
		par^-	$V^VO_2(par)^-$	$Fe^{II}(par)_2^{2-}$	$Co^{III}(par)_2^-$	$Ni^{II}(par)_2^{2-}$	$Cu^{II}(par)_2^{2-}$
$\log K_{ass}$	TMA^+	0.18 ± 0.14	0.38 ± 0.08	—	0.37 ± 0.10	—	—
$\log K_{ass}$	TBA^+	1.34 ± 0.02	1.26 ± 0.02	1.45 ± 0.06	1.47 ± 0.02	1.26 ± 0.03	1.56 ± 0.10
$\log K_B$	Brij 35	2.11 ± 0.07	1.18 ± 0.03	1.73 ± 0.07	1.78 ± 0.09	1.39 ± 0.03	1.74 ± 0.22
$\log K_B$	PEG#1000	1.27 ± 0.06	1.06 ± 0.13	1.36 ± 0.05	1.26 ± 0.06	1.26 ± 0.06	1.38 ± 0.06

a. Error: 3σ.

work.[19] Changes in the electrophoretic mobility were too slight with TMA^+ to determine the ion-association constants; only the values of the monovalent anions can be determined, because the apparent charge of the 1:1 ion associates is neutral and the electrophoretic mobility of the ion associates should be zero. The ion-association constants obtained are summarized in Table 2. From a comparison of the K_{ass} values, as well as the mobility change, TBA^+ is more reactive with the anionic par chelates than TMA^+. Such results indicate that the anionic chelates are very hydrophobic, and a hydrophobic interaction in an aqueous solution can contribute to the ion associability. In comparing the K_{ass} values of $Fe(par)_2^{2-}$ and $Ni(par)_2^{2-}$, the ion associability of the anionic chelates is on the order of $Ni(par)_2^{2-} < Fe(par)_2^{2-}$. This result is comparable to the order of the ion-associate extraction of the chelates, where benzyldimethyltetradecylammonium ion (Zephiramine) was used as an ion-association reagent {K_{ex}: $10^{11.16}$ ($Ni(par)_2^{2-}$) < $10^{12.17}$ ($Fe(par)_2^{2-}$)}.[6,10] The difference in K_{ex} between the Fe^{II} and Ni^{II} chelates is greatly larger than that in K_{ass}. This indicates that the K_D values for the Ni^{II} chelate should be larger than that for the Fe^{II} chelate.

Binding reaction between the par chelates and nonionic surfactants

Nonionic surfactants can bind to organic molecules through a hydrophobic interaction, and can be utilized for the electrophoretic separation of dansyl amino acids[24] and aromatic anions.[25] In this study, polyoxyethylene (23) lauryl ether (Brij 35) was used as an example of a nonionic surfactant, forming a micelle and polyethylene glycol with its average molecular mass of 1000 (PEG#1000) as a hydrophobic matrix; the binding reactions of the par chelates with the reagents were investigated. Typical electropherograms are shown in Figs. 1(e) and (f). Changes in the electrophoretic mobility are shown in Fig. 4. In the case of Brij 35 as the interacting reagent, the peaks for the Fe^{II} chelate diminished at Brij 35 concentrations of more than 6.67 mM {0.8%(w/v)}. A hydrophobic medium of the micelle would accelerate the decomposition of $Fe(par)_2^{2-}$. As can be seen from Fig. 1(e) and Fig. 4(a), the migration order of the par chelates significantly changed in the presence of Brij 35 in the migrating solution, while the order did not change with PEG#1000. The decrease in the apparent electrophoretic mobility is larger with Brij 35 than with PEG#1000; these results indicate that the par chelates are very reactive with Brij 35 micelle.

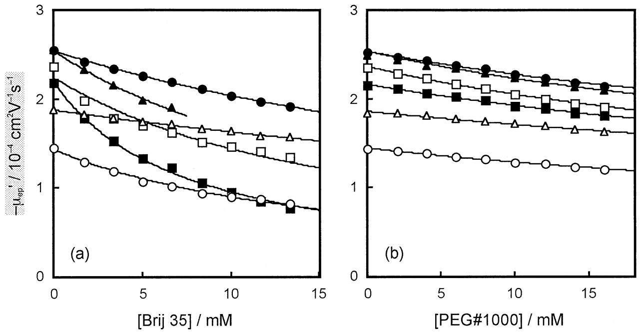

Fig. 4 Changes in the electrophoretic mobility of par and its metal chelates with increasing concentrations of nonionic surfactant. The CE conditions and the sample solution are the same in Fig. 1. Migrating solution: (a) 10 mM borate buffer (pH 9.5) + (0 – 13.3) mM Brij 35 + NaCl (I = 0.05); (b) 10 mM borate buffer (pH 9.5) + (0 – 16) mM PEG#1000 + NaCl (I = 0.05). △, V-par; ▲, Fe-par; ○, Co-par; ●, Ni-par; □, Cu-par; ■, par.

The binding constants between the metal chelates and the nonionic surfactant micelle or the polyethylene glycol are also determined using the changes in the electrophoretic mobility. The binding constant is represented as in Eq. (6) with its equilibrium (7):

$$\text{M-par}^{n-} + \text{NIS} \rightleftharpoons \text{M-par}^{n-}\text{-NIS}, \qquad (6)$$

$$K_B = \frac{[\text{M-par}^{n-}\text{-NIS}]}{[\text{M-par}^{n-}][\text{NIS}]}, \qquad (7)$$

where NIS is a nonionic surfactant (Brij 35 or PEG#1000 in this study) and K_B is a binding constant. Brij 35 and PEG#1000 are written as NIS in Eqs. (6) and (7). However, there is a definitive difference: the former can form molecular aggregates, micelles, while the latter is dispersed in an aqueous solution. The binding constants were also determined using the decreases in the electrophoretic mobility in the same manner as in our previous work;[25] the K_B values obtained are summarized in Table 2. By comparing the binding constants, it can be seen that the K_B values with Brij 35 are much larger than those of PEG#1000. Such a result can be reasonably understood from the fact that Brij 35 provides a strongly hydrophobic medium in aqueous solution as micelles.

Conclusions

In this study, the resolution of the anionic par chelates was improved by using interacting reagents. The equilibrium reactions of the par chelates in aqueous solutions including the acid dissociation, ion associations and binding reactions were clarified through measurements of the electrophoretic mobility. By comparing the equilibrium constants, the par chelates were proved to be hydrophobic through equilibrium analyses. Quaternary ammonium ions and Brij 35 were found to accelerate the decomposition of $Cu(par)_2^{2-}$ and $Fe(par)_2^{2-}$, respectively. The results obtained in this study indicate that the selectivity among the par chelates in the kinetic differentiation mode can be controlled by using a particular interacting reagent.

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