ANALYTICAL SCIENCES DECEMBER 2008, VOL. 24

Redox Potentials and HPLC Behavior of Cobalt and Iron Complexes with Pyridylazo Compounds

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The redox potentials of cobalt and iron complexes with ten pyridylazo compounds, E^0_{ML2} ($ML_2^{+/0}$; M: $Co^{III/II}$, $Fe^{III/II}$; L^-: pyridylazo compounds), have been determined in order to explore the difference in their reversed-phase HPLC behavior. The redox potentials of Co complexes were in the range of –0.62 – 0.03 V, while those of Fe complexes were –0.06 – 0.59 V relative to 0.20 V for ferricinium/ferrocene. The redox potentials of both the Co and Fe complexes were linearly correlated to the basicities of the ligands. The correlation was quantitatively explained by a difference in dependence of the stabilities of M^{III} and M^{II} complexes on the ligand basicities. The complex of $[Co^{III}L_2]^+$ or $[Fe^{II}L_2]$ with any compound injected in the reversed-phase HPLC system was detected without any change in the composition. When $[Co^{II}L_2]$ was injected, only those complexes having the highest potentials of $E^0_{CoL2} \cong 0.0$ V were detected as $[Co^{II}L_2]$, while other complexes having lower potentials gave a peak of $[Co^{III}L_2]^+$. When $[Fe^{III}L_2]^+$ was injected, only complexes having the lowest potentials of $E^0_{FeL2} \cong 0.0$ V were detected as $[Fe^{III}L_2]^+$, while others having higher potentials gave a peak of $[Fe^{II}L_2]$.

(Received August 20, 2008; Accepted October 20, 2008; Published December 10, 2008)

Introduction

Chromogenic chelating reagents are widely used for the determination of metal ions in conjunction with chromatographic systems. In postcolumn derivatization, the reagents are used only for the detection of metal ions, and those with high reactivities and sensitivities are favored. In contrast, metal ions are separated as metal complexes in precolumn derivatization, and thus the separation of metal ions as well as the analytical sensitivities depend on the structures and properties of the reagents.

Heterocyclic azo compounds react with varying metal ions to give stable and intensely colored complexes,[1,2] and have been widely used as precolumn derivatizing reagents.[3-8] When a typical compound of 1-(2-pyridylazo)-2-naphthol (*β*-PAN; L^- denotes a monoanionic tridentate ligand, including *β*-PAN) was used for simultaneous determination of Fe^{3+}, Co^{2+}, Ni^{2+}, and Cu^{2+}, the resulting complexes of $[Fe^{II}L_2]$, $[Co^{III}L_2]^+$, $[NiL_2]$, and $[CuL]^+$ as well as an excess ligand, HL were satisfactorily separated using an aqueous acetonitrile mobile phase containing thiocyanate ion,[5] which guaranteed a reproducible and appropriate retention of cationic complexes as ion-pairs, such as $[CoL_2]^+SCN^-$ and $[CuL]^+SCN^-$.[9]

Although common heterocyclic azo compounds showed similar behavior to *β*-PAN, a few exceptions were reported. In the HPLC system with 2-(5-nitro-2-pyridylazo)-1-naphthol, Co^{2+} was detected as a neutral 1:2 complex, $[Co^{II}L_2]$, without any reducing reagents.[8] In the HPLC system with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol, Fe^{3+} was detected as a cationic 1:2 complex, $[Fe^{III}L_2]^+$, without any oxidizing reagents.[10] The oxidation state of the complexes is related to the redox potentials of the cobalt and iron complexes, which have been studied only for cobalt complexes with 2-(2-pyridylazo)-1-naphthol (*α*-PAN), 1-(2-thiazolylazo)-2-naphthol (*β*-TAN), *β*-PAN,[11] and their sulfonated derivatives,[12] and not for iron complexes. In the present work, we studied the acid dissociation equilibria of ten pyridylazo compounds, the complexation equilibria with Co^{2+}, Fe^{2+}, and Fe^{3+}, and the redox potentials of cobalt and iron complexes and their relation to chromatographic behavior in reversed-phase HPLC.

Experimental

Reagents

The structures of pyridylazo compounds used in the present study are given in Table 1. Three compounds (2-(2-pyridylazo)-1-naphthol (*α*-PAN, **2**), 2-(5-nitro-2-pyridylazo)-1-naphthol (5N-*α*-PAN, **4**), and 2-(5-nitro-2-pyridylazo)-*p*-cresol (5N-PAC, **5**)) were prepared by the condensation of 2-hydrazinopyridine with 1,2-naphthoquinone or 1,2-benzoquinone, respectively.[13,14] 5N-PAC was separated from an isomer by reversed-phase HPLC (RP-HPLC) as iron(II) complexes, followed by ion-exchange with hydrochloric acid. Other compounds (1-(2-pyridylazo)-2-naphthol (*β*-PAN, **1**), 2-(2-pyridylazo)-*p*-cresol (PAC, **3**), 2-(2-pyridylazo)-5-dimethylaminophenol (PADMAP, **6**), 2-(2-pyridylazo)-5-ethylaminophenol (PAEAP, **7**), 2-(2-pyridylazo)-5-ethylamino-*p*-cresol (PAEAC, **8**), 2-(2-pyridylazo)-5-diethylaminophenol (PADEAP, **9**), and 2-(5-chloro-2-pyridylazo)-5-diethylaminophenol (5-Cl-PADEAP, **10**)) were synthesized by coupling the corresponding diazonium salt with naphthol or phenol derivatives.[1,15-17] Crude compounds were purified by extracting their chloroform solutions with acid and alkali solutions and by recrystallization from aqueous ethanol. The purities of the compounds were confirmed by RP-HPLC

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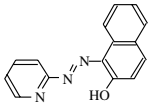
Table 1 Structures and acid dissociation constants of pyridylazo compounds



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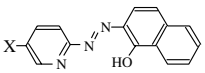

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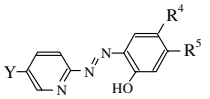

3, 5 – 10

No.	Abbreviation	Structure		$pK_{a,N(H)}$	$pK_{a,NRR'(H)}$	$pK_{a,OH}$[a]
1	β-PAN			2.9		11.6[16]
2	α-PAN	X, H		2.9		9.8[17]
3	PAC	Y, H	R^4, CH_3; R^5, H	2.6		8.9[15]
4	5N-α-PAN	X, NO_2		<1		7.2
5	5N-PAC	Y, NO_2	R^4, CH_3; R^5, H	<1		7.8
6	PADMAP	Y, H	R^4, H; R^5, $N(CH_3)_2$	1.4	4.1	11.4
7	PAEAP	Y, H	R^4, H; R^5, $NH(C_2H_5)$	1	4.2	11.4
8	PAEAC	Y, H	R^4, CH_3; R^5, $NH(C_2H_5)$	1.8	4.2	11.6
9	PADEAP	Y, H	R^4, H; R^5, $N(C_2H_5)_2$	1.4	4.2	11.3
10	5-Cl-PADEAP	Y, Cl	R^4, H; R^5, $N(C_2H_5)_2$	0.8	2.8	11.0

a. **4**, in water extrapolated from aqueous dioxane; **5**, in water extrapolated from aqueous ethanol; **6** – **10**, in 5% aqueous ethanol.

and by ^{1}H NMR measured with a Model Gemini 300 spectrometer (300 MHz, Varian, CA). Each compound was dissolved in ethanol or dioxane (5N-α-PAN and 5N-PAC) to give a 1×10^{-2} mol dm^{-3} solution for cyclic voltammetry and a 2.5×10^{-4} mol dm^{-3} solution for RP-HPLC. Standard stock solutions (1.0×10^{-2} mol dm^{-3}, pH 2) of Fe^{3+} and Co^{2+} were prepared by dissolving analytical-reagent grade nitrates.

Cyclic voltammetry

A 1-cm^3 portion of a 1×10^{-2} mol dm^{-3} reagent solution was placed in a 50-cm^3 centrifuge tube with a screw-cap and was mixed with a 1-cm^3 portion of a 1×10^{-2} mol dm^{-3} $Fe(NO_3)_3$ or $Co(NO_3)_2$ solution (pH *ca.* 2). A 1-cm^3 portion of 4×10^{-3} mol dm^{-3} ascorbic acid was added for the reduction of iron complexes, while 1 cm^3 of 1×10^{-2} mol dm^{-3} potassium periodate and 1 cm^3 of 3 mol dm^{-3} potassium perchlorate were added for the ion-pair extraction of cobalt(III) complexes. After adjusting the pH within ranges for complete extraction and adjusting the total volume to 10 cm^3, the solution was shaken with 1,2-dichloroethane (10 cm^3) for 30 min. After centrifuging at 3000 rpm for 5 min, the organic phase was evaporated under a vacuum, and the resulting precipitates were dissolved in 5 cm^3 of 1,2-dichloroethane containing 0.1 mol dm^{-3} tetra-*n*-buthylammonium perchlorate. By the above-mentioned operation, a series of 1,2-dichloroethane solutions containing 1×10^{-3} mol dm^{-3} $[Fe^{II}L_2]$ or $[Co^{III}L_2]^+ClO_4^-$ without any excess reagent, metal ion, and oxidizing or reducing agent were obtained. Cyclic voltammetry was performed using a Model P-1100 polarographic analyzer (Yanaco, Tokyo, Japan) and a Model 1120 potentio-galvanostat (Huso Electrochemical System, Kanagawa, Japan). All measurements were carried out at room temperature (25˚C) with the conventional three-electrode configuration consisting of a Model MPTE platinum microelectrode as the working electrode (25 mm i.d., BAS, Tokyo Japan), a platinum wire as a counter electrode (BAS), and a Model RE-5 nonaqueous $Ag/AgNO_3$ reference electrode (BAS). The redox potential value of the ferrocene/ferricinium couple was confirmed to be 0.20 V using this system.

Chromatographic procedure

Sample solutions for detailed studies on the chromatographic behavior of Fe and Co complexes were prepared as follows: to 1 cm^3 of a 2.5×10^{-4} mol dm^{-3} reagent solution were added 1 cm^3 of a 1×10^{-4} mol dm^{-3} $Fe(NO_3)_3$ or $Co(NO_3)_2$ solution (pH *ca.* 4), 2 cm^3 of ethanol or dioxane, and 1 cm^3 of 4×10^{-3} mol dm^{-3} ascorbic acid or 1×10^{-2} mol dm^{-3} potassium periodate solution. The resulting solutions contained an excess azo compound (1×10^{-5} mol dm^{-3}) and an oxidizing or reducing reagent as well as a metal complex (2×10^{-5} mol dm^{-3}). The HPLC system (JASCO, Tokyo, Japan) consisted of a Model PU-980 intelligent pump, a Model 965-CO column oven, and a Model MD-915 multiwavelength UV-Vis detector equipped with a Model DP-L915 data processing system. A Model L-column ODS (4.6 mm i.d. × 250 mm, particle diameter 5 μm, Chemical Evaluation Research Institute, Tokyo, Japan) was employed as a stationary phase. The mobile phase was filtered through a membrane filter (pore size, 0.2 μm; Toyo Roshi, Tokyo, Japan) and degassed before use. A 10-μl aliquot of a sample solution was placed on the column *via* a Model SVM-6U7 ceramic valve sample injector (Sanuki, Tokyo, Japan) and eluted with an aqueous acetonitrile mobile phase at a flow rate of 0.8 cm^3 min^{-1}. The absorbance at 400 – 800 nm was monitored.

Results and Discussion

Acid dissociation constants of pyridylazo compounds

The acid dissociation constants of seven pyridylazo compounds (**4** – **10**) were spectrophotometrically determined in appropriate media.[16] The results are summarized in Table 1 together with literature values for the other three compounds (**1** – **3**).[15-17] Although hydrogen-bonding of *o*-OH to the azo group and the formation of a tautomer (*o-keto* hydrazo compounds) may contribute to the basicity,[18-21] the smallest acid dissociation constant is expressed as $K_{a,OH}$ for convenience. Compared with a reference compound, β-PAN (**1**), the 1-naphthol derivative [α-PAN (**2**)] or the introduction of a methyl group at the 4-position [PAC (**3**)] of a phenol ring reduced the basicity of a phenolate group ($pK_{a,OH}$). The introduction of a nitro group at the 5-position [5N-α-PAN (**4**) or 5N-PAC (**5**)] of a pyridine ring of these compounds further decreased the basicities of both the phenolate and the pyridine nitrogen ($pK_{a,N(H)}$). In contrast, the introduction of an alkylamino group at the 5-position of a phenol ring [PADMAP (**6**), PAEAP (**7**), PAEAC (**8**) and PADEAP (**9**)] gave comparable $pK_{a,OH}$ values to that of β-PAN (**1**); the $pK_{a,N(H)}$ values were appreciably lower than that of

Table 2 Overall stability constant of iron and cobalt complexes with pyridylazo compounds

No.	Abbreviation	Overall stability constant (log β)[a]			Acid dissociation constant[a]		
		$Fe^{III}L_2^+$	$Fe^{II}L_2$	$Co^{II}L_2$	$pK_{a,N(H)}$	$pK_{a,NRR'(H)}$	$pK_{a,OH}$
1	β-PAN	29.9	27.9	24.9	1.5		12.6
3	PAC		25.5[23]				
4	5N-α-PAN	16.3	22.6	21.5	<1		9.8
9	PADEAP	33.8	30.1		1.0	3.0	12.6

a. **1**, **4**, **9**, in 60% aqueous dioxane.

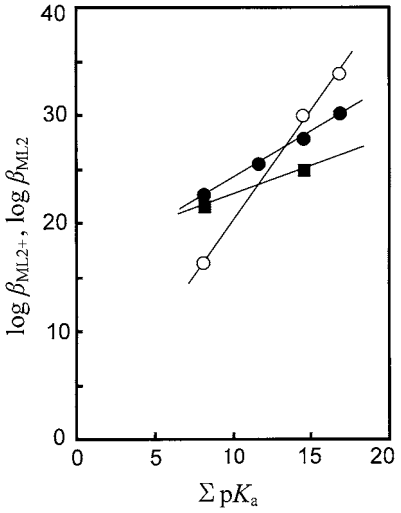

Fig. 1 Correlation between overall stability constants of iron and cobalt complexes and total basicities of ligands denoted by ΣpK_a. Metal: ●, Fe(II); ○, Fe(III); ■, Co(II).

β-PAN (**1**). The introduction of a chloro group at the 5-position of a pyridine ring [5-Cl-PADEAP (**10**)] appreciably reduced the $pK_{a,NRR'(H)}$ value.

Stability constants of iron and cobalt complexes

The stability constants ($\beta_{ML2+} = [ML_2^+]/[M^{3+}][L^-]^2$, $\beta_{ML2} = [ML_2]/[M^{2+}][L^-]^2$; M: Fe, Co) of Fe(III), Fe(II) and Co(II) complexes with selected reagents were spectrophotometrically determined in 60% aqueous dioxane as described in Ref. 22; the acid-dissociation constants were also determined in this medium for equilibrium analysis. The results are summarized together with a literature value in Table 2.[23] Figure 1 shows the relation between the logarithmic values of the β_{FeL2+}, β_{FeL2}, β_{CoL2} and ΣpK_a values as a measure of the coordinating abilities of ligands. The following linear correlations were observed:

$$\log \beta_{FeL2+} = 2.05 \times \Sigma pK_a + 0.39, \quad (1)$$

$$\log \beta_{FeL2} = 0.85 \times \Sigma pK_a + 15.57, \quad (2)$$

$$\log \beta_{CoL2} = 0.54 \times \Sigma pK_a + 17.10. \quad (3)$$

The inclusion of $pK_{a,NRR'(H)}$ in ΣpK_a was necessary to accommodate the complexes with alkylaminophenol derivatives in this correlation; the alkylamino group may contribute to coordination to metal ions in the charged quinoid structure as shown in Fig. 2.[24] It is noteworthy that the stabilities of Fe(III) complexes depend on the ligand basicities much more than those of Fe(II) and Co(II), and thus the relative stabilities of Fe(III) and Fe(II) complexes are inverted at a ΣpK_a of around 13.

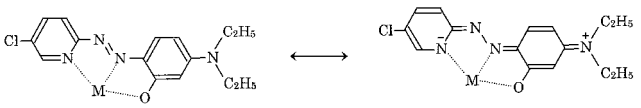

Fig. 2 Charged quinoid structure of metal complex with 5-Cl-PADEAP.

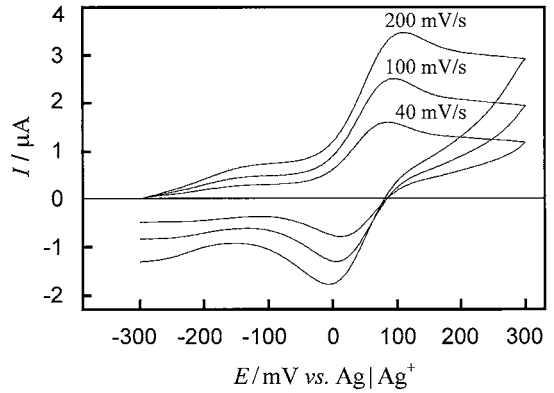

Fig. 3 Cyclic voltammograms of iron complex with 5-Cl-PADEAP. $C_{Fe} = 1 \times 10^{-3}$ mol dm^{-3}. Solvent: 1,2-dichloroethane containing 0.1 mol dm^{-3} tetra-*n*-butylammonium perchlorate.

Redox potentials of cobalt and iron complexes

Cyclic voltammograms for all of the cobalt and iron complexes with pyridylazo compounds showed a quasi-reversible, one-electron wave corresponding to the $[M^{III}L_2^+]/[M^{II}L_2]$ couple, as exemplified by the iron complex with 5-Cl-PADEAP (**10**) in Fig. 3. The peak separation, ΔE, increased with increasing the scan rate (v), and the anodic peak currents were proportional to $v^{1/2}$. The redox potentials, E^0_{ML2}, were determined as $1/2(E_p^a + E_p^c)$, where E_p^a and E_p^c were the anodic and cathodic peak potentials against Ag/0.01 mol dm^{-3} Ag^+, respectively. The redox potentials obtained for cobalt and iron complexes with 10 azo compounds are given in Table 3.

Compared with the cobalt complex with β-PAN (**1**) as a reference, the E^0_{CoL2} values of α-PAN (**2**) and PAC (**3**) complexes were greater by 0.13 V. The introduction of a nitro group (5N-α-PAN (**4**) and 5N-PAC (**5**)) further increased the E^0_{CoL2} values by about 0.3 V. In contrast, the E^0_{CoL2} values of PADMAP (**6**), PAEAP (**7**), PAEAC (**8**), and PADEAP (**9**) complexes were lower than that of the β-PAN (**1**) complex by about 0.2 V. The introduction of a chloro group (5-Cl-PADEAP (**10**)) to PADEAP increased the E^0_{CoL2} value by 0.1 V. The effects of the substitutes on the redox potential of the iron complexes were similar to those of the cobalt complexes.

The redox potentials (E^0_{ML2}) of metal complexes, $M^{III}L_2^+/M^{II}L_2$, in 1,2-dicholoroethane are related to the redox potentials ($E^0_{M3+/M2+}$) of meal ions, M^{3+}/M^{2+} by:

$$E^0_{ML2} = E^0_{M3+/M2+} - 0.0592\log(\beta_{ML2+,o}/\beta_{ML2,o}), \quad (4)$$

where $\beta_{ML2+,o}$ and $\beta_{ML2,o}$ are the overall stability constants in 1,2-dichloroethane. Provided the correlation between $\beta_{ML2+,o}/\beta_{ML2,o}$ and β_{ML2+}/β_{ML2} for $[Fe^{III}L_2^+]/[Fe^{II}L_2]$, the combination of Eqs. (1), (2), and (4) suggests that E^0_{FeL2} of iron complexes is also governed by ΣpK_a as:

Table 3 Redox potentials and HPLC behavior of cobalt and iron complexes with pyridylazo compounds

No.	Abbreviation	ΣpK_a[a]	Redox potential[b]		HPLC behavior[c]			
			E^0_{Co}	E^0_{Fe}	(Co^{II})	$(Co^{II})_{SCN}$	(Fe^{III})	$(Fe^{III})_{SCN}$
4	5N-α-PAN	8.2	0.03	0.59	Co^{II}	n.d.	Fe^{II}	Fe^{II}
5	5N-PAC	8.8	0.00	0.52	Co^{II}	n.d.	Fe^{II}	Fe^{II}
3	PAC	11.5	–0.27	0.34	Co^{III}	Co^{III}	Fe^{II}	Fe^{II}
2	α-PAN	12.7	–0.27	0.27	Co^{III}	Co^{III}	Fe^{II}	Fe^{II}
1	β-PAN	14.5	–0.40	0.19	Co^{III}	Co^{III}	Fe^{II}	Fe^{II}
10	5-Cl-PADEAP	14.6	–0.49	0.05	Co^{III}	Co^{III}	Fe^{II}	Fe^{II}
7	PAEAP	16.6	–0.58	–0.01	Co^{III}	Co^{III}	Fe^{II}	Fe^{III}
6	PADMAP	16.9	–0.62	–0.01	Co^{III}	Co^{III}	Fe^{II}	Fe^{III} + Fe^{II}
9	PADEAP	16.9	–0.60	–0.03	Co^{III}	Co^{III}	Fe^{II}	Fe^{III}
8	PAEAC	17.6	–0.61	–0.06	Co^{III}	Co^{III}	Fe^{II}	Fe^{III}

a. $\Sigma pK_a = pK_{a,N(H)} + pK_{a,NRR'(H)} + pK_{a,OH}$. b. E^0/V *vs.* Ag/0.01 mol dm^{-3} Ag^+ (AN). c. $(M^{III/II})_{SCN}$, $(M^{III/II})$: metal complexes injected in the mobile phase containing 0.01 mol dm^{-3} NH_4SCN and no salts. M^{III}, M^{II}, metal complexes detected in the HPLC system; n.d., not detected.

$$E^0_{FeL2} = (2.05 - 0.85) \times 0.0592 \times \Sigma pK_a + \text{const.} = -0.070 \times \Sigma pK_a + \text{const.} \quad (5)$$

The actual plots for Fe complexes in Fig. 4 show a linear correlation with a slope of –0.069 and an intercept of 1.135, which agree well with Eq. (5). The plots for Co complexes also show a linear relationship with a slope of –0.071 and an intercept of 0.607; the difference in the intercepts for two metal ions is 0.6 V. Again, the inclusion of $pK_{a,NRR'(H)}$ in ΣpK_a was necessary to accommodate the complexes with alkylaminophenol derivatives in this correlation. Such a correlation was reported for $Co^{III/II}$ complexes with PAN derivatives and $Cu^{II/I}$ complexes with substituted phenanthrolines.[11,12,25]

Precolumn derivatization reaction of cobalt and iron complexes

When a cobalt(II) solution in MES buffer (pH 6.5) was added to ethanol or dioxane solutions of heterocyclic azo compounds, the resulting solutions first showed colors characteristic of cobalt(II) complexes, which gradually changed to cobalt(III) complexes without any oxidizing reagents. The reagents were classified into three groups, depending on the oxidation behavior of the cobalt(II) complex: Group A (5N-α-PAN (**4**) and 5N-PAC (**5**) complexes; $E^0_{CoL2} \geq 0.00$ V), Group B (PAC (**3**) and α-PAN (**2**) complexes; $E^0_{CoL2} \cong -0.27$), Group C (β-PAN (**1**), 5-Cl-PADEAP (**10**), PAEAP (**7**), PADMAP (**6**), PADEAP (**9**), and PAEAC (**8**) complexes; $E^0_{CoL2} \leq -0.40$ V). The oxidations of cobalt(II) complexes with Group C compounds were rapid, while those of complexes with Group B compounds were slow. The cobalt(II) complexes with Group A compounds were not quantitatively oxidized to the cobalt(III) complexes.

With any compound, the cobalt(III) complexes were obtained by the addition of potassium periodate, while the cobalt(II) complexes were obtained in the presence of ascorbic acid. The cobalt(III) complexes with Group A compounds once formed were rapidly reduced by the addition of ascorbic acid, while the cobalt(III) complexes with Group B and C compounds could not be reduced by the same method.

In the reaction of Fe^{2+} in aqueous ethanol or dioxane, all of the reagents gave $[Fe^{II}L_2]$, which were not oxidized even after a few hours. In the reaction with Fe^{3+}, all of the reagents gave $[Fe^{III}L_2{}^+]$, which remained stable and could be reduced by the addition of ascorbic acid.

Chromatographic behavior of cobalt and iron complexes

A single complex of $[Co^{II}L_2]$, $[Co^{III}L_2]^+$, $[Fe^{II}L_2]$, or $[Fe^{III}L_2]^+$ was injected and eluted with aqueous acetonitrile mobile phases with and without SCN^-; the initial oxidation state of metal complexes in sample solutions were adjusted by the addition of ascorbic acid or potassium periodate. With any reagents, injected $[Co^{III}L_2]^+$ or $[Fe^{II}L_2]$ was detected without any change in the oxidation state; the peak of $[Co^{III}L_2]^+$ was sharpened by thiocyanate due to the favorable retention of an ion-pair, $[Co^{III}L_2]^+SCN^-$.[5]

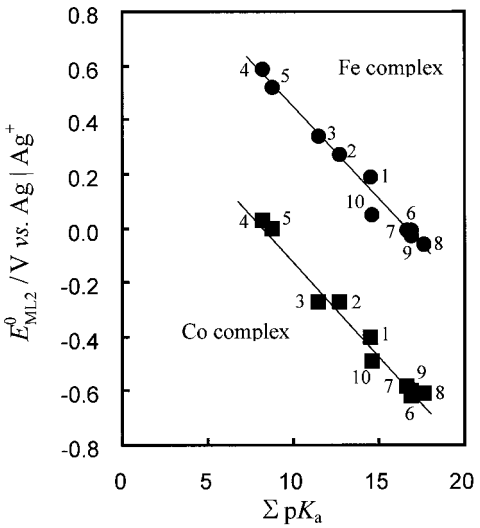

Fig. 4 Correlation between redox potentials of cobalt and iron complexes and total basicities of ligands denoted by ΣpK_a. Circles, Fe complexes; squares, Co complexes.

Table 3 summarizes the cobalt and iron complexes detected in HPLC when $[Co^{II}L_2]$ or $[Fe^{III}L_2]^+$ was injected. The cobalt(II) complexes with Group A compounds (5N-α-PAN (**4**) and 5N-PAC (**5**)) were eluted as a sharp peak with a mobile phase containing no additives by keeping the oxidation state. In Group C compounds, on the other hand, only $[Co^{III}L_2]^+$ was detected as a sharp peak; the cobalt(II) complexes were rapidly oxidized at the top of the column after separation from ascorbic acid. In Group B compounds, only $[Co^{III}L_2]^+$ was detected but with some broadening for PAC (**3**) or with slight tailing for α-PAN (**2**); the oxidation was not fast enough. These finding are consistent with the results of a systematic study on the formation and oxidation of Co(II) complex with β-PAN by Mochizuki and

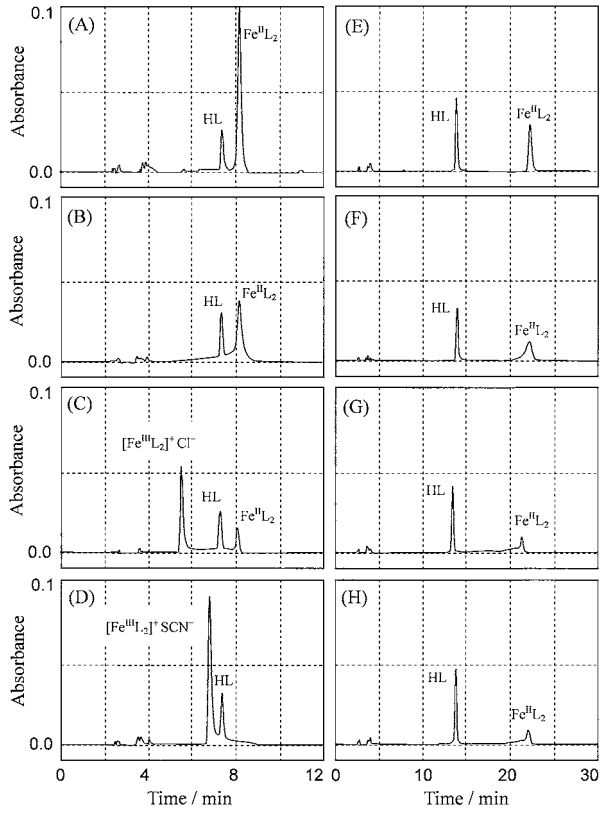

Fig. 5 Chromatographic behavior of $[Fe^{II}L_2]$ (A, E) and $[Fe^{III}L_2]^+$ (B – D, F – H) with PADEAP (A – D) and 5-Cl-PADEAP (E – H). Column, L-column ODS; eluent, acetonitrile–water (65:35, v/v) containing no additives (A, B, E, F), 1×10^{-2} mol dm^{-3} ammonium chloride (C, G), and 1×10^{-2} mol dm^{-3} ammonium thiocyanate (D, H). Flow-rate, 0.8 cm^3 min^{-1}. Column temperature, 25°C.

others: (1) in the absence of any reducing reagent, the formation of Co(III) complex could not be completely avoided, even when using a vacuum line;[26] (2) the cobalt(II) complex prepared in the presence of a reducing reagent was not oxidized in DMSO even by the bubbling of oxygen gas but was instantaneously oxidized by the addition of slight amount of water.[11]

When $[Co^{II}L_2]$ was injected into the mobile phase containing 0.01 mol dm^{-3} NH_4SCN, peaks for cobalt were lost for Group A compounds (5N-α-PAN (**4**) and 5N-PAC (**5**)) due to ligand-exchange reactions with the thiocyanate ion.

The reagents were alternatively classified into two groups depending on the chromatographic behavior of $[Fe^{III}L_2^+]$: Group X (5N-α-PAN (**4**), 5N-PAC (**5**), PAC (**3**), α-PAN (**2**), β-PAN (**1**), and 5-Cl-PADEAP (**10**) complexes; $E^0_{FeL2} \geq 0.05$ V), Group Y (PAEAP (**7**), PADMAP (**6**), PADEAP (**9**), and PAEAC (**8**) complexes; $E^0_{FeL2} \leq -0.01$ V). Typical chromatograms of $[Fe^{II}L_2]$ and $[Fe^{III}L_2^+]$ with Group Y compound of PADEAP (**9**) and Group X compound of 5-Cl-PADEAP (**10**) are shown in Fig. 5. When $[Fe^{II}L_2]$ of these compounds was injected into the mobile phase containing no additives, a sharp peak of $[Fe^{II}L_2]$ was obtained (Figs. 5(A) and (E)). When $[Fe^{III}L_2]^+$ was injected into the mobile phase containing no additives, only $[Fe^{II}L_2]$ was detected with some leading, as shown in Figs. 5(B) and 5(F). The iron(III) complexes were reduced after separation from periodate as found for other pyridylazo compounds.[10,27] When $[Fe^{III}L_2]^+$ of PADEAP (**9**) was injected into the mobile phase containing NH_4Cl, both a major peak of $[Fe^{III}L_2]^+$ and a minor peak of $[Fe^{II}L_2]$ were obtained (Fig. 5(C)). In the case of NH_4SCN, only a peak of $[Fe^{III}L_2]^+$ was obtained (Fig. 5(D)); anions of higher lipophilicities stabilize $[Fe^{III}L_2]^+$. Among the complexes with other Group Y compounds, PAEAP (**7**) and PAEAC (**8**) gave only the peak of $[Fe^{III}L_2]^+$, while PADMAP (**6**) gave both peaks. More bulky substituents on an amino group may stabilize the ion-pair of $[Fe^{III}L_2]^+$. The coordination of azo compounds with higher basicities stabilize the Fe(III) complexes against dissociation and against reduction to Fe(II) complexes to afford the peak of Fe(III) complexes.[28] In contrast, only a peak of $[Fe^{II}L_2]$ with leading was obtained for 5-Cl-PADEAP (**10**) even in the presence of NH_4Cl or NH_4SCN (Figs. 5(G) and 5(H)).

Conclusions

The redox potentials of Co and Fe complexes with pyridylazo compounds were linearly correlated to the ligand basicities; the correlation was quantitatively explained by the difference in dependence of the stabilities of $[M^{III}L_2^+]$ and $[M^{II}L_2]$ on the ligand basicities. In a reversed-phase HPLC system, cobalt could be detected as $[Co^{II}L_2]$ only with the compounds of highest E^0_{ML2} of $\cong 0.0$ V or lowest basicities, while iron could be detected as $[Fe^{III}L_2]^+$ only with the lowest E^0_{ML2} of $\cong 0.0$ V or highest basicities. The cobalt complexes with lower potentials gave only a peak of $[Co^{III}L_2]^+$, while the iron complexes with higher redox potentials gave only a peak of $[Fe^{II}L_2]$.

When a sample containing Co^{2+} and Fe^{3+} is derivatized with pyridylazo compounds, equivalent amounts of $[Co^{III}L_2]^+$ and $[Fe^{II}L_2]$ is formed by the mutual redox reaction, since the redox potential of the Fe complex is generally higher than that of the Co complex by 0.6 V. In the cases of the reagents **1** – **3** and **10**, the excess Co^{2+} or Fe^{3+} could be derivatized to $[Co^{III}L_2^+]$ or $[Fe^{II}L_2]$ by an oxidizing or reducing reagent. One of two other alternatives is a combined use of 5N-α-PAN (**4**) or 5N-PAC (**5**) with a reducing reagent to detect Co and Fe as $[Co^{II}L_2]$ and $[Fe^{II}L_2]$. The other is a combined use of PAEAP (**7**), PAEAC (**8**), or PADEAP (**9**) with an oxidizing agent to detect Co and Fe as $[Co^{III}L_2]^+$ and $[Fe^{III}L_2]^+$. A derivatizing reagent and an auxiliary reagent (oxidizing or reducing reagent or none) may be optimized, depending on the sample composition.

Acknowledgements

The authors thank Prof. Hajime Katano of Fukui Prefectural University (Fukui, Japan) for help with the CV measurement.

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