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Reversed-phase ion-pair liquid chromatographic method for determination of reaction equilibria involving ionic species: Exemplification of the method using ligand substitution reactions of ethylenediaminetetraacetatochromium(III) ion with acetate and phosphate ions

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ABSTRACT

A reversed-phase ion-pair liquid chromatographic method is presented for the determination of reaction equilibria involving ionic species of the same charge sign as reactant and product compounds. It has been demonstrated that ion-exchange chromatography or reversed-phase ion-pair chromatography is a useful tool for the determination of equilibrium constants of chemical reactions involving ionic species such as metal complexation reactions. Previous work with these methods has been based on the assumption that the limiting retention factors of the reactant and product species are constant independent of concentration of the chemical species (X) in the mobile phase, which reacts with the analyte compound. However, when all the reactant and product species are ions of the same charge sign as that of the species X, it is virtually impossible to apply these methods to the equilibrium constant determination because the retention factors of both the reactant and product species may depend on the concentration of X. In this study, an alternative approach was developed that estimates the limiting retention factors of ionic species from the dependence of the retention factor on the ionic strength of the mobile phase. Ligand substitution reactions of ethylenediaminetetraacetatochromium(III) ion with acetate and phosphate ions were used as model reactions to test this method. The equilibrium constants determined by this method are in good agreement with those obtained by a UV–visible spectrophotometric method.

1. Introduction

A lot of studies have demonstrated that HPLC is a useful technique for the determination of equilibrium constants of chemical reactions in solution such as acid–base reactions [1–9] and metal complexation reactions [9–12]. The advantages of using HPLC to determine equilibrium constants are that HPLC requires small amounts of sample at low analyte concentration and the samples need not be pure since impurities can be separated from the analytes [2].

If the reaction is much slower than the mass transfer of a solute between the mobile and stationary phases, several peaks appear in the chromatogram corresponding to the individual species, *i.e.*, the reactants and products when a sample solution in which the reaction equilibrium has been attained is injected. The equilibrium constant can be thus obtained by determining the concentration of each species through measurement of the peak area or peak height. On the other hand, in the case of labile systems, where the reaction proceeds much faster than the mass transfer between the two phases, only a single peak containing the equilibrium mixture of the individual species appears in the chromatogram. The observed retention factor, k, of an analyte A, which participates with a chemical species X in chemical equilibrium of a labile system shown below (Eq. (1)) is given by Eq. (2), where F_A and F_{AX} are stoichiometric fractions of the analyte in each of its forms and k_A and k_{AX} are the retention factors of the individual forms of the analyte.

$$A + X \rightleftarrows AX \quad (1)$$

$$k = F_A k_A + F_{AX} k_{AX} \quad (2)$$

The observed retention factor is the weighted average of the limiting retention factors of species A and AX, and can be rewritten using the equilibrium constant, K, as follows:

$$k = \frac{k_A + k_{AX} K[X]}{1 + K[X]} \quad (3)$$

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where [X] is the concentration of X in the mobile phase. It is thus possible to calculate the equilibrium constant K as well as k_A and k_{AX} by measuring the retention factor of the analyte as a function of the concentration of X in the mobile phase.

Most of the HPLC methods that have so far been presented are based on this general principle and the equilibrium constants are calculated from the dependence of the k value on the concentration of X using a non-linear least-squares analysis or a linearized form of equation. It should be noted, however, that these methods postulate that the limiting retention factors are constant independent of the concentration of X or the other component in the mobile phase added in order to change the concentration of X. For example, the acid dissociation constants are usually determined using reversed-phase liquid chromatography assuming that neither pH nor the type and/or concentrations of buffer agents affects the retention factors of the acid and base forms of the analyte compound. It may be possible in some cases to assume that the effects of the change in composition of the mobile phase on the limiting retention factors are negligibly small. However, this assumption would fail in the other cases, especially for the systems where high concentrations of the reactant X in the mobile phase are needed due to the low equilibrium constant. In such cases, the change in the concentration of X may cause the variation of the limiting retention factors.

Measurements of metal complexation equilibrium constants have been performed successfully using ion-exchange chromatography [10–13]. In these studies, cation-exchangers were exclusively used for the determination of the stability constants of complexes of metal ions with anionic ligands such as tartarate and oxalate ions. In a similar way to the methods described above, the stability constants were obtained from the dependence of the observed retention factor of a metal ion on the concentration of a ligand in the mobile phase. Therefore the concentration of a counterion, *e.g.*, sodium ion, in the mobile phase must be kept constant in order to keep the involved ion-exchange equilibria unchanged. It may be possible to compensate the variation of the counterion concentration caused by the change in the ligand concentration by adding a suitable electrolyte such as sodium perchlorate to the mobile phase. However, when all the chemical species involved in the reaction, that is, A, X and AX in Eq. (1), are ions of the same charge sign, it would be virtually impossible to assume that the limiting retention factors are constant independent of the concentration of X since the change in concentration of X inevitably causes the change in the limiting retention factors of A and AX.

One of the ways to overcome this problem is to clarify the dependence of the limiting retention factors on the concentration of X. In this paper, we present a new HPLC method for determining the equilibrium constants of chemical reactions involving ionic species as reactant and product compounds by reversed-phase ion-pair chromatography (RP-IPC). The limiting retention factors are estimated using a relationship between the retention factor of an ionic solute and the ionic strength of the mobile phase in RP-IPC. The validity of the method will be exemplified using ligand substitution reactions of ethylenediaminetetraacetatochromium(III) ion with acetate and phosphate ions, where all the chemical species involved in the equilibria are anions.

2. Theory

If one can express the limiting retention factors, k_A and k_{AX}, as a function of the concentration of X, $f_A([X])$ and $f_{AX}([X])$, respectively, the observed retention factor is given by:

$$k = \frac{f_A([X]) + K f_{AX}([X])[X]}{1 + K[X]} \tag{4}$$

Therefore the equilibrium constants can be obtained by analyzing the dependence of the observed retention factor on the concentration of X in the mobile phase provided that the functions, $f_A([X])$ and $f_{AX}([X])$, have already been known.

It has been reported that the logarithmic values of the retention factor of an ionic solute and salt concentration or ionic strength of the mobile phase indicate a linear relationship of which the slope reflects the ionic charge in RP-IPC [14–17]. On the basis of the electrostatic double layer model, Bartha and Stahlberg [15] derived the following equation for the retention factor of an analyte ion A:

$$\ln k_A = \frac{1}{2}\left(\frac{z_P z_A}{z_P^2 + 1}\right) \ln I - \left(\frac{z_P z_A}{z_P^2 + 1}\right) \ln c_P + C \tag{5}$$

where z_P and z_A are the charges of the pairing ion and the analyte ion, respectively, I is the ionic strength of the mobile phase, c_P is the concentration of the pairing ion and C is a constant depending on the respective charges and hydrophobicity of the analyte ion and the pairing ion. Zhang et al. [17] proposed another equation to describe the effect of concentration of the univalent salt, c_S, on retention of ionic solutes in RP-IPC as follows:

$$\ln k_A = A + B \ln c_S \tag{6}$$

The parameters A and B in Eq. (6) are given by the following equations:

$$A = \ln \phi + \frac{\Delta G_R}{RT} + \frac{2|z_A|}{|z_P|}\beta \tag{7}$$

$$B = \frac{2|z_A|}{|z_P|}\gamma \tag{8}$$

where ϕ is the phase ratio, ΔG_R the non-electrostatic contribution to the Gibbs free energy change of retention, R the gas constant, T the absolute temperature and β and γ are the constants under the given conditions.

The equation presented by Bartha and Stahlberg and that by Zhang et al. are not identical, but both of them indicate that the plots of the logarithmic values of the retention factor of an analyte ion against the ionic strength or the salt concentration should be linear and the slope of the plots depends only on the charge of the analyte ion when the type of the salt added to the mobile phase is unchanged. This means that the limiting retention factors of A^{m-} and $AX^{(m+n)-}$ at a given concentration of X^{n-} in the chemical equilibrium shown below (Eq. (9)), for example, can be represented as a function of I as given by Eqs. (10) and (11), respectively:

$$A^{m-} + X^{n-} \rightleftarrows AX^{(m+n)-} \tag{9}$$

$$\log k_A = a_A + b_m \log I \tag{10}$$

$$\log k_{AX} = a_{AX} + b_{m+n} \log I \tag{11}$$

The ionic strength is related to the concentration of X^{n-} when the mobile phase only contains the salt of X^{n-}. For example, the ionic strength of the solution of Na_nX is given by:

$$I = \frac{n+1}{2}[X^{n-}] \tag{12}$$

Consequently the equilibrium constant can be obtained by analyzing the dependence of the observed retention factor on the ionic strength of the mobile phase using Eqs. (4), (10) and (11).

The equilibrium constants may also be determined by a similar method using ion-exchange chromatography because the retention factor and the concentration of the counter ion bear a linear relationship to each other in ion-exchange chromatography as well. However, the ion-exchange capacity of the column is fixed so that the retentivity cannot be easily modified when the type and/or concentration range of the counter ion to be used are limited. In RP-IPC, on the other hand, one can adjust the retention of analyte ions by choosing a suitable type and/or concentration of the pairing ion. Therefore it may be preferable to use RP-IPC for determining the equilibrium constants precisely and rapidly.

3. Experimental

3.1. Apparatus

The chromatographic equipment comprised a Uniflows (Tokyo, Japan) Model DG-6310 degasser, a JASCO (Tokyo, Japan) Model PU-2080i intelligent HPLC pump, a Rheodyne (Cotati, CA) Model 9725 loading injector fitted with a 10 μL sample loop and a GL Science (Tokyo, Japan) Model GL-7452 photodiode array detector. Data analysis was carried out on GL Science Chrom Merge. The column temperature was maintained at 35 °C with a GL-Science Model 556 LC column oven. For spectrophotometric determination of the equilibrium constants, a JASCO Model V630 UV-VIS spectrophotometer was used.

3.2. Reagents

All reagents used in this study were of analytical reagent grade unless otherwise stated. Tetrabutylammonium hydroxide was purchased from Tokyo Chemical Industry (Kumamoto, Japan). Disodium dihydrogen ethylenediaminetetraacetate (Na_2H_2edta), Cu(II)-edta, Ni(II)-edta, Fe(III)-edta and 2-morpholinoethanesulfonic acid (MES) were obtained from Dojindo Laboratories (Tokyo, Japan). Cr(III)-edta [18] and Co(III)-edta [19] were prepared according to the literature. Water was purified subsequently with an ion-exchange cartridge PF-III H10 (Organo, Tokyo, Japan) and an Arium 611 DI (Sartorius, Tokyo, Japan). The column used was an L-column ODS (5 μm, 100 mm × 4.6 mm I.D.) obtained from Chemical Evaluation and Research Institute (Tokyo, Japan).

3.3. Chromatographic measurements

The mobile phases used were aqueous solutions of acetate, phosphate or MES buffer containing 10 mM tetrabutylammonium ion (TBA^+). The acetate, phosphate and MES buffer solutions were prepared using the pairs, CH_3COOH/CH_3COONa, NaH_2PO_4/Na_2HPO_4 and MES/NaOH, respectively. All the mobile phases prepared were filtered through a 0.45 μm membrane filter and degassed ultrasonically before use. Elutions were carried out at constant flow rate of 1.0 mL min^{-1}. The analyte solutions were prepared by dissolving the compounds in the mobile phase solution to be used and were filtered through a 0.45 μm membrane filter. The mobile phase volume of the column was determined by the method presented by Shibukawa and Ohta [20]. Each measurement on a sample was carried out at least in triplicate. The repeatability of the retention volumes was satisfactory; the relative standard deviation in each case was less than 0.7%.

3.4. Spectrophotometric measurements

Spectrophotometric measurements of the equilibrium constant for ligand substitution reactions of Cr(III)-edta with phosphate ion were performed at 35 °C and ionic strength of 0.5 M according to the method presented by Ogino et al. [21]. The solution containing 2.5 mM Cr(III)-edta, a phosphate buffer solution (pH 5.2 or 6.5) and sodium perchlorate added to adjust the ionic strength was prepared and transferred into a quartz optical cell with a light path of 1 cm in length. For measurement of the absorption spectrum for Cr(III)-edta in a solution free from a complexation ligand, MES buffer was used instead. The temperature of the optical cell was controlled to 35.0 ± 0.1 °C by circulating thermostated water through the cell holder.

4. Results and discussion

Substitution inertness of chromium(III) complexes is well documented. However, it has been reported that one of the coordination sites of Cr(III)-edta is relatively labile and undergoes rapid substitution reactions with some anions as shown in the following reaction [21–25].

$$[Cr(edta)]^- + L^{n-} \rightleftarrows [Cr(edta)L]^{(n+1)-} \quad (13)$$

The liquid chromatographic methods that have so far been presented cannot be used for the determination of the equilibrium constant of this substitution reaction since all the species involved in this reaction are anions and the retention factors of $[Cr(edta)]^-$ and $[Cr(edta)L]^{(n+1)-}$ may depend on the concentration of L^{n-} in the mobile phase. We have thus chosen the substitution reactions of $[Cr(edta)]^-$ with acetate and phosphate ions as model reactions for evaluating the validity of the present method.

The retention factor of the equilibrium mixture of $[Cr(edta)]^-$ and $[Cr(edta)L]^{(n+1)-}$, k_{Cr}, is represented by the following equation:

$$k_{Cr} = F_{Cr(edta)}k_{Cr(edta)} + F_{Cr(edta)L}k_{Cr(edta)L} \quad (14)$$

where $F_{Cr(edta)}$ and $F_{Cr(edta)L}$ are the stoichiometric fractions and $k_{Cr(edta)}$ and $k_{Cr(edta)L}$ are the limiting retention factors of $[Cr(edta)]^-$ and $[Cr(edta)L]^{(n+1)-}$, respectively. Using the equilibrium constant, K, and the concentration of anion $[L^{n-}]$, Eq. (14) can be rewritten by:

$$k_{Cr} = \frac{K[L^{n-}]}{1+K[L^{n-}]}k_{Cr(edta)} + \frac{1}{1+K[L^{n-}]}k_{Cr(edta)L} \quad (15)$$

where

$$K = \frac{[[Cr(edta)L]^{(n+1)-}]}{[[Cr(edta)]^-][L^{n-}]} \quad (16)$$

It may be possible to calculate the K value together with the a and b values in Eqs. (10) and (11) for $[Cr(edta)]^-$ and $[Cr(edta)L]^{(n+1)-}$ by non-linear regression, but many data points are needed to precisely determine the K value. We have thus estimated the limiting retention factor for $[Cr(edta)]^-$ and the b value for $[Cr(edta)L]^{(n+1)-}$ in the following manner and then calculated the equilibrium constant together with the a value for $[Cr(edta)L]^{(n+1)-}$.

It has been demonstrated that the separation factor or the ratio of the retention factors of two ions which have the same charge can be assumed to be constant irrespective of the type of the salt in the mobile phase in RP-IPC [14,15] as well as in partition chromatography [26,27]. At pH 3.5–6.5 Co(III)-edta and Cr(III)-edta have the same charge and similar structures, *i.e.*, $[Co(edta)]^-$ and $[Cr(edta)]^-$ in a solution free from a ligand anion [28] and $[Co(edta)]^-$ is unreactive to acetate and phosphate ions [24,29]. Therefore the limiting retention factor of $[Cr(edta)]^-$ in acetate or phosphate solution system can be obtained by the following equation:

$$k_{Cr(edta)} = \frac{k_{Co(edta)}k^{MES}_{Cr(edta)}}{k^{MES}_{Co(edta)}} \quad (17)$$

where $k_{Co(edta)}$ is the retention factor of $[Co(edta)]^-$ determined in an acetate or phosphate eluent system and $k^{MES}_{Cr(edta)}$ and $k^{MES}_{Co(edta)}$ are the retention factors of $[Cr(edta)]^-$ and $[Co(edta)]^-$ measured in a MES buffer eluent system, respectively. Here we can assume that MES has no coordinating ability to metal ions.

Figs. 1–3 show the chromatograms obtained at various ionic strengths for the metal-edta complexes using the eluents of acetate, phosphate and MES buffer solutions of pH 5.2 containing 10 mM TBA^+, respectively. As can be seen, Cr(III)-edta exhibits remarkably broad peaks in the phosphate eluent system, which is attributable to the relative slowness of ligand substitution reaction of Cr(III)-edta with phosphate ion [24]. Fe(III)-edta also shows peaks slightly

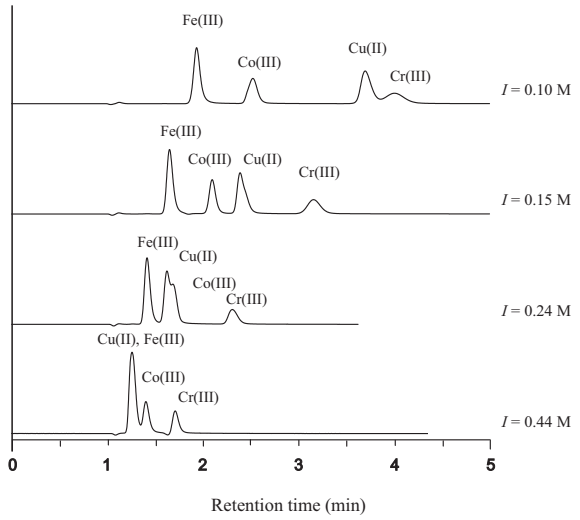

Fig. 1. Chromatograms of the metal-edta complexes in pH 5.2 acetate buffer eluent system at various ionic strengths. Other chromatographic conditions as in Section 3.

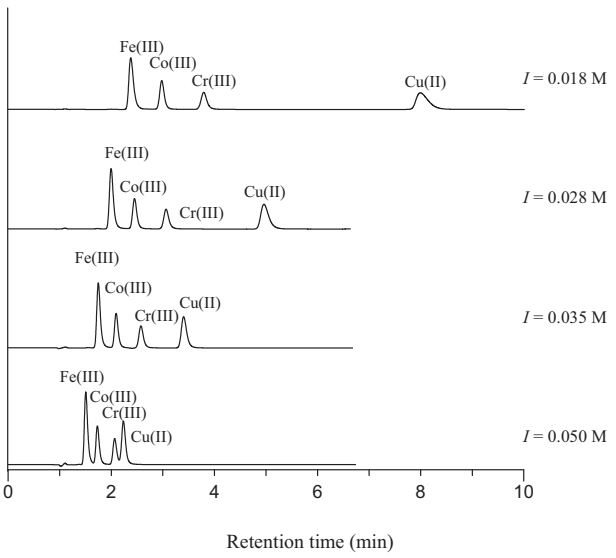

Fig. 3. Chromatograms of the metal-edta complexes in pH 5.2 MES buffer eluent system at various ionic strengths. Other chromatographic conditions as in Section 3.

broader than those for Co(III)-edta and Cu(II)-edta in the phosphate eluent system. Although it is conceivable that this is also caused by ligand substitution reaction of Fe(III)-edta with phosphate ion, the mechanism has not been fully elucidated due to the complexity of the structure of Fe(III)-edta, which may have six coordinate and seven-coordinate structures in solution [25].

Figs. 4–6 show the plots of $\log k$ vs. $\log I$ for the edta complexes of Co(III), Cr(III) and Cu(II) in an acetate (pH 5.2) and phosphate (pH 5.2 and 6.5) buffer eluent systems, respectively. On the other hand, the plot obtained in a MES buffer eluent system (pH 5.2) is shown in Fig. 7. The plot for $[Fe(CN)_6]^{4-}$ obtained in the pH 6.5 phosphate buffer system is also shown in Fig. 6. It should be noted that all the plots exhibit good linearity ($r > 0.998$) and the slopes of the plots correspond to the charges of the complexes. The plots for Cr(III)-edta in Figs. 4–6 should not be linear but convex because the mean charge varies with the concentration of acetate or phosphate ion in the eluent. However we observed approximately linear relationships between $\log k$ and $\log I$. This is probably because the change in ligand concentration we adopted is not so large.

In the MES buffer eluent system the slope of the plot for Cr(III)-edta is approximately the same as that for Co(III)-edta. On the other hand, in the acetate and the phosphate eluent systems, the slope of the plot for Cr(III)-edta is larger than that for Co(III)-edta. This reveals that the negative mean charge of the Cr(III)-edta complex is larger than −1 by coordination of acetate, monohydrogenphosphate or dihydrogenphosphate ion.

The b values in Eqs. (10) and (11) can be assumed to depend only on the charge but not on the other properties of the analyte ion as described above. Fig. 8 shows the $\log k$ vs. $\log I$ plots for $[Cu(edta)]^{2-}$, $[Ni(edta)]^{2-}$ and $S_2O_3^{2-}$ in the pH 5.2 phosphate buffer system. The slopes of the plots for these divalent anions can be regarded as being identical. The values of the slopes of the plots for the univalent anions $[Co(edta)]^-$ and $[Cr(edta)]^-$, observed in the MES buffer eluent system are also the same as each other as shown in Fig. 7. These results indicate that the slopes of the $\log k$ vs. $\log I$ plots for

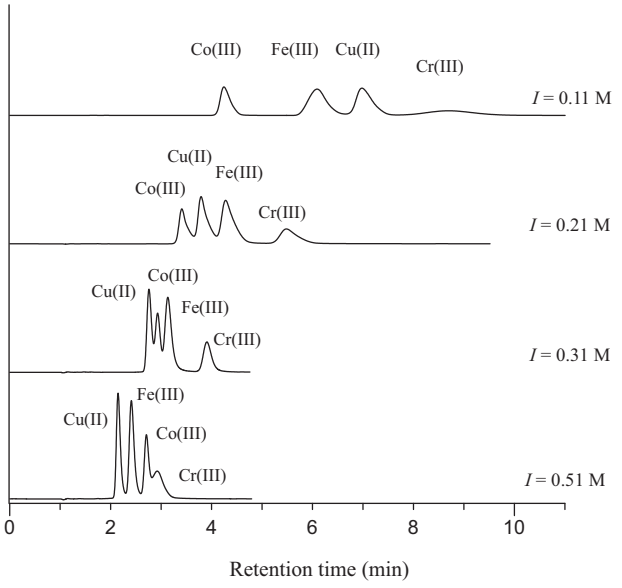

Fig. 2. Chromatograms of the metal-edta complexes in pH 5.2 phosphate buffer eluent system at various ionic strengths. Other chromatographic conditions as in Section 3.

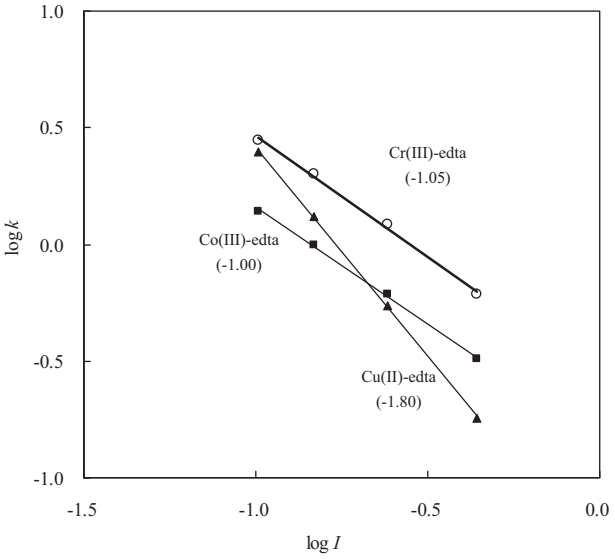

Fig. 4. Plots of $\log k$ vs. $\log I$ for the metal-edta complexes in pH 5.2 acetate buffer eluent system. Values in parentheses are the slopes of the plots.

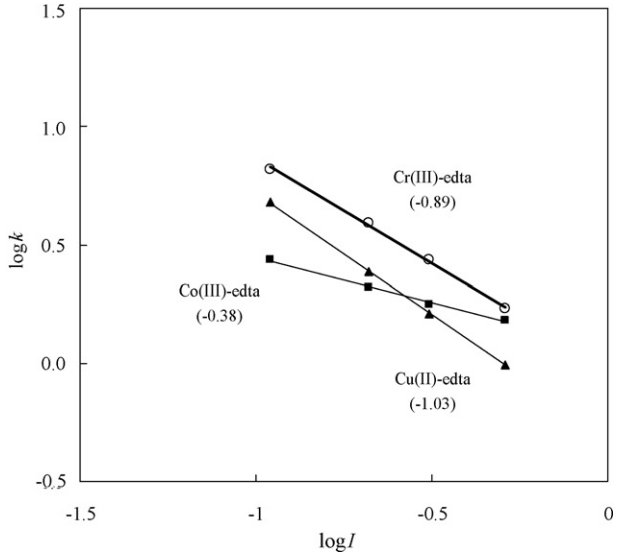

Fig. 5. Plots of log k vs. log I for the metal-edta complexes in pH 5.2 phosphate buffer eluent system. Values in parentheses are the slopes of the plots.

the ions having the same charge can be assumed to be identical, which means that the b value for $[Cr(edta)L]^{(n+1)-}$ can be estimated from the slope of the plot for a reference ion arbitrarily chosen, of which the charge is $(n+1)^-$.

Since the mixed ligand complex of Cr(III)-edta with acetate ion should have the charge of −2, $[Cr(edta)(CH_3COO)]^{2-}$, the slope of the plot of $\log k_{[Cr(edta)(CH_3COO)]}$ vs. log I should be the same as that of the plot for $[Cu(edta)]^{2-}$. Now that the values of $k_{Cr(edta)}$ and the b value or the slope of the $\log k_{[Cr(edta)(CH_3COO)]}$ vs. log I plot have been obtained, one can determine the equilibrium constant together with the a value of the $\log k_{[Cr(edta)(CH_3COO)]}$ vs. log I plot from the data shown in Fig. 4. We have determined the equilibrium constant by obtaining the K value which gives the $\log k_{[Cr(edta)(CH_3COO)]}$ vs. log I plot with the slope of −1.80, the same as the slope for the plot of $[Cu(edta)]^{2-}$. The result obtained by calculation is shown in Table 1.

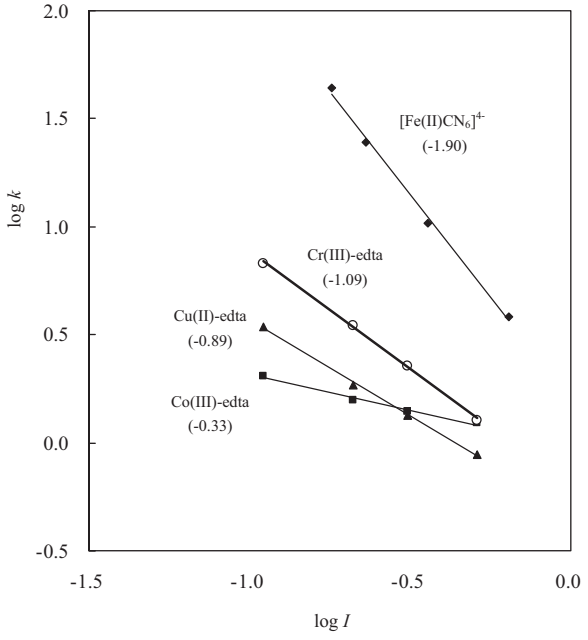

Fig. 6. Plots of log k vs. log I for the metal-edta complexes and $[Fe(II)CN_6]^{4-}$ in pH 6.5 phosphate buffer eluent system. Values in parentheses are the slopes of the plots.

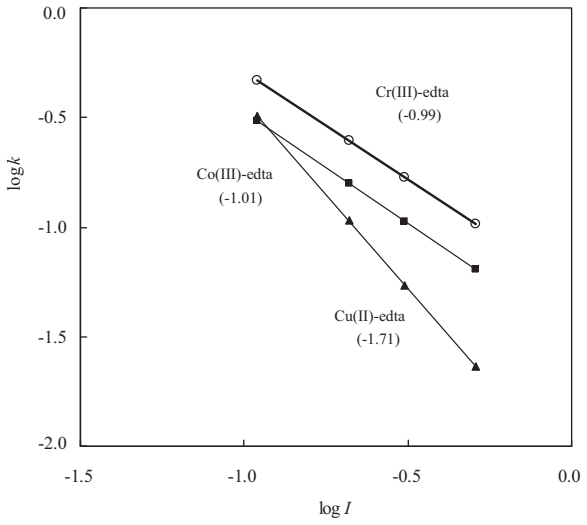

Fig. 7. Plots of log k vs. log I for the metal-edta complexes in pH 5.2 MES buffer eluent system. Values in parentheses are the slopes of the plots.

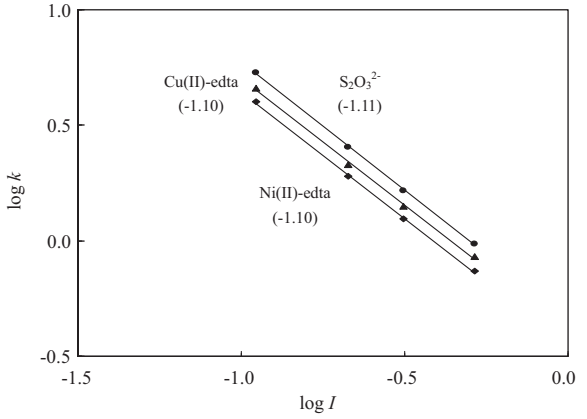

Fig. 8. Plots of log k vs. log I for $[Cu(II)edta]^{2-}$, $[Ni(II)edta]^{2-}$ and $S_2O_3^{2-}$ in pH 5.2 phosphate buffer eluent system. Values in parentheses are the slopes of the plots.

Phosphate ion may coordinate to the central metal, Cr(III), as $H_2PO_4^-$, HPO_4^{2-} or PO_4^{3-}. The slope of the plot for Cr–edta complex obtained in the phosphate eluent system at pH 5.2 shown in Fig. 5 is smaller than that for $[Cu(edta)]^{2-}$, while the plot obtained at pH 6.5 exhibits the slope larger than that for $[Cu(edta)]^{2-}$. This indicates that the negative mean charge of the Cr–edta complex at pH 6.5 is larger than −2 so that the mixed ligand complex produced may be $[Cr(edta)(HPO_4)]^{3-}$ or $[Cr(edta)(PO_4)]^{4-}$. The concentration of PO_4^{3-} is so low at pH 6.5 and cannot be directly determined but can only be calculated from the concentration of HPO_4^{2-} using acid dissociation constant. We have thus determined the equilibrium

Table 1
Comparison of the mixed ligand complex formation constants for Cr(III)-edta with acetate and phosphate ions measured by the RP-IPC method with those by the spectrophotometric method.

Anion	pH	I (M)	log k[a]	
			RP-IPC	Spectrophotometry
CH_3COO^-	5.2	0.10–0.44	−0.18 ± 0.02	−0.21 ± 0.02[b]
$H_2PO_4^-$	5.2	0.11–0.51	1.34 ± 0.03	1.11 ± 0.02
HPO_4^{2-}	6.5	0.11–0.51	1.73 ± 0.03	1.64 ± 0.02

[a] Mean ± standard deviation.
[b] I = 1 M, 25 °C [21].

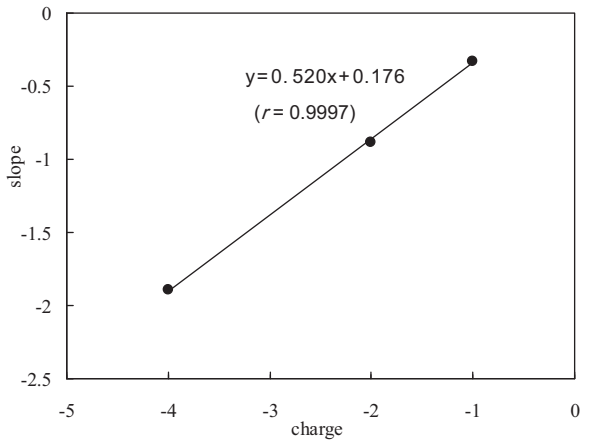

Fig. 9. Relationship between the slopes of the $\log k$ vs. $\log I$ plots and the charges of $[Co(III)edta]^-$, $[Cu(II)edta]^{2-}$ and $[Fe(II)CN_6]^{4-}$ in pH 6.5 phosphate buffer eluent system.

constant assuming that the reaction can be represented as:

$$[Cr(edta)]^- + H_2PO_4^- \rightleftarrows [Cr(edta)(H_2PO_4)]^{2-} \quad \text{at pH 5.2} \tag{18}$$

$$[Cr(edta)]^- + HPO_4^{2-} \rightleftarrows [Cr(edta)(HPO_4)]^{3-} \quad \text{at pH 6.5} \tag{19}$$

The slope of the $\log k$ vs. $\log I$ plot for $[Cr(edta)(HPO_4)]^{3-}$, a trivalent anion, in the pH 6.5 phosphate eluent system was estimated from the relationship between the slopes of the plots and the charges of $[Co(edta)]^-$, $[Cu(edta)]^{2-}$ and $[Fe(CN)_6]^{4-}$, because $[Fe(CN)_6]^{3-}$, which we selected as a reference trivalent anion that exhibits a moderate retention, was reduced to $[Fe(CN)_6]^{4-}$ in the eluents used. Fig. 9 demonstrates that the data for these anionic complexes follow a straight line. From this linear relationship the slope of the $\log k$ vs. $\log I$ plot for $[Cr(edta)(HPO_4)]^{3-}$ was estimated to be −1.38. Then the equilibrium constant for the reaction was calculated in a similar manner to that described for the determination of the equilibrium constant of the reaction of $[Cr(edta)]^-$ and acetate ion. The equilibrium constants obtained for reactions 18 and 19 are also shown in Table 1.

Ogino et al. studied equilibria of the reactions of *N*-substituted ethylenediamine–*N*,*N*′,*N*′-triacetatochromium(III) complexes including $[Cr(edta)]^-$ with acetate ion and determined the equilibrium constants using a UV–visible spectrophotometric method [21]. However, the equilibrium constant of the reaction of $[Cr(edta)]^-$ with phosphate ion has never been reported. Therefore we determined the equilibrium constant of the reaction of $[Cr(edta)]^-$ with phosphate ion according to the spectrophotometric method presented by Ogino et al. on the basis of the spectral change due to the formation of the mixed ligand complex. The equilibrium constants obtained are shown in Table 1 together with the literature value for the reaction with acetate ion.

The equilibrium constants determined by the RP-IPC method are in good agreement with the corresponding values obtained by the spectrophotometric method. Determination of the equilibrium constants by the present method is based on the measurement of dependence of the retention factors on ionic strength. Therefore the activity coefficients of the ionic species involved in the reaction cannot be assumed to be constant throughout the experiment so that the thermodynamic equilibrium constants could not be obtained. However, within the range in the ionic strength adopted in this study, 0.10–0.44 M for the acetate system and 0.11–0.51 M for the phosphate system, the variation in the activity coefficient quotient, q, given by the following equation is not very large.

$$q = \frac{y_{[Cr(edta)L]}}{y_{[Cr(edta)]}y_L} = \frac{K_{th}}{K} \tag{20}$$

where y is the molar ionic activity coefficient and K_{th} is the thermodynamic equilibrium constant. The q values estimated for the equilibrium constant of the formation reaction of $[Cr(edta)(CH_3COO)]^{2-}$ according to the Davies equation [30] are 0.62 at $I = 0.10$ M and 0.51 at $I = 0.44$ M, while the values for the formation of $[Cr(edta)(HPO_4)]^{3-}$ are given as 0.36 at $I = 0.11$ M and 0.25 at $I = 0.51$ M. The Davies equation is represented by:

$$\log y_i = -0.52 z_i \left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.20I \right) \tag{21}$$

This indicates that the present method gives reasonably accurate values of conditional equilibrium constants of chemical reactions involving ionic species in aqueous solutions.

5. Conclusions

An RP-IPC method was developed for the determination of the equilibrium constants of the reaction involving ionic species as reactants and products. It is difficult or impossible to determine the chemical equilibria by the ion-exchange chromatographic methods that have so far been presented when the chemical species of reactants and products are ions of the same charge sign as that of the chemical species reacting with the analyte because the limiting retention factors for the species cannot be assumed to be constant. The present method enables one to determine the equilibrium constants for such reactions by estimating the limiting retention factors using a relationship between the retention factor of an ionic solute and the ionic strength of the mobile phase in RP-IPC. The equilibrium constants determined for the substitution reactions of $[Cr(edta)]^-$ with acetate and phosphate ions are in fairly good agreement with the corresponding values obtained by a UV–visible spectrophotometric method. The spectrophotometric method hinges on the reactant and product species having different spectra. A liquid chromatographic method is useful when the spectral difference between the reactant and product species is very small and/or pure sample cannot be obtained.

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