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Anomalous bandspreading of ethylenediaminetetraacetato–chromium(III) ion in reversed-phase high-performance liquid chromatography

An example of slow equilibrium kinetics

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ABSTRACT

As part of a more general study of the high-performance liquid chromatography of ethylenediaminetetraacetate (EDTA) complexes of transition metal ions, it was noted that the EDTA complex of chromium(III) (Cr(III)–edta) gave broad, but symmetrical, peaks in reversed-phase chromatography on alkyl-modified silica (Hypersil C_8), whereas other metal–edta complexes exhibited normal narrow peaks. The plots against linear flow velocity for Cr(III)–edta are straight lines with slopes which depend strongly on the nature and the concentration of the electrolyte used as buffer. The results obtained are interpreted in terms of the Giddings' model for slow reactions leading to equilibration and indicate that the relative slowness of ligand substitution reactions of Cr(III)–edta with anions in the mobile phase and stationary phases is the dominant contributor to the axial dispersion of this solute. Rate constants and activation energies for ligand substitution are estimated.

INTRODUCTION

It is well known that slow kinetic processes involved in sorption and desorption may contribute to spreading of solute bands. Giddings [1,2] has derived several useful equations based on various kinetic schemes which may be relevant in real chromatographic systems. However, very few, if any, real examples of slow equilibration leading to significant excess band dispersion have been reported.

In a wider study of the high-performance liquid chromatography (HPLC) of ethylenediaminetetraacetate (EDTA) complexes of transition metal ions, it was noted that the EDTA complex of chromium(III), (Cr(III)–edta), gave extremely broad, but symmetrical, peaks in reversed-phase ion-pair chromatography. These were in striking contrast to the narrow peaks exhibited by the other metal–edta complexes.

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We felt that this characteristic phenomenon most probably reflected the slow kinetics of some reaction of Cr(III)–edta and that this system provided an opportunity to demonstrate the effect of slow chemical change on the spreading of the solute bands in line with Giddings' theory. It will be shown in the present paper that the bandspreading of Cr(III)–edta can indeed be interpreted in terms of the Giddings' model and that the experimental results are consistent with predictions based upon known equilibrium and kinetic data.

EXPERIMENTAL

Materials

$[Cr(Hedta)(H_2O)]$ [3], $Na[Co(edta)]$ [4], $Na_2[Co(edta)]$ [4], $Na[Fe(edta)(H_2O)]$ [5] and $[Cu(H_2edta)]$ [6] were prepared according to literature. The compounds were identified by their UV and visible absorption spectra. Cetrimide (cetyltrimethylammonium bromide) was obtained from BDH (Poole, UK), HPLC-grade acetonitrile from FSA Laboratory Supplies (Loughborough, UK) and doubly distilled water was used to prepare the sample solutions and the eluent.

Chromatographic conditions

The chromatographic equipment comprised a Waters Assoc. (Croydon, UK) Model M6000 pump, a Rheodyne injection valve, a 125 × 5 mm I.D. stainless-steel column (Shandon Southern Products, Runcorn, UK) and a Kratos (NJ, USA) Spectroflow 773 UV absorbance photometer. The detection signal was fed into a Hewlett-Packard reporting integrator Model 3390A (Bracknell, UK).

Columns were slurry packed in the laboratory with 5-μm octyl silica, Hypersil C_8 (Shandon Southern Products), at 400 kg cm^{-2} using 2-propanol followed by water–methanol (50:50, v/v). The column was thermostatted by an air-circulated oven (Shandon Southern Products).

Eluents were 10% (w/v) acetonitrile–water containing 0.1 m*M* cetrimide. pH and ionic strength were adjusted using phosphate (H_3PO_4, NaH_2PO_4 and Na_2HPO_4) or acetate (CH_3COOH and CH_3COONa). Sample solutions (0.1–4 mM) were prepared by dissolving the metal–edta complexes in solutions whose compositions were the same as those of the eluents used.

Portions (5 μl) of the sample solutions were injected into the column. Preliminary experiments showed that neither retention volume nor height equivalent to a theoretical plate (HETP) depended upon the volume injected in the range between 1 and 10 μl. The volumetric flow-rate, f_v (ml min^{-1}), was accurately measured using the burette designed to prevent vaporazation of solvent.

Determination of column parameters

The volume of the empty column, V_{column}, was determined to be 2.41 ml by filling the column with methanol from a burette. The extra column volume, V_{extra}, was determined to be 0.076 ml by measuring the elution volume of a sample solute through the system from which the column had been removed.

The mobile phase volume, V_m, was assumed to be the smallest retention volume of Cu(II)–edta. This was obtained by elution with 10% (w/v) acetonitrile–water containing 0.1 *M* NaCl and gave $V_m = 1.48$ ml.

RESULTS AND DISCUSSION

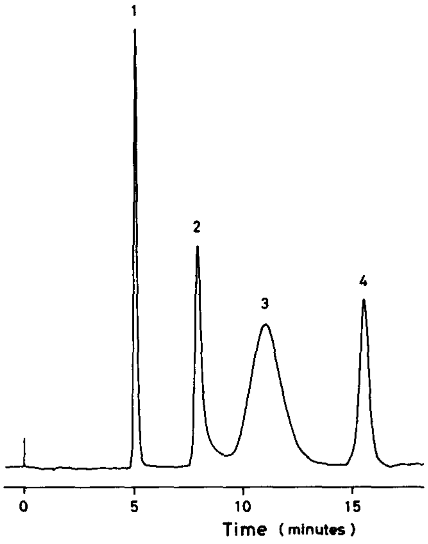
Figs. 1 and 2 show chromatograms of several metal(II,III)–edta complexes obtained by elution with eluents containing phosphate buffers of pH 4.5 and 7.0, respectively. As can be seen, Cr(III)–edta exhibits remarkably broad peaks which are in a striking contrast to the normal narrow peaks of the other analytes.

In order to clarify the dominant factor contributing to the bandspreading of Cr(III)–edta, the HETP was measured as a function of the linear flow velocity, u (mm s^{-1}), which was calculated by eqn. 1.

$$u(\mathrm{mm\,s^{-1}}) = \frac{f_v}{a_m} = \frac{f_v(V_{\mathrm{column}} + V_{\mathrm{extra}})}{a_{\mathrm{column}}V_m} = 1.43 f_v\,(\mathrm{ml\,min^{-1}}) \qquad (1)$$

where a_{column} and a_m are the cross-sectional areas of the column and the mobile phase within the column, respectively.

Fig. 3 illustrates plots of HETP against u for Co(III)–edta and Cu(III)–edta; both give normal curves having a minimum. The HETP of these metal–edta complexes are nearly independent of pH, ionic strength, temperature (30–50°C) and the nature of the electrolyte used as buffer.



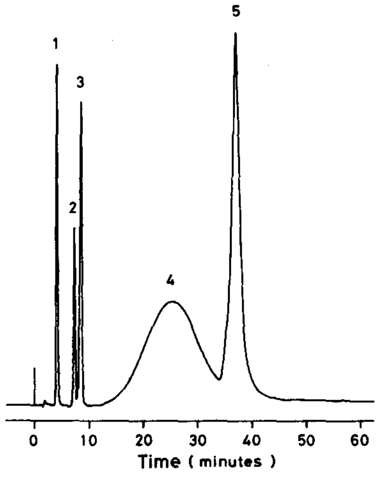


Fig. 1. Chromatogram of metal(II,III)–edta complexes. Eluent, 0.1 mM cetrimide and 10% (w/v) acetonitrile in aqueous phosphate buffer (pH 4.5, ionic strength, I = 0.1 M); flow-rate, 1.03 ml min^{-1}; detection wavelength, 230 nm; temperature, 30°C. Peaks: 1 = Co(III)–edta; 2 = Fe(III)–edta; 3 = Cr(III)–edta; 4 = Cu(II)–edta.

Fig. 2. Chromatogram of metal(II,III)–edta complexes. Eluent, 0.1 mM cetrimide and 10% (w/v) acetonitrile in aqueous phosphate buffer (pH 7.0, I = 0.1 M); flow-rate, 1.03 ml min^{-1}; detection wavelength, 230 nm; temperature, 30°C. Peaks: 1 = Co(III)–edta; 2 = Co(II)–edta; 3 = Cu(II)–edta; 4 = Cr(III)–edta; 5 = Fe(III)–edta.

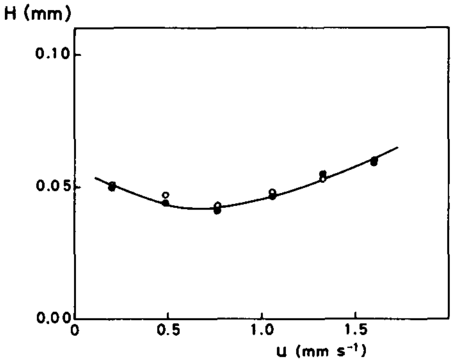
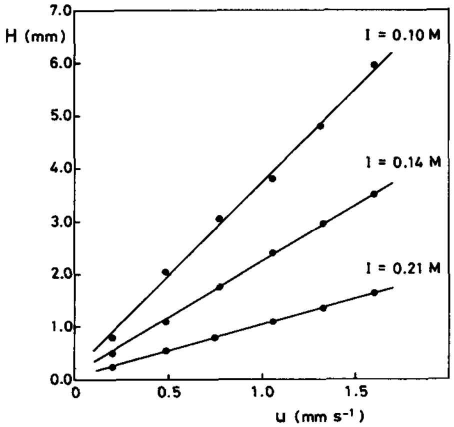


Fig. 3. Plots of HETP *vs.* linear flow velocity for Co(III)–edta and Cu(II)–edta. Eluent, 0.1 m*M* cetrimide and 10% (w/v) acetonitrile in aqueous phosphate buffer (pH 7.0, *I* = 0.1 *M*); temperature, 30°C. ● = Co(III)–edta; ○ = Cu(II)–edta.

By contrast, the plots for Cr(III)–edta were straight lines in almost all cases whose slopes depended strongly upon the composition of the eluent. Figs. 4 and 5 show the effects of ionic strength, *I*, and pH on the plots of HETP against *u* for Cr(III)–edta, while Fig. 6 shows that the slope depends on the nature of the buffer.

In general, a linear relationship must exist between the HETP and the linear velocity for any contribution to HETP arising from slow equilibration of the analyte between the mobile and stationary phases. Such contributions are, of course, covered by the C-term in the van Deemter equation. Giddings [1] has indicated that there are six slow processes which may produce dispersion of solute zones, namely: (i) single-step sorption and desorption; (ii) diffusion through the stationary or mobile phase; (iii) adsorption on heterogeneous surfaces; (iv) simultaneous partition and adsorption; (v)



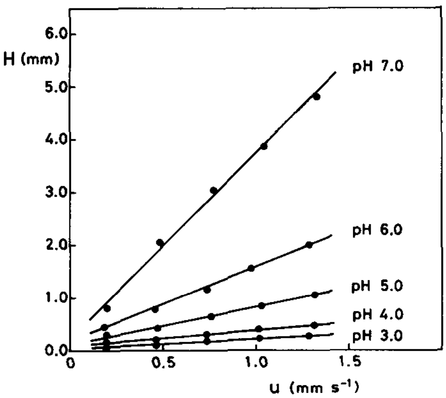


Fig. 4. Effect of ionic strength on the plot of HETP *vs.* linear flow velocity for Cr(III)–edta. Eluent, 0.1 m*M* cetrimide and 10% (w/v) acetonitrile in aqueous phosphate buffer (pH 7.0); temperature, 30°C.

Fig. 5. Effect of pH on the plot of HETP *vs.* linear flow velocity for Cr(III)–edta. Eluent, 0.1 m*M* cetrimide and 10% (w/v) acetonitrile in aqueous phosphate buffer (*I* = 0.1 *M*); temperature, 30°C.

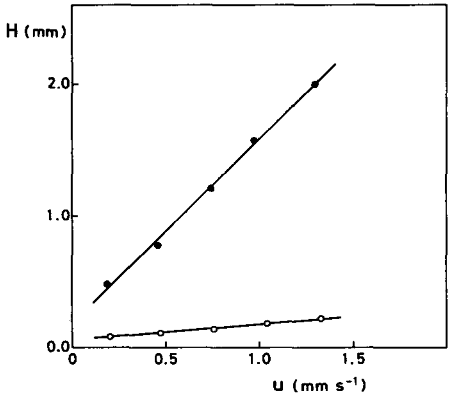


Fig. 6. Effect of the type of the electrolyte used as buffer on the plot of HETP *vs.* linear flow velocity for Cr(III)–edta. Eluent, 0.1 m*M* cetrimide and 10% (w/v) acetonitrile in aqueous buffer (pH 6.0, *I* = 0.1 *M*); temperature, 30°C. ● = Phosphate; ○ = acetate.

adsorption of large molecules; and (vi) chemical reactions not directly related to sorption.

It is not likely that any of the processes (i)–(v) plays a predominant role in the spreading of Cr(III)–edta zones because Cr(III)–edta is considered to have basically a size and structure similar to other metal(III)–edta complexes, especially as Co(III)–edta whose peaks are narrow. Slow chemical reaction (vi) is thus the most likely cause.

Generally, aqueous Co(III) and Cr(III) ions are both kinetically inert. However, it has been found in recent years that one of the coordination sites of Cr(III)–edta is relatively labile and undergoes unexpectedly rapid substitution reactions [7–9] with some anions as shown in reaction A.

$$[\mathrm{Cr(edta)}]^- + \mathrm{L}^{n-} \rightleftharpoons [\mathrm{Cr(edta)L}]^{(n+1)-} \qquad \text{(A)}$$

Similar ligand substitution reactions have also been observed for several other metal(III)–edta or related complexes, such as Fe(III) [9] and Co(III) [10,11]. But while the reactions of the Fe(III) complex are too fast to cause spreading of solute zones in chromatography [9], the substitution reactions of the Co(III) complex are much too slow [11–14]. In addition, aqueous solutions of $[\mathrm{Co(edta)L}]^{(n+1)-}$ are thermodynamically unstable and eliminate L^{n-} forming $[\mathrm{Co(edta)}]^-$ [10] so that the ligand substitution reaction of $[\mathrm{Co(edta)}]^-$ may be ignored in the present context.

Accordingly, it is most likely that process (vi), that is, the slowness of the substitution reactions of Cr(III)–edta with co-existing anions in the eluent such as $H_2PO_4^-$ and CH_3COO^- is responsible for the spreading of Cr(III)–edta zones.

The general scheme of chemical reaction and sorption–desorption of Cr(III)–edta complexes can be represented by Fig. 7 (charges on ions are omitted) where k'_W and k'_L are the distribution coefficients (capacity ratios) for the forms $[\mathrm{Cr(edta)H_2O}]^-$ and $[\mathrm{Cr(edta)L}]^{(n+1)-}$ while K_M and K_S are the equilibrium ratios for the two forms of the complex (reaction A) in the mobile and stationary phases, respectively. Definitions of

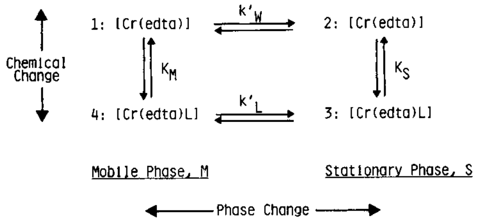


Fig. 7. Phase and chemical equilibria involved in the chromatography of Cr–edta complexes.

the various equilibrium and rate constants used in this paper are given in Table I. The following definitions apply where X_1, X_2, X_3 and X_4 refer to the fractions of analyte (the Cr–edta complex) in each of the four possible forms (numbered 1: to 4:) shown in the reaction scheme:

$$\text{Capacity ratios} \qquad k'_W = X_2/X_1 \tag{2}$$

$$k'_L = X_3/X_4 \tag{3}$$

TABLE I

DEFINITIONS OF EQUILIBRIUM AND RATE CONSTANTS

Equilibrium constants	
Chromatographic distribution equilibria	
k'	Overall capacity ratio of Cr(edta) irrespective of ligand (eqn. 6)
k'_W	Capacity ratio of uncomplexed Cr(edta) between mobile and stationary phases (eqn. 2)
k'_L	Capacity ratio of Cr(edta)L between mobile and stationary phases (eqn. 3)
Chemical equilibria	
K_M	Equilibrium distribution ratio between Cr(edta) and Cr(edta)L in mobile phase, *i.e.*, K_M = [Cr(edta)L]/[Cr(edta)] (eqn. 4)
K_S	Equilibrium distribution ratio between Cr(edta) and Cr(edta)L in stationary phase (eqn. 5)
Rate constants	
k_M	First-order rate constant for conversion of Cr(edta) to Cr(edta)L in mobile phase (eqn. 8)
k_S	First-order rate constant for conversion of Cr(edta) to Cr(edta)L in stationary phase (eqn. 8)
k_f	Second-order rate constant for reaction B in forward direction
k_b	First-order rate constant for reaction B in reverse direction

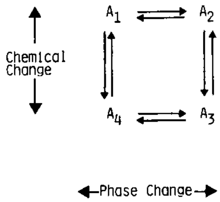


Fig. 8. Giddings' scheme for slow chemical and interphase equilibration.

Chemical equilibria $$K_M = X_4/X_1 \tag{4}$$

$$K_S = X_3/X_2 \tag{5}$$

The overall k' value for the Cr(III)–edta complex as a whole is then

$$k' = \frac{X_2 + X_3}{X_1 + X_4} = \frac{k'_W + K_M k'_L}{1 + K_M} \tag{6}$$

and the relative migration rate is

$$R = \frac{1}{1 + k'} = (X_1 + X_4) \tag{7}$$

Giddings [1,2] considered several chemical systems involving slow chemical equilibration including the combination of slow mass transfer of analyte between phases and slow equilibration of two forms of analyte within the mobile and stationary phases. The relevant equilibria are given in Fig. 8, which is seen to be formally identical to our Fig. 7 for the Cr(III)–edta system.

When mass transfer between phases is rapid, as we assume in our case, but chemical equilibration is slow, Giddings' formulation gives a contribition to the plate height, H, represented by eqn. 8 where the X_i, as defined above, are the fractions of analyte in the forms A_i and where k_M and k_S are the forward rate constants for the substitution reactions in the mobile and stationary phases (*i.e.*, k_{14} and k_{23}, respectively, in Giddings' original formulation).

$$H = \frac{2(X_1X_3 - X_2X_4)^2}{R(k_M X_1 + k_S X_2)} u \tag{8}$$

It is then a simple matter to replace the X_i values using eqns. 2–7 to give

$$H = \frac{2}{(1 + k')^2} \frac{K_M^2}{(1 + K_M)^3} \frac{(k'_W - k'_L)^2}{(k_M + k_S k'_W)} u \tag{9}$$

The denominator in the final factor, $k_M + k_S k'_W$ is the overall rate constant based upon mobile phase concentrations for the substitution reaction occurring in both phases. In other words, the combined reaction rate in the two phases is given as $(k_M + k_S k'_W) q_M$ where q_M is the quantity of analyte in the mobile phase.

In order to correlate the above expression for H with the experimental data, the various constants must either by measured experimentally or estimated. Only k', the overall capacity factor, can be measured experimentally; however, k_M and k_S, K_M, k'_W and k'_L may be estimated from literature data, as discussed below.

Values of K_M and k_M

Ogino and co-workers [7,9] have determined the equilibrium constant, K_B, and the rate constants, k_f and k_b, of the reaction of Cr(III)–edta with an acetate ion in aqueous solution at 25°C and with $I = 1.0\ M$ adjusted using $NaClO_4$ (reaction B).

$$[Cr(edta)]^- + CH_3COO^- \underset{k_b}{\overset{k_f}{\rightleftharpoons}} [Cr(edta)(CH_3COO)]^{2-} \tag{B}$$

($K_B = 0.62\ M^{-1}$; $k_f = 3.3 \pm 0.4\ M^{-1}s^{-1}$; $k_b = 5.4 \pm 0.6\ s^{-1}$)

It is impossible to use the ClO_4^- ion to adjust ionic strength of the eluent in the present chromatographic system because the cetyltrimethylammonium ion added to the eluent as an ion-pairing agent precipitates when ClO_4^- is added. We have thus assumed that the equilibrium constant and the rate constants which Ogino and co-workers determined are applicable to the present system where the mobile phase consists of 10% (w/v) acetonitrile–water containing acetate buffer at an ionic strength of 0.1 M. Consequently, K_M is calculated as follows

$$K_M = K_B[CH_3COO^-] = 0.1K_B = 0.062 \tag{10}$$

Unless the stationary phase accelerates the ligand substitution reaction, k_M is given by

$$k_M = k_f[CH_3COO^-] = 0.1k_f = 0.33\,s^{-1} \tag{11}$$

Capacity factors k'_W and k'_L

We have formulated the Cr(III)–edta complex as $[Cr(edta)]^-$ although there remains a problem concerning the structure of Cr(III)–edta complex in an aqueous solution of *ca.* pH 5: whether $edta^{4-}$ in the Cr(III)–edta complex acts as a quinquedentate ligand or as a sexidentate ligand [15–19]. It is well known that the Cr(III)–edta complex has two acid dissociation steps, which was interpreted as the loss of a proton from the uncoordinated carboxylic acid group followed by the loss of a proton from the coordinated water molecule, as shown in reaction C.

$$[Cr(Hedta)(H_2O)] \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} [Cr(edta)(H_2O)]^- \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} [Cr(edta)(OH)]^{2-} \tag{C}$$

($pK_{a1} = 1.8$; $pK_{a2} = 7.39$ [7])

Thus, it was believed that the $edta^{4-}$ remained coordinated to Cr(III) as a quinquedentate ligand over a wide pH range. Recently, however, Wheeler and Legg [16] showed by deuterium NMR spectroscopy that $edta^{4-}$ forms a sexidentate complex with Cr(III) between pH 3.5 and 6.5. Their view has been supported by the recent work carried out by a variety of analytical methods [17–19].

At pH 4–6 Co(III)–edta and Cr(III) have the same charge, −1, and therefore similar structures, *i.e.*, $[Co(edta)]^-$ and $[Cr(edta)]^-$. Because it seems reasonable to assume that the affinity of $[Cr(edta)]^-$ to the stationary phase is nearly the same as that of $[Co(edta)]^-$ we assume

$$k'_W = k'_{[Co(edta)]^-} \tag{12}$$

TABLE II

ESTIMATED EQUILIBRIUM CONSTANTS AND RATE CONSTANTS RELATING TO THE CHEMICAL CHANGE AND THE SORPTION OF Cr(III)–edta COMPLEX IN ACETATE BUFFER SYSTEM

pH	k'_W	k'_L	K_M	k'	k_M (s^{-1})
5.0	2.40	23	0.062	3.6	0.33
6.0	2.60	31	0.062	4.3	0.33

On the other hand, since the overall k' is obtained experimentally, k'_L can be calculated from k', k'_W and K_M from eqn. 6 as

$$k'_L = \frac{k'(1 + K_M) - k'_W}{K_M} \qquad (13)$$

Values of k_M and k_S

The rates of substitution in the mobile and stationary phases are expected to be similar and we have assumed that $k_M = k_S k'_W$, that is, that the rates are identical and therefore that $k_M + k_S k'_W = 2k_M$. If they are not identical we would replace $2k_M$ by αk_M ($\alpha > 1$), where α would be a constant which could be adjusted to obtain the best fit between direct experiment and literature prediction.

The estimated equilibrium constants and the rate constants relating to the chemical change and the sorption of Cr(III)–edta complexes at pH 5.0 and 6.0 are summarized in Table II. Substituting these values into eqn. 9, we can obtain the expected slopes of the plots of HETP *vs.* *u*. The values thus obtained are listed in Table III, together with the experimental values obtained from the plots shown in Fig. 9. The experimental values are somewhat smaller than the corresponding calculated ones, but

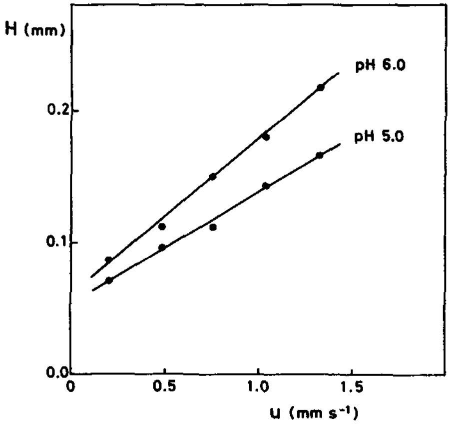


Fig. 9. Effect of pH on the plot of HETP *vs.* linear flow velocity for Cr(III)–edta. Eluent, 0.1 m*M* cetrimide and 10% (w/v) acetonitrile in aqueous acetate buffer (I = 0.1 *M*); temperature, 30°C.

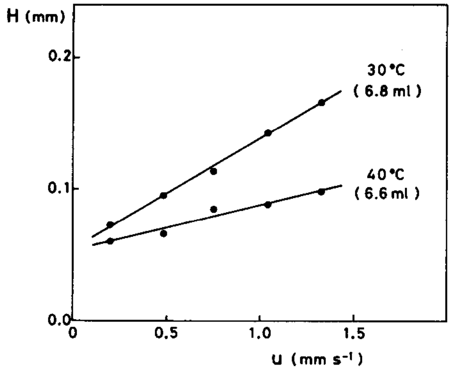


Fig. 10. Effect of temperature on the plot of HETP *vs.* linear flow velocity for Cr(III)–edta. Eluent, 0.1 mM cetrimide and 10% (w/v) acetonitrile in aqueous acetate buffer (pH 5.0, I = 0.1 M). Values in parentheses are the retention volumes of Cr(III)–edta.

this discrepancy mainly results from the difference in temperature: the experimental values were obtained at 30°C while the calculated values are for 25°C.

Fig. 10 shows the effect of temperature on the plot of HETP *vs.* u. Provided the capacity factors of $[\mathrm{Cr(edta)}]^-$ and $[\mathrm{Cr(edta)(CH_3COO)}]^-$ and the equilibrium constant of the ligand substitution reaction K_M are independent of temperature, T, we can write as follows

$$H = (\mathrm{const}/k_M)\,u \tag{14}$$

where both H and k_M are temperature dependent. It is reasonable to assume that the capacity factors of $[\mathrm{Cr(edta)}]^-$ and $[\mathrm{Cr(edta)(CH_3COO)}]^-$, namely k'_W and k'_L, and K_M are approximately independent of temperature between 30 and 40°C because the retention volume of Cr(III)–edta at 40°C (6.6 ml) is nearly the same as at 30°C (6.8 ml). This means that the activation energy, E_a, can be estimated by eqn. 15

$$E_a = R\left(\frac{313 \times 303}{313 - 303}\right)\ln\left[\frac{\mathrm{H(40°C)}}{\mathrm{H(30°C)}}\right] \tag{15}$$

TABLE III

COMPARISON OF THE SLOPES OF THE PLOTS OF HETP vs. u CALCULATED BY EQN. 10 WITH THE EXPERIMENTALLY OBTAINED VALUES

pH	Calcd. (25°C)	Exptl. (30°C)[a]	Exptl. (25°C)[b]
5.0	0.20	0.084	0.14
6.0	0.28	0.12	0.19

[a] Obtained from the plots shown in Fig. 9.

[b] Values corrected for temperature (see text for details).

where R is the gas constant. E_a at pH 5.0 is then 71 kJ mol^{-1}. From this E_a value, the slope of the H *vs.* u plot at 25°C can be estimated. The value at pH 6.0 was calculated by assuming that the activation energy of the reaction is the same at pH 6.0 as at 5.0. The estimated values are given in Table III and are in fairly good agreement with those calculated from literature. Accordingly, the spreading of Cr(III)–edta zones in reversed-phase HPLC can be interpreted in terms of the Giddings' model for slow reactions leading to equilibration where the slowness of ligand substitution reactions of Cr(III)–edta is the main contributor to the axial dispersion of this solute.

$[\mathrm{Cr(edta)}]^-$ has been found to react not only with CH_3COO^- but also with some other anions such as SCN^-, CrO_4^{2-} and MoO_4^{2-} [9]. Although the equilibrium constants and the rate constants relating to the reaction of Cr(III)–edta with $H_2PO_4^-$ have not yet been determined, similar ligand substitution reactions may be responsible for the bandspreading of Cr(III)–edta in the phosphate buffer system. We have found evidence that the average charge on Cr(III)–edta in a phosphate buffer solution of pH 7.0 is not -1 but -2 [20]. This appears to indicate that Cr(III)–edta undergoes the ligand substitution reaction with $H_2PO_4^-$ or HPO_4^{2-}.

The dependence of the slope of the HETP *vs.* u plot on the ionic strength can be explained qualitatively as follows. The increase in the concentration of L^{n-} added as buffer salt results in the increase both in K_M and k_M according to eqns. 10 and 11, respectively. Furthermore, the capacity factors of $[\mathrm{Cr(edta)}]^-$ and $[\mathrm{Cr(edta)L}]^{(n+1)-}$ and then the difference between these two capacity factors ($k'_L - k'_W$) decrease with increase in ionic strength. Consequently, the slope decreases with an increase in the ionic strength as shown in Fig. 4.

The effect of pH on the plots shown in Fig. 5 may be explained to some extent. It has been reported that the rate constant of the reaction of $[\mathrm{Cr(Hedta)(H_2O)}]$ with an acetate ion is much higher than that of $[\mathrm{Cr(edta)}]^-$ [7]. Therefore, the overall rate constant of this kind of ligand substitution reaction is expected to increase with increase in the concentration of $[\mathrm{Cr(Hedta)(H_2O)}]$ under pH 5. At pH 5 or above, however, the concentration of $[\mathrm{Cr(Hedta)(H_2O)}]$ is so small that the effect of the reaction of the reaction of $[\mathrm{Cr(Hedta)(H_2O)}]$ on the overall reaction rate is negligible even if its high rate constant is taken into account. Changes in concentration of phosphate species in the eluent, *i.e.*, $H_2PO_4^-$ and HPO_4^{2-}, as pH is changed may cause the result shown in Fig. 5. In order to quantitatively evaluate the effect of pH on the bandspreading of Cr(III)–edta, K_M for the reaction of $[\mathrm{Cr(edta)}]^-$ with $H_2PO_4^-$ and HPO_4^{2-} should be determined by means of some other method.

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