CHROM. 8812

GEL CHROMATOGRAPHIC BEHAVIOUR OF EDTA COMPLEXES ON SEPHADEX COLUMNS

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(Received October 14th, 1975)

SUMMARY

Ethylenediaminetetraacetate complexes of cobalt(II), nickel(II), copper(II), chromium(III), iron(III), cobalt(III) and bismuth(III) were eluted with sodium chloride solution on Sephadex columns. The elution volumes of the complexes (MY^{n-}) were measured as functions of the concentration and pH of the eluents, sample concentration and gel porosity.

The following results were obtained. The concentration dependence of the sample could be classified into two types, $M^{II}Y^{2-}$ and $M^{III}Y^{-}$. The elution volumes were dependent on the concentration of the eluent: in the region of sample concentrations in which the elution volumes of the complexes were maintained constant, these volumes increased with an increase in eluent concentration. The elution volumes were dependent on the pH values of the eluent: the variations in the elution volumes were similar to the variation in the mean charge of the complexes with pH. It seems that the effective radii of the hydrated complexes increase with an increase in the charge of the complexes.

INTRODUCTION

In recent years, gel chromatography has been applied to the investigation of small inorganic compounds[1]. The behaviour of ethylenediaminetetraacetate (EDTA) complexes (abbreviated to MY^{n-}) in gel chromatography has also been studied[2–5]. In a previous paper[2], it was demonstrated that univalent and divalent EDTA complexes of some metal ions were separated on a Sephadex G-15 column using sodium chloride as eluent. Concerning the elution of inorganic ions, some workers[6–8] have pointed out that both the sample concentration and background electrolyte concentration play important roles in the gel chromatographic behaviour of inorganic ions.

In the present investigation, fundamental information on the gel chromatographic behaviour of univalent and divalent EDTA complex ions was sought. Although the molecular sieve effect is a major factor in the elution mechanism of inorganic ions, side effects such as ion exclusion must also be considered in some instances[7,8]. In order to estimate the contribution of the side effects, elution volumes for EDTA complexes were measured as functions of the concentration and pH of the

eluent, sample concentration and gel porosity, using Sephadex G gels and sodium chloride solution as the eluent, *i.e.*, background electrolyte.

EXPERIMENTAL

Sample solution

All reagents used were of guaranteed grade obtained from Wako (Osaka, Japan) unless otherwise stated. EDTA complexes such as $KCo^{III}Y$ and $HCr^{III}Y$ were prepared by the method of Dwyer *et al.*[9] and Hamm[10], respectively. The compounds were identified by their absorption spectra. Sample solutions were prepared by dissolving $Na_2Ni^{II}Y$, $Na_2Co^{II}Y$, $Na_2Cu^{II}Y$, $HCr^{III}Y$, $NaFe^{III}Y$, $KCo^{III}Y$ and $NaBi^{III}Y$ in the same solution as the eluents. Solutions of Blue Dextran 2000 (Pharmacia, Uppsala, Sweden; 0.2%), used as a standard for K_{av} values, were prepared in a manner similar to that described above.

Eluent

The eluents used were aqueous solutions of sodium chloride with various concentrations. Some of these solutions were adjusted to definite pH values with hydrochloric acid, acetic acid, sodium acetate or sodium hydroxide.

Preparation of Sephadex columns

Sephadex G-10, G-15 or G-25 (Pharmacia, dry particle size 40–120, 40–120 and 20–80 μm, respectively), which is a cross-linked dextran gel, was used as the bed material. The dry powder was suspended in the eluent and allowed to swell for 2 days. A slurry of the prepared gel was poured into the column, consisting of suitable length of glass tubing with a porous polystyrene disc at the bottom. A small disc of filter-paper served to reduce disturbances at the top of the gel bed. After packing, the eluent (5 times the bed volume) was passed through the column in order to settle the gel bed.

Procedure

A 1-ml volume of the sample solution was placed on the top of the bed just as the last few drops of the eluent soaked into the bed, and the eluent was applied when the last portion of the sample solution passed into the bed. The effluent was collected in fractions by means of a drop-count fraction collector at a flow-rate of 15–20 ml/h. The elution was carried out at 20–25°.

The amount of solute in the fractions was determined as follows. The concentration of Blue Dextran 2000 was determined colorimetrically by measurement of the absorbance at 620 nm. The concentration of the EDTA complexes was determined colorimetrically by the method described by Ueno and Sakaguchi[11] with some modifications, and the lower concentrations of $Cu^{II}Y^{2-}$ and $Co^{II}Y^{2-}$ were determined by atomic-absorption spectrophotometry at 324.8 and 240.7 nm, respectively.

Calculation of K_{av} values

The K_{av} value is defined by the equation[12]
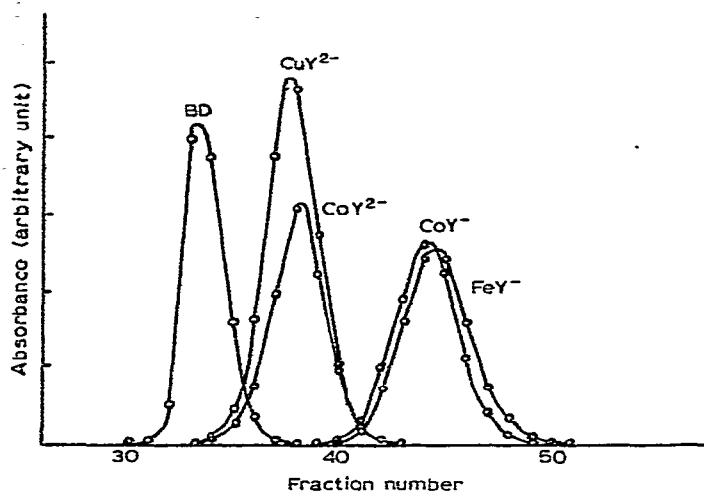
$$K_{av} = (V_e - V_0) / (V_t - V_0)$$

Fig. 1. Elution curves of EDTA complexes on Sephadex G-10. Column: 95.0 × 1.5 cm. Eluent: 0.1 M NaCl solution. Sample: 0.025 M of each complex. One fraction: 1.83 ml.

where V_t is the total bed volume, V_0 is the void volume outside the gel particles and V_e is the elution volume.

RESULTS AND DISCUSSION

Effect of gel porosity on elution volume

When solutes of lower molecular weight are chromatographed by the molecular sieve technique, a highly cross-linked gel must be used, and Sephadex G-10, G-15 and G-25 were used in these experiments. The results are shown in Figs. 1–3. The elution volume of each EDTA complex increases with increasing porosity of the
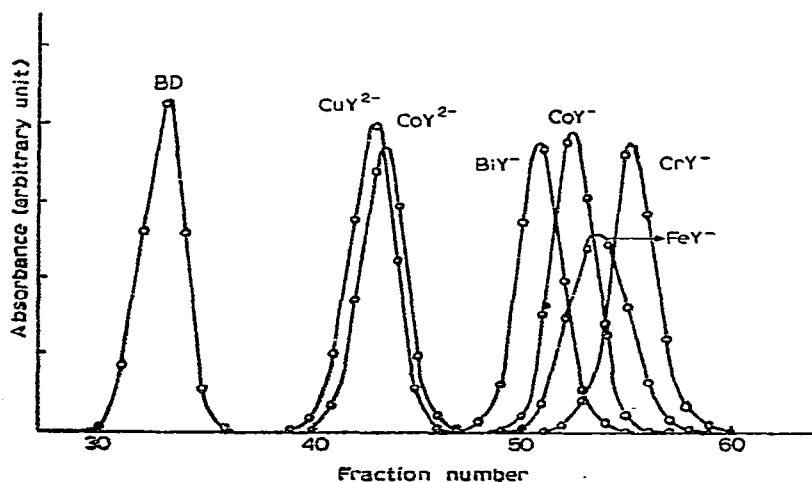

Fig. 2. Elution curves of EDTA complexes on Sephadex G-15. Column: 95.2 × 1.5 cm. Eluent: 0.1 M NaCl solution. Sample: 0.025 M of each complex. One fraction: 1.84 ml.
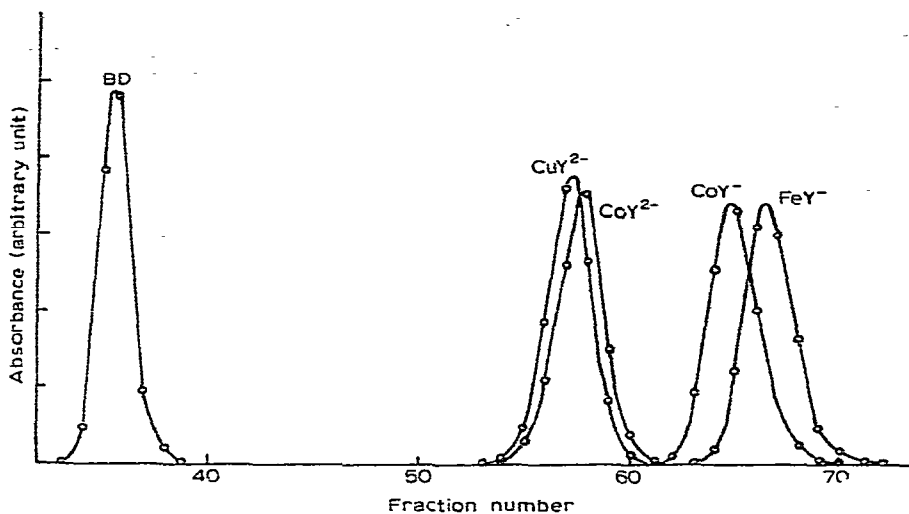
Fig. 3. Elution curves of EDTA complexes on Sephadex G-25. Column: 95.0 × 1.5 cm. Eluent: 0.1 M NaCl solution. Sample: 0.025 M of each complex. One fraction: 1.94 ml.

gels, as expected from molecular sieve effect. Sephadex G-15 is the preferred gel for obtaining the maximum difference in elution volumes between MY^{2-}- and MY^{-}-type complexes.

We have pointed out previously[2] that MY^{2-}- and MY^{-}-type complexes (0.025 M) were completely separated on a Sephadex G-15 column (41.7 × 1.5 cm) using 0.1 M sodium chloride as the eluent. Fig. 4 shows the elution pattern obtained with a longer column (95.0 × 1.5 cm) of Sephadex G-15 using the same procedure. It is apparent that $Bi^{III}Y^{-}$ and $Cr^{III}Y^{-}$ could be separated more effectively with the longer column.
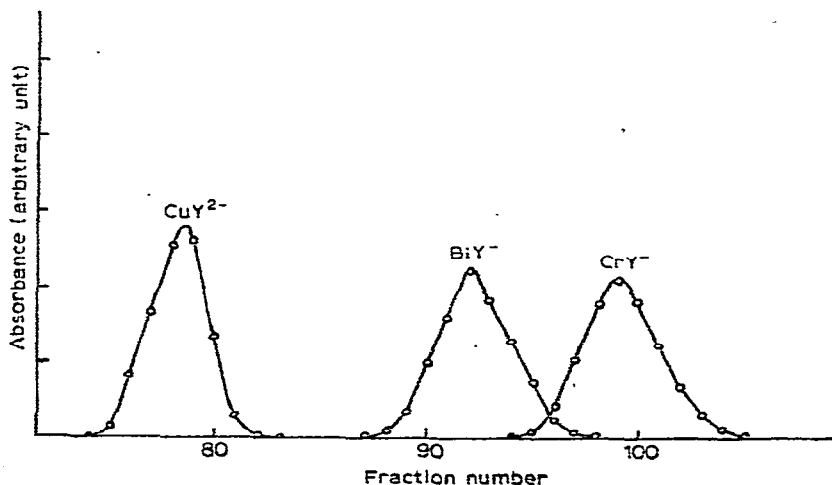

Fig. 4. Elution curves of a mixture of CuY^{2-}, BiY^{-} and CrY^{-}. Column: Sephadex G-15 (95.0 × 1.5 cm). Eluent: 0.1 M NaCl solution. Sample: 1 ml of a mixture containing 0.025 M each of CuY^{2-}, BiY^{-} and CrY^{-} in 0.1 M NaCl solution. One fraction: 1.00 ml.

By considering the effects of the gel porosity and the results presented previously (the K_d values of the EDTA complexes are less than unity)[2], it appears that the migration of the complexes may be caused mainly by the sieving effect. Yoza et al.[6], however, pointed out that in order to evaluate the contribution of side effects, it is necessary to consider the concentration dependence of the samples.

Effects of sample and eluent concentrations

The concentration dependences of the samples of $Cu^{II}Y^{2-}$, $Co^{II}Y^{2-}$, $Fe^{III}Y^-$ and $Bi^{III}Y^-$ at various eluent concentrations were examined. The sample solutions were eluted with 0.1 M sodium chloride solution, and the effects of sample concentration on the elution curves of $Cu^{II}Y^{2-}$ and $Fe^{III}Y^-$ are illustrated in Figs. 5 and 6, respectively. When the concentration was higher than *ca.* 10^{-2} M $Cu^{II}Y^{2-}$, unsymmetrical elution curves with skewed leading edges (L-shaped) were observed. The elution peak positions increased with an increase in the sample concentration. However, when the sample concentration was lower than *ca.* 10^{-2} M $Cu^{II}Y^{2-}$, the elution curves were almost symmetrical (S-shaped), and the elution peak positions did not change. On the other hand, all of the elution curves of $Fe^{III}Y^-$ at sample concentrations lower than 10^{-1} M were symmetrical, and the elution peak positions did not change. The sample concentration dependences of $Co^{II}Y^{2-}$ and $Bi^{III}Y^-$ with 0.1 M sodium chloride solution as the eluent seemed to be similar to those for $Cu^{II}Y^{2-}$ and $Fe^{III}Y^-$, respectively.
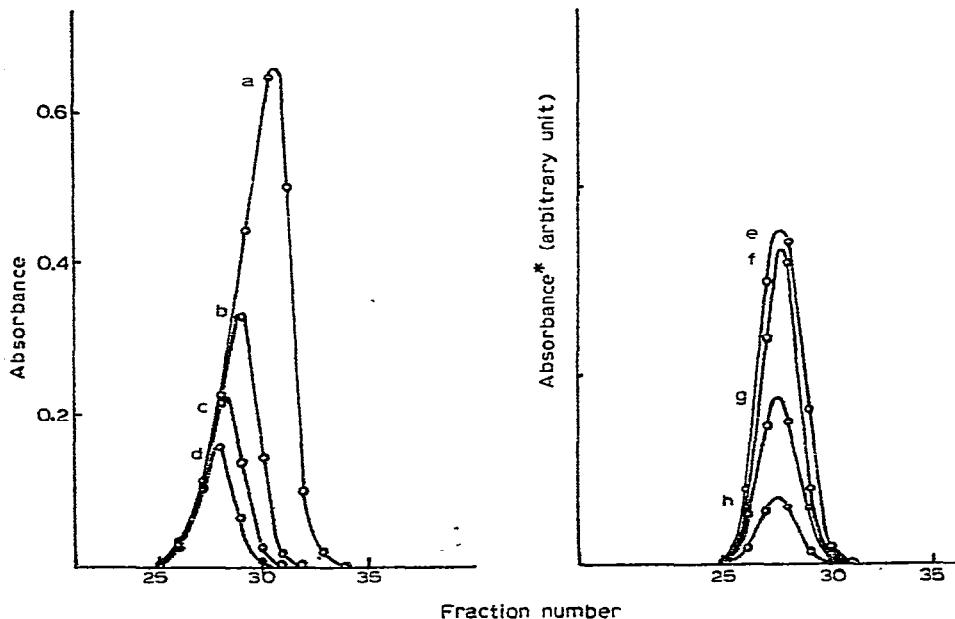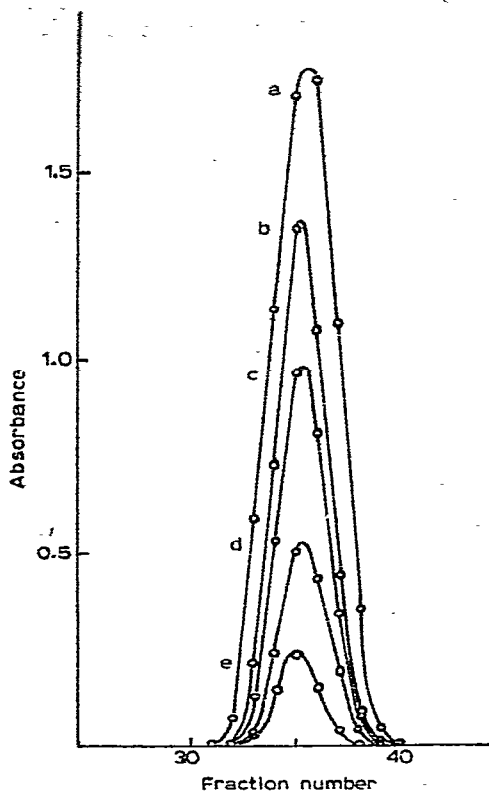

Fig. 5. Concentration dependence of elution curves of CuY^{2-}. Column: Sephadex G-15 (40.8 × 1.5 cm). Eluent: 0.1 M NaCl solution. Sample concentration: a, 10^{-1} M; b, 5×10^{-2} M; c, 2.5×10^{-2} M; d, 10^{-2} M; e, 5×10^{-3} M; f, 10^{-3} M; g, 5×10^{-4} M; h, 10^{-4} M. One fraction: 1.19 ml.
* Atomic-absorption spectrophotometry.

Fig. 6. Concentration dependence of elution curves of FeY^-. Column: Sephadex G-15 (40.8×1.5 cm). Eluent: 0.1 M NaCl solution. Sample concentration: a, 10^{-1} M; b, 5×10^{-2} M; c, 2.5×10^{-2} M; d, 1.25×10^{-2} M; e, 6.2×10^{-3} M. One fraction: 1.20 ml.

Figs. 7–10 show the sample concentration dependences of $Cu^{II}Y^{2-}$, $Co^{II}Y^{2-}$, $Fe^{III}Y^-$ and $Bi^{III}Y^-$ at various eluent concentrations. The V_e value (the elution peak position) of the solute at each sample concentration is plotted on the ordinate ($\log[MY^{n-}]$). The slopes of the V_e versus $\log[MY^{n-}]$ plots suggest that the elution curves can be classified qualitatively as S- or L-shaped when the value of $dV_e/d\log-[MY^{n-}]$ is zero or greater than zero, respectively[6]. As shown in Figs. 9 and 10, at concentrations of the sample higher than the concentration of each eluent, the elution patterns of $Fe^{III}Y^-$ and $Bi^{III}Y^-$ are L-shaped and the V_e values increase with increase in sample concentration. At concentrations of the sample lower than the concentration of each eluent, the patterns are S-shaped and the V_e values do not change. Similar effects have also been reported[6,7] for the concentration dependences of ions such as magnesium, triphosphate and hypophosphate. As shown in Figs. 7 and 8, the elution patterns of $Cu^{II}Y^{2-}$ and $Co^{II}Y^{2-}$ were also similar to those of the MY^- types. However, the V_e values of the MY^{2-} type of complexes seem to become almost constant when the concentration of the sample is less than about one tenth of the eluent concentration. Thus, the chromatographic behaviours of MY^{2-}- and MY^--type complexes are different. The different elution patterns of MY^{2-}- and MY^--type complexes are caused by the difference in the charges of the two types of complexes.
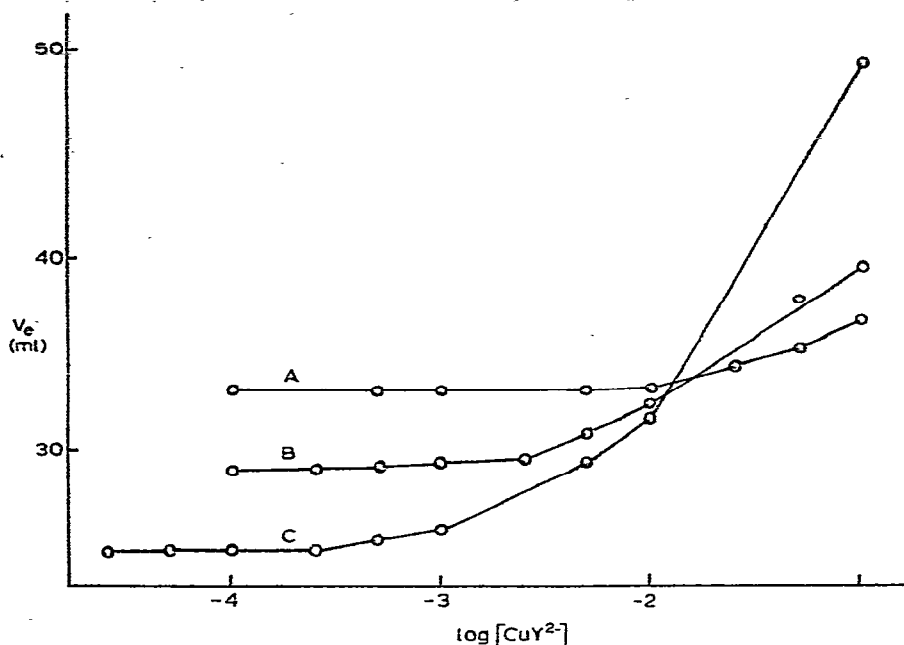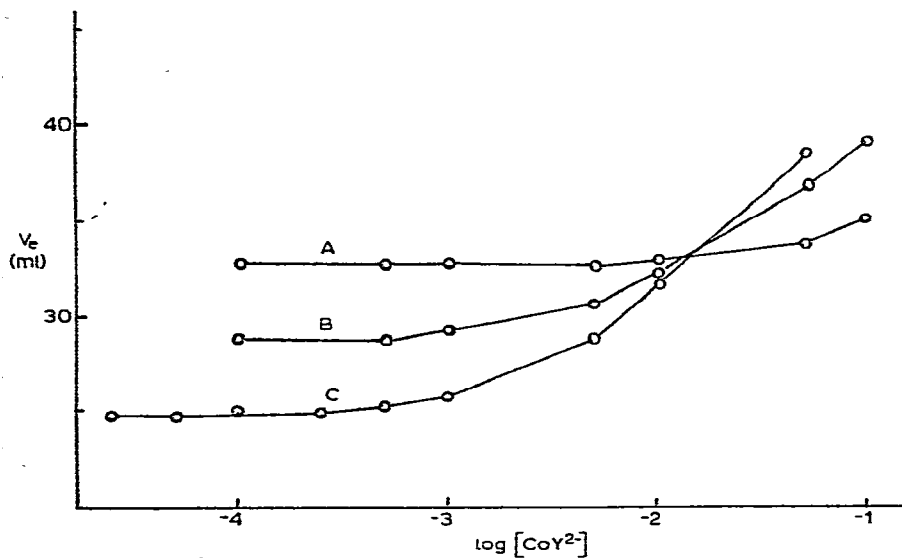
Fig. 7. Sample and eluent concentration dependence of elution volumes of CuY^{2-}. Column: Sephadex G-15 (40.8 × 1.5 cm). NaCl eluent concentration: A, 10^{-1} M; B, 10^{-2} M; C, 10^{-3} M.

Fig. 8. Sample and eluent concentration dependence of elution volumes of CoY^{2-}. Column: Sephadex G-15 (40.8 × 1.5 cm). NaCl eluent concentration: A, 10^{-1} M; B, 10^{-2} M; C, 10^{-3} M.
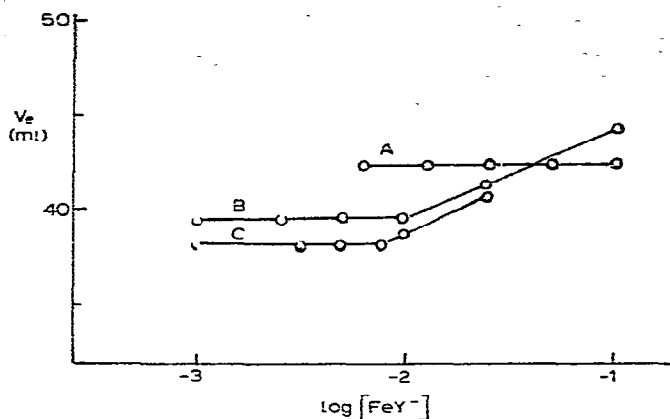
Fig. 9. Sample and eluent concentration dependence of elution volumes of FeY$^-$. Column: Sephadex G-15 (40.8 × 1.5 cm). NaCl eluent concentration: A, 10^{-1} M; B, 10^{-2} M; C, $5 × 10^{-3}$ M.

The MY^{2-}-type complexes were more strongly affected than MY$^-$-type complexes by the gel matrix, that is, the ion exclusion effect with the former may be predominant.

At sample concentrations where the V_e values of the EDTA complexes are maintained constant, these V_e values increase with increase in the eluent concentration (Figs. 7–10). These results indicate that the effective sizes of the EDTA complexes in the media decrease with increase in the eluent concentration. It has been pointed out by Eaker and Porath[13] that the effective size of charged solutes can be decreased substantially by including high concentrations of small electrolytes. This view agrees with our results.

Effect of pH of eluent solutions

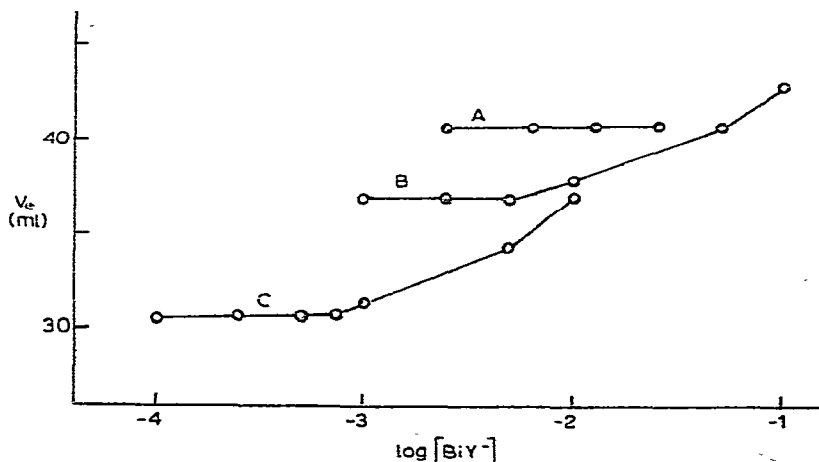As mentioned above, the different behaviours of MY^{2-}- and MY$^-$-type com-

Fig. 10. Sample and eluent concentration dependence of elution volumes of BiY$^-$. Column: Sephadex G-15 (40.8 × 1.5 cm). NaCl eluent concentration: A, 10^{-1} M; B, 10^{-2} M; C, 10^{-3} M.
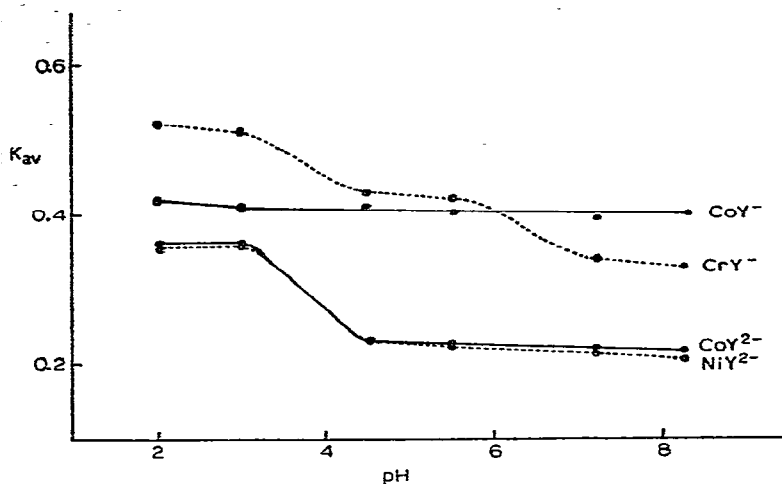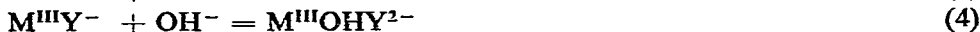
Fig. 11. pH dependence of the K_{av} values of CoY^{2-}, NiY^{2-}, CoY^- and CrY^-. Column: Sephadex G-15 (95.3 × 1.5 cm). Sample: 0.025 M of each complexes. Eluent: sodium chloride solutions were adjusted to a definite pH with hydrochloric acid, acetic acid, sodium acetate or sodium hydroxide (ionic strength 0.1).

plexes may be due to their effective radii of hydration caused by the different charges of the complex anions.

It has been reported that acidic or basic EDTA complexes are formed as follows[14]:

$$M^{II}Y^{2-} + H^+ = M^{II}HY^- \tag{1}$$
$$M^{II}Y^{2-} + OH^- = M^{II}OHY^{3-} \tag{2}$$
$$M^{III}Y^- + H^+ = M^{III}HY \tag{3}$$
$$M^{III}Y^- + OH^- = M^{III}OHY^{2-} \tag{4}$$

These equations show that the charge of the complex changes as the pH of the solution varies. Fig. 11 shows the effect of pH on the K_{av} value of the complex anions. From eqns. 1–4, the radii of acidic and basic EDTA complexes with hydrogen or hydroxide ions would be greater than those of the EDTA complexes themselves. Therefore, in gel chromatography, these effects may lead to a decrease in the K_{av} values. This tendency occurred at the higher pH region in Fig. 11, but the increase in K_{av} values at the lower pH region could not be explained by this concept. Moreover, $Co^{III}Y^-$ shows almost constant K_{av} values over the whole pH range tested.

Fig. 12 shows a plot of the mean charge of each complex anion against pH values. The mean charges were calculated as follows. From eqns. 1–4, the formation constants, K_{MHY}^H and K_{MOHY}^{OH}, of the addition complexes are

$$K_{MHY}^H = \frac{[MHY^{(n-1)-}]}{[MY^{n-}][H^+]} \tag{5}$$

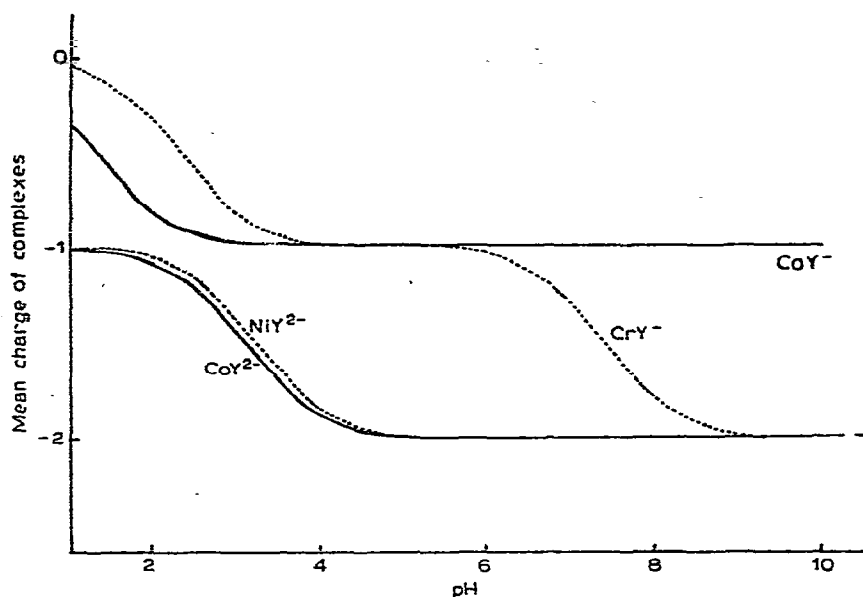$$K_{MOHY}^{OH} = \frac{[MOHY^{(n+1)-}]}{[MY^{n-}][OH^-]} \tag{6}$$

Fig. 12. Mean charges of CoY^{2-}, NiY^{2-}, CoY$^-$ and CrY$^-$ complexes *versus* pH.

If the total concentration of MY^{n-} is C_{MY}, then

$$C_{MY} = [MY^{n-}] + [MHY^{(n-1)-}] + [MOHY^{(n+1)-}] \tag{7}$$

Eqn. 7 is based on the assumption that the additional acidic and basic complexes of MY^{n-} only are formed, and the concentrations of free metal ions in the equilibrium are negligible under the conditions of these experiments. The acidic complexes of Co^{II}Y^{2-}, Ni^{II}Y^{2-}, Cr^{III}Y$^-$ and Co^{III}Y$^-$ are known to occur as MHY^{n-1-}, in the case of Cr^{III}Y$^-$, the basic complex, MOHY^{n+1-}, is also formed[14]. Therefore, eqn. 7 is obtained.

If the function a is defined as the ratio of the total concentration of the EDTA complex, C_{MY}, to the equilibrium concentration of each complex species, then

$$\alpha_{MHY} = \frac{C_{MY}}{[MHY^{(n-1)-}]} \tag{8}$$

$$\alpha_{MY} = \frac{C_{MY}}{[MY^{n-}]} \tag{9}$$

$$\alpha_{MOHY} = \frac{C_{MY}}{[MOHY^{(n+1)-}]} \tag{10}$$

When eqns. 7, 5 and 6 are substituted successively into eqns. 8, 9 or 10, the following equations are obtained:

$$\alpha_{MHY} = 1 + \frac{1}{K_{MHY}^H \cdot [H^+]} + \frac{K_{MOHY}^{OH} \cdot K_w}{K_{MHY}^H \cdot [H^+]^2} \tag{11}$$

$$\alpha_{MY} = 1 + K^H_{MHY} \cdot [H^+] + \frac{K^{OH}_{MOHY} \cdot K_w}{[H^+]} \tag{12}$$

$$\alpha_{MOHY} = 1 + \frac{K_w}{K^{OH}_{MOHY} [H^+]} + \frac{K^H_{MHY} \cdot [H^+]^2}{K^{OH}_{MOHY} \cdot K_w} \tag{13}$$

where K_w is the ionization product of water and the K^H_{MHY} or K^{OH}_{MOHY} values of $Co^{II}Y^{2-}$, $Ni^{II}Y^{2-}$, $Cr^{III}Y^-$ and $Co^{III}Y^-$ from ref. 14 were used. The mean charges, Z_m, of univalent and divalent EDTA complexes are calculated from eqns. 14 and 15, respectively:

$$Z_m(MY^{2-}) = \frac{1}{\alpha_{MHY}}(-1) + \frac{1}{\alpha_{MY}}(-2) + \frac{1}{\alpha_{MOHY}}(-3) \tag{14}$$

$$Z_m(MY^-) = \frac{1}{\alpha_{MY}}(-1) + \frac{1}{\alpha_{MOHY}}(-2) \tag{15}$$

The variations of the curves in Fig. 12 are similar to those of the curves corresponding to the complexes in Fig. 11. Thus the elution volumes are dependent on the pH of the eluent, and the variation of the elution volumes is similar to that of the mean charges of the complexes with pH. For the dependences on the pH of the eluent for compounds such as NaH_2PO_2, Na_2HPO_3 and Na_3PO_4 on the Sephadex G-15 column, analogous phenomena have also been reported[15]. Therefore, it can be concluded that the effective radii of the hydrated complexes increase with increase in the mean charge of the complexes. Even if the mean charges of the different EDTA complexes at appropriate pH regions are equivalent (for example, the mean charges of $Co^{III}Y^-$ and $Cr^{III}Y^-$ at pH 4–6 are equivalent), the observed K_{av} values of the complexes are not equal. This result suggests that the size of the unhydrated complex must be considered.

ACKNOWLEDGEMENTS

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