GEL CHROMATOGRAPHIC BEHAVIOR OF SOME METAL IONS

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SUMMARY

Some metal ions were chromatographed on Sephadex G-15 columns. Solutions of potassium chloride, sodium sulfate, sodium nitrate, and sodium perchlorate were used as eluting agents, *i.e.*, as background electrolytes. It was found that the elution volumes of the metal ions depend very much on the nature of the background electrolytes employed. These phenomena were explained in terms of the penetrating ability of the background electrolytes into the gel phase and direct and indirect interaction between the metal ions and the gel matrix.

INTRODUCTION

Gel chromatography has been developed as an extremely useful tool for the separation of macromolecules and the estimation of their molecular weights. Some recent investigations[1-13] have demonstrated that highly cross-linked gels are useful for the separation of small inorganic ions. Although the molecular-sieve effect due to the size difference of solutes is the main factor controlling the separation mechanism in gel chromatography, side effects such as adsorption[2,11], ion exclusion[5,6] and restricted diffusion[14] of solutes must be considered in some cases, especially for the separation of inorganic ions.

SAUNDERS AND PECSOK[1] have shown that the observed distribution coefficients of strong electrolytes in gel chromatography can be divided into additive cation and anion contributions. EGAN[2], and NEDDERMEYER AND ROGERS[3] have observed the effects of counter ions on the elution volumes of inorganic cations and anions.

The present investigation was undertaken to clarify the effect of the background electrolytes on the gel chromatographic behavior of various metal ions. Solutions of potassium chloride, sodium sulfate, sodium nitrate, and sodium perchlorate were used as eluting agents, *i.e.*, as background electrolytes.

EXPERIMENTAL

Sample solutions

Sample solutions of sodium, potassium, magnesium, calcium, strontium, barium

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manganese(II), cobalt(II), nickel(II), zinc, and cadmium ions were prepared by dissolving their chlorides in a solution of the same composition as that of the eluent used for the column operation. The sulfate of the copper(II) ion was used. The metal concentration of the sample solutions was 0.01 M.

0.1 M solutions of sodium nitrate and sodium perchlorate were prepared by a similar method. Solutions of Blue Dextran 2000 (Pharmacia Fine Chemicals, 0.1–0.2%) and tritiated water (Radiochemical Center) prepared by a similar method were used as standard materials of $K_d = 0$ and 1, respectively.

Eluents

Eluent I: 0.1 M potassium chloride–0.01 M hydrochloric acid solution. Eluent II: 0.1 M sodium sulfate–0.005 M sulfuric acid solution. Eluent III: 0.1 M sodium nitrate–0.01 M nitric acid solution. Eluent IV: 0.1 M sodium perchlorate–0.01 M perchloric acid solution.

Columns

Sephadex G-15 (Pharmacia Fine Chemicals, dry particle size 40–120 μ), which is a cross-linked dextran gel, was used as the bed material. The columns were 1.5 × 60 cm and 1.5 × 90 cm glass tubes (Shoei Glass).

The dry gel powder was suspended in an eluent and allowed to swell for two days. Undesirable fine particles were removed by decantation. The suspension of the swollen gel was deaerated under reduced pressure before use. The gel suspension prepared in this manner was poured into a vertical glass tube which had been partially filled with the eluent. After the gel bed had reached a height of about 5 cm, the outlet at the bottom was opened to allow the eluent to flow at a rate of approximately 30 ml/h. The addition of the gel suspension was continued until the gel bed reached the desired height. Bed volumes were adjusted to 100 or 150 ml. A disk of filter paper was placed on the top of the gel bed to protect the surface from disturbance. After packing, 500 ml of the eluent was passed through the column to settle the gel bed.

Procedure for elution

One milliliter 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. As soon as the solution vanished into the bed, about 4 ml of the eluent were added and elution was started at a constant flow rate of 25–35 ml/h. The column temperature was kept at 20°. The effluent was collected in fractions of 1 ml with a Toyo Kagaku drop count fraction collector. Some fractions were chosen arbitrarily and their volumes were measured so that the fraction volume could be determined.

Concentrations of the samples or the standard substances in the effluents were determined as follows. Sodium ion: measurement of the radioactivity of the ^{22}Na used as a tracer. Potassium ion: flame photometric measurement. Other metal ions: chelatometric titration with EDTA. Chloride ion: argentometric titration. Blue Dextran 2000: measurement of absorbance at 630 mμ. Tritiated water: measurement of the radioactivity with a liquid scintillation counter.

Elution curves for nitrate, perchlorate and ammonium ion were recorded on a Japan Electron Optics liquid chromatograph Model JLC-2A equipped with detection columns packed with Sephadex G-15.

RESULTS AND DISCUSSION

In a previous paper[4] it was demonstrated that the degree of peak skewing of alkaline earth metal ions chromatographed on a Sephadex column decreases on using an acidic eluent containing both sodium chloride and hydrochloric acid. Therefore, acidic solutions of the background electrolytes were used as an eluent in this work. Distribution coefficients and elution orders of the metal ions are summarized in Fig. 1. Some of the elution curves of the metal ions obtained with eluents I, II, III, and IV are illustrated in Figs. 2, 3, 4 and 5, respectively. All of the columns used for the measurements of these elution curves had a bed volume of 100 ml. The distribution coefficient K_d is defined by eqn. 1,

$$V_e = V_0 + K_d \cdot V_i \tag{1}$$

where V_e is the elution volume of a given solute, V_0 the void volume outside the gel particles, and V_i the internal volume within the gel phase.
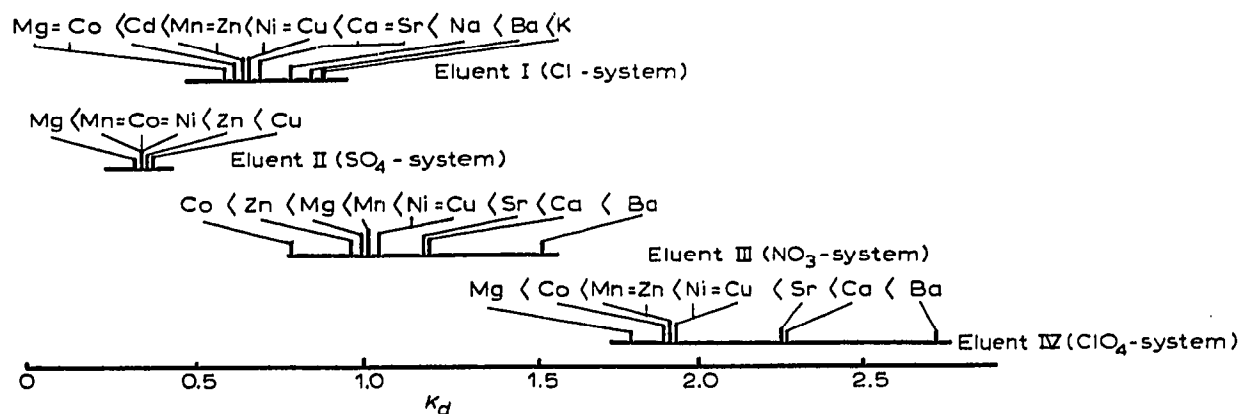

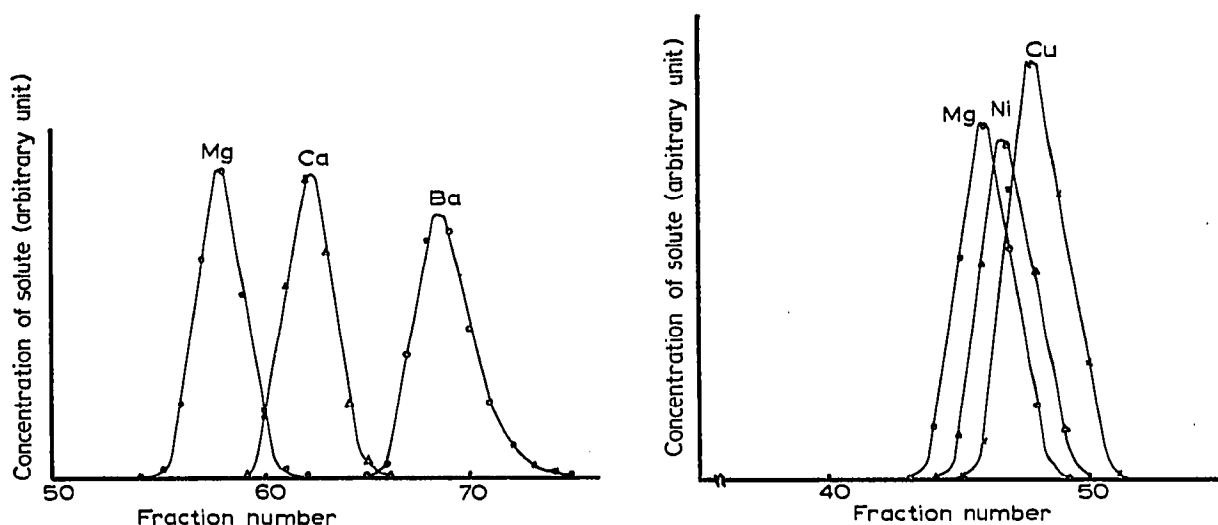Fig. 1. K_d values of metal ions obtained by gel chromatography with eluents I, II, III and IV.

Fig. 2. Chromatograms obtained with eluent I. One fraction: 1.01 ml.

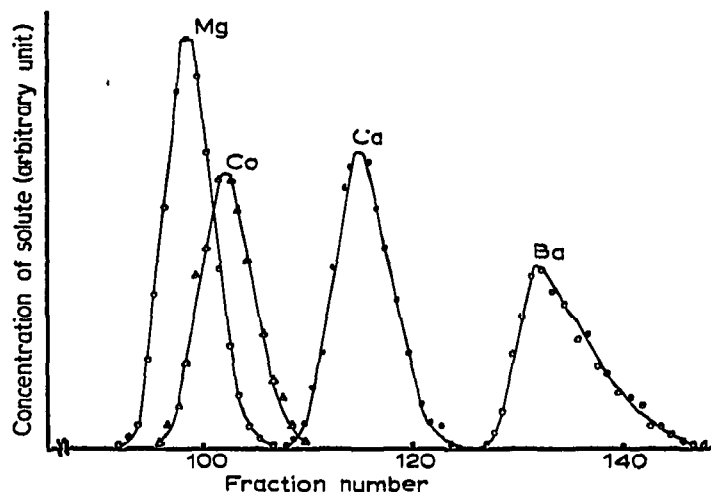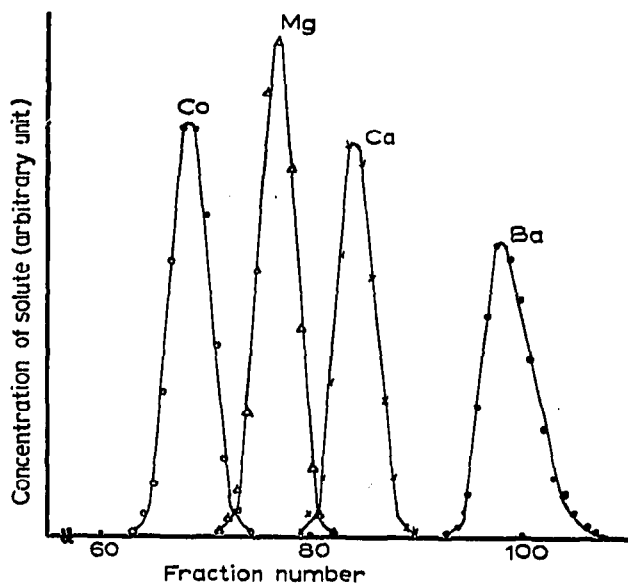Fig. 3. Chromatograms obtained with eluent II. One fraction: 1.08 ml.

Fig. 4. Chromatograms obtained with eluent III. One fraction: 1.01 ml.

Fig. 5. Chromatograms obtained with eluent IV. One fraction: 1.11 ml.

When eluent I was used, all of the metal ions examined were eluted within the volume of $V_0 + V_t$, and their elution curves were symmetrical with the exception of the barium ion. As shown in Fig. 2, of the alkaline earth metal ions, magnesium, calcium and barium ion were separated from each other in this chloride system. However, the strontium ion was eluted at the same position as the calcium ion. When potassium chloride was used as a background electrolyte the elution peak positions of the potassium and chloride ion were determined by adding pure water as a sample and measuring the negative peaks of both the potassium and chloride ion.

When eluent II was used, magnesium, manganese (II), cobalt(II), nickel(II), zinc, and copper(II) ion were eluted very rapidly as compared with the results obtained with eluent I. All of their elution curves were symmetrical. However, as shown in Figs. 1 and 3, the separation of these metal ions was very poor in this sulfate system.

When eluent III was used, all of the metal ions examined were eluted slowly as compared with the results obtained with eluent I. Zinc, magnesium, manganese(II), nickel(II) and copper(II) ions were eluted nearly at the volume of $V_0 + V_t$, and the strontium, calcium, and barium ions were eluted more slowly. All of the elution curves of these metal ions except that of the barium ion were symmetrical. As shown in Fig. 4, the separation of the cobalt(II), magnesium, calcium and barium ions was almost complete in this nitrate system.

When eluent IV was used, all of the metal ions tested were eluted extremely slowly as compared with the results obtained with eluent I. All of the elution curves of the metal ions except that of the barium ion were also symmetrical. As shown in Fig. 5, magnesium, calcium and barium ions were completely separated in this perchlorate system.

In gel chromatography the molecular-sieve effect due to the size difference of solutes is the main factor controlling the separation mechanism. However, sometimes side effects such as adsorption will accompany the molecular-sieve effect. If there are no such side effects, the elution order of the metal ions should not be altered by variation of the composition of background electrolytes. The results obtained by the present work, as shown in Fig. 1, indicate the remarkable dependence of the elution order of the metal ions on the background electrolytes employed.

In the sulfate system the K_d values of the metal ions were very small. These phenomena may be closely connected with the gel chromatographic behavior of sulfate ion. It was found that sulfate ion is eluted faster than any of the metal ions employed in this work from a Sephadex G-15 column with eluent I. This result suggests that sulfate ion may be excluded from the gel to a considerably greater extent than that expected from the radius of its hydrated ion, 3.79 Å, reported by NIGHTINGALE[15]. Although the behavior of sulfate ion on a gel column cannot be explained quite satisfactorily at the present, the following discussion based on the idea of the poly-functional character of the gel phase[4] will be possible. According to this idea the gel phase is divided into two regions, phase I and phase II. A background electrolyte can penetrate into phase II but not into phase I. When sodium sulfate is used as a background electrolyte, the volume of phase I is expected to become much larger than that in the chloride system. Since the metal ions accompanying the sulfate ion as a counter ion cannot penetrate into phase I, the K_d values of the metal ions must be considerably smaller than those obtained in the chloride system.

When eluents III and IV were used, the K_d values of most of the metal ions were larger than unity. These phenomena demonstrated that a side effect such as adsorption may play an important role in these systems. The adsorption effect may be explained in two ways. First, a given metal ion is directly adsorbed on the gel matrix. Second, the metal ion in question is adsorbed by counter ions which are preliminarily adsorbed on the gel matrix. If adsorption of the first type takes place, the similar results should also be observed when eluents I and II are used. The experimental results were contrary to the above assumption. The second type of adsorption seems to be similar to ion exchange. The gel chromatographic behavior of the metal ions in the nitrate and the perchlorate system can be explained by adsorption of this type. Sodium nitrate or perchlorate is adsorbed on the gel matrix and then a part of the sodium ions is exchanged for the metal ions in question.

Nitrate and perchlorate ion were eluted from a Sephadex G-15 column more slowly than the chloride ion when eluent I was employed. Since the radii of hydrated nitrate and perchlorate ions are larger than that of the hydrated chloride ion, the above fact suggests that nitrate and perchlorate ion are strongly adsorbed on the gel matrix. A similar adsorption effect can be seen in the behavior of the borate ion, which is strongly adsorbed on dextran gels[11].

The present authors have found that the K_d value of the magnesium ion obtained by its elution from a Sephadex G-25 column with eluent IV is nearly equal to that obtained on a Sephadex G-15 column. This can be explained by adsorption of the second type. The elution orders of the metal ions excepting the cobalt(II) and zinc ions in both the nitrate and the perchlorate system are very similar to each other. This fact also supports the above explanation.

The skewness of the elution curve of the barium ion can be interpreted in terms

of the interaction of the barium ion with the gel matrix as has been pointed out previously[4].

LAURENT AND KILLANDER[16] have used another distribution coefficient, K_{av}, defined as

$$K_{av} = \frac{V_e - V_0}{V_t - V_0} = K_d \cdot \frac{V_i}{V_i + V_g} \tag{2}$$

where V_t is the total volume of the gel bed, and V_g the volume occupied by the gel matrix. Using an expression derived by OGSTON[17], they give

$$K_{av} = \exp\left[-\pi L(R_s + R_r)^2\right] \tag{3}$$

where L is the length of the gel skeleton per unit volume, and R_s and R_r denote the radii of a given solute and the gel skeleton, respectively. Eqn. 3 can be rewritten as

$$(-\log K_{av})^{1/2} = A \cdot R_s + B \tag{4}$$

where A and B are the constants for a given gel column.

The $(-\log K_{av})^{1/2}$ values of some metal ions obtained with eluent I are plotted against the R_s values of these ions reported by NIGHTINGALE[15] in Fig. 6, which shows a straight line without significant deviation for potassium, ammonium, sodium, nickel and magnesium ion. These metal ions do not form any chlorocomplexes. The K_{av} value for the chloride ion also falls on the same line. The plots for the barium, strontium, and calcium ions, however, do not fall on the straight line in spite of the lower stability constants of their chlorocomplexes. This may be ascribed to the adsorption of these ions on the gel matrix.
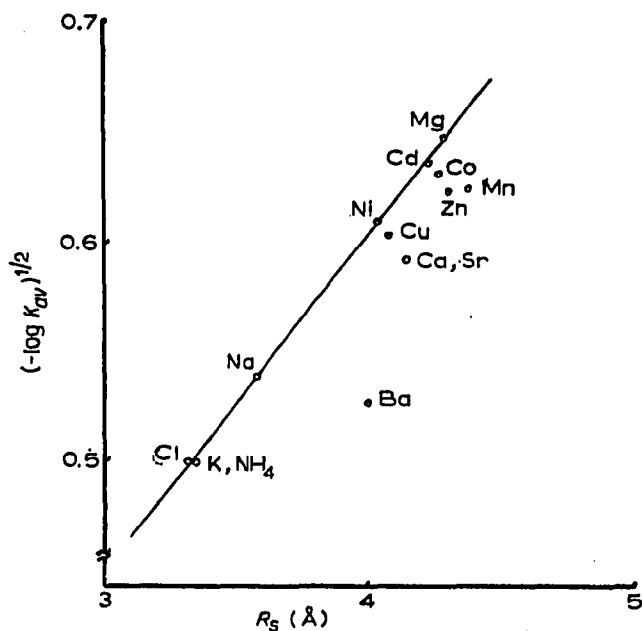

Fig. 6. Relationship between $(-\log K_{av})^{1/2}$ and R_s.

Since the properties of both the gel matrix and the solute ions are affected by the nature of the background electrolyte, it is necessary to satisfy the following requirements if one wants to correlate gel chromatographic data for sample ions with the sizes of their hydrated ions. (1) There are no side effects such as adsorption in the gel chromatographic process. (2) The background electrolyte can penetrate into the gel phase to a larger extent than the sample ions. (3) Both the cation and anion of the background electrolyte are not adsorbed on the gel matrix. (4) The sample ions do not form any complexes with counter ions of the background electrolyte.

Taking into consideration the above requirements it can be suggested that potassium chloride will be an excellent background electrolyte for gel chromatographic studies on various ions.

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