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THE EFFECT OF SAMPLE CONCENTRATION ON THE GEL CHROMATOGRAPHIC BEHAVIOR OF ALKALINE EARTH METAL IONS ON SEPHADEX COLUMNS

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SUMMARY

Magnesium, strontium and barium ions were chromatographed on a Sephadex G-15 column with a 0.1 *M* sodium chloride solution as an eluent. The shapes of elution curves and K_d values were found dependent on sample concentrations. This was explained in terms of the interaction of the solutes with the gel matrix and the variation in effective sizes of the solutes with the variation in ionic strength of the surrounding medium. The general features of the concentration dependence were discussed on the basis of two representative types of ion, the Mg-type and the Sr- or Ba-type. The former type is influenced by only a negligibly slight adsorption effect over a wide range of sample concentrations, while the latter type is markedly influenced by the adsorption effect, especially at lower sample concentrations.

INTRODUCTION

It has been demonstrated that gel chromatography on Sephadex columns is a useful technique for the investigation of inorganic compounds such as polyphosphates[1-6], polymeric ferric hydroxides[7,8], hydrated metal ions[2,6,9-14] and metal complexes[10,14]. The basic principle of this method has been explained in terms of steric exclusion or a sieving effect in the gel phase[15]. Larger molecules can penetrate into a smaller fraction of the internal volume of the gel phase and therefore are eluted earlier than smaller molecules.

However, the acceptance of exclusion principle alone as an explanation for the separation mechanism does not interpret all phenomena which are observed during elutions of inorganic compounds on columns packed with highly cross-linked Sephadex[2,9,11,12]. Other side effects resulting from the interaction of solutes with the gel matrix must be considered in some cases. The present work was undertaken to observe the sample-concentration dependence of elution curves of alkaline earth metal ions and followed by evaluation of the contribution of such side effects as adsorption to their chromatographic behavior. Magnesium, strontium and barium ions were chromatographed on a Sephadex G-15 column with a 0.1 *M* sodium chloride solution as eluent. The effect of sample concentrations on the chromatographic behavior was explained in terms of the interaction of the metal ions with the gel matrix and the variation in the effective sizes of the solutes with the ionic strength of the media. The extent of the contribution of adsorption was found to be dependent on both sample concentration and the type of metal ion. Magnesium ions are influenced by only a negligibly slight adsorption effect over a wide range of sample concentrations, while strontium and barium ions are markedly influenced by the adsorption effect, especially at lower sample concentrations. The general aspects can be discussed on the basis of two representative types, the Mg-type and the Sr- or Ba-type, which are different in their affinity for the gel matrix.

EXPERIMENTAL

Sample solutions

All reagents used were of guaranteed grade from Wako Chemicals, unless otherwise stated. Sample solutions for magnesium, strontium, barium and EDTA ions were prepared by dissolving magnesium chloride, strontium chloride, barium chloride and disodium dihydrogen ethylenediaminetetraacetate in 0.1 *M* sodium chloride solution, respectively. Barium-133 available from the Radiochemical Center (Amersham, Great-Britain) was used as a tracer for barium ions.

Two standard solutions of Blue Dextran 2000 (Pharmacia Fine Chemicals 0.2%) and tritiated water (Radiochemical Center, used as a tracer) were also prepared by a similar method.

Eluent

The eluent used was 0.1 *M* sodium chloride solution.

Preparation of Sephadex columns

Sephadex G-15 (Pharmacia Fine Chemicals, particle size 40–120 μ) was suspended in the solution to be used as eluent and allowed to swell for 2 days. Undesirable fine particles were eliminated by repeated treatments *viz.* suspension, settling and decantation. A slurry of the prepared gel was poured into a column consisting of a glass tube, 1.5 × 60 cm, with a porous polystyrene disc at the bottom. A third of the column volume should be filled with the eluent before pouring the gel. After the gel had settled by allowing 500 ml of the eluent to flow through the column, excess gel above the desired height of the gel was removed, and a disc of filter paper (Tokyo Roshi 5 B) was placed on the top of the bed as a stabilizer. About 30 ml of a 0.01 *M* EDTA solution (disodium dihydrogen ethylenediaminetetraacetate in the eluent) was passed through the column to remove adsorbed impurities, and the bed was then washed again with 100 ml of the eluent before use. The total volume of the gel bed was adjusted to be 100 ml.

Procedure

One milliliter of the sample solution or standard solution was placed on the gel bed just as the last layer of the eluent soaked into the bed. Then the eluent was applied when the last portion of the sample solution vanished into the bed. The effluent was collected in fractions of 1.02 ml for magnesium, barium and EDTA ions and of 1.09 ml for strontium ions, at a flow rate of 20–30 ml/h maintained by hydrostatic pressure, with a Toyo Kagaku Drop Count fraction collector.

Unless otherwise stated all elutions were carried out at 20 ± 1°.

The amounts of solute in the fractions were determined as follows:

Blue Dextran. Measurement of absorbance at 620 mμ.

Alkaline earth metals. Complexometric titration with an EDTA solution, with the exception of the colorimetric determination[16] of magnesium at lower concentrations.

EDTA. Complexometric titration with a magnesium chloride solution.

Barium-133. Radioactivity measurement with a G. M. counter.

Tritium. Radioactivity measurement with a liquid scintillation counter.

Calculation of K_d values

The distribution coefficient, K_d, was calculated according to eqn. 1 (see also ref. 12),

$$K_d = \frac{V_e - V_0}{V_i} = \frac{V_e - V_e^{\mathrm{BD}}}{V_e^{\mathrm{THO}} - V_e^{\mathrm{BD}}} \tag{1}$$

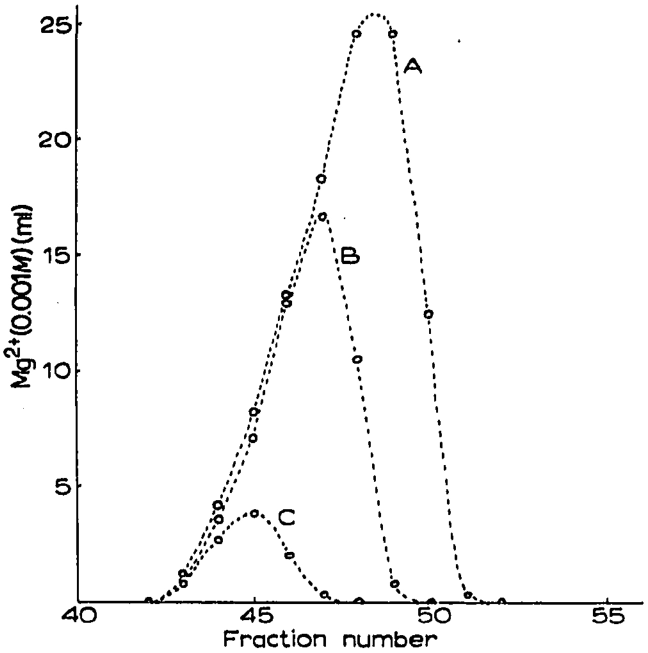
where V_0 is the void volume outside the gel particles, V_i the internal volume within the gel particles and V_e the elution volume of the sample corresponding to the maximum concentration of the elution peak. V_e^{BD} and V_e^{THO} represent the elution volumes of Blue Dextran (molecular weight = 2,000,000) and tritiated water, which were used as standard materials of K_d = 0 and 1, respectively.

RESULTS

In the course of a study[10] concerning the gel chromatographic behavior of magnesium and EDTA ions the present authors noticed that the elution patterns of magnesium and EDTA ions are dependent on the sample concentrations. Magnesium and EDTA ions gave symmetrical elution curves when 0.01 *M* sample solutions were eluted individually on a Sephadex G-15 column at room temperature (about 28°) with a 0.1 *M* sodium chloride solution. The K_d values were 0.62 and 0.22 for magnesium and EDTA ions, respectively. With increasing sample concentrations, however, the peak skewness for both ions became more marked and their elution volumes increased. An example is shown in Fig. 1 for EDTA ions.

It was also pointed out in a previous paper[12] that the chromatographic behavior of magnesium ions was different from that of calcium, strontium and barium ions. The appearance of the unsymmetrical elution curves for the latter three ions was explained in terms of the interaction of the solutes with the gel matrix. Therefore, magnesium, strontium and barium ions seem to be suitable samples for characterizing the interaction of the solutes with the gel matrix. Calcium is not included in this work, because it showed exceptionally marked peak skewing which could not be explained satisfactorily.

As shown in Fig. 2, the elution curve for magnesium ions at 0.01 *M* is symmetrical (S-shape). However, the K_d values increase with the increase of sample concentration. The appearance of unsymmetrical elution curves with sharp back edges (leading or L-shaped) is consistent with the preliminary observation for magnesium and EDTA ions described above. At extremely high sample concentrations shoulders



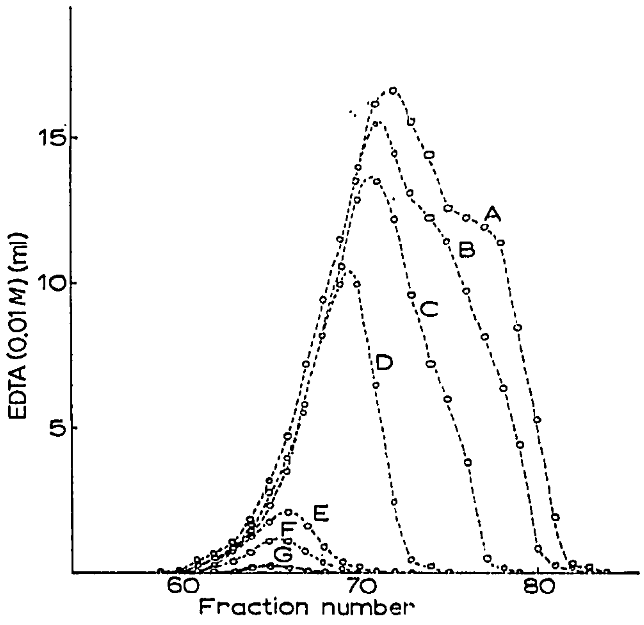


Fig. 1. Concentration dependence of elution curves of EDTA ions. Sample concentrations: A = 10^{-1} *M*; B = 5×10^{-2} *M*; C = 10^{-2} *M*.

Fig. 2. Concentration dependence of elution curves of magnesium ions. Sample concentrations: A = 2 *M*; B = 1.5 *M*; C = 1 *M*; D = 5×10^{-1} *M*; E = 10^{-1} *M*; F = 5×10^{-2} *M*; G = 10^{-2} *M*.

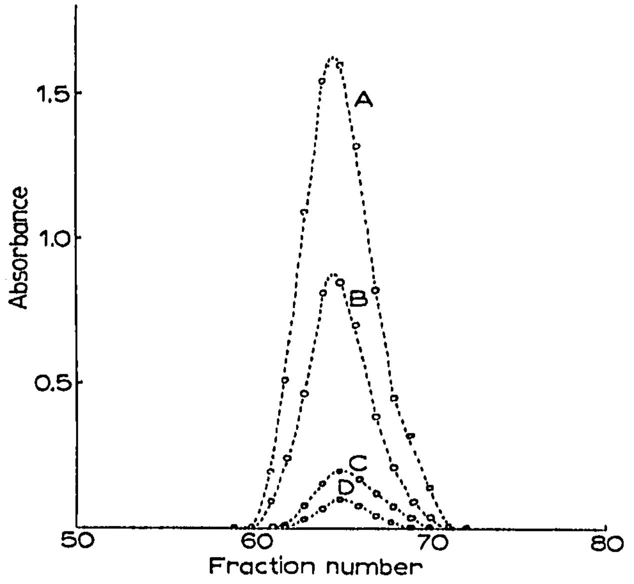


Fig. 3. Concentration dependence of elution curves of magnesium ions. Sample concentrations: A = 10^{-2} *M*; B = 5×10^{-3} *M*; C = 10^{-3} *M*; D = 5×10^{-4} *M*.

appear on the descending sides of the elution curves. On the other hand all elution curves at lower sample concentrations than 0.01 *M* are symmetrical and their elution positions remained unchanged in spite of the considerable variation in sample concentrations (Fig. 3).

The variation in the elution patterns for strontium and barium ions with sample concentrations is more complicated by the appearance of unsymmetrical elution curves with sharp front edges (tailing or T-shaped) at lower sample concentrations. As shown in Figs. 4 and 5 the elution curves for strontium ions belong to the T-shape in the concentration range of 10^{-1}–10^{-3} M and their K_d values increase with the decrease of sample concentrations. At higher sample concentrations than 0.1 M, however, the K_d values increase with the increase of sample concentrations, which is similar to the behavior of magnesium ions at sample concentrations higher than 0.01 M. Figs. 6 and 7 show the concentration dependence of barium ions. The experiments for barium ions in Fig. 7 were carried out using equal amounts of radioactive Ba-133 for each run. The general trend of variation in the elution patterns of barium ions is identical with that of strontium ions over a wide range of sample concentrations. However, the degree of variation in the K_d values of strontium ions at lower sample concentrations is somewhat less pronounced than that of barium ions. The elution curves for barium ions at higher sample concentrations appear as a whole to be composed of the T-shape. However, it becomes evident from quantitative analysis of the elution curves that such T-shape elution curves arise from the combined contributions of both T- and L-shapes. The front-to-back ratio[2], which is defined as the ratio of the front portion to the back portion of the peak width should be unity for a symmetrical elution peak, greater than unity for an L-shape elution peak and smaller than unity for a T-shaped elution peak. The front-to-back ratio of curve A in Fig. 6 is greater than unity for the top portion of the peak but, on the other hand is, smaller than unity for the lower portion.

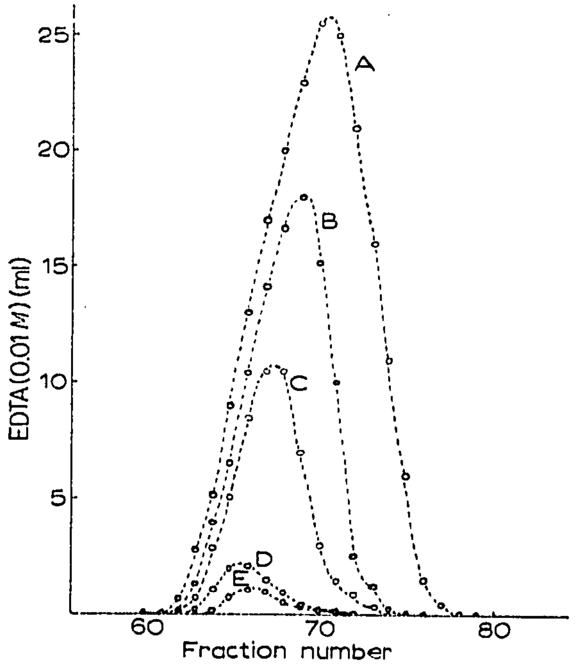


Fig. 4. Concentration dependence of elution curves of strontium ions. Sample concentrations: A = 2 M; B = 1 M; C = 5×10^{-1} M; D = 10^{-1} M; E = 5×10^{-2} M.

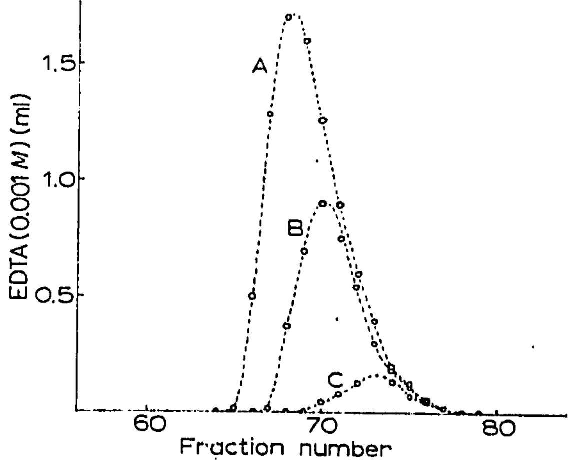
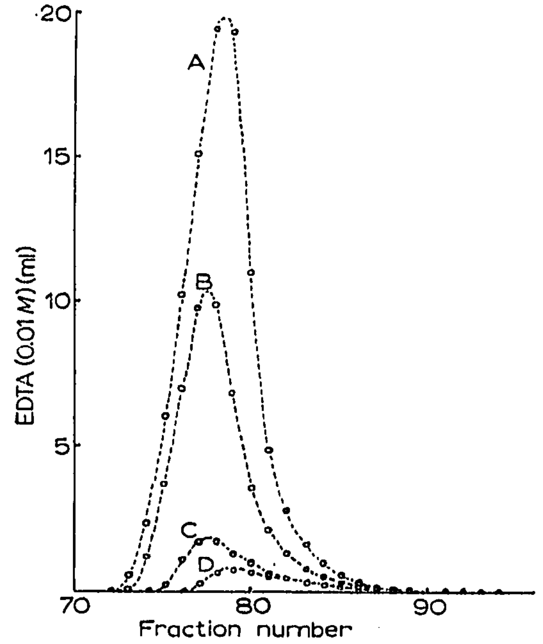


Fig. 5. Concentration dependence of elution curves of strontium ions. Sample concentrations: A = 10^{-2} M; B = 5×10^{-3} M; C = 10^{-3} M.



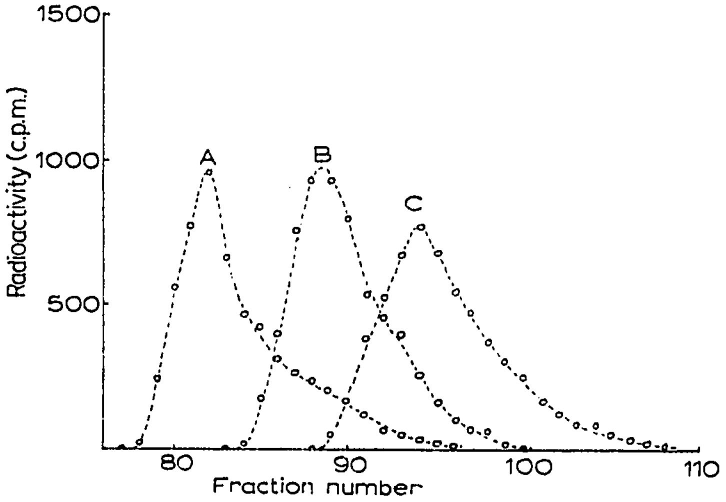


Fig. 6. Concentration dependence of elution curves of barium ions. Sample concentrations: A = 1 M; B = 5×10^{-1} M; C = 10^{-1} M; D = 5×10^{-2} M.

Fig. 7. Concentration dependence of elution curves of barium ions. Sample concentrations: A = 10^{-2} M; B = 10^{-3} M; C = 10^{-4} M.

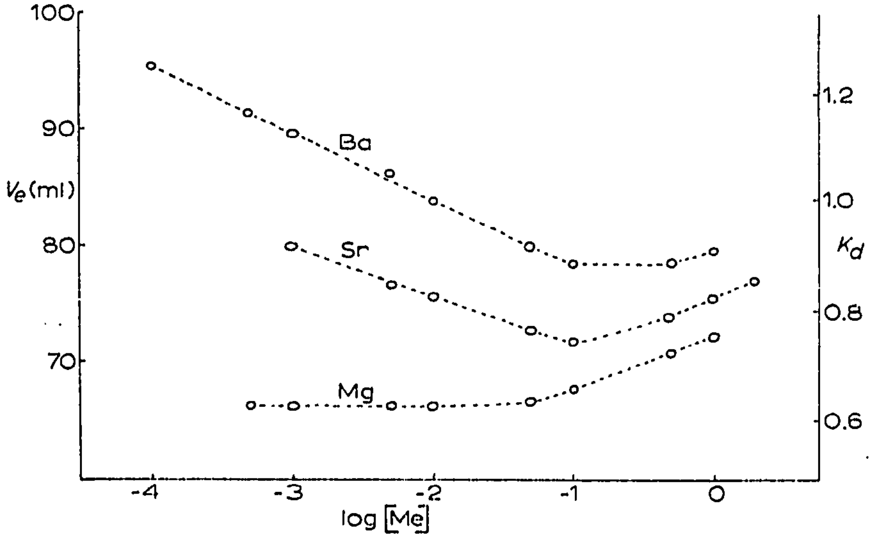


Fig. 8. Concentration dependence of the elution volumes or K_d values of magnesium, strontium and barium ions.

Therefore it is concluded that, although the peak skewing is still observed to a considerable extent, over the lower portion of the descending sides of the curves, even at relatively high sample concentrations, the top portion of the curve is primarily controlled by the effect which promotes the formation of the L-shape.

The overall picture for the three metals can be seen in Fig. 8, where the elution volumes were plotted against the logarithms of the concentrations of the metal ion, [Me]. Barium ions at very low concentrations are eluted at the positions greater than the total liquid volume of the bed ($V_i + V_0 = 84$ ml). The slopes of V_e–log[Me] plots in Fig. 8 can be qualitatively correlated with the shapes of the corresponding elution curves in the following way:

$$\frac{dV_e}{d \log [\text{Me}]} = 0; \text{ S-shape}$$

$$\frac{dV_e}{d \log [\text{Me}]} < 0; \text{ T-shape}$$

$$\frac{dV_e}{d \log [\text{Me}]} > 0; \text{ L-shape}$$

Thus, the chromatographic behavior of magnesium ions can be described in terms of the combination of the S-shape and the L-shape, while strontium and barium ions are combinations of the T-shape and L-shape. It is a characteristic of strontium and barium ions that the minima appear in the intermediate region where two conflicting factors counteract to a comparable extent.

DISCUSSION

In most cases the gel chromatographic behavior of inorganic solutes can be explained in terms of the sieving effect based on their ionic sizes. A number of examples, however, are known which cannot be interpreted satisfactorily by the sieving effect alone. The possible side effects which may affect the chromatographic behavior of solute ions are classified into two categories. The first is ion exclusion which results in the faster elution of an ionic solute than that which could be expected from its ionic size. This has been successfully interpreted[2,11] in terms of the Donnan exclusion principle due to the presence of small amounts of negative charge (carboxylate ions) fixed on the Sephadex gel. Such phenomenon, however, can be eliminated by use of a suitable eluent containing a background electrolyte such as sodium chloride employed in this work. Secondly are the factors which cause the retardation of the solute on a column for a longer period than that which would be assumed from its ionic size. Such factors may be: (1) Coulombic interaction between the ionic solute and the fixed charge in the gel phase (ion exchange), and (2) chemical or physical adsorption of the solute on to the gel matrix (adsorption). Such interactions of the solute with the gel matrix may play an important role in determining the chromatographic behavior of the alkaline earth metal ions. Thus it is necessary to take into consideration the contribution of more than one factor in order to characterize the experimental results described above.

If only the sieving effect is operative and the effective size of the solute to be eluted is independent of the sample concentration one can expect the following experimental results: (a) The elution curve is symmetrical, corresponding to a linear isotherm. (b) The K_d value is independent of the sample concentration and always satisfies the relationship $0 \leqq K_d \leqq 1$. Most of the elution curves of the alkaline earth metal ions appear in the range of $0 \leqq K_d \leqq 1$ but it does not necessarily mean the absence of the side effects. The contribution of the side effects can be estimated from the variation in both the K_d values and the shapes of elution curves. Since, as shown in Fig. 8, the general picture of the elution patterns varies greatly at concentrations near or around 0.1 M, the effect of sample concentrations will be discussed separately in the two sections concerning lower and higher sample concentrations below.

The effect at lower sample concentrations

Magnesium ions give the elution pattern expected from the contribution of the sieving effect alone. The effective size of the magnesium ions probably does not vary with sample concentration, because the total ionic strength of the medium is almost controlled by the contribution due to sodium chloride (0.1 M) in the eluent.

It is of interest that strontium and barium ions at lower sample concentrations give T-shaped elution curves and their elution volumes vary inversely with sample concentration. Such T-shape elution curves have more commonly been observed in adsorption chromatography[17] and accepted as evidence of a nonlinear isotherm of the convex type which can be related to the faster migration of the higher concentration portion of a solute band on the column. It therefore seems reasonable to discuss the gel chromatographic behavior of strontium and barium ions in terms of the interaction of the solutes with the gel matrix.

It has been reported[2,18] that, when pure water with no background electolyte was used as an eluent, the K_d values of inorganic electrolytes such as sodium chloride and calcium chloride were relatively small and increased with the increase of sample concentration, and moreover all the elution curves observed were unsymmetrical and L-shaped. From the additional fact that the elution patterns of uncharged substances such as glucose are not significantly dependent on sample concentrations, such a phenomenon was ascribed to the Donnan exclusion, resulting from the presence of negatively charged groups in the gel phase which exhibit a tendency to exclude anions from the gel phase, especially when small amounts of samples are applied to the gel bed. It is evident that the concentration dependence of strontium and barium ions in Figs. 5 and 7 is in marked contrast with the above observation. It can be ascribed to the difference in ionic strengths of the eluents used in both experiments. Since the eluent used in this work contains a sufficient amount of background electrolyte (0.1 M NaCl) to depress the action of the ion exchange sites, the variation in the K_d values of strontium and barium ions with sample concentrations cannot be ascribed to coulombic interaction between the solute ions and the gel matrix alone. Therefore, another mechanism of solute–gel interaction is required to explain the retardation of strontium and barium ions on the Sephadex column.

Some workers[18,19] have described a number of observations concerning the striking affinity of the dextran gel for aromatic compounds. Such abnormal adsorption of aromatic compounds has been explained in terms of the π-electron interaction between the solute molecules and the gel matrix. Of the inorganic oxo-acids hitherto investigated in our laboratory sodium salts of various oxo-acids of phosphorus[6] did not show any noticeable affinity for the gel matrix, showing a concentration dependence similar to that of magnesium ions. On the other hand molybdate and molybdophosphate[20] were markedly adsorbed on the Sephadex column. Thus, since the retardation of the solutes on the Sephadex column takes place not only for cations but also for anions, the authors feel that such solute–gel interaction may not be based on the coulombic force in the main but on less electrostatic forces such as hydrogen bonding which is greatly dependent on the chemical structure of the solute. The hydroxy groups in the structures and the polarized water molecules in the hydration spheres of both solutes and Sephadex gel may play an important role in such an interaction. However, it is difficult at present to explain the difference between the adsorptive properties of such simple hydrated ions as alkaline earth metal ions. Therefore, it may be worthwhile to study the correlation between chemical structures and adsorptive properties of more complex inorganic compounds, such as has been discussed for aromatic compounds. The difference between elution behavior of phosphate and that of molybdate and molybdophosphate seems to be an interesting example to be characterized in the future.

The effect at higher sample concentrations

When sample concentrations became comparable with or larger than that of the eluent, the effect which promotes the formation of the L-shape (corresponding to a non-linear isotherm of the concave type) became predominant and the K_d values of the alkaline earth metal ions increased with sample concentration. Such a trend was also observed for anions such as EDTA, triphosphate and hypophosphate[6]. The general picture at higher concentrations is opposite to that at lower concentrations. Since the limiting K_d value or maximum K_d value at extremely high concentrations approaches but does not exceed unity, a primary contribution due to the sieving effect seems to be probable. It has been assumed[11,18] that there is a layer of immobile water molecules that firmly hydrate the gel matrix and are no longer able to act as a solvent available to ionic solutes. If the structure of the hydration layer breaks owing to the increase in ionic strength of the surrounding medium the effective internal volume available to the solutes becomes larger with sample concentration, which will consequently result in an increase in K_d values.

Another possibility is the variation in the effective sizes of the hydrated metal ions. It has also been assumed by EAKER AND PORATH[18] that the thickness of the hydration layer of an ionic solute or the effective size is dependent on the ionic strength of the medium. The fact that the extent of the variation in the K_d values of the alkaline earth metal ions decreases in parallel with the decreasing order of their sizes[21], Mg > Sr > Ba, can be explained on the basis of an additional assumption that the larger the thickness of the hydration layer of the metal ion, the greater the extent of the structure-breaking taking place.

It seems to be less probable that an alteration in the gel phase is a main factor. An increment, ΔV_i, in the internal volume may lead to an increment, $\Delta V_i \cdot K_d$, in the elution volume of a given metal ion. Therefore, the elution volume of a smaller metal ion might be expected to vary to a greater extent compared with that of a larger metal ion. However, no positive evidence for such an assumption can be seen in Fig. 8.

The appearance of the shoulders in Fig. 2 is probably attributable to the presence of multicomponents in solution. The formation of a magnesium chlorocomplex such as $[MgCl_n]^{2-n}$ seems to be likely, though little information is available as to the chemical state of magnesium in such a concentrated solution. The effective size of the magnesium chlorocomplex is assumed to be smaller than that of the corresponding hydrated ion, owing to the structure-breaking action of the ligands on the hydration layer.

It is concluded that sample concentration greatly affects the K_d values and the shape of the elution curves and that such concentration dependence can be classified into two representative types, the Mg-type and the Sr- or Ba-type, which differ in their affinity for the gel matrix .

Thus, if one tries to make a calibration curve between elution volumes and ionic sizes it is at first necessary to observe the sample-concentration dependence and to evaluate the contribution of side effects. The employment of an elution volume such as was obtained under the conditions corresponding to the region with negative slope in Fig. 8 should be avoided. A desirable criterion for such a purpose is that the elution curve is symmetrical and the slope in the V_e–log [Me] plot is zero.

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