CHROM. 5297

THE EFFECT OF ANIONS ON THE GEL CHROMATOGRAPHIC BEHAVIOR OF MAGNESIUM IONS

TAKAYUKI OGATA, NORIMASA YOZA AND SHIGERU OHASHI
Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka (Japan)
(Received February 1st, 1971)

SUMMARY

Magnesium ions were chromatographed on a Sephadex G-15 column using sodium chloride, sodium sulfate, sodium nitrate, sodium perchlorate and sodium triphosphate as eluting agents. In order to evaluate the solute–gel interaction and then to estimate the contribution of the anions, the variation in elution curves of magnesium ions with sample concentrations was observed in various eluent systems. It was concluded that the elution volume of the magnesium ions is dependent on the sizes, the complexing ability and the adsorptive property of the anions in the elution system.

INTRODUCTION

Gel chromatography is a technique for separating solute molecules according to their sizes in solution. Larger solute molecules are eluted earlier than smaller solute molecules. In most cases the gel chromatographic behavior of inorganic compounds on a Sephadex column has been explained in terms of steric exclusion or a sieving effect in the gel phase[1–11]. However, it has also been reported that side effects arising from solute–gel interactions, such as ion exclusion (Donnan effect), ion exchange and adsorption, play an important role in some cases[11–16].

In addition to the solute–gel interaction the solute–solute interaction between solute molecules and the eluting agent may affect the chromatographic behavior of the solute in question. Some investigators[2,13,16] have demonstrated that the elution behavior of metal ions was greatly dependent on the chemical form of the samples and the kind of eluting agent. However, the interpretation of the mechanism of such a solute–solute interaction is still inadequate. This work was undertaken to investigate the effect of the anion on the gel chromatographic behavior of magnesium ions on a Sephadex G-15 column using sodium chloride, sodium sulfate, sodium nitrate, sodium perchlorate and sodium triphosphate as eluting agents. In order to evaluate the solute–gel interaction and then to estimate the contribution of the anion, the variation in elution curves of the magnesium ions with sample concentration was observed in various eluent systems. It was concluded that the elution volume of magnesium ions is dependent on the size, the complexing ability and the adsorptive property of the anions in the elution system.

EXPERIMENTAL

Unless otherwise stated, all experiments were carried out according to our previous paper[15]. It should be noted that the total bed volume of a Sephadex G-15 column was adjusted so that it was 100 ml and the volume of each fraction was 0.98 ml.

Determination of triphosphate

Triphosphate was determined colorimetrically with an Mo(V)–Mo(VI) reagent[6].

Determination of magnesium

Magnesium ions were determined by the method of MANN AND YOE[17] with some modification. To each fraction 2.0 ml of borate buffer (0.08 *M*) and 6.0 ml of deionized water were added. Then 5.0 ml of Dotite XB-1 reagent (150 mg in 1 l of 95 % ethyl alcohol) was added and the total volume was adjusted to 25 ml with 95 % ethyl alcohol. The absorbance at 510 mμ was measured.

In case of the presence of triphosphate the effluent in each fraction was adjusted to pH 4.2 with 0.01 *M* hydrochloric acid and the total volume was adjusted to 10 ml with deionized water. 1 g of dry ion-exchange resin (Dowex 1 X 8, Cl^--form, 100–200 mesh)was added to remove triphosphate ions by ion-exchange adsorption. The magnesium was then determined according to the procedure mentioned above.

RESULTS

Preliminary experiment

Fig. 1 shows the elution pattern of magnesium chloride (0.01–2.0 *M*) eluted on a Sephadex G-15 column with a 0.1 *M* sodium sulfate solution. At lower sample concentrations symmetrical elution curves appear and their elution volumes are smaller than those of magnesium ions eluted with 0.1 *M* sodium chloride solution[15]. With increasing sample concentration, however, the peak skewness becomes remarkable and the elution band spreads irregularly to give a complicated elution curve with

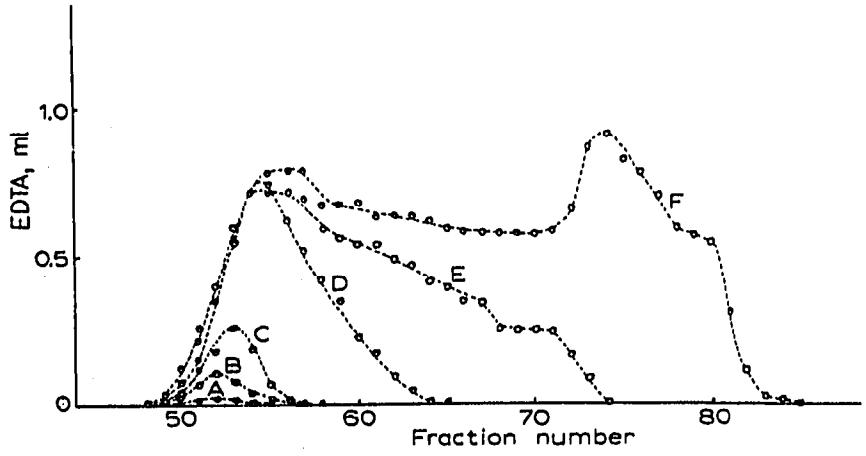

Fig. 1. Concentration dependence of the elution curves in the $MgCl_2$–Na_2SO_4 system. Eluent: 0.1 *M* Na_2SO_4. Concentrations of sample ($MgCl_2$): A = 0.01 *M*; B = 0.05 *M*; C = 0.1 *M*; D = 0.5 *M*; E = 1.0 *M*; F = 2.0 *M*.

a plateau. At extremely high concentration, 2.0 *M*, a secondary peak appears in a position corresponding to the elution volume of magnesium ions eluted with a 0.1 *M* sodium chloride solution.

The above fact suggests that not only the chloride ions in the sample solution but also the sulfate ions in the eluent play an important role in determining the gel chromatographic behavior of magnesium ions. In order to characterize this effect due to the anions four "homogeneous" sample–eluent systems were investigated, *viz.*, $MgCl_2$–NaCl, $MgSO_4$–Na_2SO_4, $Mg(ClO_4)_2$–$NaClO_4$ and $Mg(NO_3)_2$–$NaNO_3$ systems. In such homogeneous systems the anions of both the sample and the eluent are common. In addition, two "heterogeneous" systems, $MgCl_2$–(NaCl + Na_2SO_4) and $MgCl_2$–(NaCl + $NaClO_4$), were examined. The heterogeneous systems contain more than one kind of anion.

The homogeneous $MgCl_2$–NaCl system

This system has already been discussed in our previous paper[15].

The homogeneous $MgSO_4$–Na_2SO_4 system

Fig. 2 shows the elution behavior of magnesium sulfate eluted with a 0.1 *M* sodium sulfate solution. The elution volumes of the magnesium ions in this system are smaller than those in the $MgCl_2$–NaCl system and the general features of the variation in the elution curves with sample concentration is identical to that at lower sample concentrations in Fig. 1.

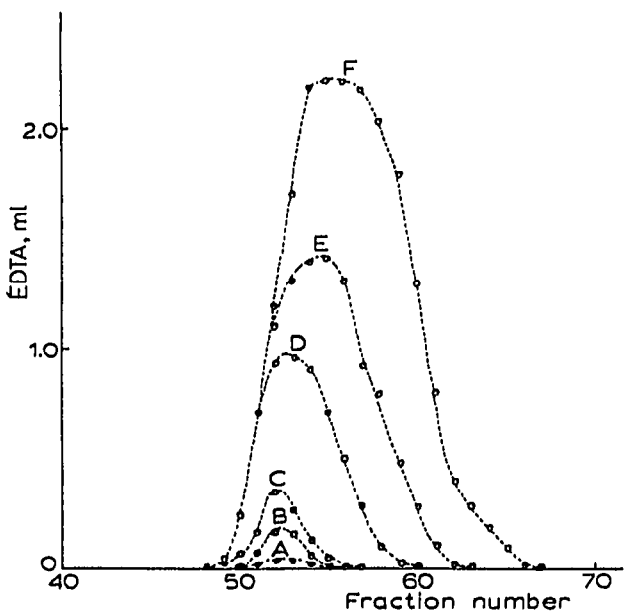

Fig. 2. Concentration dependence of the elution curves in the $MgSO_4$–Na_2SO_4 system. Eluent: 0.1 *M* Na_2SO_4. Concentrations of sample ($MgSO_4$): A = 0.01 *M*; B = 0.05 *M*; C = 0.1 *M*; D = 0.5 *M*; E = 1.0 *M*; F = 2.0 *M*.

The homogeneous $Mg(ClO_4)_2$–$NaClO_4$ system

Figs. 3(a) and 3(b) show the concentration dependence of the elution curves of magnesium perchlorate eluted with 0.1 *M* sodium perchlorate solution. In contrast to the elution behavior in the $MgCl_2$–NaCl and the $MgSO_4$–Na_2SO_4 systems the K_d

values are larger than unity and decrease with the increase in the sample concentrations. The elution curves are unsymmetrical and of a T-shape (tailing)[15] over a wide range of sample concentrations, which is identical to the elution pattern of barium ions in the $BaCl_2$–NaCl system.

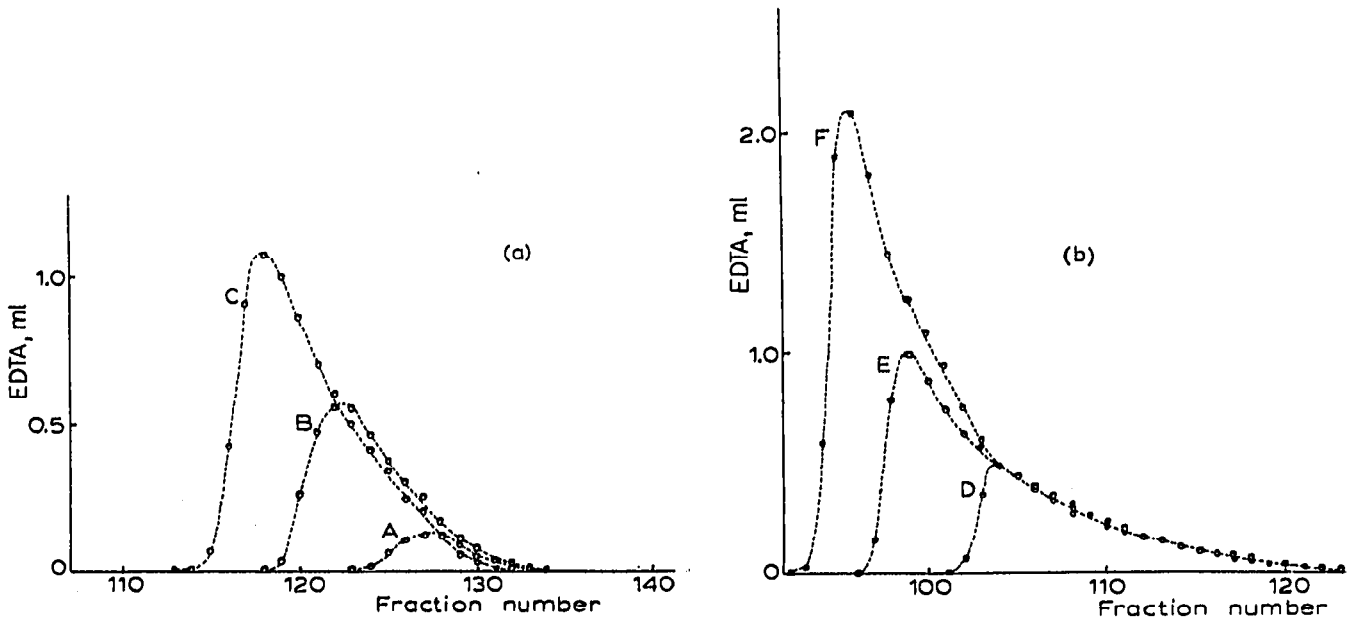

Fig. 3. Concentration dependence of the elution curves in the $Mg(ClO_4)_2$–$NaClO_4$ system. Eluent: 0.1 M $NaClO_4$. Concentrations of sample ($Mg(ClO_4)_2$): (a) A = 0.01 M; B = 0.05 M; C = 0.1 M. (b) D = 0.5 M; E = 1.0 M; F = 2.0 M.

The homogeneous $Mg(NO_3)_2$–$NaNO_3$ system

Figs. 4(a) and 4(b) show the elution behavior of magnesium nitrate eluted with a 0.1 M sodium nitrate solution. The elution volumes are somewhat larger than those

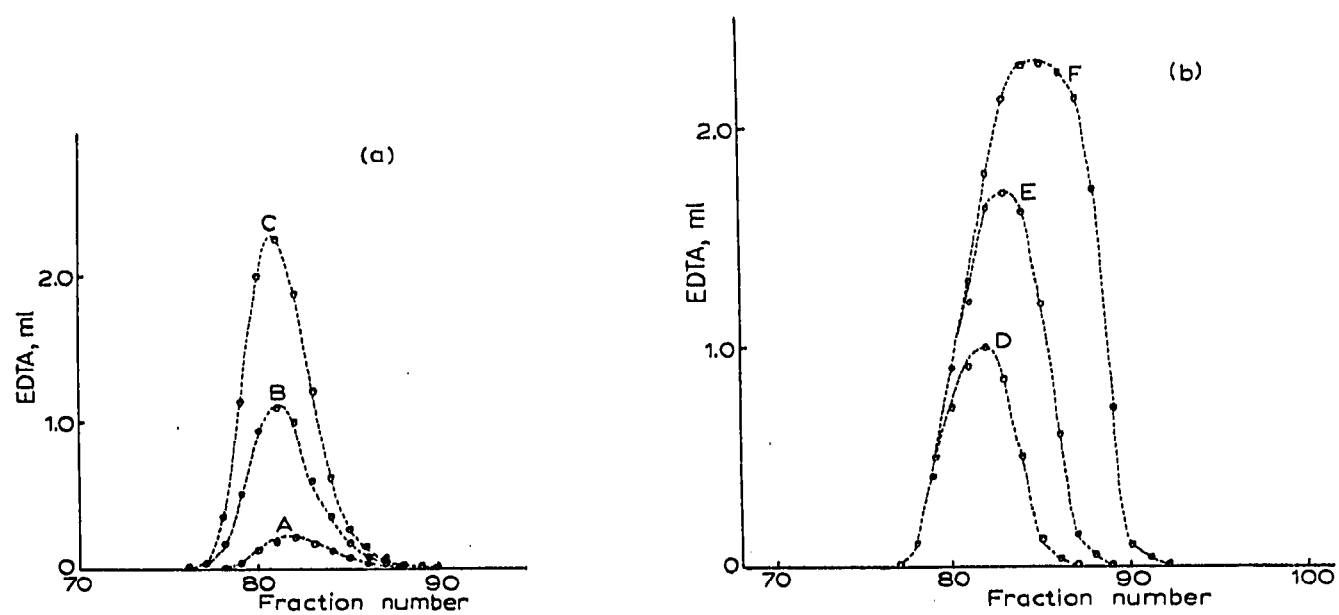

Fig. 4. Concentration dependence of the elution curves in the $Mg(NO_3)_2$–$NaNO_3$ system. Eluent: 0.1 M $NaNO_3$. Concentrations of sample ($Mg(NO_3)_2$): (a) A = 0.01 M; B = 0.05 M; C = 0.1 M. (b) D = 0.5 M; E = 1.0 M; F = 2.0 M.

in the $MgCl_2$–NaCl system and increase with the increase in sample concentration. Some tailing is observed at the lower portion of the descending sides of the elution curves in Fig. 4(a).

The overall feature for the elution volumes (the elution-peak positions) of magnesium ions in the four homogeneous systems can be seen in Fig. 5, in which the elution volumes were plotted against the concentrations of magnesium ions. It shows that magnesium ions in the $Mg(ClO_4)_2$–$NaClO_4$ system are eluted at positions far behind the total liquid volume of the column ($V_i + V_0 = 84$ ml).

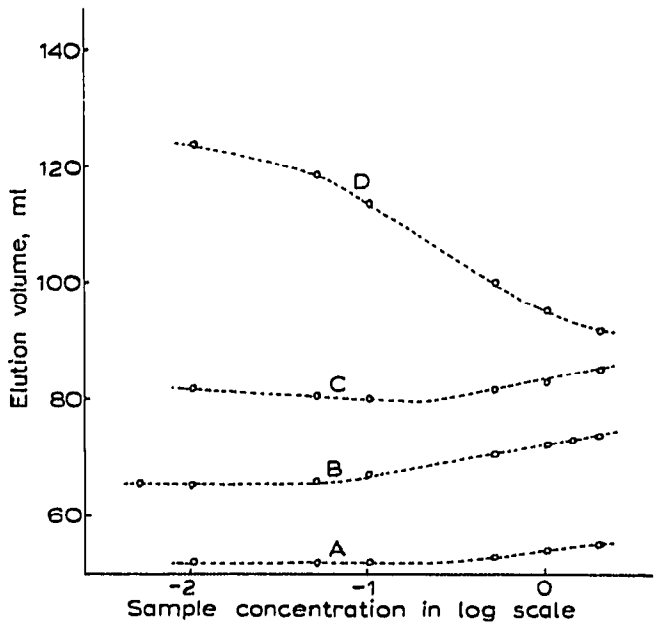

Fig. 5. Concentration dependence of the elution volumes in the four homogeneous systems. A = $MgSO_4$–Na_2SO_4 system; B = $MgCl_2$–NaCl system; C = $Mg(NO_3)_2$–$NaNO_3$ system; D = $Mg(ClO_4)_2$–$NaClO_4$ system.

The heterogeneous $MgCl_2$–(NaCl + Na_2SO_4) and $MgCl_2$–(NaCl + $NaClO_4$) systems

Figs. 6 and 7 show the variation in the elution volumes of magnesium ions with the variation in the compositions of the eluents. In these systems the total molar concentrations of the eluents were kept constant, 0.1 *M*. The elution volumes increase

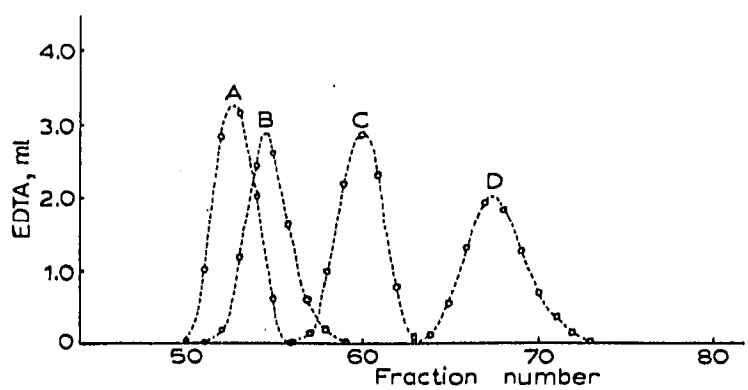

Fig. 6. The effect of the composition of the eluent on the elution volumes in the $MgCl_2$–(NaCl + Na_2SO_4) system. Sample: 0.01 *M* $MgCl_2$. Composition of the eluents: A = 0.1 *M* Na_2SO_4; B = 0.075 *M* Na_2SO_4 + 0.025 *M* NaCl; C = 0.05 *M* Na_2SO_4 + 0.05 *M* NaCl; D = 0.1 *M* NaCl.

with the increase of the molar ratio, $NaCl/Na_2SO_4$, in the $MgCl_2$–($NaCl + Na_2SO_4$) system but decrease with the increase of the ratio, $NaCl/NaClO_4$, in the $MgCl_2$–($NaCl + NaClO_4$) system. All elution peaks appear in the intermediate regions between the elution positions of magnesium ions in the $MgCl_2$–NaCl and the $MgSO_4$–Na_2SO_4 or in the $MgCl_2$–NaCl and the $Mg(ClO_4)_2$–$NaClO_4$ systems mentioned above.

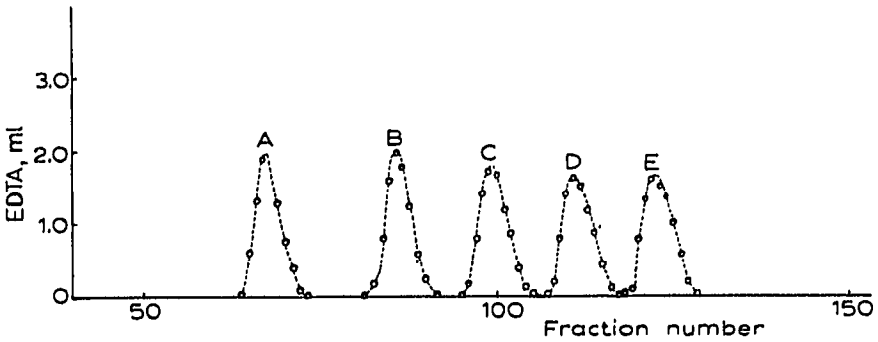

Fig. 7. The effect of the composition of the eluent on the elution volumes in the $MgCl_2$–($NaCl + NaClO_4$) system. Sample: 0.01 *M* $MgCl_2$. Composition of the eluents: A = 0.1 *M* NaCl; B = 0.075 *M* NaCl + 0.025 *M* $NaClO_4$; C = 0.05 *M* NaCl + 0.05 *M* $NaClO_4$; D = 0.025 *M* NaCl + 0.075 *M* $NaClO_4$; E = 0.1 *M* $NaClO_4$.

DISCUSSION

It is evident from Figs. 2–5 that the elution volume of magnesium ions is greatly dependent on the type of counter anion. As has been described in a previous paper[16], this can be discussed with respect to the elution behavior of counter anions. It was found, by using an automatic chromatograph with a thermal detector[18], JLC-2A (Japan Electron Optics Laboratory), that, when 0.1 *M* solutions of sodium salts of the four counter anions were eluted on a Sephadex G-15 column (bed volume = 110 ml) with a 0.1 *M* sodium chloride solution as eluent, the elution volumes increased in the order of $Na_2SO_4 < NaCl < NaNO_3 < NaClO_4$. Sodium nitrate appeared at a position somewhat larger than the total liquid volume of the column. Sodium perchlorate was eluted more slowly, about 50 ml behind the elution position of sodium nitrate. The shapes of the elution curves for both nitrate and perchlorate were unsymmetrical and of a T-shape, which suggests the interaction of the solute anions with the gel matrix.

Steric exclusion

Sulfate ions and chloride ions seem to have no direct interaction with the gel matrix. Therefore, the behavior of magnesium ions in both the $MgCl_2$–NaCl and the $MgSO_4$–Na_2SO_4 systems can be expected to be based on steric exclusion considerations. In order to explain the difference between the elution patterns of magnesium ions in the $MgCl_2$–NaCl and the $MgSO_4$–Na_2SO_4 systems it seems appropriate to introduce the idea of the polyfunctional character of the gel phase[11]. For convenience of interpretation the features of polyfunctional character are shown schematically in Fig. 8. If a background electrolyte, *e.g.*, sodium chloride, can only penetrate to a limited extent into the internal volume because of steric exclusion the gel phase is considered to be divided into two phases, phases I and II in Fig. 8A, resulting from the presence

or absence of the background electrolyte. The degree of the penetration of the background electrolyte, *i.e.*, the magnitude of phase II, will be primarily determined by the larger one of the two constituent ions because of the requirement of the electrical neutrality. Since the effective sizes[16] of chloride, sodium and sulfate ions have been estimated to increase in this order, the magnitude of phase II in the sulfate system (Fig. 8B) may be smaller than that in the chloride system.

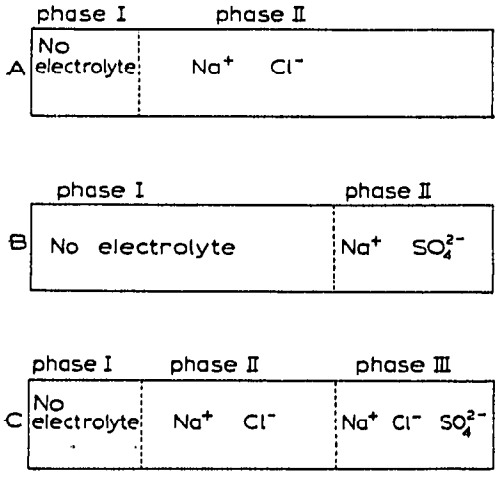

Fig. 8. Schematic representation of polyfunctional character in the gel phase.

In a homogeneous sample–eluent system the elution volume of magnesium ions is dependent on the penetrability of counter anions in phase II, because magnesium ions must be present near their counter anions in order to satisfy the principle of electrical neutrality. In the $MgCl_2$–NaCl system magnesium ions can penetrate freely according to their ionic size because counter anions, chloride ions, are smaller than the magnesium ions. On the other hand the penetration of magnesium ions in the $MgSO_4$–Na_2SO_4 system is restricted by the sulfate ions whose effective size is larger than that of the magnesium ions. Thus the available volumes or K_d values of the magnesium ions in the $MgSO_4$–Na_2SO_4 system become smaller than those in the $MgCl_2$–NaCl system.

When a mixed solution of sodium chloride and sodium sulfate is used as an eluent the internal liquid phase is considered to be divided into three regions (Fig.8C). An additional, new phase III is composed of sodium cations and chloride and sulfate anions. If magnesium ions do not form any complex with the sulfate ions these ions can distribute both in phases II and III according to their ionic size. The elution volume of magnesium ions in the $MgCl_2$–(NaCl + Na_2SO_4) system is expected to be comparable to that in the $MgCl_2$–NaCl system. Since the formation of a sulfato-complex of magnesium is well known, however, it would rather be expected that magnesium ions would tend to be retained preferentially in phase III. Therefore, the elution volume decreases with the increase in the ratio of Na_2SO_4/NaCl, which is consistent with the experimental results in Fig. 6.

The preliminary observation in Fig. 1 can be explained in the same way. When the sample concentration is low, *i.e.*, the molar ratio of chloride ions to sulfate ions is small, magnesium ions in the $MgCl_2$–Na_2SO_4 system behave as in the homogeneous

$MgSO_4$–Na_2SO_4 system. With an increase of sample concentration the elution system in the sample zone is considered to become heterogeneous as represented in Fig. 8C. When the concentration of magnesium ions is larger than that of sulfate ions or, in other words, the sample is loaded over the capacity of phase III (the sulfate anion phase is regarded as a sort of liquid cation-exchanger) a portion of magnesium ions will be distributed over not only phase III but also phase II, which results in the slower migration of the magnesium ions. Magnesium ions thereby remove into an adjacent plate (behind the sample zone) successively to be re-equilibrated. The sample band spreads through such a successive re-equilibration of magnesium ions in the elution process. At extremely high concentrations, the large excess of magnesium ions behave as in the $MgCl_2$–NaCl system to give a secondary peak.

Complex formation

In order to confirm the effect of complex formation on the gel chromatographic behavior of magnesium ions, an additional experiment was done using sodium triphosphate instead of sodium sulfate in the heterogeneous $MgCl_2$–(NaCl + Na_2SO_4) system. The gel chromatographic behavior[7] and the complex formation[19] of triphosphate have been well characterized. Fig. 9(A) shows the elution behavior of magnesium obtained by eluting a 0.004 *M* magnesium chloride solution on a Sephadex G-15 column. The eluent is composed of a mixed solution of 0.1 *M* NaCl + 0.01 *M* $Na_5P_3O_{10}$, adjusted to pH 7.2. Fig. 9(B) shows a negative peak for triphosphate anions obtained by eluting pure water as a sample with the same eluent and determining the triphosphate colorimetrically. Magnesium ions in the $MgCl_2$–(NaCl + $Na_5P_3O_{10}$) system appear at the same position as the triphosphate ions that is about 22 ml before the elution volume of magnesium ions in the $MgCl_2$–NaCl system. The fact that the elution volume of magnesium ions is greatly influenced by the presence of smaller amounts of triphosphate ions than sulfate ions in the $MgCl_2$–(NaCl + Na_2SO_4) system is attributable to the larger formation constant of the magnesium triphosphate

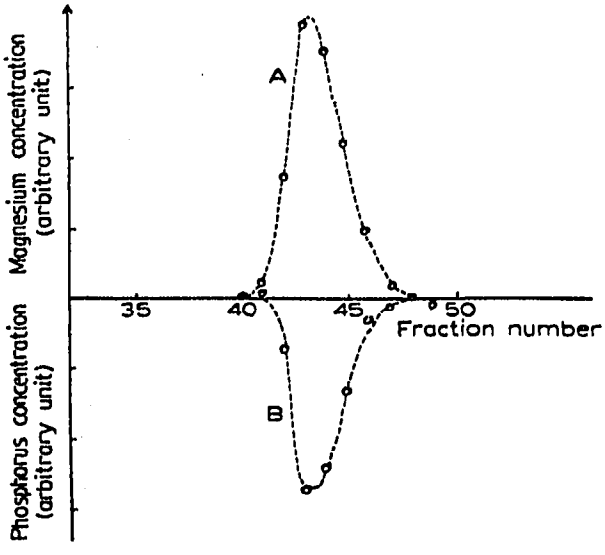

Fig. 9. Elution curves of magnesium and triphosphate ions in the $MgCl_2$–(NaCl + $Na_5P_3O_{10}$) and the H_2O–(NaCl + $Na_5P_3O_{10}$) systems. Samples: A = 0.004 *M* $MgCl_2$; B = H_2O. Eluent: 0.1 *M* NaCl + 0.01 *M* $Na_5P_3O_{10}$ (pH 7.2).

complex. Such complex formation may increase the probability of the retention of magnesium ions in phase III, which results in a decrease of the apparent elution volume of the magnesium ions. It can be assumed from Fig. 9 that the effective size of magnesium triphosphate may be comparable to that of free triphosphate ions.

Adsorption

In a previous paper[11] it was observed that when a sodium chloride solution was used as eluent the shape of the elution curves for magnesium ions at lower sample concentrations was symmetrical and its elution volume did not vary with sample concentration, while strontium and barium ions gave elution curves of the T-shape and their elution volumes varied inversely with sample concentrations. The elution behavior of strontium and barium ions was interpreted in terms of the interaction of the solutes with the gel matrix. On the other hand, it was concluded that magnesium ions are not influenced by such an adsorption effect.

In the $Mg(ClO_4)_2$–$NaClO_4$ system, however, magnesium ions give elution patterns which are similar to the behavior of barium ions mentioned above. As has already been discussed in a previous paper[16] it is suggested that a side effect such as solute–gel interaction may play an important role in this system. Since direct adsorption of magnesium ions on the gel matrix was concluded to be unlikely, another possible mechanism is that magnesium ions are adsorbed electrostatically by perchlorate anions which were preliminarily adsorbed on the gel matrix. This assumption can be expressed by eqns. 1 and 2.

$$R + ClO_4^- \rightarrow R\text{–}ClO_4^- \quad (1)$$

$$R\text{–}ClO_4^- + Mg^{2+} \rightarrow R\text{–}ClO_4\text{–}Mg^+ \text{ or } (R\text{–}ClO_4)_2Mg \quad (2)$$

where R represents the gel matrix.

The mechanism of direct adsorption of perchlorate anions is not yet clear. However, the fact that the elution curve of sodium perchlorate is of a T-shape which is typical in adsorption chromatography[15] and the heat of interaction of sodium perchlorate with a Sephadex gel[18] is extraordinarily large compared with those of sodium chloride and sodium sulfate indicates the predominant adsorption of perchlorate anions. PECSOK AND SAUNDERS[13] suggested the formation of hydrogen bonds between perchlorate anions and the amide hydrogens of Bio-Gel P-2. It is not unlikely that the hydroxy groups in the Sephadex gel skeleton and the polarized water molecules in the hydration sphere of the Sephadex gel may interact with perchlorate anions to form hydrogen bonds.

With increasing concentration of perchlorate anions in the eluent the concentration of $R\text{–}ClO_4^-$ in the gel phase increases according to eqn. 1 and the apparent distribution coefficient of magnesium ions would be expected to increase correspondingly. According to such an expectation the elution volume of magnesium ions increases with the increase in the concentration of perchlorate anions at constant ionic strength.

Nitrate anions are considered to interact with the Sephadex gel to a much smaller extent so that these ions do not affect the chromatographic behavior of magnesium ions so markedly as perchlorate anions.

REFERENCES

1 S. OHASHI, N. YOZA AND Y. UENO, *J. Chromatogr.*, 24 (1966) 300.
2 P. A. NEDDERMEYER AND L. B. ROGERS, *Anal. Chem.*, 40 (1968) 755.
3 P. A. NEDDERMEYER AND L. B. ROGERS, *Anal. Chem.*, 41 (1969) 94.
4 A. FELTER, G. DIRHEIMER AND J. P. EBEL, *J. Chromatogr.*, 35 (1968) 207.
5 J. H. STEWARD AND M. E. TATE, *J. Chromatogr.*, 45 (1969) 400.
6 Y. UENO, N. YOZA AND S. OHASHI, *J. Chromatogr.*, 52 (1970) 469.
7 Y. UENO, N. YOZA AND S. OHASHI, *J. Chromatogr.*, 52 (1970) 481.
8 R. A. HENRY AND L. B. ROGERS, *Separ. Sci.*, 3 (1968) 11.
9 T. TARUTANI, *J. Chromatogr.*, 50 (1970) 523.
10 N. YOZA, T. OGATA, Y. UENO AND S. OHASHI, *Bull. Chem. Soc. Japan*, 41 (1968) 2550.
11 N. YOZA AND S. OHASHI, *J. Chromatogr.*, 41 (1969) 429.
12 B. J. EGAN, *J. Chromatogr.*, 43 (1968) 382.
13 R. L. PECSOK AND D. SAUNDERS, *Separ. Sci.*, 3 (1968) 325.
14 C. H. LOCHMULLER AND L. B. ROGERS, *Anal. Chem.*, 41 (1969) 173.
15 N. YOZA, T. OGATA AND S. OHASHI, *J. Chromatogr.*, 52 (1970) 329.
16 Y. UENO, N. YOZA AND S. OHASHI, *J. Chromatogr.*, 52 (1970) 321.
17 C. K. MANN AND J. H. YOE, *Anal. Chem.*, 28 (1956) 202.
18 N. YOZA, T. OGATA AND S. OHASHI, to be published.
19 L. G. SILLEN AND A. R. MARTELL, *Stability Constants*, Chemical Society, London, Spec. Publ., 1964.