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THE APPLICATION OF A THERMAL DETECTOR TO THE GEL CHROMATOGRAPHY OF INORGANIC COMPOUNDS

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SUMMARY

A liquid chromatograph with a thermal detector was applied to the gel chromatography of inorganic compounds. A thermal detection column packed with Sephadex G-15 was found to be sensitive for the detection of inorganic solutes. The sensitivity increased in the order LiCl < NaCl < CsCl < RbCl < KCl and Na_2SO_4 < NaH_2PO_4 < < NaCl < $NaNO_3$ < $NaClO_4$. In addition to a sample peak, some pseudo-peaks were recorded, which were concluded to be caused by the exclusion of an eluting agent from the sample zone or by the slower migration of hydroxyl ions that are in equilibrium with phosphate ions.

INTRODUCTION

With the advanced application of gel chromatography to the investigation of inorganic compounds, the automatic recording of the chromatographic behaviour is becoming increasingly necessary. Since gel chromatography is a type of liquid chromatography, the conventional liquid chromatographs which have commonly been used in ion-exchange and adsorption chromatographic systems can be employed for gel chromatography without any significant modification. The detection methods possible in liquid chromatography fall into several categories[1]: photometric, refractometric, gas ionization, radiometric, electrochemical and thermal detection.

In most cases, a refractometric method has been employed as a flow detector in the gel chromatography of inorganic compounds[2,3]. SAUNDERS AND PECSOK[4] recommended the use of a conductometric detector. NAGASAWA[5], who attempted the automatic recording of the gel chromatographic behaviour of ethylene glycol and its oligomers, reported the high sensitivity of a thermal detector packed with dextran gel (Sephadex G-15).

The present work was undertaken to examine the applicability of a liquid chromatograph with a thermal detector, JLC-2A (Japan Electron Optics Laboratory), to the gel chromatography of inorganic compounds. A thermal detection column packed with Sephadex G-15 was found to be sensitive for the detection of inorganic

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solutes. The sensitivity was greatly dependent on the composition of the samples and, for example, increased in the order LiCl < NaCl < CsCl < RbCl < KCl and Na_2-EDTA < Na_2SO_4 < NaH_2PO_4 < NaCl < $NaNO_3$ < $NaClO_4$. When several sodium salts were eluted, in addition to a sample peak, some pseudo-peaks were recorded, which was concluded to be caused by the exclusion of an eluting agent from the sample zone or by the slower migration of hydroxyl anions that are in equilibrium with phosphate anions.

EXPERIMENTAL

The liquid chromatograph and its operation

An automatic liquid chromatograph with a thermal detector, JLC-2A (Japan Electron Optics Laboratory), was employed. A schematic diagram of the equipment

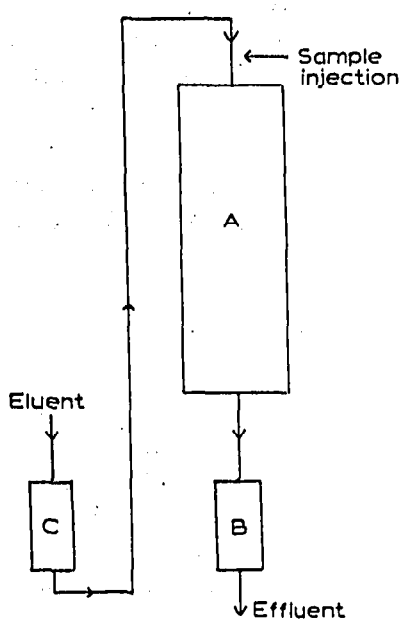


Fig. 1. Schematic diagram of the chromatographic equipment. A = separation column; B = detection column; C = reference column.

is shown in Fig. 1. A separation column (bed volume = 1.5 cm diameter × 60.5 cm high) and a pair of detection and reference columns (bed volume = 0.8 cm diameter × 7 cm high) were packed with Sephadex G-15 (40–120 μm, Pharmacia Fine Chemicals). The equipment was placed in a room kept at 20 ± 1°. A temperature of 25° was selected for the detection column. The detector sensitivity can be adjusted to a full-scale deflection of 0.1, 0.03, 0.01, 0.003 or 0.001°.

1 ml of a sample solution was injected into the separation column connected with the detection column or directly into the detection column. As shown in Fig. 2b, the thermal elution diagram is recorded in terms of the differential form of the concentration profile (Fig. 2a) (ref. 6). The signal height, $h_1 + h_2$, in Fig. 2b is represented as a peak height in the subsequent figures.

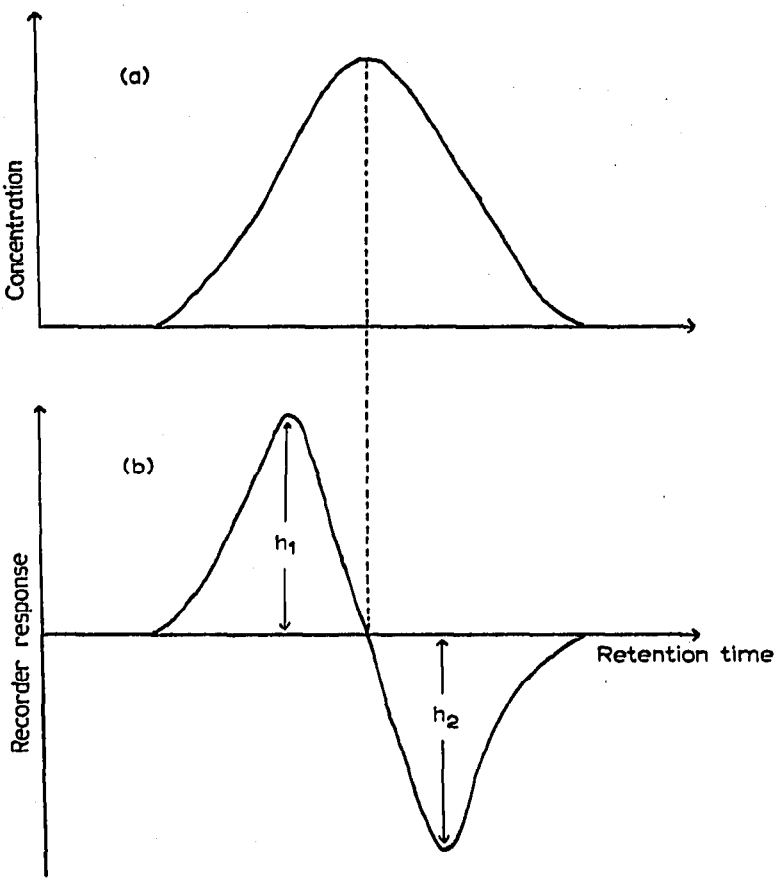


Fig. 2. Relationship between the thermal diagram (b) and the concentration distribution curve (a).

Sample solutions

All reagents used were of guaranteed grade from Wako Chemicals. A sample solution was prepared by dissolving each compound in pure water or 0.1 *M* sodium chloride solution.

RESULTS AND DISCUSSION

Selection of the packing material

The basic principle of the thermal detection method is based on the measurement of the variation in temperature due to the heat of interaction between a sample and a packing material in the detection column[6]. A sample component in the effluent emerging from the bottom of the separation column is then introduced into the detection column which is equipped with a sensitive thermistor and packed with a suitable packing material. The thermal sensitivity of the detector[5–9], which is dependent on the kind of the packing material in the detection column, is generally considered to be highest in ion-exchange chromatography, moderate in adsorption chromatography and lowest in partition chromatography.

One of the possible elution systems in gel chromatography is a combination of a separation column packed with a dextran gel and a detection column packed with an ion exchanger. In many cases, however, the separation effects in gel and ion-

exchange chromatographic columns act contrary to one another. For example, consider the elution of orthophosphate (P_1), diphosphate (P_2) and triphosphate (P_3) in a chromatographic system that consists of a separation column with a dextran gel (Sephadex G-15) and a detection column with an ion-exchange resin (Dowex 1-X8). When a sodium chloride solution is used as eluent, the orders of the distribution coefficients (or elution volumes) of the three phosphates on the two columns are known to be contrary to one another[10-12]:

separation column (molecular sieve): $P_1 > P_2 > P_3$

detection column (ion exchange): $P_1 < P_2 < P_3$.

Consequently, the peak resolution of the sample components in such an elution system is expected to become poor because of the contribution of the counteracting effects in the gel and ion-exchange chromatographic processes.

Since the distribution coefficient in ion-exchange chromatography is, in general, larger than that in gel chromatography, the gel chromatographic behaviour of sample

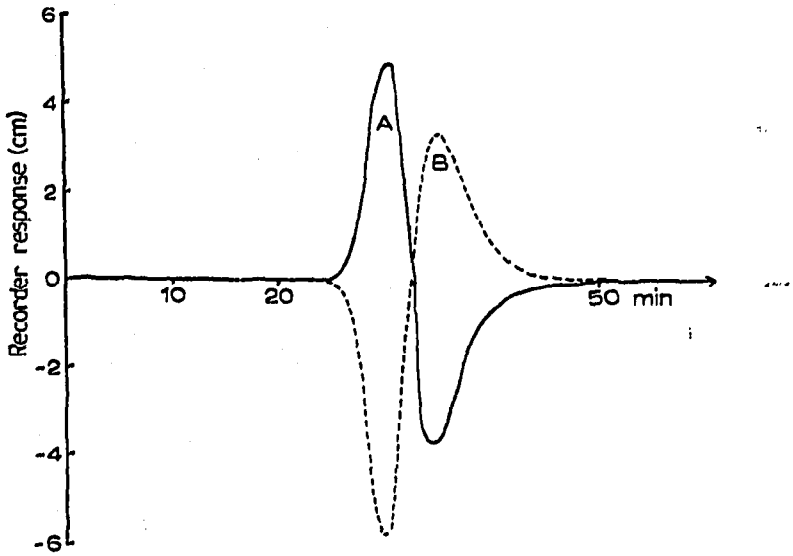


Fig. 3. Thermal diagrams for sodium chloride and pure water. Sample, A = 0.2 *M* NaCl; B = water. Eluent, 0.1 *M* NaCl. Flow rate, 0.37 ml/min. Detector sensitivity, 0.03°. Chart speed, 120 mm/h.

components on the separation column cannot be observed precisely, even when the orders of the distribution coefficients in both columns are the same. The chromatogram observed in such a system is ascribed rather to the predominant contribution of the separation effect on the detection column and not on the separation column. Therefore, it is undesirable to use an ion-exchange resin as a packing material for the detection column in the gel chromatographic system.

In this work, a Sephadex gel was examined as a packing material for both the detection and separation columns. Since gel chromatography is based primarily on the sieving effect in the gel phase, which means a lower interaction of a sample with the gel matrix, high sensitivity of the detection column could not be expected. As mentioned in the following section, however, the thermal detection column packed with a Sephadex gel was found to be sensitive enough to detect all the compounds tested in this work.

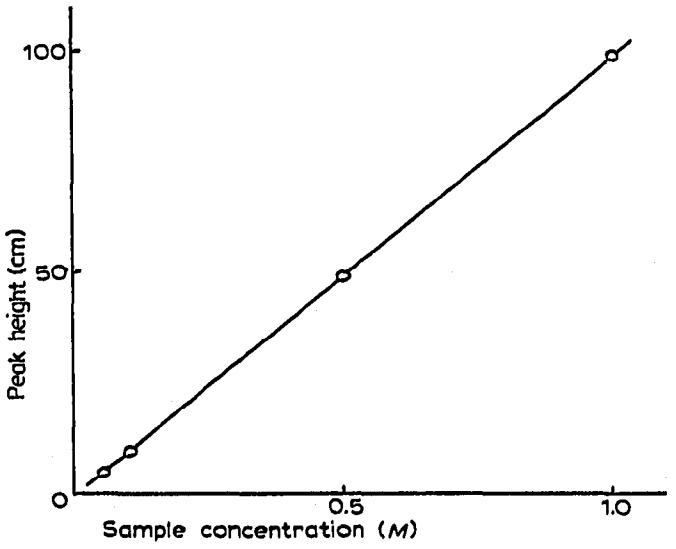


Fig. 4. Relationship between the sample concentration and the peak height for sodium chloride. Eluent, water. Detector sensitivity: calculated on the basis of 0.03°.

The sensitivity of the detection column

Unless otherwise stated, the experiments in this section were carried out by direct application of 1 ml of a sample solution into the detection column, without the use of the separation column. The thermal diagrams for two samples of a 0.2 *M* sodium chloride solution and pure water are shown in Fig. 3. Since a 0.1 *M* sodium chloride solution was used as eluent, the relative sample concentrations of the 0.2 *M* sodium chloride solution and the pure water are regarded as +0.1 *M* and −0.1 *M*, respectively. Therefore, as expected, the positive peak for the sodium chloride solution and the negative peak for pure water were symmetrical with respect to the base line.

As shown in Fig. 4, a linear relationship was observed between the sample concentration and the peak height of sodium chloride.

The sign and the shape of a thermal diagram are dependent on the sample composition. Three types of signal were observed: type A for sodium chloride (positive signal, exothermic), type B of a reverse sign (negative signal, endothermic) and type C

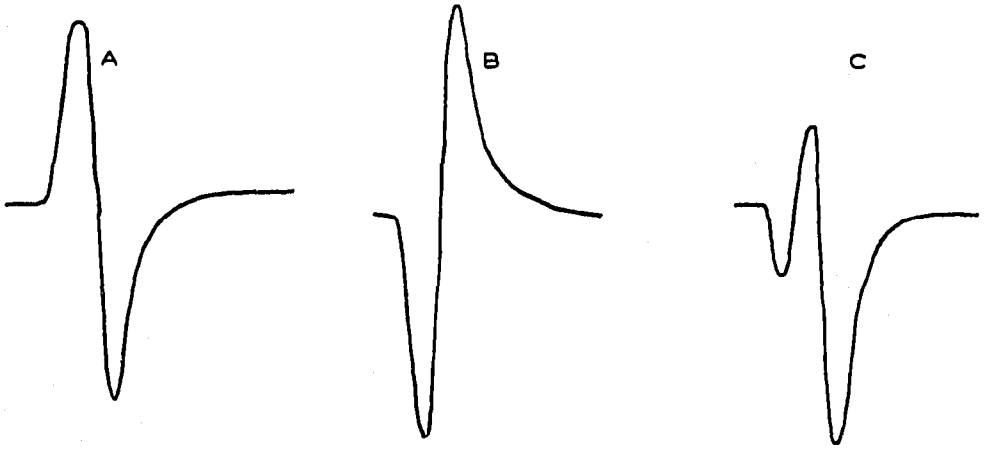
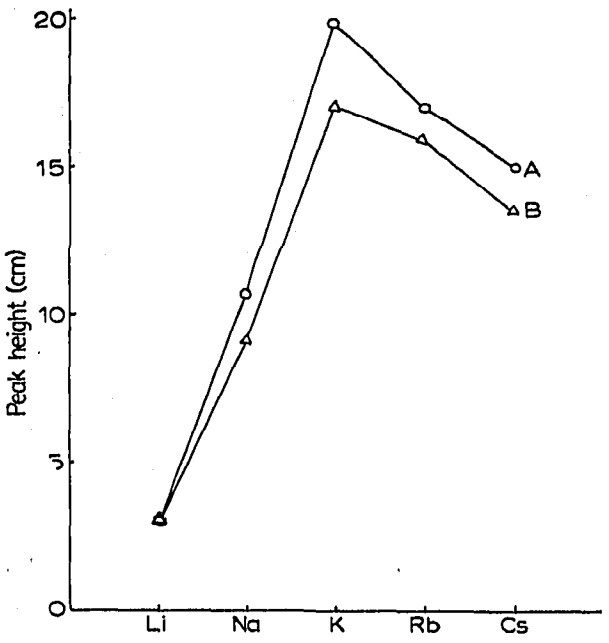


Fig. 5. Three types of thermal diagrams.



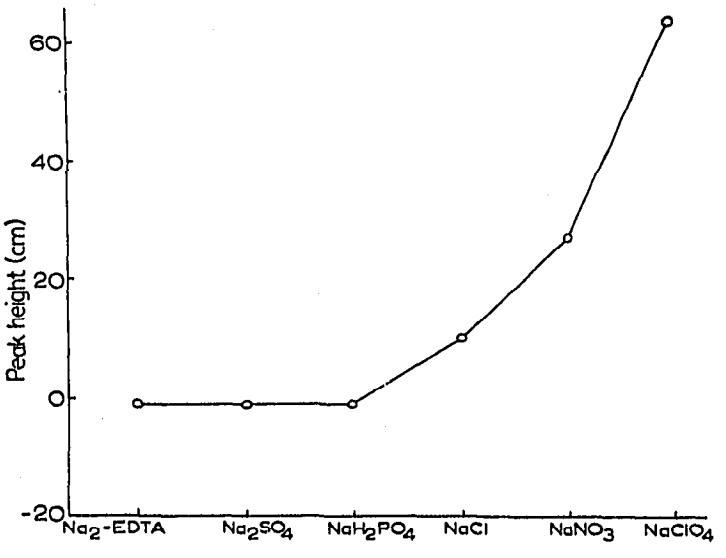


Fig. 6. The sensitivity of alkali chlorides. Sample, 0.1 *M* solution. Eluent, A = water; B = 0.1 *M* NaCl. Detector sensitivity: calculated on the basis of 0.03°.

Fig. 7. The sensitivity of several sodium salts. Sample, 0.1 *M* solution. Eluent, water. Detector sensitivity: calculated on the basis of 0.03°.

of a complicated form (Fig. 5). The compounds examined in this work can be classified into these three types.

Type A: HCl, NaOH, LiCl, NaCl, KCl, RbCl, CsCl, $MgCl_2$, $CaCl_2$, $SrCl_2$, $BaCl_2$, $NaNO_3$, $NaClO_4$, $Mg(NO_3)_2$, $Mg(ClO_4)_2$.

Type B: H_2SO_4, Na_2SO_4, Na_2-EDTA, CH_3COCH_3, CH_3OH, C_2H_5OH, $(CH_3)_2CHOH$, $(CH_3)_2CHCH_2OH$.

Type C: Na_3PO_4, Na_2HPO_4, NaH_2PO_4, KH_2PO_4.

The sensitivity is also dependent on the composition of the sample. For example, the peak heights for alkali chlorides increase in the order Li < Na < Cs < Rb < K (Fig. 6). The effect of anions on the sensitivities of several sodium salts can be seen in Fig. 7. Negative values in Fig. 7 correspond to the peak heights for the negative signals of types B and C in Fig. 5.

The interpretation of the mechanism of exothermic and endothermic processes in the detection column is at present not clear. As mentioned above, the basic principle of gel chromatography has been explained in terms of the sieving effect in the gel phase. If the chromatography of a sample solute on the Sephadex column is governed only by the sieving effect in the gel phase, *i.e.* there is no interaction between the sample solute and the gel matrix, then the heat of dilution of the sample solute is probably represented by the variation in temperature in the detection column. However, the experimental results cannot be correlated simply with the available thermochemical data on heats of dilution. For example, the heats of dilution of alkali chlorides at low concentrations are exothermic and decrease in the order Li > Na > K > Rb > Cs. Such a trend is not always in parallel with their sensitivities in the detection column (Fig. 6). The appearance of a maximum sensitivity in Fig. 6 may be

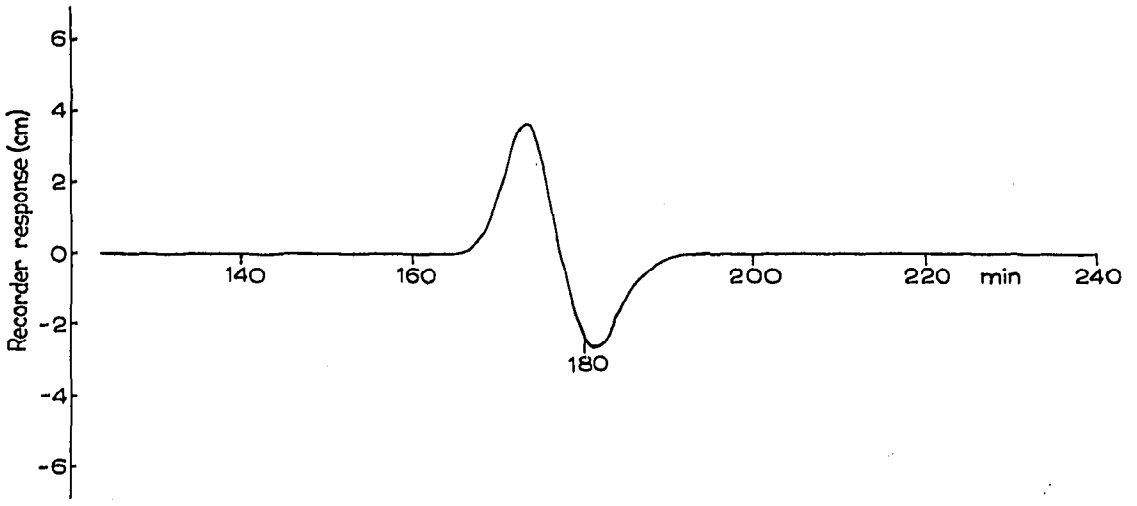


Fig. 8. Elution curve for sodium chloride. Sample, 0.2 *M* NaCl. Eluent, 0.1 *M* NaCl. Flow rate, 0.49 ml/min. Detector sensitivity, 0.01°. Chart speed, 120 mm/h.

explained qualitatively by assuming that there are two conflicting factors which counteract each other to a comparable extent. The relatively high sensitivities of sodium nitrate and sodium perchlorate in the series of the sodium salts shown in Fig. 7 may be ascribed to the solute–gel interaction, which is in agreement with the conclusion from the chromatographic data. The contribution of more than one factor must be taken into account in order to achieve a complete understanding of all the thermal diagrams of types A, B and C in Fig. 5. This problem will be discussed in detail elsewhere.

The appearance of pseudo-peaks

The separation column packed with a Sephadex G-15 gel was connected with the detection column in order to observe the gel chromatographic behaviour of several sodium salts. Unless otherwise stated, a sample solution was prepared by dissolving

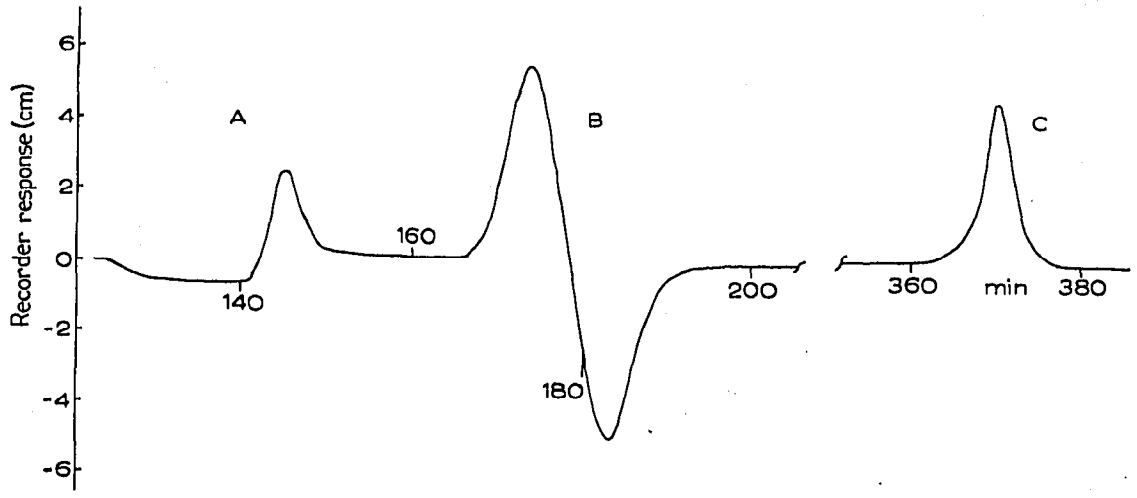


Fig. 9. Elution curve for trisodium phosphate. Sample, 0.2 *M* Na_3PO_4 (in 0.1 *M* NaCl). Eluent, 0.1 *M* NaCl. Flow rate, 0.49 ml/min. Detector sensitivity: 0.01° for A and B, 0.1° for C. Chart speed, 120 mm/min.

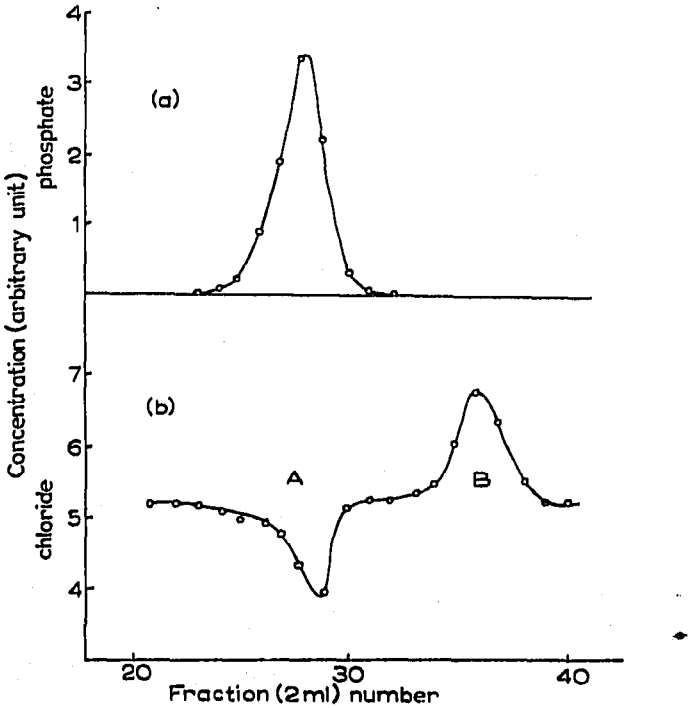


Fig. 10. Concentration distribution of (a) phosphate and (b) chloride ions obtained by colorimetric and argentometric methods. Sample, 0.2 *M* Na_3PO_4 (in 0.1 *M* NaCl). Eluent, 0.1 *M* NaCl. Column, Sephadex G-15 (1.5 × 55 cm).

each sodium salt in a 0.1 *M* sodium chloride solution. As shown in Fig. 8, sodium chloride gave a single elution peak. On the other hand, three peaks were observed for trisodium orthophosphate (Fig. 9). The peak height increases in the order A < B < C. The elution position of peak A does not change significantly with the sample concentration, while its shape is greatly dependent on the sample concentration. A symmetrical peak B appears at the same position as that of sodium chloride in Fig. 8 and its peak height varies proportionally with the sample concentration. A skewed peak C, that is assumed to correspond to an asymmetrical (tailing) concentration profile,

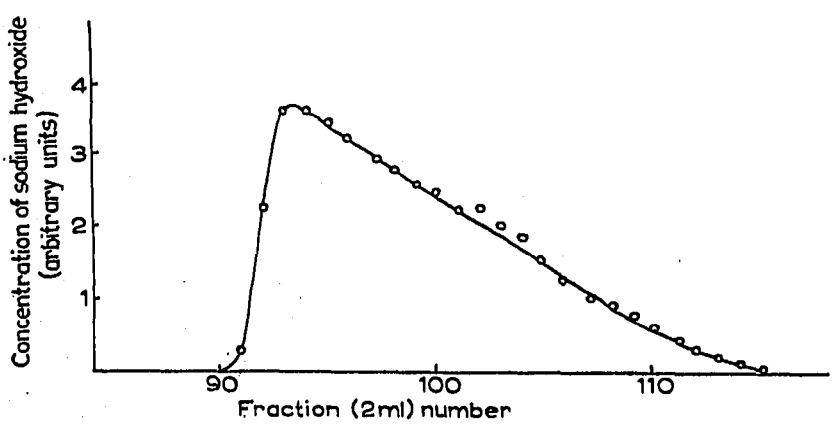


Fig. 11. Concentration distribution of sodium hydroxide obtained by titration with hydrochloric acid. Sample, 0.2 *M* Na_3PO_4 (in 0.1 *M* NaCl). Eluent, 0.1 *M* NaCl. Column, Sephadex G-15 (1.5 × 55 cm).

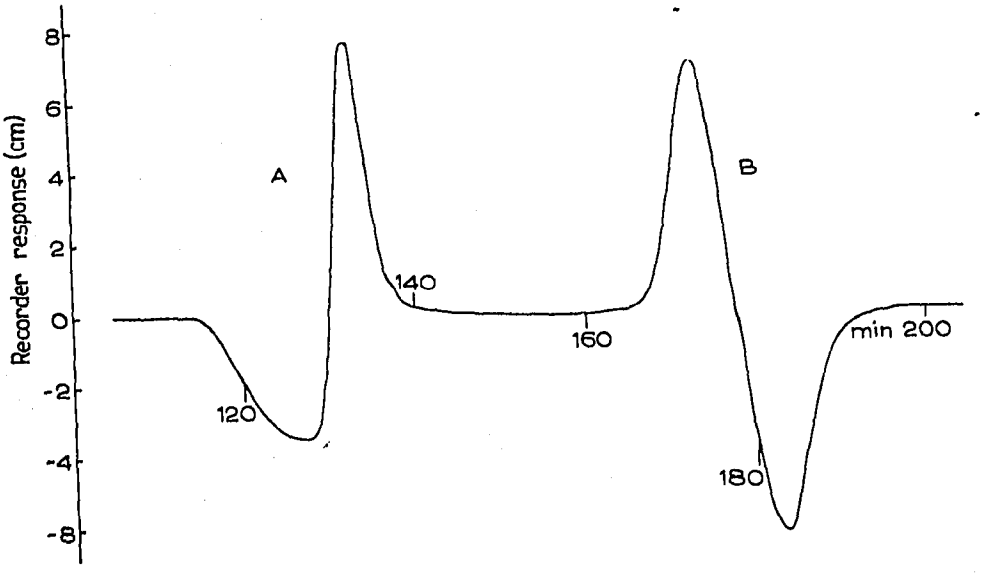


Fig. 12. Elution curve for sodium sulphate. Sample, 0.1 *M* Na_2SO_4 (in 0.1 *M* NaCl). Eluent, 0.1 *M* NaCl. Flow rate, 0.49 ml/min. Detector sensitivity, 0.003°. Chart speed, 120 mm/h.

always appears far behind the total liquid volume of the column. The peak height increases and the peak position shifts towards the lower elution volume with the increase in the sample concentration.

It was verified by chemical analysis of the effluents that peaks B and C in Fig. 9 correspond to positive peaks for sodium chloride (B in Fig. 10b) and sodium hydroxide (Fig. 11), respectively, and peak A in Fig. 9 includes not only a positive peak for phosphate (Fig. 10a) but also a negative peak for sodium chloride (A in Fig. 10b). Sodium chloride is excluded from the sample zone to give a pair of negative and positive peaks of sodium chloride. Similar exclusion of sodium chloride was also observed when disodium phosphate and monosodium phosphate were applied as samples, to such an extent that the amount of sodium chloride excluded decreased in the order $Na_3PO_4 > Na_2HPO_4 > NaH_2PO_4$.

Peak C in Fig. 9 may be produced by the slower migration of hydroxyl ions that are in equilibrium with phosphate ions according to eqn. 1.

$$PO_4^{3-} + H_2O \rightleftarrows HPO_4^{2-} + OH^- \qquad (1)$$

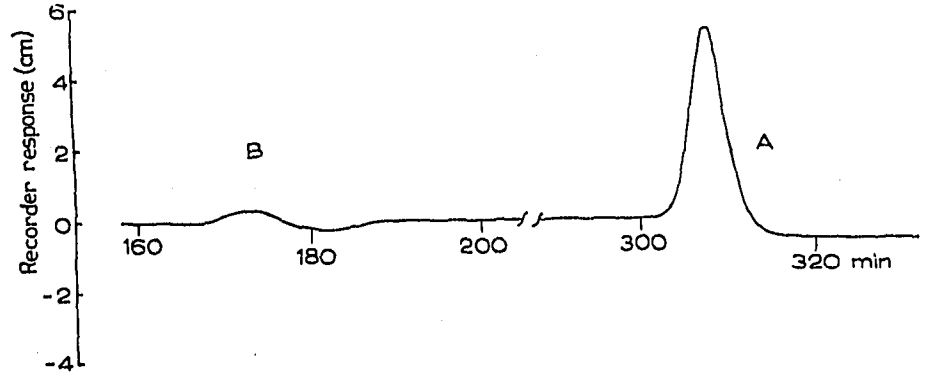


Fig. 13. Elution curve for sodium perchlorate. Sample, 0.1 *M* $NaClO_4$ (in 0.1 *M* NaCl). Eluent, 0.1 *M* NaCl. Flow rate, 0.49 ml/min. Detector sensitivity, 0.03°. Chart speed, 120 mm/h.

Such a concentration dependence of peak C in Fig. 9, where the peak position shifts towards the lower retention time with an increase in the sample concentration, is in good agreement with the chromatographic behaviour of sodium hydroxide eluted under the same conditions. Hydroxyl ions are considered to be adsorbed on the Sephadex gel to give an asymmetrical concentration profile with a sharp front edge[13], which is also reflected in the relatively high thermal sensitivity of hydroxyl ions, as can be seen in Fig. 9.

The appearance of the positive peaks for sodium chloride was also observed when sodium sulphate, sodium nitrate, sodium perchlorate and sodium hydroxide were eluted. Two examples for sodium sulphate and sodium perchlorate are shown in Figs. 12 and 13. In all cases, positive peaks for sodium chloride appeared at the same position as that for sodium chloride in Fig. 8, *i.e.* at the position of peak B in Figs. 12 and 13, in spite of the different elution positions of the respective sample zones. The sample zone for sulphate corresponds to peak A in Fig. 12. On the other hand, the sample peaks for sodium nitrate, perchlorate and hydroxide appear behind the elution peak for sodium chloride, at positions such as peak A in Fig. 13 for perchlorate. Their retention times increase in the order sulphate < nitrate < perchlorate < hydroxide, when each 0.1 *M* sample solution is applied. Such a trend has been reported in connection with the effect of sulphate, nitrate and perchlorate anions on the gel chromatographic behaviour of magnesium ions[14] and can also be correlated with their thermal sensitivities in the detection column. Perchlorate and hydroxide are assumed to interact strongly with the Sephadex gel to give the asymmetrical elution curves. Nitrate seems to interact to a lesser extent, whilst for sulphate no appreciable interaction seems to take place.

When a 0.05 *M* aqueous trisodium orthophosphate solution is eluted with a 0.1 *M* sodium chloride solution, a negative peak for sodium chloride (peak B in Fig. 14) appears at the same position as that of positive peak B in Fig. 9. With the increase in

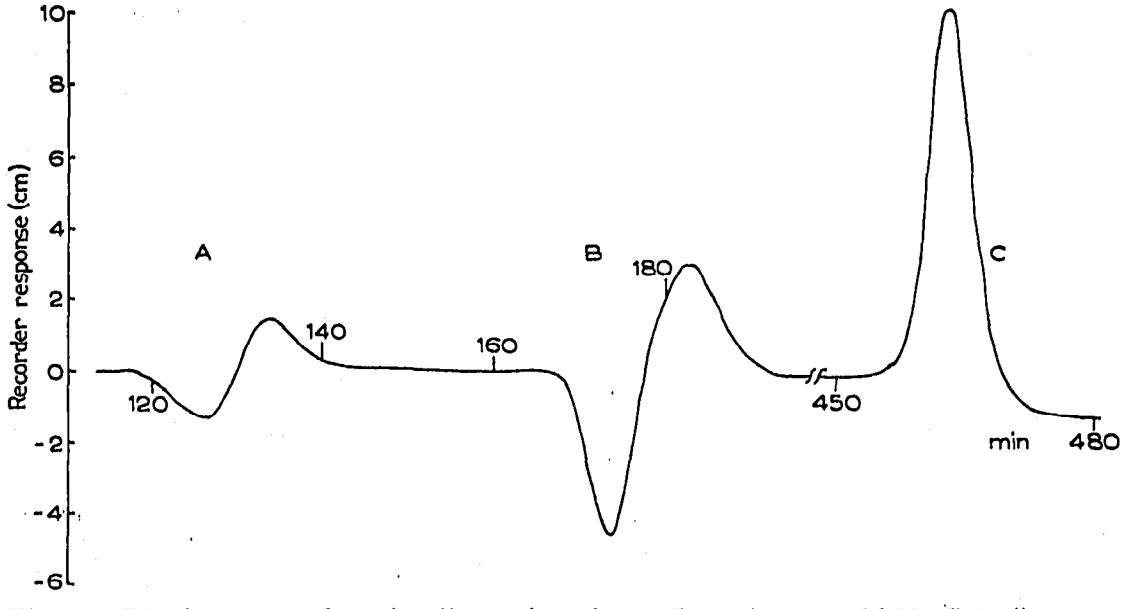


Fig. 14. Elution curve for trisodium phosphate. Sample, 0.05 *M* Na_3PO_4 (in water). Eluent, 0.1 *M* NaCl. Flow rate, 0.49 ml/min. Detector sensitivity, 0.003° for A and B, 0.01° for C. Chart speed, 120 mm/min.

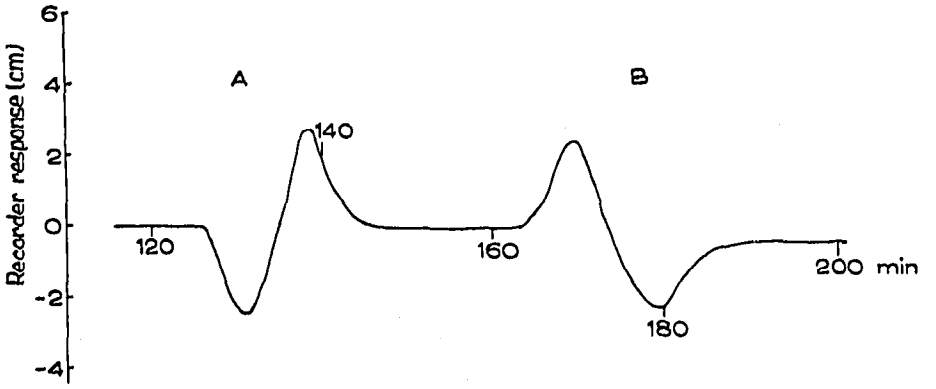


Fig. 15. Elution curve for trisodium phosphate. Sample, 0.2 *M* Na_3PO_4 (in water). Eluent, 0.1 *M* NaCl. Flow rate, 0.49 ml/min. Detector sensitivity, 0.01°. Chart speed, 120 mm/h.

the sample concentration, however, peak B becomes positive (Fig. 15), which indicates the exclusion of a considerable amount of sodium chloride from the sample zone, in spite of the absence of sodium chloride in the sample solution applied. An analogous observation has been described by NEDDERMEYER AND ROGERS[2]. As shown in Fig. 14, peak C corresponding to sodium hydroxide appeared expectedly.

These experimental results lead to the conclusion that, in addition to a sample peak, some pseudo-peaks are caused by the exclusion of the eluting agent from the sample zone or by the exclusion of hydroxyl ions which are in equilibrium with phosphate ions but which tend to migrate more slowly than the sample zone. The retention time of the pseudo-peak due to the exclusion of the eluting agent is independent of the sample composition but its peak height is greatly dependent on both the composition and the concentration of the sample. For the pseudo-peak due to the slower migration of hydroxide ions, however, not only the peak height but also the rention time is dependent on the composition and the concentration of the sample.

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