CHROMATOGRAPHIC BEHAVIOUR OF INORGANIC ANIONS ON A SEPHADEX G-15 COLUMN

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(Received August 13th, 1976)

SUMMARY

Citrate, sulphate, oxalate, chromate, carbonate, fluoride, acetate, formate, iodate, bicarbonate, bromate, chloride, nitrite, chlorate, bromide, nitrate, iodide, perchlorate and thiocyanate (each with sodium as counter-ion) were applied to a column of Sephadex G-15, and elution was carried out with sodium chloride solution. The elution behaviour of the anions could be followed by examining the concentration distribution of the background electrolyte (sodium chloride) by argentimetry; the elution order of the anions was in accord with the lyotropic series. The elution mechanism and the possibility of quantitative separation of the anions by gel chromatography are discussed.

INTRODUCTION

In recent years, gel chromatography has been applied to the investigation of small inorganic compounds[1], and the behaviour of simple inorganic anions during gel chromatography has also been dealt with[2-9]. In these studies, pure water was generally used as eluent[2-4,6,9]. However, Neddermeyer and Rogers[4] pointed out that, when sodium chloride was eluted with pure water, the elution volume increased with increase in the sample concentration, and the elution curves were all of the leading-edge type. They attributed the phenomenon to the Donnan salt-exclusion effect, which restricts the penetration of anions into the charged gel matrix, and suggested that the exclusion effect could be eliminated by the presence of sufficient electrolyte in the eluent[4].

One of us has effectively separated halide anions on a Sephadex G-15 column with an electrolyte as eluent[8]. When sodium fluoride was eluted with sodium chloride solution, the elution curve obtained by argentimetry showed a pair of positive and negative peaks. An analogous phenomenon was also observed when phosphate was eluted with sodium chloride solution[5]. These positive peaks are pseudo-peaks (due to chloride anion) caused by exclusion of the eluting agent from the sample zones, and the negative peaks correspond to the sample anions[5,8]. In this paper, the elution behaviour of a simple anion during elution with sodium chloride solution has been studied by ascertaining the positions of the negative peaks on the elution curves ob-

tained by determining the concentration distribution of the background electrolyte (sodium chloride) in the eluate by argentimetry. From the position and area of the negative peak, the separability could be estimated and the anion could be determined. The behaviour of the anions could be reasonably interpreted by reference to the lyotropic numbers generally accepted in colloid science.

EXPERIMENTAL

Sample solution

All reagents used were of analytical grade. Sample solutions of citrate, sulphate, oxalate, chromate, carbonate, fluoride, acetate, formate, iodate, bicarbonate, bromate, chloride, nitrite, chlorate, bromide, nitrate, iodide, perchlorate and thiocyanate ions were prepared by dissolving their sodium salts in 0.1 M sodium chloride (the eluent). A 0.2% solution of Blue Dextran 2000 (Pharmacia, Uppsala, Sweden) used as a standard of K_{av} was prepared in a similar manner.

Eluent

The eluent used was 0.1 M sodium chloride.

Column and procedure for elution

Sephadex G-15 (Pharmacia; dry particle size 40–120 μm) which is a cross-linked dextran gel, was used as column packing. The column was prepared in a 1.5 × 50-cm glass tube (Shoei Glass, Tokyo, Japan) in the way described previously[8].

A 0.5-ml portion of sample solution was placed on top of the gel bed just as the last few drops of eluent soaked into the bed. The sample solution was allowed to soak into the gel and was then washed further in with two portions (each *ca.* 0.5 ml) of eluent; the eluent vessel was attached to the top of the column, and elution was carried out in the usual manner. The eluate was collected in fractions of *ca.* 1 ml with a drop-count fraction collector, the volumes of some arbitrarily chosen fractions were measured so that the fraction volume could be determined.

The concentrations of solute in the eluate fractions were determined by argentimetric titration unless otherwise stated. The concentration of Blue Dextran was determined colorimetrically at 620 nm.

Calculation of the K_{av} value

The K_{av} value is defined by the equation,[10]

$$K_{av} = (V_e - V_0)/(V_t - V_0) \tag{1}$$

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

Identification of negative peaks on the elution curves

Figs. 1 and 2 show the elution profiles of sulphate and nitrate chromatographed individually. The curves marked B in each figure were obtained by argentimetry, whereas those marked A were obtained by spectrophotometry; sulphate[11] was deter-
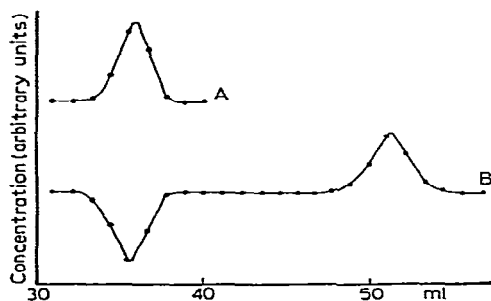
Fig. 1. Concentration distribution of sulphate (A) and chloride (B). Sample: 0.5 ml of 0.05 M sodium sulphate. $V_t = 73.5$ ml.
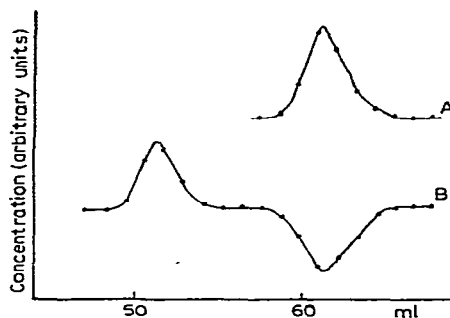
Fig. 2. Concentration distribution of nitrate (A) and chloride (B). Sample: 0.5 ml of 0.05 M sodium nitrate. $V_t = 73.5$ ml.

mined colorimetrically at 530 nm with use of Dotite CLB tablets (barium chloranilate; Dojine, Kumamoto, Japan), and nitrate[12] was determined from the absorbance at 296 nm. The negative peaks on the curves marked B correspond to sulphate and nitrate, respectively, and the positive peaks to chloride excluded by the sulphate and nitrate. Therefore, in this work, the elution volume of an anion was determined from the negative peak on the elution curves obtained by argentimetry. However, the elution curves for iodide[8], bromide[8] and thiocyanate showed two positive peaks, the second ones indicating the sample zones.

Behaviour of anions on Sephadex G-15 column

Fig. 3 shows examples of the elution curves obtained; the distribution coefficients (K_{av}) of the anions were calculated by using eqn. 1 and are listed in Table I. The magnitude of each K_{av} value is in accord with the order of the lyotropic number[13] in colloid chemistry rather than with the radii of the hydrated ions[14]; these numbers (derived from the concentrations of the sodium salts of the different anions required to flocculate agar) are also listed in Table I.

The value of the lyotropic number would depend on the extent of dehydration of the colloid due to hydration of these anions; in other words, the strength of hydration of the anion should increase with decrease in the lyotropic number. Therefore, the order of the K_{av} values should be comparable to that of the strength of hydration. Epton *et al.*[9] established a correlation between the distribution coefficient on Enzacryl gel and the charge-transfer-to-solution (CTTS) wavelength maxima. Our results indicate considerable correlation between K_{av} values and CTTS spectra, but the correlation with lyotropic number was better.

Figs. 4 and 5 show the concentration dependence of sample solutions of sulphate and nitrate. With sulphate, the elution curves in the lower concentration region were symmetrical, but this symmetry gradually changed to give a leading-edge curve as the concentration increased and the elution volume became larger. These tendencies have also been observed for metal–EDTA complexes, and an analogous discussion would be applicable in this instance[15]. Namely, the migration of sulphate in the lower concentration region will be caused mainly by a sieving effect. Previously[8],
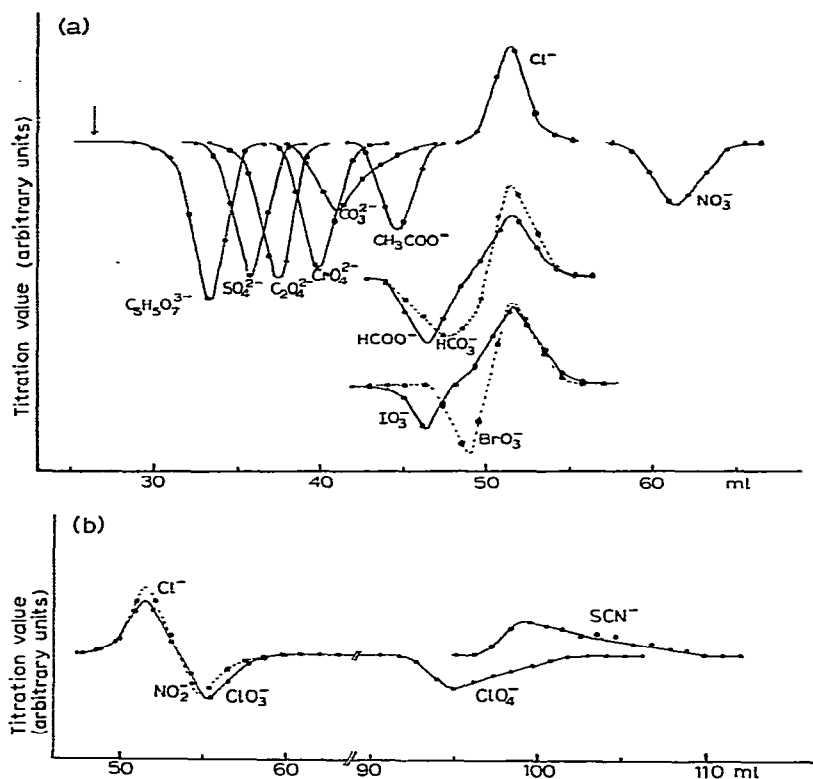
Fig. 3. Elution curves for some anions. Sample: 0.5 ml of 0.05 M solution of each anion. $V_t = 73.5$ ml. The arrow indicates the elution position of Blue Dextran.

it was pointed out that the behaviour of fluoride, chloride and bromide in gel chromatography could be attributed to a sieving effect.

On the other hand, the elution curves of nitrate showed tailing over the entire concentration range studied, the elution volumes decreasing with increase in the

TABLE I

VALUES OF K_{av} AND LYOTROPIC NUMBERS OF SOME ANIONS

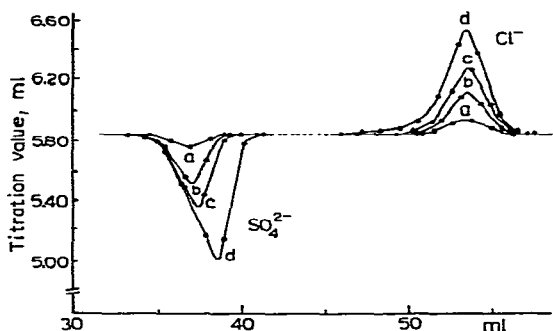| Anion	K_{av}	N^*	Anion	K_{av}	N^*
Citrate	0.15	—	Bromate	0.49	9.7
Sulphate	0.20	2.0	Chloride	0.54	10.0
Oxalate	0.24	—	Nitrite	0.60	10.1
Chromate	0.29	—	Chlorate	0.61	10.7
Carbonate	0.31	—	Bromide	0.71	11.5
Fluoride	0.35	4.8	Nitrate	0.74	11.6
Acetate	0.39	—	Iodide	1.27	12.5
Formate	0.43	—	Perchlorate	1.45	11.8
Iodate	0.43	6.3	Thiocyanate	1.54	13.3
Bicarbonate	0.46	—			

* Lyotropic number[13].

Fig. 4. Concentration dependence of elution curves of sodium sulphate. Sample: 0.5 ml of each concentration of sulphate; a = 0.01 M, b = 0.03 M, c = 0.05 M, d = 0.1 M. V_t = 76.3 ml.
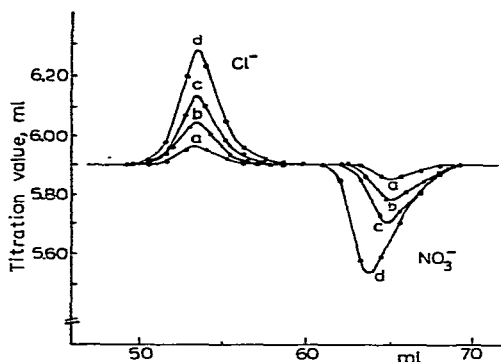

Fig. 5. Concentration dependence of elution curves of sodium nitrate. Sample: 0.5 ml of each concentration of nitrate; a = 0.01 M, b = 0.03 M, c = 0.05 M, d = 0.1 M. V_t = 76.3 ml.

sample concentration. This phenomenon can be explained by partial adsorption. The concentration dependence of iodide[8] showed the same tendency as that of nitrate, and this was explained in terms of adsorption. Sinibaldi and Lederer[7] have demonstrated the hydrophobic adsorption of iodide on a Sephadex gel. The K_{av} values for perchlorate and thiocyanate are much larger than those for the other anions, and their elution curves showed excessive tailing. Recently, some workers pointed out that perchlorate[16] and thiocyanate[7] were adsorbed on a gel of the Sephadex G type; our experiments support their results. It is considered that hydrophobic anions (anions with weak hydration) have a large capacity for interaction with the gel matrix, so causing pronounced adsorption.

From the above considerations, the sieving effect may predominate for anions with lower lyotropic numbers (hydrophilic anions) and the adsorption effect for those with larger lyotropic numbers (hydrophobic anions).

Separation and determination of components of the sample solution

Fig. 6 is an elution curve for a mixture of sulphate, acetate and nitrate; the anions were separated completely. The third positive peak on the curves is a pseudo-peak caused by exclusion of the eluting agent from the sample-anion zones. In this
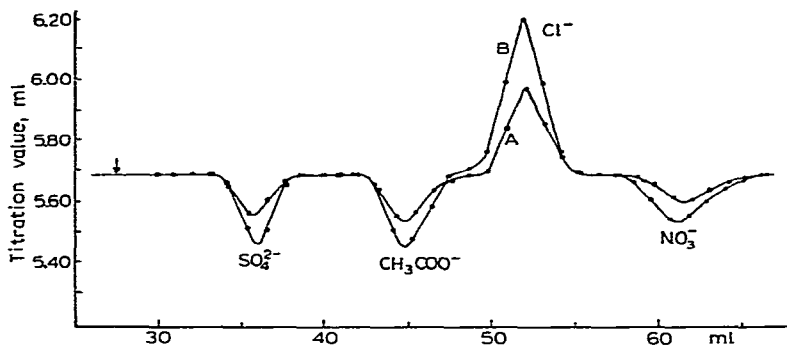
Fig. 6. Elution curves of mixtures of sulphate, acetate and nitrate. A: 0.5 ml of a mixture 0.01 M in sulphate and 0.02 M in both acetate and nitrate; B: 0.5 ml of a mixture 0.02 M in sulphate and 0.04 M in both acetate and nitrate. $V_t = 73.5$ ml. The arrow indicates the elution position of Blue Dextran. The positive peak is the peak for chloride caused by exclusion of the eluting agent from zones for the sample anions.

instance, the K_{av} values of the anions were the same as that of each anion eluted alone. Some anions, therefore, could be separated by utilising the differences in their elution volumes.

Fig. 7 shows the relationship between the areas of the negative peaks and the concentrations of some of the anions; the areas were rectilinearly proportional to anion concentration. Thus, the concentration of an anion could be indirectly determined by measuring the area of the corresponding negative peak.

Fig. 8 shows the relation between the areas of the positive peaks (representing the sodium chloride excluded by the anions in the sample solutions) and the concentration of the anions; this relationship was also rectilinear. The areas of the positive and the negative peaks for sulphate and nitrate were coincident with each other within the limits of experimental error. However, for citrate, the positive and negative areas were apparently different from each other; this difference may be caused by formation of the silver–citrate complex during argentimetric titration (recoveries of bromide and iodide were not quantitative because of co-precipitation with silver
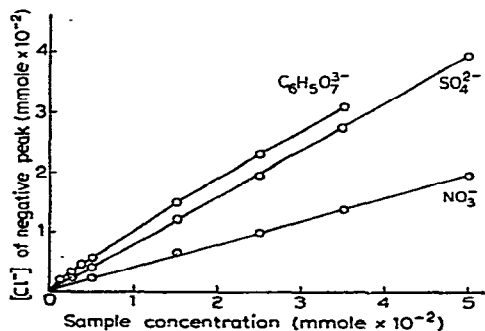

Fig. 7. Effect of sample concentration on area of negative peak.
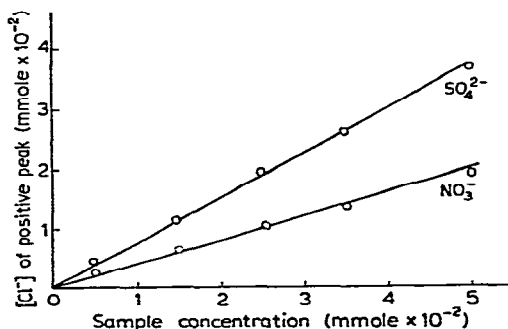
Fig. 8. Effect of sample concentration on the area of positive peak.

chloride[8]). Thus, possible interference by the sample anions during argentimetry should be borne in mind.

As shown in Figs. 7 and 8, the concentrations of sample anions were not consistent with that of the chloride excluded (negative peak area). These phenomena may be interpreted in terms of the Donnan equilibrium.

CONCLUSIONS

Our work on the behaviour of simple anions on a column of Sephadex G-15, with 0.1 M sodium chloride as eluent, leads to the following conclusions.

(1) The behaviour of the anions in the sample solution can be followed by determining the concentration distribution of the background electrolyte by argentimetry. This suggests possible automation of the procedure by selectively determining the anion of the background electrolyte (e.g., with an ion-selective electrode).

(2) The elution order of the anions followed the order of the lyotropic series; the more hydrophilic the anion, the more easily it was eluted from the column. Moreover, the more hydrophobic anions were more strongly adsorbed on the gel matrix.

(3) Some anions (for example, sulphate, acetate and nitrate) could be separated because of the differences in their elution volumes.

(4) When dilute sodium chloride solution was used as eluent, the concentrations of the anions in the sample solution could be indirectly determined by measuring the concentration of chloride in the eluate.

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