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CHROMATOGRAPHIC BEHAVIOUR OF INORGANIC SALTS ON HYDROXYETHYL METHACRYLATE GELS*

JINDŘICH BORÁK

Research Institute of Pure Chemicals, Lachema, 621 33 Brno (Czechoslovakia)

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SUMMARY

The behaviour of electrolytes in chromatography on hydroxyethyl methacrylate gels in aqueous media is considered. Experiments were carried out at different temperatures and the influence of different electrolytes in the mobile phase on the elution volumes of salts and on the chromatographic behaviour of water and heavy water was studied. The exclusions of most of the ions studied from the gel pores depended mainly on the type of anions, on the temperature during separation and on the composition of the mobile phase. The results are discussed on the basis of the microheterogeneity of electrolyte solutions and the structural properties of water near the surface of amphiphilic hydroxyethyl methacrylate gels.

INTRODUCTION

Recently, it was demonstrated that chromatography on neutral highly cross-linked polysaccharide, polyacrylamide or poly(acryloylmorpholine) gels is a useful technique for the separation of low-molecular-weight species as small hydrated inorganic ions¹⁻¹⁹. The chromatographic behaviour of inorganic ions was originally interpreted as an adsorption phenomenon or on the basis of steric exclusion. However, many facts contradict the steric exclusion of ions. The sizes of pores even in very tightly crosslinked chromatographic gels are still too great in comparison with the sizes of hydrated ions. Moreover, the hydration of ions may not be considered as a permanent bonding of water molecules. Ionic exclusion on the principle of Donnan equilibria seems to be well founded as most of the "neutral" chromatographic gels contain ionogenic, usually carboxylic, groups. However, their content is usually too small to cause this effect in all instances. Therefore, new mechanisms for the separation were suggested, applying specific structural features of polymeric gels. According to Gelotte¹, part of the water is needed for the hydration of polysaccharide chains of swollen Sephadex gels and its volume is no longer available for unrestricted distribution of the solute. Hence, small ions are eluted earlier than

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expected. Some other workers explained the separation on the principle of physical sorption. According to Lindqvist², the separation of inorganic ions on Sephadex G-25 might be due to the more or less bound ions on the gel matrix which give the gel the character of an amphoteric ion exchanger in its relationship to the mobile ions outside the gel.

The separation of inorganic solutes on Enzacryl K 1 gel was explained by Epton *et al.*¹¹ by the phenomenon of "additional exclusion". This can be regarded as adverse micropartition of solutes between regions within the individual gel beads where the constrained solution concentration of poly(acryloylmorpholine) chains is high and regions consisting of relatively pure solvents^{11,17}.

In the systematic investigation of the chromatographic properties of hydroxyethyl methacrylate gels (SpheronTM) the behaviour of electrolytes was also studied. As the Spheron gels are macroporous and contain small proportions of ionogenic groups, most attention was paid to the structural features of the gel phase, which is known to have an amphiphilic structure due to the presence of both hydrophilic and hydrophobic groups²⁰⁻²². The structure of water near the gel surface, influenced by the presence of hydrophobic sites, is considered to be very important for the explanation of the chromatographic behaviour of various solutes. This paper attempts to explain the different retentions of inorganic ions on hydroxyethyl methacrylate gels by their different effects on various "water structures" in the system.

EXPERIMENTAL

Materials

Spheron 300 hydroxyethyl methacrylate gel of particle size 10–25 μm (Lachema, Brno, Czechoslovakia) was washed before use with ethanol, benzene and acetone, using 250 ml of each solvent for 30 g of gel. This material contained 0.015 mequiv./g of carboxylic groups.

Glycerol, glucose, urea, and the solvents used for washing the gel (all from Lachema) were of analytical-reagent grade. Deuterium oxide (99.8% D) was obtained from SIC (Geneva, Switzerland) and Dextran T 2000 from Pharmacia (Uppsala, Sweden).

Apparatus

Experiments were carried out on a liquid chromatograph (Waters Assoc., Milford, Mass., U.S.A.) with a Model 401 refractometric detector. The stainless-steel chromatographic column (100 \times 0.7 cm) was provided with a septum injector and a jacket with circulating water from a thermostated bath. For packing the column, an MC 706-300 pump (Mikrotechna, Prague, Czechoslovakia) with a pulse damper was used.

Method

The column was packed with Spheron 300 suspended in a 40% aqueous solution of ammonium sulphate by the slurry technique²³ using a 60 \times 0.7 cm adapter. After 200 ml of the salt solution had passed through at a rate of 2 ml/min, the column was washed with distilled water until the test for sulphates in the eluate was negative.

In most instances water or 0.1–0.5 M solutions of inorganic salts were used as

mobile phases. Measurements were made at flow-rates of the mobile phases of 0.5–0.6 ml/min. Samples (50 μ l) were applied as 0.02–0.5 M aqueous solutions. Chromatographic separations were carried out at temperatures of 5°, 27°, 51° and 80°.

RESULTS

Elution volumes (V_e) and distribution coefficients (K_D) measured for different substances at various temperatures and in different mobile phases are summarized in Tables I–IV. The distribution coefficient is defined as $K_D = (V_e - V_0)/(V_{HDO} - V_0)$, where V_0 is the void volume of the column determined by means of Dextran T 2000, V_{HDO} is the elution volume of heavy water and V_e is the elution volume of the substance being tested.

Fig. 1 shows a chromatogram of a mixture of Dextran T 2000, glucose and heavy water. According to the data obtained, for glucose $K_D = 0.80$, which suggests that part of the volume of the gel pores is not accessible to glucose molecules. A similar

TABLE I

V_e AND K_D VALUES OF VARIOUS COMPOUNDS IN WATER AT DIFFERENT TEMPERATURES

Compound	Temperature (°K)							
	278		300		324		353	
	V_e (ml)	K_D	V_e (ml)	K_D	V_e (ml)	K_D	V_e (ml)	K_D
D ₂ O	28.1	1	28.0	1	27.9	1	27.7	1
Na ₂ SO ₄	23.7	0.73	23.8	0.74	23.7	0.74	23.7	0.75
NaF			24.3	0.78	24.1	0.77	24.2	0.78
NaOAc	24.9	0.80	25.0	0.82	24.6	0.79		
NaCl	25.2	0.82	25.0	0.82	24.7	0.81	24.6	0.80
NaNO ₂	25.9	0.87	25.7	0.86	24.9	0.82	25.1	0.83
NaBr	26.2	0.89	25.7	0.86	25.2	0.83	24.9	0.82
NaNO ₃	27.0	0.93	26.4	0.90	25.5	0.85	25.1	0.83
NaN ₃	26.8	0.92	26.3	0.90	25.4	0.85	25.1	0.83
NaI	29.3	1.07	27.9	0.99	26.6	0.92	25.8	0.88
NaSCN	32.3	1.25	30.0	1.12	27.9	1	26.9	0.94
LiCl			25.0	0.82				
CsCl			25.0	0.82				
UO ₂ (NO ₃) ₂			26.3	0.90				
Glycerol	26.1	0.88						
Urea	29.8	1.10						

TABLE II

K_D VALUES OF VARIOUS INORGANIC SALTS IN 0.1 M Na₂SO₄ AT 27°

Compound	K_D	Compound	K_D
Na ₂ SO ₄	0.76	KBr	0.96
NaF	0.78	NaN ₃	1.01
NaOAc	0.84	NaNO ₃	1.02
NaCl	0.87	NaI	1.36
NaNO ₂	0.94	NaSCN	2.15

TABLE III

V_e VALUES OF NaSCN IN 0.1 M SOLUTIONS OF DIFFERENT ELECTROLYTES AS ELUENTS

Eluent (0.1 M)	V_e (ml)	Eluent (0.1 M)	V_e (ml)
Na ₂ SO ₄	47.0	NaI	31.9
NaCl	37.1	NaSCN	30.1
NaNO ₃	34.8	H ₂ O	30.0

TABLE IV

V_e VALUES OF WATER AND HEAVY WATER IN DIFFERENT ELUENTS AT 27°

Compound	Eluent						
	0.1 M Na ₂ SO ₄	0.1 M NaCl	0.5 M NaCl	0.1 M NaNO ₃	0.1 M NaI	0.1 M NaSCN	H ₂ O
D ₂ O	28.1	28.2	28.3	28.1	28.2	28.0	28.0
H ₂ O	23.9	25.3	25.1	26.5	28.1	29.7	

effect was also observed for most of the salts chromatographed (Table I). The temperature dependence of the retention of various salts on Spheron 300 is clearly shown in Fig. 2, where $\ln K_D$ is plotted against $1/T$.

Figs. 3 and 4 illustrate the separations of inorganic salts at 27° (Fig. 3) and 5° (Fig. 4). For a better comparison, in Fig. 3 the elution times of heavy water are shown. In Fig. 4 a typical adsorption shape of the peak of sodium thiocyanate (which is eluted after heavy water) can be observed.

Other examples of separations of salts with a common counter ion (Na⁺) are shown in Fig. 5 (80°) and Fig. 6 (27°). An increase in temperature resulted in a decrease in the elution times of all salts except sulphate. The influence of temperature was greatest for sodium thiocyanate (peak 5), the adsorption of which was completely suppressed at 80°. In Fig. 6 the full line corresponds to the separation of a mixture of 0.5 M salt solutions and the broken line to that of 0.1 M solutions. The elution times

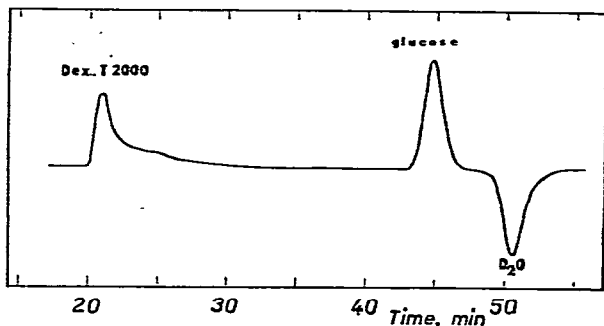


Fig. 1. Chromatogram of a mixture of Dextran T 2000, glucose and D₂O on Spheron 300 in water as eluent. Column, 100 × 0.7 cm; particle size, less than 25 μm; flow-rate, 0.55 ml/min; temperature, 27°; pressure, 30 atm. Sample: 50 μl of 0.5% solution of Dextran T 2000 and 1% glucose in mixture of D₂O and H₂O (1:1).

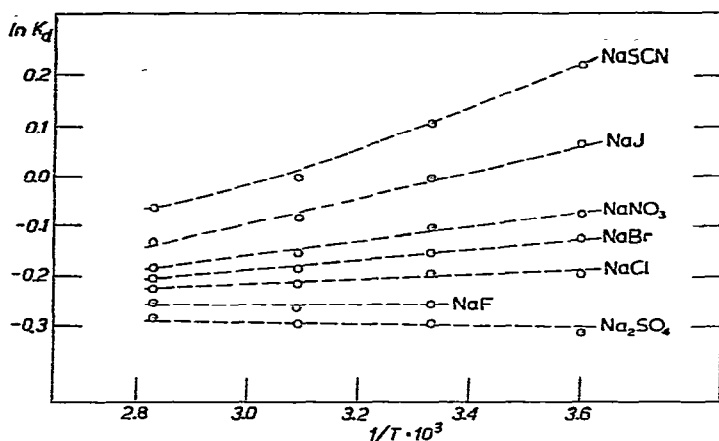


Fig. 2. Temperature dependence of distribution coefficients of inorganic salts on Spheron 300 in water.

of individual peaks are identical or very similar on both chromatograms in Fig. 6, except for thiocyanate (peak 5). When more dilute salt solutions (0.02–0.08 *M*) were used, the peaks were highly asymmetric, with a small slope at the front, and the positions of the maxima depended largely on concentration. On the other hand, at concentrations above 0.5 *M* the elution times did not change.

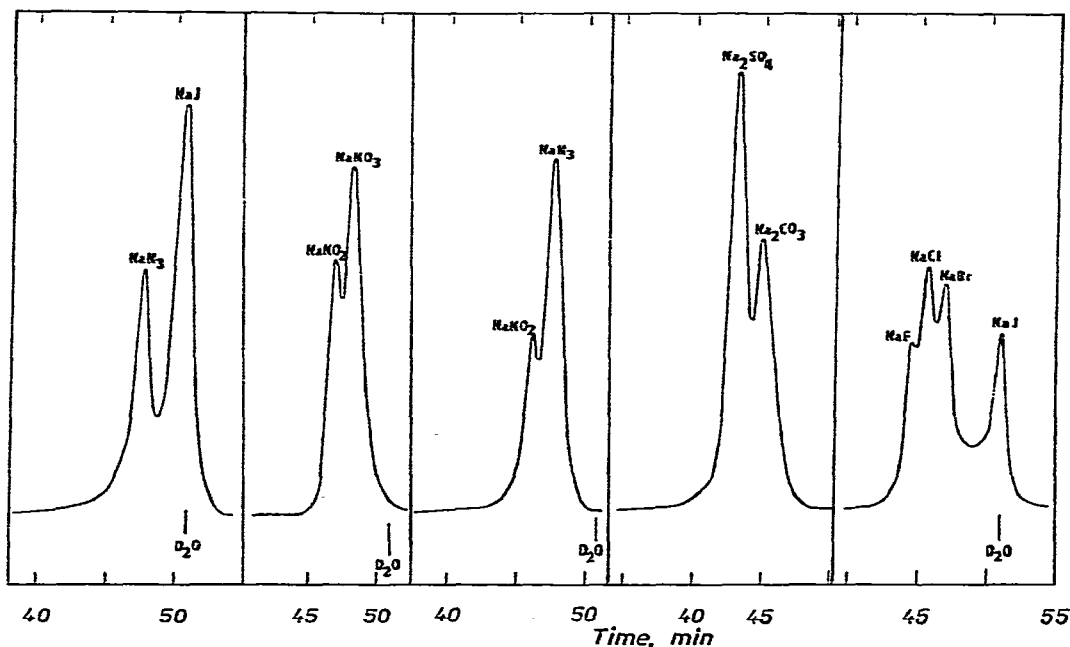


Fig. 3. Separation of some mixtures of inorganic salts. Samples: 50 μ l of the mixture of 0.5 *M* solutions of particular salts with the exception of the last example, when 50 μ l of a mixture of 0.5 *M* NaF, 0.3 *M* NaCl, 0.2 *M* NaBr and 0.2 *M* NaI was applied. Column, packing and operating conditions as in Fig. 1.

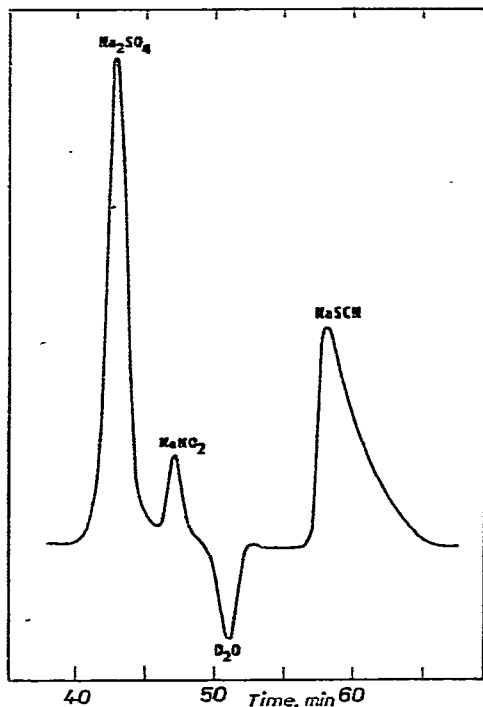


Fig. 4. Chromatogram of a mixture of some inorganic salts and heavy water. Sample: 50 μ l of a mixture of 0.5 M solutions of salts in 50% D_2O . Temperature, 5°. Column, packing and other operating conditions as in Fig. 1.

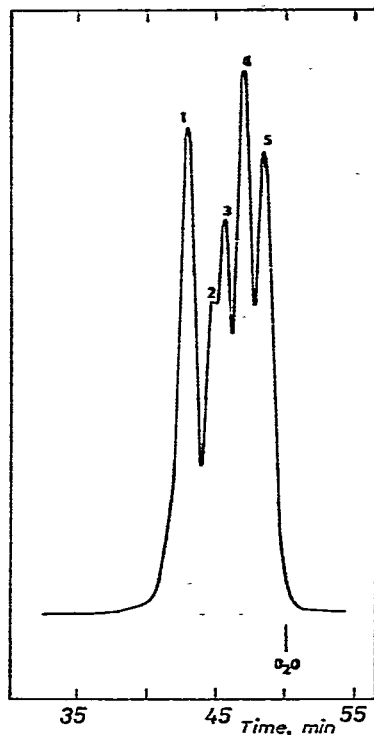


Fig. 5. Separation of a mixture of sodium salts in water at 80°: 1, sulphate; 2, acetate; 3, nitrate; 4, iodide; 5, thiocyanate. Sample: 50 μ l of a mixture of 0.5 M salts in water. Column and operating conditions as in Fig. 1.

The influence of electrolytes in the mobile phase on the retention of anions on Spheron 300 is apparent from Tables II and III and Fig. 7. In Table II the K_D values of inorganic salts in 0.1 M sodium sulphate solution as the mobile phase are given. An example of a separation in sodium sulphate solution is shown in Fig. 7, where the adsorption character of the peaks of nitrate, iodide and thiocyanate can be observed. It should be mentioned that the peak of sodium sulphate is much higher than would correspond to the concentration of sodium sulphate in the sample used.

The elution volumes of sodium thiocyanate in different 0.1 M electrolyte solutions as mobile phases are given in Table III. From these data it is obvious that the elution volume of the thiocyanate increases in the order H_2O , 0.1 M NaI, 0.1 M $NaNO_3$, 0.1 M NaCl, 0.1 M Na_2SO_4 .

Interesting effects were observed when the influence of the electrolytes in the mobile phase on the chromatographic behaviour of water and heavy water was studied. Table IV summarizes the elution volumes of normal and heavy water in different solutions. Fig. 8 shows an example of the different behaviour of normal and heavy water in chromatography on Spheron 300 in 0.1 M sodium sulphate solution.

In addition to Spheron 300, other Spheron gels differing in their porosities

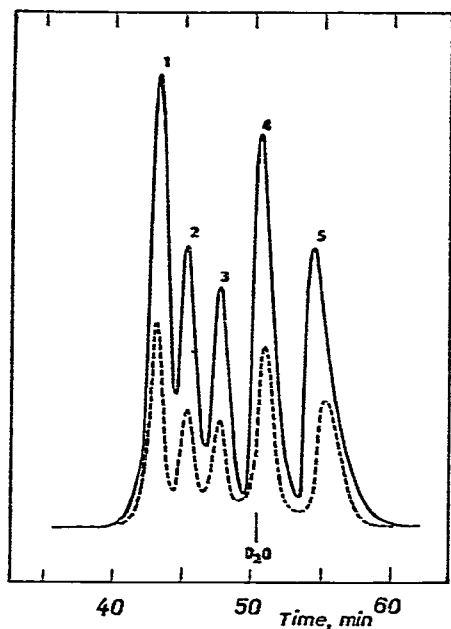


Fig. 6. Separation of a mixture of sodium salts in water at 27°C: 1, sulphate; 2, acetate; 3, nitrate; 4, iodide; 5, thiocyanate. Samples: full line, 50 μ l of a mixture of 0.5 M salts in water; broken line, 50 μ l of a mixture of 0.1 M salts in water. Column and operating conditions as in Fig. 1.

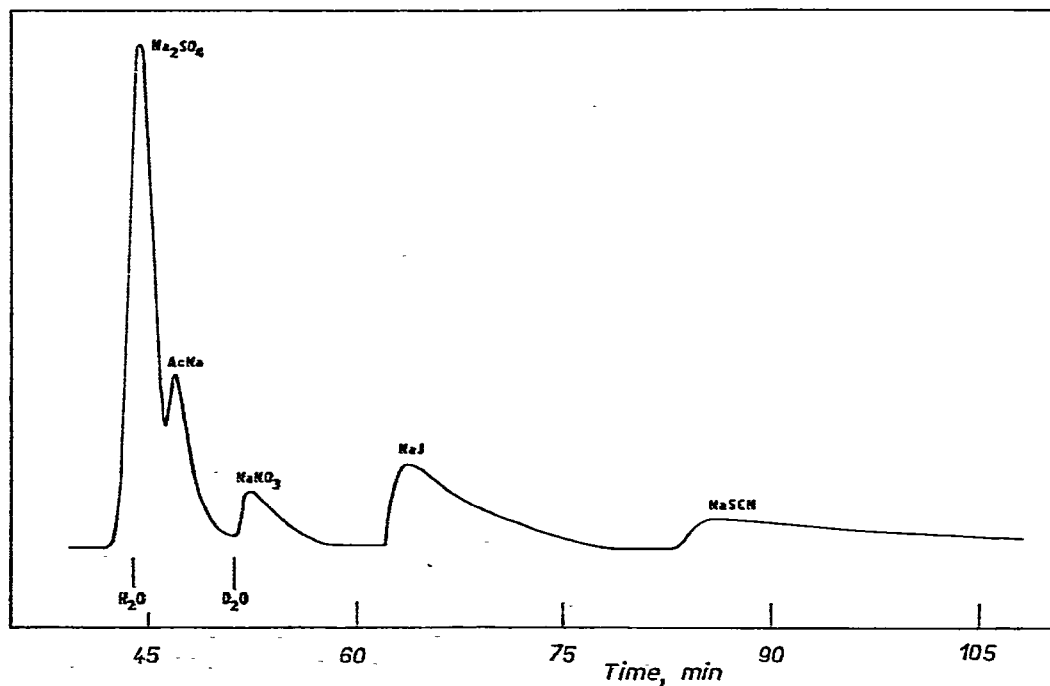


Fig. 7. Separation of a mixture of various sodium salts in 0.1 M Na_2SO_4 solution as eluent. Sample: 50 μ l of a mixture of 0.5 M solutions in 0.1 M Na_2SO_4 solution. Column, packing and operating conditions as in Fig. 1.

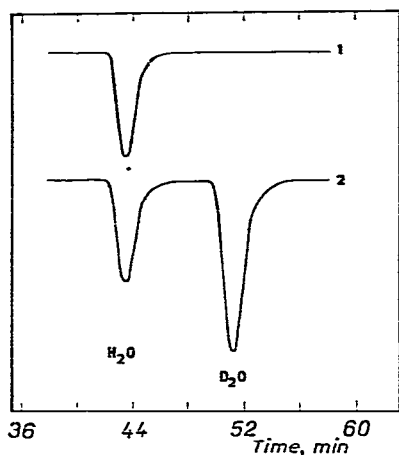


Fig. 8. Peaks recorded after injection of normal water (1) and heavy water (2). Eluent: 0.1 M Na₂SO₄ solution. Sample: 50 μ l of water or heavy water. Column, packing and operating conditions as in Fig. 1.

were also tested for separations of inorganic salts. The results obtained are not given here, but they were very similar to those for Spheron 300.

DISCUSSION

When interpreting the results of chromatographic measurement, heavy water is assumed to penetrate completely into the pores of hydroxyethyl methacrylate gel. Moreover, it is assumed that the heavy water is not retained due to adsorption or isotopic exchange with hydrogen atoms of the surface hydroxyl groups. This can be deduced from a paper by Marsden²⁴, who determined the pore volumes of Sephadex G-10 and G-15 using tritiated water and water labelled with oxygen-18. With Sephadex G-10 the value of the correction factor $[= (V_e^{\text{THO}} - V_0)/(V_e^{\text{H}_2^{18}\text{O}} - V_0)]$ was found to be 1.09, which corresponds approximately to a 4–5% higher elution volume of tritiated water in comparison with the elution volume of water labelled with oxygen-18. The content of hydroxyl groups in the Spheron gels is several times lower than that in tightly crosslinked Sephadex gels. Therefore, it can be expected that the retention of deuterium oxide on the Spheron surface due to isotope exchange would be lower than that with Sephadex, and that this effect could be neglected. This is also suggested by the independence of the elution volume of heavy water on temperature and the type of the electrolyte in the mobile phase. As can be seen in Tables I and IV, the latter vary only in the ranges of experimental errors.

It follows from the distribution coefficients given in Table I that most of the ions investigated are excluded to different degrees (negative adsorption) from the pores of the Spheron gel. At ambient temperature only sodium thiocyanate is positively adsorbed, while at 5° sodium iodide is also adsorbed. Adsorption of some ions on tightly crosslinked Sephadex gels was studied by several workers^{8,10,18}. The adsorption of iodide, perrhenate, dichromate and chloroaurate ions on Sephadex G-25 and LH-20 was explained by the interactions of anions with hydrophobic

centres on the gel surface¹⁰, and the adsorption of thiocyanate ions on soluble poly(2-hydroxyethyl methacrylate) was explained in a similar manner²⁵. Interactions of an analogous type may also be postulated in the adsorption of iodide ions on Spheron 300 at 5° and in the adsorption of thiocyanate ions at temperatures under 50° (see Table I). Interactions of these ions with hydrophobic centres on the surface of Spheron gel are suggested also by the increase in adsorption in the presence of electrolytes in the mobile phase, as can be seen in Figs. 6 and 7 and Tables I-III.

Studies on the exclusion of strong electrolytes on neutral gels^{1-9,11-17} show that this phenomenon cannot be explained merely by the Donnan equilibria. With respect to the low content of ionogenic, mainly carboxylic, groups, for neutral gels the Donnan exclusion of ions^{26,27} was observed only in the chromatography of very dilute aqueous solutions^{5,11,12}. With Spheron 300 containing 0.015 mequiv./g of carboxylic groups this effect was observed when 0.02-0.08 *M* salt solutions were applied and water was used as the mobile phase. For more concentrated solutions the influence of Donnan forces is suppressed and the mechanism of salt exclusion is apparently different.

When considering the decisive influence of a macromolecular backbone of Spheron gels on their interactions with ions, it is necessary to interpret the results on the basis of the character of these materials. When the interactions of aliphatic alcohols with the surface of Spheron gels in aqueous solutions were studied, the adsorption was found to increase with temperature in the range 5-60° (ref. 21). At room temperature the adsorption is endothermic and the main reason for this process is a "hydrophobic interaction". Hydrophobic sites on the gel surface in aqueous media are accessible to the hydrocarbon chains of aliphatic alcohols, thus permitting adsorption due to an increase in the entropy of the system. Hydrophobic groups of the gel in aqueous medium are in contact with water molecules, the arrangement (structure) and also the properties of which therefore differ from those of bulk water molecules. Changes in the water structure near non-polar species have been investigated by several workers (*e.g.* refs. 28-37). Useful results were also obtained in studies of the stability of fine hydrophobic suspensions in water and in electrolyte solutions^{38,39}. A considerable influence on the properties of water by hydrophobic crosslinking agents was observed by means of NMR spectroscopy with tightly cross-linked polyacrylamide gels and with Sephadex G-25 (ref. 15).

From Table I, it is obvious that the elution volumes of salts depend only on the type of anions. The elution volumes of chlorides were identical for lithium, sodium and caesium counter ions. Similarly with nitrates, the elution volumes remained constant when sodium was replaced with uranyl. These results are in accordance with several recent investigations^{3,10,13}. A considerable influence of anions compared with cations was also found in a study of the swelling of poly(2-hydroxyethyl methacrylate) in aqueous solutions of electrolytes²⁵.

Workers investigating the stability of hydrophobic suspensions³⁸⁻⁴⁰ explained the decisive importance of the anion as a consequence of different orientations of water molecules near the hydrophobic surface. This concept is based on the results of measurements of potentials in the water-air interface⁴¹, suggesting that the molecules of water adjacent to a non-polar phase are oriented mainly with the negative part of the dipoles toward the non-polar surface. Hence, a similar orientation of water is also probable in the proximity of hydrophobic groups of the Spheron hydroxyethyl methacrylate gels. Owing to the asymmetric orientation near the gel

surface, the molecules of water are directed with their hydrogen atoms and therefore also with their partial positive charges towards the bulk phase, thus enhancing the interaction of negative ions and making difficult a closer contact between the positive ions and the water near the surface. It is manifested in a higher sensitivity of the surface structure of water towards particular anions.

The elution sequence of anions on Spheron 300 gel does not change with temperature, and corresponds to the lyotropic series or the so-called lyotropic number of anions. The lyotropic number is derived from the concentration of sodium salts of different anions required to flocculate agar⁴². In this respect, it is useful to compare the exclusion and adsorption of ions on neutral gels with salting-out and salting-in of organic substances in aqueous solutions. Spheron hydroxyethyl methacrylate gels and also tightly crosslinked polysaccharide and polyacrylamide gels are fairly rigid, thus preventing substantial deformation of their macromolecular backbone owing to the presence of salts in the aqueous phase. Therefore, the minimization of free energy in the gel-aqueous solution system of the electrolyte may be accomplished by the exclusion of ions from the environment of rigid organic macromolecules, *i.e.*, by their exclusion from part of the gel pores. In a system with low-molecular-weight organic substances this is accomplished by separating a new phase, salting-out from the solution. On the other hand, the salting-in ability of ions leads to their adsorption on the gel but for low-molecular-weight organic substances to an increase in their solubility.

It is obvious from Fig. 2 that the retentions of sodium sulphate and sodium fluoride are not significantly dependent on temperature. For other substances, the retention decreases with increasing temperature. This is remarkable, especially for iodide and thiocyanate with the highest distribution coefficients. From the temperature dependence of the distribution coefficients of NaCl, NaBr, NaNO₃, NaI and NaSCN, it can be deduced that the transfer of these salts to the gel surface is accompanied by a decrease in entropy. Therefore, the transition of these ions into the surface phase is controlled by the enthalpy term of the Gibbs-Helmholtz equation, *i.e.*, the energy of the interaction between ions and water molecules close to the gel surface is higher than that between ions and water molecules at greater distances from the surface. The entropy change accompanying this process may be caused by the decrease in entropy of the bulk phase as a consequence of the higher degree of ordering of water molecules in pure water in comparison with that in solutions of ions with low charge densities (especially I⁻ or SCN⁻)^{29,43}, the so-called hydrophobic or chaotropic ions.

In explaining the principle of ion exclusion from the pores of hydroxyethyl methacrylate gels, it is useful to apply some models assuming a microheterogeneous structure of water and aqueous solutions of electrolytes^{28-33,34,35,43,44}. Water molecules in closest contact with ions are in a different energetic state from those which are distant from the solute. Different ions affect the structure of water in different ways²⁹. Ions with a high charge density (SO₄²⁻, F⁻) cause a higher degree of ordering of water molecules, while those with a low charge density (I⁻, SCN⁻) affect water structure in a destructive way, *i.e.*, the entropy of the system in comparison with pure water is increased. Samoilov⁴³ suggested an interpretation of the specificity of aqueous solutions of electrolytes based on a diverse influence of ions on the translational movement of adjacent molecules of water or water aggregates. Transport phenomena in aqueous solutions of electrolytes are accomplished by means of activated exchange

between different equilibrium states characterized by particular values of the activation energy. The so-called "kinetic" or transport properties of ions in aqueous solutions depend mainly on the type and intensity of their contacts with adjacent water molecules⁴³.

When taking into account the decisive influence of the so-called "proximate hydration" on the properties of electrolyte solutions, it is possible to consider them as a sum of microstructural elements involving aggregates of water molecules similar to those which exist in pure water and microstructural elements consisting of ions and adjacent water molecules. If in the solution of an electrolyte an amphiphilic gel is present, then the structure of water in the system is additionally influenced by the surface of the gel. The system is in a dynamic state characterized by a permanent exchange among the particular microstructures. This exchange needs a reorientation of water molecules, *i.e.*, adaptation to that microstructure into which they enter. It may be expected that the process of reorientation determines the rate of transition of water molecules from one microstructure to another and, hence, it may be expressed in terms of the activation energy of water transfer (as Samoilov⁴³ did for his model of electrolyte solutions). Values of these activation energies depend on temperature and on the type of microstructural elements in the system, *i.e.*, on the type of ions and the structure of water in the interface adjacent to the gel surface. The transfer of hydrated ions to the gel surface is likewise assumed to be accompanied by an exchange of water molecules or aqueous aggregates among the microstructures in the neighbourhood of ions and structural elements of water influenced by the gel surface. Simultaneously with this process, however, a permanent water exchange among the microstructures of bulk water and microstructures influenced both by the ion and by the surface occurs. Eventually, in this model it is necessary to consider also the transition of water molecules and aqueous aggregates among individual microstructures of bulk water, *i.e.*, those, which are not adjacent to ions or the surface of the gel. All of these processes may be characterized by particular values of the activation energy.

A quantitative theoretical treatment of this model will be very difficult, but some qualitative consequences can be discussed. Exclusion of ions will probably occur if the activation energy of the transfer of water molecules among ion and surface microstructures is higher than those among bulk phase microstructures and microstructures adjacent to the ion or gel surface, respectively. This is probably the case with sulphate ions which, owing to their high charge density, stabilize the water structures in their neighbourhood so that the reorientation of water molecules becomes more difficult. Iodide or thiocyanate ions show a reverse effect and therefore at lower temperatures these ions penetrate to the gel surface and may be adsorbed there.

An increase in temperature results in a decrease in the activation energies of the transfer of water molecules among microstructural elements of bulk water partly destroyed by a higher thermal movement on the one hand and water aggregates adjacent to the ions or to the gel surface on the other. Assuming that the temperature change influences the structure of water aggregates in bulk water more than those in the nearest neighbourhood of ions and in the surface interface, we can explain in this way the higher exclusion effect of ions at increased temperatures observed in this study (see Fig. 2 and compare the chromatograms in Figs. 5 and 6).

As can be seen in Table II, the presence of sulphate ions in the mobile phase

greatly suppresses the exclusion of chromatographed ions and increases their adsorption on the gel. This may be explained on the basis of the proposed model by influencing the structure of the bulk water by means of sulphate ions. Here, the activation energy of the transfer of water molecules among microstructures of particular, *e.g.*, thiocyanate, ions and other types of water aggregates in the system increases. An increase in this activation energy due to the presence of a foreign ion is probably the cause of incompatibilities of water microstructures of ions of different types in the solution which leads to "repelling" of ions from the bulk phase to the gel surface. This effect may also be the cause of the so-called "false" peaks corresponding to the electrolyte in the mobile phase observed in the chromatography of salts on Sephadex gels in electrolyte solutions^{5,7,13}. The same applies to the peak of sodium sulphate (Fig. 7), which is more intense than that corresponding to its amount in the sample.

Table III shows that the effect of repelling the thiocyanate ions from the bulk phase decreases in the same order as the decreasing salting-out ability of electrolytes in the mobile phase. The ability of electrolytes to increase the degree of ordering of water molecules in solutions decreases in the same order. This is probably manifested also in the corresponding influence on the activation energies of water exchange among individual microstructural elements.

As is shown in Table I, the distribution coefficient of glycerol found at 5° is less than 1, which means that glycerol is excluded from hydroxyethyl methacrylate gel pores. On the other hand, urea exhibits a weak adsorption on the gel. It is known that glycerol increases the degree of ordering of water molecules in solutions, whereas urea affects aqueous aggregates in a destructive manner. Hence, the exclusion of glycerol from pores of hydroxyethyl methacrylate gels and the adsorption of urea may be explained in an analogous manner to the exclusion and adsorption of ions.

Table IV shows that the elution volume of heavy water is almost the same in all solutions used whereas the elution volume of normal water changes and its values are identical with those of electrolytes used in chromatography with water as the mobile phase. The different behaviours of normal and heavy water in chromatography on Spheron with the use of mobile phases containing electrolytes (see Table IV and Fig. 8) suggest different affinities of ions for normal and heavy water. Investigations by Tauber and co-workers^{45,46}, who studied the isotopic compositions of H₂¹⁸O in electrolyte solutions, showed that the isotopic equilibrium composition of water in the neighbourhood of ions is different from that in the remainder of the bulk phase and depends mainly on the type of electrolyte in the solution. Similar effects probably apply also during the chromatography of normal and heavy water on Spheron gel in solutions of different electrolytes as eluents. In the nearest environment of ions the interaction with H₂O is preferred. Therefore, the molecules of heavy water are excluded from this area and the elution volume of heavy water is virtually independent of the type of the electrolyte in the mobile phase (see Table IV). Smaller elution volumes of normal water in eluents with Na₂SO₄, NaCl and NaNO₃ suggest its exclusion from the surface interface of the gel. This may follow from the suppression of the rates of exchange of water molecules among bulk and surface water microstructures caused by interaction of the electrolyte with water in the bulk phase.

CONCLUSION

In a similar manner to other neutral amphiphilic materials, hydroxyethyl methacrylate gels can be used for the separation of species as small as inorganic ions. Differences in the chromatographic behaviour of anions are caused by the different degree and type of water arrangement in the vicinity of the ions and the amphiphilic surface. Structure-breaking anions are adsorbed on the gel surface whereas structure-forming anions are squeezed out from the pores of gels. Similar effects may also be observed for organic substances that are able to break down the structure of water aggregates (urea) or, on the contrary, to stabilize it (glycerol). The adsorption of ions is increased if the mobile phase contains an electrolyte. A similar increase in retention also applies in the chromatography of substances that are able to interact with the hydrophobic centres of amphiphilic gels. In both instances the magnitude of this effect depends on concentration and the type of anion.

The study of the chromatographic behaviour of anions may contribute to the explanation of the mechanisms of the so-called "hydrophobic chromatography". The results of such a study may serve in the elucidation of some phenomena in the transport of ions and other materials through artificial (desalination) and biological membranes.

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