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ALKALI CATION SELECTIVITY OF SEPHADEX G-25 IN WATER AND AQUEOUS MIXTURES OF METHANOL, ETHANOL AND *tert.*-BUTANOL

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SUMMARY

The column chromatographic partitioning of some alkali salts in Sephadex G-25 and the divinyl sulphone cross-linked dextran gel DVS-9 in water and in aqueous methanol, ethanol and *tert.*-butanol in G-25, is described. In water the order of elution is $\text{Li}^+ < \text{Na}^+ < \text{Cs}^+ < \text{Rb}^+ < \text{K}^+$ in G-25 and $\text{Li}^+ < \text{Na}^+ < \text{Rb}^+ < \text{Cs}^+ < \text{K}^+$ in DVS-9; the selectivity in G-25 is poor and somewhat less than in DVS-9. Comparison of the two gels suggests that they have an affinity for the alkali salts. The cation distribution coefficients (G-25) are anion-dependent, and affinity in this quasi-electroneutral gel presumably involves ion pairs.

In all the binary solvent mixtures studied, the alkali cation selectivity was much enhanced and, at least in methanol, the complete aqueous order was preserved. In both methanol and ethanol but not in *tert.*-butanol the distribution coefficient of Li^+ alone of the cations was little affected. In methanol separation of all five lower cations is possible on a column of only moderate length. Preliminary studies also indicated that alkaline earth cation selectivity was considerably enhanced in water-methanol mixtures.

Using data on the composition of the imbibed solvent it was shown that for the mixed solvent system partitioning due to the different solubilities of the alkali salts in the solvent compartments inside and outside the gel cannot account for the observed values. In analogy with other systems, the elution order suggests, as concluded for the pure aqueous systems, that affinity for the gel is also involved at least in the aqueous methanol system. Aqueous *tert.*-butanol seems to be a special case as this alcohol was almost completely excluded from the gel, and it is suggested that at least in this mixture a decreased swelling pressure of the gel may contribute in part to the higher alkali cation affinity.

INTRODUCTION

The highly cross-linked but otherwise unsubstituted dextran gels display moderate selectivity to both simple anions and cations when water is the sole solvent¹⁻⁷. Not unexpectedly, substitution of alcohols for water has marked effects on cation

selectivity: thus, partial replacement of water by methanol results in considerable enhancement⁸⁻¹⁰. This, it has been suggested, is a consequence of partitioning due to differences between the solubilities of the salts in the two solvents. Solubility-based partitioning depends on differences both in the solubilities of the electrolytes in the two solvents and in the solvent compositions inside and outside the gel beads.

This paper examines the partitioning of some alkali salts when water is the sole solvent and also in water-methanol, water-ethanol and water-*tert.*-butanol mixtures. Finally, partitioning in the binary systems is discussed in relation to the differences in solvent composition inside and outside the gel beads.

Although the more highly cross-linked Sephadex G-10 has a higher alkali cation selectivity than the less highly cross-linked gel G-25, the latter was used in the mixed solvent systems since in these solvent mixtures G-10 exhibits undesirable chromatographic effects such as peak broadening and tailing.

EXPERIMENTAL

Materials

Sephadex[®] G-25, Batch No. 5336 (water regain = 2.08) and a divinyl sulphone cross-linked dextran gel, DVS-9 (water regain = 0.85), dextran[®] 500, $M_w = 450,000$ daltons and ficoll[®], $M_w = 400,000$ daltons (Pharmacia, Uppsala, Sweden), a generally ¹⁴C-labelled dextran, $M_w = 77,000$ daltons (Argonne National Lab., U.S.A.), tritiated water, [¹⁴C]methanol and [¹⁴C]ethanol (Radiochemical Centre, Amersham, U.K.) and [¹⁴C]*tert.*-butanol (NEN, Dreieich, F.R.G.). All other chemicals were of analytical grade.

Analyses

Dextran and ficoll were determined by an anthrone-sulphuric acid method¹¹. ¹⁴C and tritium activities were measured by liquid scintillation (CPM 200, Beckman, CA, U.S.A.). The alkali metal cations were determined by flame photometry (Beckman B). When alcohol was present, the weighed sample was first evaporated to dryness and then redissolved in a known weight of water in order to eliminate the unstable flame-enhancing effect of the alcohol.

Methods

Column chromatography. Dextran gels were allowed to swell for at least 24 h in a large excess of deionized water, the latter being changed several times. When a binary mixture was used, the water-wetted gel was transferred to the desired solvent mixture for at least a further 24 h. This solvent was also changed several times, the final external solvent composition being checked by pycnometry. The binary mixtures had the following compositions in weight fractions of the alcohol: methanol (0.43 and 0.68), ethanol (0.42) and *tert.*-butanol (0.44). The solvated gels were packed into columns 60 × 1 cm I.D. or 15 × 1 cm I.D. The prewashing and eluting solutions were in each case the same and contained Tris (2-amino-2-hydroxymethyl-1,3-propanediol), together with an acid to give a pH of 7.6. The common anion was varied in the aqueous systems but was either chloride or acetate in the binary mixtures. A column was always washed with at least three bed volumes of eluent immediately before an experiment began.

The sample volume loaded on to the 60-cm columns was 0.5 ml (less than 1% of bed volume) of a solution containing 0.05 or 0.1 mol dm⁻³ of each of the alkali salts.

Reference solutes. In the aqueous systems dextran was used as the void volume (V_0) indicator. In some experiments the generally labelled ^{14}C dextran and in others dextran 500 were used. In the binary systems the polysucrose, ficoll, was used because it is more soluble than dextran in these systems. Tritiated water and ^{14}C -labelled alcohols were also used in some experiments to determine the elution behaviour of these solvents.

Alkali cation recoveries. These were performed by eluting a 0.3-ml sample of an alkali chloride in Tris-HCl through a 15-cm column for both pure aqueous and binary solvent systems. A single sample of effluent was collected; its volume was estimated to be large enough to include all the alkali cation contained in the eluted peak. The subsequent 3 ml was also collected; if this sample contained any alkali cation the experiment was rejected. In order to reduce uncertainty due to a non-linear calibration curve, flame photometric measurements were made on test and standard solutions with approximately the same concentration, *i.e.* 1 mmol dm⁻³. Thus, the effluent sample was first diluted to give very nearly this concentration. Three 0.3-ml samples of the loading solution were delivered from a micrometer-operated syringe and diluted with the same buffer solution to give an alkali cation concentration of 1 mol dm⁻³. When alcohol was present all samples were evaporated before dilution and then redissolved in the required amount of deionized water.

Calculations

Solvent regain. The solvent regain (S_r) is approximately equal to the product of the weight fraction of water (f_0^w) in the binary mixture (A. B. Krantz, personal communication) and the water regain value (W_r), *i.e.*

$$S_r = f_0^w \cdot W_r \quad (1)$$

and has been calculated as such.

Chromatographic data. For aqueous systems, the distribution coefficient for component i (K_d^i) is calculated from the peak elution weights of i (m^i), a void volume indicator (m^0), in this case ficoll or dextran 500, and tritium of tritiated water (m^T). Owing to hydrogen isotope exchange (HIE) with the hydroxyl groups in the gel, a correction is required for the discrepancy between the elution volumes of the tritium activity and water¹². Thus,

$$K_d^i = K_d^T \frac{m^i - m^0}{m^T - m^0} \quad (2)$$

where the correction factor, K_d^T , is the distribution coefficient of tritium when the latter is loaded on to the column on a molecule such as water on which it is labile, *viz*:

$$K_d^T = \frac{m^T - m^0}{m^w - m^0} \quad (3)$$

where m^w is the elution weight of water labelled with a non-exchanging oxygen isotope, e.g. ^{18}O . For DVS-9 and G-25, the K_d^T values are 1.135 and 1.060, respectively.

In alcohol-water mixtures there is no unique indicator for the internal solvent space of the gel, but both the solvent and solute distribution coefficients and the internal solvent composition can be evaluated if isotopically labelled, small samples of the alcohol and tritiated water are eluted through the gel bed¹³. The ratio ($K_d^{T/s}$) between the chromatographic distribution coefficients of tritium (tritiated water) and alcohol can be calculated as follows:

$$K_d^{T/s} = \frac{m^T - m^0}{m^s - m^0} \quad (4)$$

where m^s is the elution weight of a labelled sample of the alcohol.

In view of the different solvent mixtures within and outside the gel particles, a distinction will be made between distribution coefficients expressed in terms of weight fractions (K_d) and mol fractions [$K_d(X)$], which are different. The distribution coefficient of the alcohol in eqn. 4 is defined as the ratio between the weight fractional concentrations of the alcohol in the solvent mixtures inside and outside the gel, *i.e.*

$$K_d^s = f_g^s / f_0^s \quad (5)$$

where f_g^s and f_0^s are the weight fractions of alcohols in 1 kg of imbibed and external solvent, respectively.

It should be noted that K_d^T is a complex distribution coefficient which is really a mole ratio being the ratio of the concentrations of the HIE sites inside and outside the gel. In a binary solvent it thus reflects not only the fixed HIE sites on the gel matrix but also the different compositions of protic solvents inside and outside the gel particles¹³. The ratio between the tritium and alcohol distribution coefficients ($K_d^{T/s}$ in eqn. 4) can thus be used to calculate the weight fraction of alcohol in the gel (f_g^s) as described previously¹³.

Since f_0^s is known, K_d^s can then be calculated and the distribution coefficient of the solute obtained, *viz.*:

$$K_d^i = K_d^s \cdot K_d^{i/s} \quad (6)$$

since by analogy with eqn. 4:

$$K_d^{i/s} = \frac{m^i - m^0}{m^s - m^0} \quad (7)$$

Finally, it is perhaps more meaningful to express a distribution coefficient in a binary system as the ratio of the mole fractions in the two solvent compartments, *viz.*:

$$K_d^i(X) = \frac{X_g^i}{X_0^i} \quad (8)$$

where

$$K_d^i(X) = K_d^i \frac{f_0^s (M^w - M^s) + M^s}{f_g^s (M^w - M^s) + M^s} \quad (9)$$

where M^w and M^s are the molecular weights in daltons of the water and alcohol, respectively.

TABLE I

ALKALI CHLORIDE SOLUBILITIES (MOLE FRACTIONS) AT 25°C

Figures in parentheses refer to the references listed below. When more than one reference is given, this indicates that the published values are similar, or if different, that the divergence is small and the arithmetic mean is given. In other cases more than one value is given. (1) N. V. Sidgwick, *The Chemical elements and their compounds*, Vol. 1, University Press, Oxford, 1949, p. 96. (2) A. Seidell, *Solubility of Inorganic, Metalorganic and Organic Compounds*, 3rd ed., McGraw-Hill, New York, 1940. (3) H. Stephen and T. Stephen, *Solubilities of Inorganic and Organic Compounds*, Vol. 1, *Binary Systems*, Pergamon, Oxford, 1963. (4) W. E. Turner and C. C. Bissett, *J. Chem. Soc.*, 103 (1913) 1904. (5) E. Lloyd, C. B. Brown, D. Glynwyn, R. Bonnell and W. J. Jones, *J. Chem. Soc.*, (1928) 658. (6) T. Pavlopoulos and H. Strehlow, *Z. Phys. Chem.*, 202 (1953) 475. (7) E. R. Kirn and H. E. Dunlop, *J. Amer. Chem. Soc.*, 53 (1931) 391. (8) R. G. Larson and H. Hunt, *J. Phys. Chem.*, 43 (1939) 417. (9) N. A. Izmailov and V. S. Chernii, *Trudi Komissii, Anal. Khim.*, II (XII) AN.SSR., (1958) 44. (10) H. E. Patten and W. R. Mott, *Z. Phys. Chem.*, 8 (1904) 153.

	<i>Water</i>	<i>Methanol</i>	<i>Ethanol</i>
Li ⁺	0.261 (1, 2)	0.243 (1, 4, 5, 6)	2.71 · 10 ⁻² (10) 0.212 (4)
Na ⁺	9.99 · 10 ⁻² (1, 2, 3)	7.61 · 10 ⁻³ (6, 7*, 8)	5.12 · 10 ⁻⁴ (4, 8) 9.17 · 10 ⁻³ (7*)
K ⁺	7.96 · 10 ⁻² (1, 2, 3)	2.26 · 10 ⁻³ (4, 6) 7.81 · 10 ⁻³ (7*)	1.36 · 10 ⁻⁴ (4) 1.82 · 10 ⁻⁴ (8) 8.19 · 10 ⁻⁴ (7*)
Rb ⁺	0.122 (1, 3)	3.63 · 10 ⁻³ (4, 6)	—
Cs ⁺	0.168 (1, 3)	5.43 · 10 ⁻³ (6, 9)	—

* Interpolated to 25°C.

Solubilities. In Table I the respective alkali chloride solubilities in water and alcohol (S_w^i , S_s^i) are given in mole fractions and for comparison (see Tables VIII and IX) the distribution coefficients $K_s^i(X)$ which would arise from ideal solubility, partitioning have been calculated as follows:

$$K_s^i(X) = \frac{S_s^i X_g^s + S_w^i X_g^w}{S_s^i X_0^s + S_w^i X_0^w} \quad (10)$$

where X_g^s and X_0^s are the mole fractions of alcohol inside and outside the gel, respectively, and X_g^w and X_0^w the corresponding values for water. In a binary system:

$$X_i^s + X_i^w = 1 \quad (11)$$

Selectivities. The selectivities are given as the relevant values normalized to that of lithium, viz:

$$S_d = K_d^i(X)/K_d^{Li}(X) \quad (12a)$$

$$S_s = K_s^i(X)/K_s^{Li}(X) \quad (12b)$$

$$S_{sdw} = K_s^i(X)/K_s^{Li}(X) \cdot K_d^i(X)/K_d^{Li}(X) \quad (12c)$$

TABLE II

DISTRIBUTION COEFFICIENTS OF ALKALI CHLORIDES AND SOME NON-ELECTROLYTES IN THE TWO DEXTRAN GELS WHEN WATER IS THE SOLE SOLVENT

Eluent, Tris-HCl 0.05 mol dm⁻³, pH 7.6.

	G-25*	DVS-9**
Li ⁺	0.89	0.68
Na ⁺	0.94	0.84
K ⁺	1.02	1.07
Rb ⁺	1.01	0.99
Cs ⁺	1.00	1.01
Glucose	0.81	0.59
Sucrose	0.72	0.43
Maltotriose	0.58	0.36
1-Heptanol	1.21	1.37

* Room temperature (ca. 22°C).

** 25 ± 0.1°C.

where S_d is the ratio of the distribution coefficients [$K_d(X)$], S_s is the ratio of the calculated solubility-based distribution coefficients and S_{sdw} is the product $S_s S_{dw}$.

RESULTS AND DISCUSSION

With water as the sole solvent

As shown in Table II, Sephadex gels G-25 and DVS-9 exhibit only a small degree of selectivity to the five lower alkali cations, which have the following order of elution volumes in G25: $Li^+ < Na^+ < Cs^+ < Rb^+ < K^+$. This order is the same in DVS-9, except for a change of places between Rb^+ and Cs^+ . These are neither orders of ascending crystal radii nor descending hydrated radii¹⁴ but are, in fact, two of the eleven orders predicted by Eisenman¹⁵ in cation-selective glasses. He postulated that cationic hydration and electrostatic interactions with the matrix determined the order¹⁶.

Comparison of the cationic K_d -values on G-25 and the less hydrated gel DVS-9 suggests that steric hindrance does not apparently contribute significantly to their partitioning as it does for polar non-electrolytes such as saccharides¹⁷. Thus, a particular saccharide is more excluded from a less water-swollen gel. The latter behaviour, which is clearly evident for saccharides in Table II, is the reverse of that for K^+ and Cs^+ whose K_d values increase with increasing matrix concentration; this suggests that the gels have an affinity for these cations. In this respect the pattern of cation behaviour is similar to that of weakly polar solutes, as exemplified by 1-heptanol in Table II, although the cause of the affinity must be different in the two cases.

As with simple anions, the distribution coefficients of the alkali cations were essentially independent of the eluent (background) electrolyte concentration in the range 10–500 mmol dm⁻³ (Table III). Similar findings have been reported in the more highly cross-linked Sephadex G-10⁶.

Since the Sephadex gels contain low concentrations of negatively charged carboxylate groups at the usually prevailing pH values, Donnan exclusion effects occur if the eluent concentration is too low⁶, i.e. in Sephadex G-10 below ca. 10 mmol dm⁻³. In G-10 these charged groups do not, however, contribute significantly to

TABLE III

DISTRIBUTION COEFFICIENTS OF THREE ALKALI CHLORIDES IN SEPHADEX G-25 ELUTED WITH DIFFERENT ELECTROLYTE CONCENTRATIONS AND WITH WATER AS THE SOLE SOLVENT AT 22°C

Eluent concentration (mmol dm ⁻³)	Li ⁺	Na ⁺	K ⁺
10	0.865	0.918	—
20	0.861	0.916	—
50	0.831	0.911	—
100	0.851	0.887	0.96
200	—	—	1.03
500	0.847	—	1.05

* Eluent cation was K⁺ in the case of Li⁺ and Na⁺; Tris-HCl was used to elute K⁺.

partitioning since the K_d values were essentially the same⁶ in the presence of either 10⁻³ molar NaCl or HCl in the eluting electrolytes (10⁻¹ molar NaCl). Similar considerations must also apply to gels G-25 and G-15 which contain even fewer charged groups.

A background electrolyte is mandatory if single cation K_d values are to be measured. Only with an excess of eluting electrolyte can ions migrate essentially independently of counter-ions. This does not imply, of course, that cation K_d values are anion-independent. Since it is not the charged groups and thus not an ion-exchange process which determines partitioning, conservation of electroneutrality demands the presence of pairs of ions at "sorption" sites. Thus, in addition to any attraction the cation itself has for the gel, the distribution coefficient will also depend on both the affinity of the anion for the gel and of the cation and anion for each other.

As Table IV shows, the K_d values of Na⁺ and K⁺ were highly dependent on the eluent anion whose order of increasing affinity, *i.e.* SO₄²⁻ < acetate < Cl⁻ < NO₃⁻ is the same as that of their effect on cationic K_d values. Thus, K⁺ has a higher K_d value when eluted with Cl⁻ than when SO₄²⁻ is the eluent anion. The opposite occurs with anions; Br⁻, for example, has a higher K_d value when SO₄²⁻ is the eluent than in the presence of Cl⁻ (Marsden, unpublished results).

Table IV also shows that, in two cases at least, the K_d ratios of Na⁺ and K⁺ were similar when eluted with different anions, *i.e.* Cl⁻ and acetate. This underlines

TABLE IV

DISTRIBUTION COEFFICIENTS OF Na⁺ AND K⁺ WHEN ELUTED WITH DIFFERENT ANIONS THROUGH SEPHADEX G-25 WITH WATER AS THE SOLE SOLVENT

Temperature, 25°C; eluent, Tris-HX pH 7.6.

X	Na ⁺	K ⁺	Ratio K ⁺ /Na ⁺
SO ₄ ²⁻	—	0.88	—
Acetate	0.89	0.94	1.06
Cl ⁻	0.97	1.05	1.08
NO ₃ ⁻	—	1.14	—

TABLE V

DISTRIBUTION COEFFICIENTS OF K^+ ELUTED WITH DIFFERENT ANIONIC MIXTURES (Tris-HX, 0.2 mol dm⁻³, pH 7.6) WITH WATER AS THE SOLE SOLVENT AT 22°C

Anions	Anionic mole ratio	K_d	H^*
SO_4^{2-}	—	0.884	—
Cl^-	—	1.03	—
NO_3^-	—	1.14	—
SO_4^{2-}, Cl^-	1:1	0.96	0.95
SO_4^{2-}, NO_3^-	1:1	0.98	1.00
Cl^-, NO_3^-	1:1	1.09	1.08
SO_4^{2-}, NO_3^-	3:1	0.92	0.94
Cl^-, NO_3^-	1:3	1.12	1.11
SO_4^{2-}, Cl^-, NO_3^-	1:1:1	1.02	1.01

* Weighted harmonic mean of K_d values of single anions.

the influence of the anion on cation partitioning. The anion influence is also additive as is evident from Table V, which shows partitioning data of K^+ when eluted with anionic mixtures. The weighted harmonic mean of the K_d values when eluted with single anions gave the best fit to the observed values in the binary and ternary anionic mixtures. A similar additivity has been reported for the polyacrylamide gel, Biogel^R P-2¹⁸.

As pointed out by St. Pierre and Jencks¹⁹, affinity does not necessarily indicate binding to a specific site on the matrix as opposed to a decreased activity coefficient in the solvent in the vicinity of some part of the matrix. Affinity, however, has been reported to be associated with a particular chemical group. Thus, Von Hippel and co-workers^{20,21} concluded that the partitioning of halides in a polyacrylamide gel was due to the influence of the amide groups modulated by the vicinal non-polar groups. An activity coefficient effect implies that two (or more) fluid compartments are involved in partitioning, they may be the internal and external compartments and the former may be complex. In this context it is perhaps relevant that an altered vicinal water structure has been postulated as responsible for the striking affinity of the hydrophilic gels for non-polar solutes¹⁷.

However, as regards the question of association of a cation or ion-pair with Sephadex, there is considerable evidence of complex formation between electrolytes and non-ionic compounds similar to the structural elements of these gels. Thus, association between alkali salts and both cyclic carbohydrates²²⁻²⁶ and linear oligo- and polyethers²⁷⁻³³ and macrocyclic ethers³⁴⁻³⁷ has been reported.

The remarkable binding capacity of the linear and crown ethers for ions³⁸ has recently acquired special relevance with regard to the affinity of the Sephadex gels. In an elegant and painstaking analysis of Sephadex G-25 hydrolyzed to split the glycosidic but not the ether linkages, Holmberg and Lindqvist demonstrated an extensively dioxane-like nature of the matrix³⁹. It was found that very frequently cross-linking occurred between two carbons on the same anhydroglucose residue to form a 1,4-dioxane ring.

The alkali cation recoveries were all within $100 \pm 2\%$ in both aqueous and mixed solvent systems.

Alkali cation partitioning in binary mixtures

Water-methanol. As reported previously from this laboratory⁸ and also by Ortner and Spitzzy^{9,10}, there is considerable enhancement of alkali cation selectivity in this binary system. Fig. 1 shows a chromatogram of the five lower alkali cations eluted with a 32:68 (w/w) water-methanol mixture, the eluent anion being chloride. The selectivity increases with increasing fractional methanol concentration (Table VI). Moreover, as shown in Fig. 2, the logarithm of the alkali cation distribution coefficient [$K_d^i(X)$] was related approximately linearly to the mole fractional ratio between methanol and water. It should be noted in particular that the K_d value of Li^+ is essentially independent of the methanol concentration.

As Table VII shows, the K_d values of Na^+ and K^+ were higher when chloride was the eluent anion than with acetate. This suggests that, as when water is the sole solvent, the cationic K_d values are highly dependent on the nature of the eluent anion in methanol-water systems. The fact that Li^+ actually had a slightly higher K_d value in acetate does not necessarily invalidate this conclusion in view of the essential insensitivity of this cation to changes in the methanol concentration and the nearness of its K_d value to unity when Cl^- was the eluent anion (Table VI).

A great difference in selectivity between Li^+ and Na^+ has also been reported in methanol-water-hydrochloric acid systems with cellulose⁴⁰ and a polystyrene-divinylbenzene sulphonic acid anion exchanger⁴¹, Dowex 50-X8. With the latter resin the same alkali cation elution order as in Sephadex G-25 was obtained with 80% (v/v) aqueous methanol. The order $\text{Cs}^+ < \text{Rb}^+ < \text{K}^+$ depended on the concentration of hydrochloric acid, and reversal ($\text{K}^+ < \text{Rb}^+ < \text{Cs}^+$) occurred when the hydrochloric acid concentration was below *ca.* 1 mol dm^{-3} . However, although the elution order in cellulose was also very dependent on the hydrochloric acid concentration, the actual Sephadex order was never observed.

In view of the similar orders in Sephadex and Dowex 50-X8, it is unlikely that

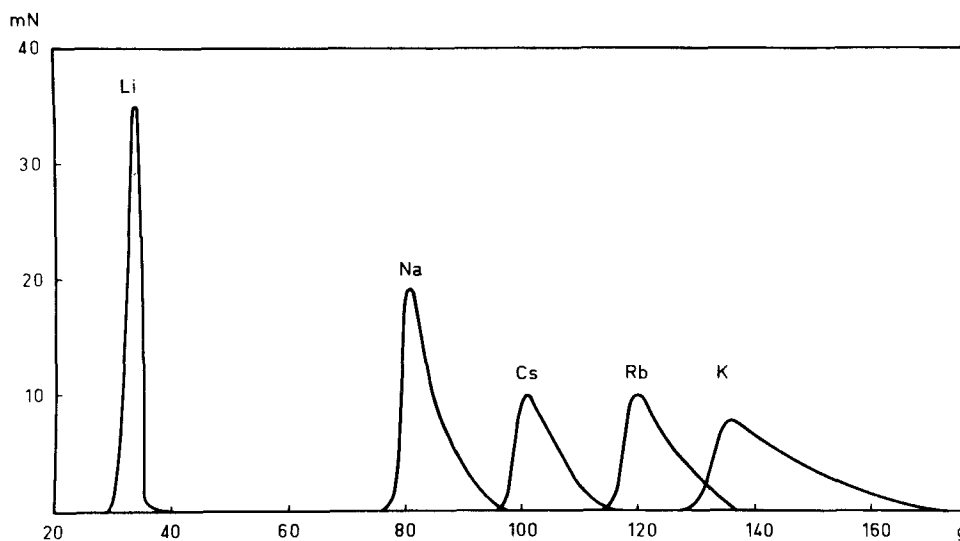


Fig. 1. Elution of alkali cations through Sephadex G-25 at 25°C by a methanol-water (68:32, w/w) mixture. Eluent electrolyte, Tris-HCl (0.05 mol dm^{-3} , pH 7.6); column, $60 \times 1 \text{ cm}$ I.D.

TABLE VI

SEPHADEX G-25 IN WATER AND TWO WATER-METHANOL MIXTURES AT 25°C

Internal solvent composition, distribution coefficients [$K_d(X)$] of solvents and alkali chlorides and selectivities (S_d) of latter.

	Water*		Water-methanol			
			4:6, v/v		1:3, v/v	
S_r^{**}	2.08		0.99		0.67	
f_0^s	0		0.52		0.68	
f_g^s	0		0.46		0.53	
X_0^s	0		0.38		0.54	
X_g^s	0		0.33		0.39	
	$K_d(X)$	S_d	$K_d(X)$	S_d	$K_d(X)$	S_d
CH ₃ OH	0.89	—	0.86	—	0.72	—
H ³ HO	1.06	—	1.23	—	1.44	—
H ₂ O	1.00	—	1.08	—	1.33	—
Li ⁺	0.89	1.00	1.02	1.00	0.87	1.00
Na ⁺	0.94	1.06	1.89	1.85	3.85	4.43
K ⁺	1.02	1.15	2.60	2.54	7.44	8.55
Rb ⁺	1.01	1.13	2.47	2.42	6.28	7.22
Cs ⁺	1.00	1.12	2.10	2.06	5.10	5.86

* These methanol data were obtained with trace amounts of [¹⁴C] methanol.

** S_r = solvent regain; f_0^s and f_g^s are the weight fractions of alcohol (methanol) outside and within the gel beads. X_0^s and X_g^s are the corresponding mole fractions, $K_d(X)$ is the distribution coefficient expressed as a mole fractional ratio (eqn. 9), S_d is the selectivity (eqn. 12a).

cation elution is very sensitive to differences in the structure of the matrix surface. The dissimilar behaviour in the structurally much more closely related cellulose would also appear to suggest a complex relation between elution order and matrix structure although it must be noted that Sephadex G-25 with its frequent 1,4-dioxane rings is considerably different from cellulose.

Water-ethanol and water-tert.-butanol. As shown in Table VIII there was also considerably enhanced selectivity, although somewhat less than with methanol, in both these mixtures. The selectivity ratios (S_d in Table VIII) were roughly similar in both mixtures although the actual K_d values were higher in *tert.-butanol*. In the case of Li⁺, ethanol resembles methanol in that the K_d value is not far from unity whereas in *tert.-butanol* it is significantly higher.

Group IIA cations

Not unexpectedly, the methanol-water induced enhancement of cation selectivity is not unique to Group Ia cations. The alkaline earths are also affected similarly. Thus a mixture of calcium, strontium and barium chlorides were nearly completely resolved on a short 15-cm column of Sephadex G-25 when eluted with a methanol-water (50:50, v/v) mixture at pH 7.6 (Tris-HCl, 0.05 mol dm⁻³). The order of elution was the same as in water, *i.e.* Ca²⁺ < Sr²⁺ < Ba²⁺.

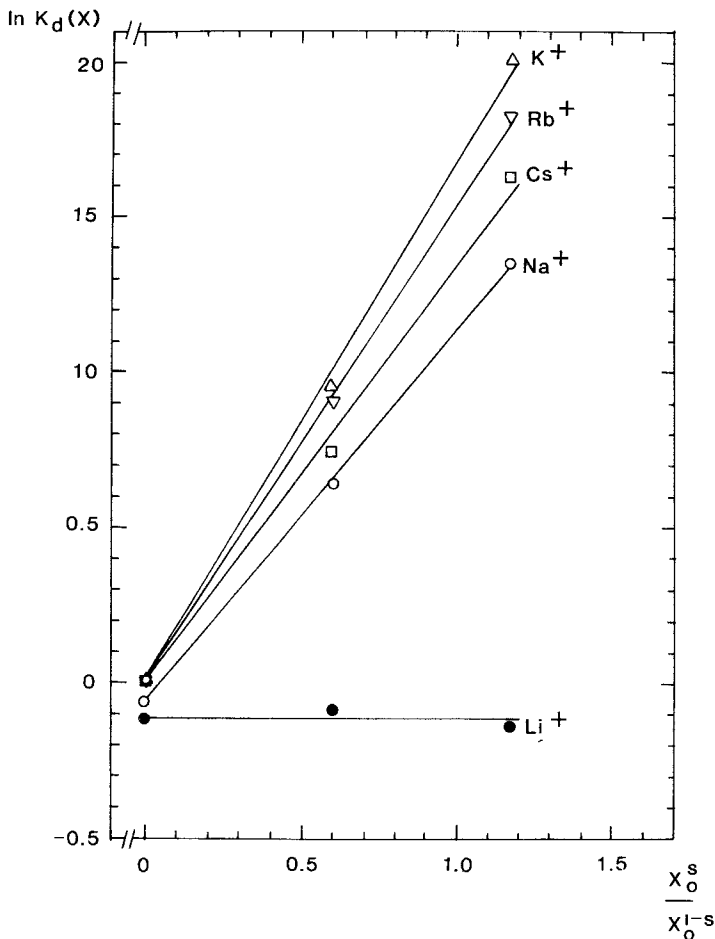


Fig. 2. Plot of $\ln K_d(X)$ values of alkali cations against the methanol-water mole ratio outside the gel beads.

The relation between partitioning and solubility

Solubility-based partitioning depends on differences in both the electrolyte solubilities in the two solvents and in the solvent compositions of the internal and

TABLE VII

DEPENDENCE OF ALKALI CATION DISTRIBUTION COEFFICIENTS $[K_d(X)]$ ON NATURE OF ELUENT ANION IN A WATER-METHANOL (1:3, v/v) MIXTURE AT 25°C

	<i>Eluent anion</i>	
	<i>Chloride</i>	<i>Acetate</i>
Li ⁺	0.87	1.04
Na ⁺	3.85	2.73
K ⁺	7.44	3.80

TABLE VIII

ELUTION AT 25°C OF ALKALI CHLORIDES IN WATER-ETHANOL AND WATER-*TERT.*-BUTANOL MIXTURES

Notation as in footnote to Table VI, also $K_s(X)$ is the calculated distribution coefficient from solubility partitioning alone (eqn. 10), S_s is the selectivity due to solubility-based partitioning (eqn. 12b) and S_{sdw} is the product of S_s and S_{dw} (the observed selectivity when water is the sole solvent, eqn. 12a).

	<i>Water</i>		<i>Ethanol</i>		<i>tert.-Butanol</i>	
S_r	2.08		1.20		1.17	
f_0^s	0		0.42		0.44	
f_g^s	0		0.31		0.017	
X_0^s	0		0.22		0.17	
X_g^s	0		0.15		0.004	

	$K_d(X)$	S_d	$K_d(X)$	S_d	$K_s(X)$	S_s	S_{sdw}	$K_d(X)$	S_d	$K_s(X)$	S_s	S_{sdw}
ROH	0.89		0.68				0.025					
H ³ HO	1.06		1.29				1.78					
H ₂ O	1.00		1.09				1.19					
Li ⁺	0.89	1.00	1.10	1.00	1.046*	1.00	1.000	1.66	1.00	1.16	1	1.00
Na ⁺	0.94	1.06	1.64	1.49	1.084	1.036	1.10	2.36	1.42	1.16	1	1.06
K ⁺	1.02	1.15	1.98	1.80	1.089	1.041	1.20	2.84	1.71	1.16	1	1.15

* Mean of two values from Table I, *i.e.* 1.014 and 1.078.

external solvent compartments. The internal solvent compositions in aqueous mixtures of these three alcohols have been reported^{4,2} and are given in Tables VI and VIII.

Alkali chloride solubilities in water, methanol and ethanol are given in Table I. Although, in some cases, the data are divergent the calculated solubility-based distribution coefficients for sodium chloride and potassium chloride in methanol and ethanol are not subject to significant uncertainty. This is because the solubility is so much lower in the alcohol than water that partitioning is determined mainly by the ratio of the water concentrations (X_g^{1-s}/X_0^{1-s}) in the gel and external compartments.

TABLE IX

ALKALI CHLORIDES IN TWO WATER-METHANOL MIXTURES IN SEPHADEX G-25 AT 25°C

Calculated ideal solubility based distribution coefficients (K_s) and selectivities (S_s) together with the observed aqueous selectivities (S_d) from Table VI and calculated S_{sdw}^* values.

	<i>Water-methanol (4:6, v/v)</i>				<i>Water-methanol (1:3, v/v)</i>			
	$K_s(X)$	S_s	S_d	S_{sdw}	$K_s(X)$	S_s	S_d	S_{sdw}
Li ⁺	1.004	1.000	1.00	1.00	1.011	1.00	1.00	1.00
Na ⁺	1.077	1.073	1.06	1.14	1.278	1.264	1.06	1.34
K ⁺	1.083	1.079	1.15	1.24	1.308	1.294	1.15	1.49
Rb ⁺	1.083	1.079	1.13	1.22	1.307	1.293	1.13	1.46
Cs ⁺	1.083	1.079	1.12	1.21	1.304	1.290	1.12	1.45

* S_{sd} is the product of S_s and S_d when water is the sole solvent.

In the case of the water-ethanol mixture X_g^{1-s}/X_0^{1-s} is 0.85/0.78, *i.e.* 1.090. The K_s values calculated from the solubility data in Table I are thus, with the ethanol solubilities given in parentheses: for Li^+ , 1.079 ($2.71 \cdot 10^{-2}$) and 1.014 (0.212); for Na^+ , 1.089 ($5.12 \cdot 10^{-4}$) and 1.079 ($9.17 \cdot 10^{-3}$); for K^+ , 1.090 ($1.36 \cdot 10^{-4}$), 1.089 ($1.82 \cdot 10^{-4}$) and 1.089 ($8.19 \cdot 10^{-4}$).

The chromatographic and solubility-based distribution coefficients in the water-methanol mixture are given in Table IX. Apart from the enhanced selectivity, what is perhaps most striking about the alkali cation K_d values in the water-methanol mixture is that the aqueous elution order is maintained. This suggests that the mechanisms involved are similar and that the presence of methanol produces only a quantitative change. Solubility-based partitioning would, however, give only a poor discrimination (S_s in Table IX) between Li^+ and Na^+ and little or none between the other three cations, although the aqueous order is maintained if the solubility ratios (S_s) are multiplied by the ratios ($K_d^i/K_d^{i'}$) of the distribution coefficients in water alone (S_{sdw} in Table IX). The product S_{sdw} combines the effects of solubility-based partitioning and the aqueous distribution coefficient. Similarly as Table VIII shows the solubility-based distribution coefficient cannot account for the increased selectivity in the water-ethanol mixture; the S_d values are much greater.

In the case of *tert.*-butanol selectivity was considerably enhanced, notwithstanding the almost complete exclusion of this alcohol from the internal solvent. In the absence of solubility data, solubility-based partitioning due to this alcohol can probably be correctly approximated by assuming that alkali chloride solubilities in *tert.*-butanol are much lower than in water. All the K_s values will thus be essentially equal to the ratio of the mole fractions of water inside and outside the gel and will be the same for all three cations (Table VIII). The selectivity would thus not be enhanced at all, in stark contrast to what is observed.

Although there appears to be no correlation with the solubilities, it is nevertheless probable that partitioning is greatly influenced by the degree of compatibility between the solvent structure in the interstices of the gel and that immediately enveloping both the cations and anions⁴³, *i.e.* ionic hydration, is likely to be of great importance.

In view of the much lower solubility of glucose in methanol than in water, there will be presumably a considerable over-representation of water at the matrix surface, which may be of significance. If the enhancement for the alkali cation in methanol-water mixtures involves a methanol-water transfer process, the order of affinity of, at least, Li^+ , Na^+ and K^+ is that which would be expected⁴⁴.

In the aqueous methanol system (Fig. 2), the $\ln K_d$ values of the alkali cations are related approximately linearly to the methanol-water mole ratio outside the gel. There was, however, only a very poor linear correlation with the mole ratio of water between the gel and the external solvent, $(1 - X_g^s)/(1 - X_0^s)$.

Common to all the binary systems is a reduced hydration. Enhancement of selectivity is, however, unlikely to be due simply to a lower water content or to the consequently increased matrix concentration. Thus, in the *tert.*-butanol system (Table VIII) the imbibed solvent is almost pure water and present in about the same amount as in the more highly cross-linked Sephadex gel G-15 when the latter is swollen in water as the sole solvent. However, in aqueous *tert.*-butanol, G-25 has a considerably greater cation selectivity, which is unlikely to be due simply to the small

structural differences in the matrices between G-15 and G-25^{4,5}. The hydration conditions are, of course, not comparable: G-15 is fully hydrated, in a binary system, whereas G-25 is not. The lower hydration is presumably due to two factors: the lower dielectric constant of the organic solvent and/or the ability of the latter to enter the interstices of the gel. The latter factor appears to be the only one operative in the case of *tert.*-butanol, but it may also be responsible, in part, for the lower concentrations of methanol and ethanol in the gel. In all three solvent mixtures the solvents within and outside the gel have ideally different osmotic pressures, but in view of the far from ideal behaviour of alcohol-water mixtures, these calculated values may be very misleading⁴⁴. However, in the case of *tert.*-butanol, which is essentially excluded, there must be an osmotic pressure difference between the internal and external solvent compartments. The case of *tert.*-butanol thus appears to be analogous to the usual situation in paper chromatography or TLC. However, in the latter, selectivity is correlated with the ratio of a mole fractions of water inside and outside the support matrix^{47,48}.

All, or at least most, of the *tert.*-butanol is, in fact far removed from all but a very small part of the general matrix, and cation selectivity may be enhanced by a mechanism different from that with penetrant solvents. Thus the external concentration of *tert.*-butanol ($f_g^s = 0.44$) is 5.95 mol/dm^{-3} , corresponding ideally to an osmotic pressure of *ca.* 146 atm at 25°C; this is of the same order as the swelling pressure of highly cross-linked gels^{49,50}. The swelling pressure of G-25 should thus be much reduced, which is in accord with its lower solvent regain and this will have a K_d increasing effect on penetrating solutes which might contribute, in part, to the enhancement observed¹⁷. This effect, however, is not unique to the *tert.*-butanol systems; in both methanol (84 atm) and ethanol (160 atm) the excess alcohol outside the gel should exert an osmotic pressure and reduce swelling. The ideal values given in parentheses may, however, be gross overestimates of the real situation since the reflexion coefficients are not known.

The use of mixed solvent systems in these gels may have some practical advantages in that separation are achieved without varying the composition of the eluent.

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