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# THE BEHAVIOUR OF SOME SEPHADEX GELS IN DIOXANE–WATER MIXTURES

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## SUMMARY

The uptake of dioxane and water by Sephadex G-25 and Sephadex LH-20 from mixtures of dioxane and water is determined, and the elution characteristics of sodium chromate and *p*-benzoquinone are reported on columns prepared from the two gels swollen in mixtures of the two solvents. The results are used to formulate a method of design of gel filtration systems using mixed solvents which will be free from adsorption and partition effects. The merits of use of gels for partition chromatography are discussed.

## INTRODUCTION

It is well known that separations on synthetic xerogels such as Sephadex do not occur solely by a simple exclusion mechanism; adsorption and partition effects also play a part, especially with the more highly cross-linked gels and additionally when mixed solvents are used. On account of the varying solubility characteristics of different substrates the use of mixed solvents is sometimes desirable; further, using mixed solvents, it is possible to cause only partially swelling of a gel so that its fractionation range is altered. Various attempts have been made to elucidate adsorption and partition effects[1–6] and it has recently been shown fairly conclusively that adsorption is by hydrogen bonding between the solute and ether or hydroxyl groups on the Sephadex gels[7,8]. The question of adsorption of solvent has been examined in order to find true values for the volume of the imbibed solute ($V_t$) to allow true distribution coefficients ($K_D$) to be established from a knowledge of solvent regain, since if part of the imbibed solvent is adsorbed to the gel matrix, it is unlikely that it will be available

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for dissolution of solutes within the gel matrix. In none of the previous studies was the actual composition of the imbibed solvent determined and hence variations in its composition could not be used to explain the observed phenomena; this is the purpose of the present work.

Sephadex G-25 and LH-20 were chosen for study because their degree of cross-linking and exclusion characteristics are similar. The latter gel is a propylene oxide derivative of the former, the degree of substitution of the hydroxyl groups being approximately 60% (ref. 9). The solvents examined were dioxane and water and their mixtures. The chromatographic characteristics of the gels were determined using low molecular weight substances of widely disparate polarity, namely sodium chromate and benzoquinone.

## EXPERIMENTAL

### *Determination of composition of imbibed solvent*

The gel dried at 70° was allowed to swell in a large volume of the solvent or solvent mixture being examined and the regain was determined by the method employed by PEPPER *et al.*[10] and modified by GRANATH AND FLODIN[11]. The centrifuged gel was allowed to equilibrate with a large volume of "Specially Dried" methanol (Hopkin and Williams Ltd.); the methanolic solution was then analysed for water by the Karl Fischer method. The dioxane content of the imbibed solvent was found by difference from the total solvent regain and the water content.

### *Chromatographic conditions*

Columns were prepared from the swollen gel (column dimensions: 1 × 20 cm)[12] allowed to settle while a flow of the solvent used for swelling was maintained (0.2 ml/min) for 6 h, and the bed volume was then measured. The void volume ($V_0$) for the column was determined using Blue Dextran (Pharmacia Fine Chemicals Ltd.) and also for water-rich solvents with colloidal carbon (obtained by dilution of Indian ink).

The elution volume ($V_e$) of aliquots (0.3 ml) of sodium chromate (0.3 $M$) and benzoquinone (0.3 $M$) each dissolved in the solvent used for swelling the sample was measured. The volume as the centre of the yellow zone emerged from the column was taken to be $V_e$. Apparent $K_D$ values for the two solutes in the various systems were calculated by assuming that $V_i$ for the column equalled the weight of dry gel in the column multiplied by the solvent regain for the particular system.

## RESULTS AND DISCUSSION

The reproducibility of the method for the determination of solvent regain was tested using a standard centrifugation speed of 6000 r.p.m. with a 45° microangle centrifuge of radius 4 cm.

### *Behaviour of G-25*

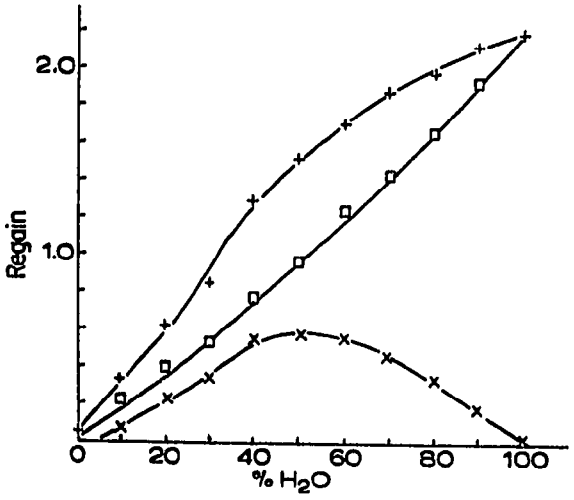
Fig. 1 shows the variation of solvent regain with changing solvent composition for G-25 Coarse and the corresponding variation of dioxane and water content whilst Fig. 2 shows the variation in solvent composition inside the gel with composition outside the gel. It is seen that there is always an excess of water inside the gel; this

TABLE I

WATER REGAIN OF BATCHES OF SEPHADEX GELS

| *Gel* | *Regain (g/g dry gel)* | *S.D (20 results each) (g)* |
|---|---|---|
| G-25 Fine | 1.98 | ± 0.12 |
| G-25 Coarse Lot No. To 6249c | 2 00 | ± 0.07 |
| G-25 Coarse Lot No. 8298 | 2 16 | ± 0.06 |
| LH-20 Coarse | 1.90 | ± 0.15 |

excess is plotted against external solvent composition in Fig. 3. Extrapolation of the curve shows that there is a permanently retained quantity of water, of the order of 0.2 g/g of dry gel, at all solvent compositions. This represents a layer of water molecules hydrating the hydroxyl groups in the gel. Extrapolation to 100% water gives a value of 0.24 g/g of dry gel, but at 100% dioxane the value is lower. This may be explained by the fact that the gel shrinks in dioxane-rich solvents so that hydrogen bonding can occur between adjacent hydroxyl groups on the gel thus excluding water. The repeating unit of the polymer[4] has a molecular weight of 1028 and contains 17 hydroxyl groups; if one molecule of water is bonded to each hydroxyl group this would correspond to 0.29 g/g of dry gel, which agrees reasonably with the observed value, 0.24 g/g, and also with the figure suggested by LATHE AND RUTHVEN[1]. The increase in the excess water in mixed solvent compositions can be explained because it is probable that the hydrogen-bonded water will itself bond to more water to form domains around the hydroxyl groups. It has been shown that these domains around the hydroxyl groups would be expected to be broken up by sodium chromate at the concentrations employed in these experiments[3]. The water hydrogen-bonded directly to the



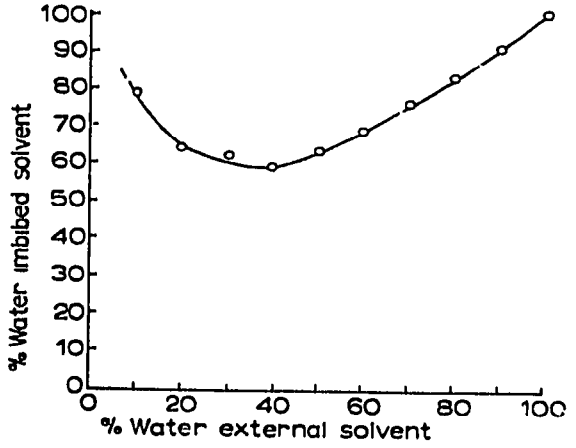
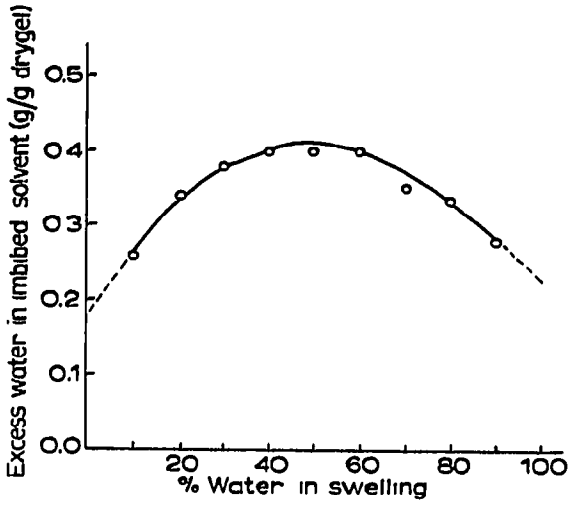




Fig. 1. Total regain (+), water regain (□), and dioxane regain (×) for different swelling solvent compositions.

Fig. 2. Variation of internal solvent composition with external solvent composition for Sephadex G-25.



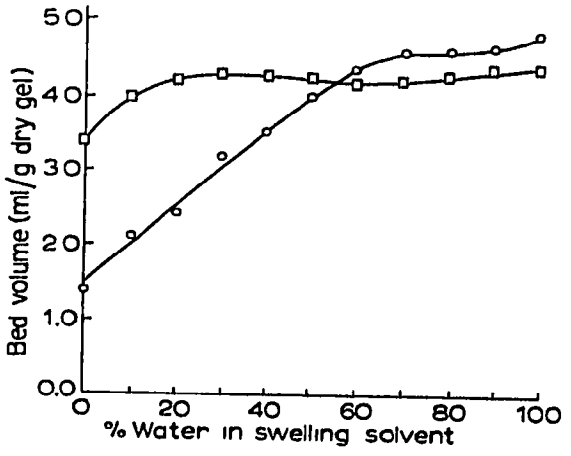
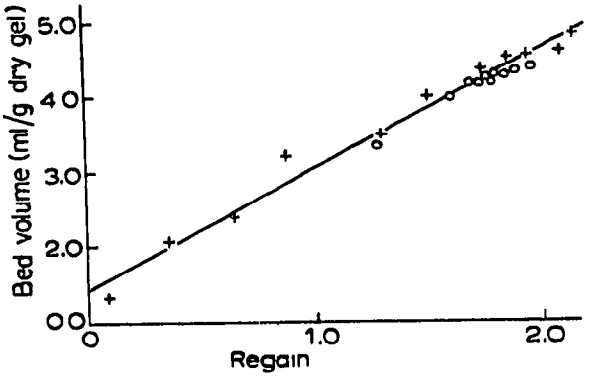




Fig. 3. Variation of excess water of swelling solvent for G-25.

Fig. 4. Variation of bed volume with composition of swelling solvent ○, G-25, □, H-20

gel would not be available to this solute for solution, so that a discrepancy may be expected between the $V_t$ of a column determined from solvent regain experiments and that obtained by elution of sodium chromate from a column. $K_D$ for sodium chromate was found to be 0.88 using solvent regain data (Fig. 6). Correction for the unavailable water of 0.24 g/g of dry gel gives a value of 0.98. It is therefore concluded that for determination of true $K_D$ values on G-25 gel in water, the solvent regain should be corrected by 0.24 g/g of dry gel to find the true value of the volume of imbibed solvent available to solutes; if very weak solutions are employed so that the ordered regions are not destroyed by the solute this correction factor should be increased and Fig. 3 shows the order of the correction factors to be applied.



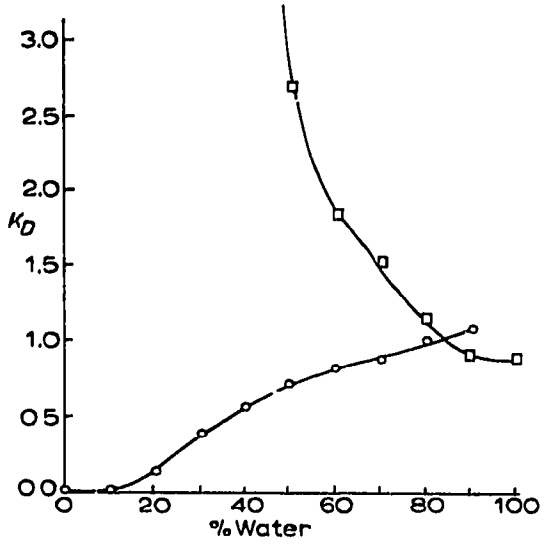




Fig. 5. Variation of bed volume with regain. +, G-25, ○, LH-20

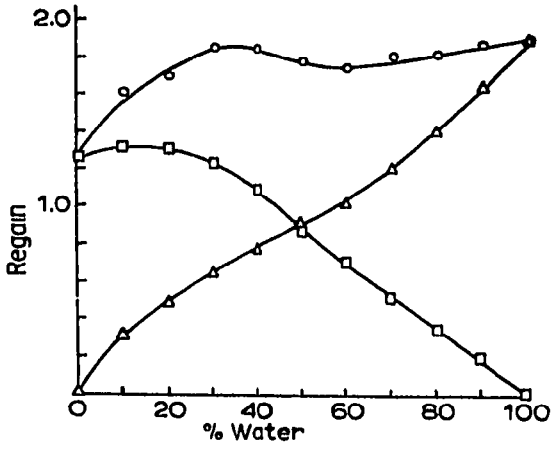
Fig. 6. Elution characteristics of *p*-benzoquinone (○) and sodium chromate (□) on G-25 in different solvent compositions.

Fig. 4 shows the variation of bed volume with composition of the swelling solvent and Fig. 5 shows the variation of bed volume with solvent regain. It is clear from Fig. 5 that determination of bed volume (a relatively simple procedure) can be used to determine solvent regain to a first approximation (slope of line 1.72 ± 0.2 ml $g^{-1}$).

Fig. 6 gives the elution characteristics of the two solutes in varying solvent compositions. As expected, the $K_D$ value of the sodium chromate increases as the excess water inside the gel increases indicating straight phase partitioning, until, in compositions containing less than 40% water, the chromate ceases to move down the column. The quinone behaves conversely and below 10% water, no quinone enters the gel at all. The $K_D$ value of quinone at 100% water (extrapolated) indicates that it has some affinity for the gel itself. True exclusion chromatography for both substances appears to occur with solvent compositions in the region of 85% water in dioxane.

*Behaviour of LH-20*

The variation of bed volume with solvent composition for LH-20 is observed to be quite different from that of G-25 (Fig. 5) as is the change in regain with solvent composition (Fig. 7). It is apparent that the gel has an affinity for both the polar and the non-polar solvent, and consequently domains of each solvent can be expected to form around hydrophilic and hydrophobic sites, respectively, in the gel. There is in addition a third effect which must be taken into account, especially with lipophilic–hydrophilic gels such as LH-20; this is the ordering effect which hydrophobic solutes have upon water[13]. Water could be expected to be forced into domains both by this effect, and by hydrogen-bonding to hydroxyl and ether groups on the gel. Dioxane is thus forced into the region of the hydrophobic sites. When an ionised solute is introduced into such a system, it is difficult to predict its behaviour quantitatively; however, the elution behaviour of the two solutes (Fig. 8) follows the trends which would be expected from an inspection of the internal and external solvent compositions which are shown in Fig. 7.



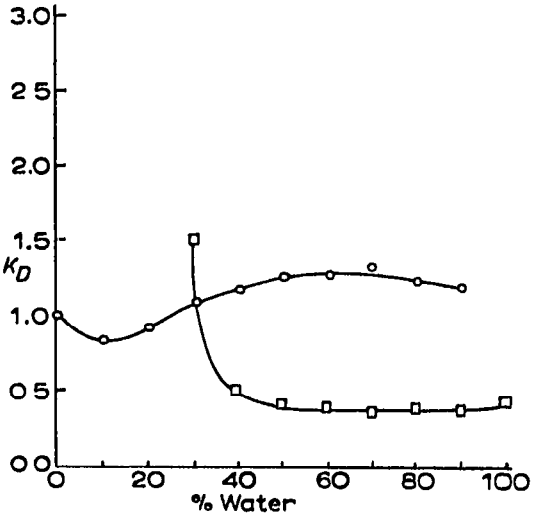




Fig. 7. Total regain (○), water regain (△), and dioxane regain (□) for different swelling solvent compositions.

Fig. 8. Elution characteristics of *p*-benzoquinone (○) and sodium chromate (□) on LH-20.

## CONCLUSIONS

The results show that attempts to isolate individual effects in separations on xerogels and to attribute them to exclusion, partition or adsorption, as a comparison of gel filtration with adsorption and partition chromatography would suggest, are likely to fail. The results reported do, however, suggest a practical procedure which may be followed in order to design a system using mixed solvents to separate series of chemically similar compounds according to their molecular weight alone. The simplest model for a gel filtration system would be to regard it as a pure partition system, where the stationary phase is a stationary solution of the gel in the solvent, and the mobile phase is the solvent or solvent mixture. More highly cross-linked gels will give more concentrated stationary solutions than looser gels, so that less polar solutes will be expected to partition from an aqueous mobile phase into tightly cross-linked gels to a greater extent than into looser gels, regardless of the molecular sieve mechanism. In order to counteract this effect the mobile phase may be made less polar by incorporation of a miscible non-polar solvent such as dioxane. However, this non-polar solvent will itself then become distributed between the two phases until equilibrium is reached; chromatography of a solute will then be between a stationary solution of the gel plus the non-polar solvent plus water, and the mobile solution of the non-polar solvent plus water; large molecules will thus be subjected to exclusion effects as well as to partition effects. In order to design a system which will produce separation by the exclusion mechanism alone, it is not necessary to carry out the whole procedure reported earlier in this paper. All that is required is the preparation of a series of columns from gel swollen in a range of solvent mixtures; $V_t$ for each column may then be estimated with sufficient accuracy for this purpose from a measurement of the bed volume for a given weight of dry gel whilst determination of $V_0$ may be made using Blue Dextran. The variation of the $K_D$ value with solvent composition for a small molecule of similar polarity to the series of molecules to be separated may be observed on the columns, and a plot similar to Figs. 6 and 8 obtained. Where the resultant curve crosses the $K_D = 1$ ordinate the system is balanced, *i.e.* the chemical affinity of the solute for the stationary solution and the chemical affinity for the mobile solution are equal, and separations due to molecular sieving alone can be expected. As gel columns are frequently employed for repeated investigations which require separations of large numbers of samples whose composition varies only slightly, the effort required to design the system properly as outlined above would be well worth while; accurate determination of $V_t$ from solvent regain at each solvent composition near to the balance point, as roughly determined using the $V_t$ to bed volume relationship, would allow the balance point to be found precisely.

The employment of synthetic xerogels such as Sephadex for partition chromatography represents only a minor part of their present total application; partition chromatography has been carried out in the main either on cellulose or on non-adsorptive supports such as celite. When a swollen xerogel is visualised as a stationary solution instead of as a rigid inert support such as celite, its merits compared to inert supports become clear. No loading of the stationary phase is necessary, the gel is simply swollen in the proposed mobile phase and once swollen, its solution properties will differ from those of the swelling solvent, particularly if a mixed swelling solvent was employed with a tightly cross-linked gel. When a column is packed, high chro-

matographic efficiency is achieved due to the uniform particle shapes and sizes of synthetic gels. The whole column will be involved in the chromatographic process so that the capacity of the columns will be higher using a gel than using a conventional system with an inert support. We have also observed that when a gel is swollen in a solvent which gives maximum regain, a column packed with such a gel retains its bed volume when a solvent which swells the gel slightly less is substituted for the original solvent on the column[14]. Hence, if after one chromatographic run separation is unsatisfactory, the mobile solvent composition may be changed slightly to improve separation. Using a conventional partition system such solvent changes are limited by the need to maintain immiscibility between the two phases: with xerogels, miscibility is impossible. Such an application of synthetic xerogels has great potential in organic chemical research. Gel chromatography has as yet not found wide application in this field because separation is required not according to molecular weight, as in much biochemical work, but according to functional group. PORATH has shown that retention chromatography on Sephadex gels compares favourably with gas chromatography as a generally applicable technique[15]. Partition chromatography with mixed solvents on highly cross-linked gels, together with suitable pumps and detectors, compares even more favourably with gas chromatography for organic chemical separations, particularly when working with sensitive or involatile samples. A liquid chromatograph comprising a peristaltic pump, a small xerogel column (20–30 × 1 cm), a simple light-absorbing or refractive index detector, and a fraction collector may in the future well find as many uses as a gas chromatograph in the organic chemical laboratory.

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