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HYDROPHOBIC INTERACTION IN GEL ADSORPTION CHROMATOGRAPHY

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SUMMARY

The hydrophilic/lipophilic material, Sephadex LH-20, shows strong retention of lipophilic molecules in aqueous media. The major retention force is believed to be entropic hydrophobic bonding. This interpretation is supported by the following experimental results: (a) separation as a function of the length of the hydrocarbon chain of the molecule; (b) stronger retention as the temperature rises or (c) when electrolyte is added to the eluant; and (d) the opposite behavior of solutes in the hydrophilic material, Bio-Gel. In organic solvents, however, hydrogen bonding and a little understood polar interaction are the dominating adsorptive forces.

INTRODUCTION

Gel chromatography is a method for separating molecules according to size differences. It is assumed that no interaction of the solutes with the gel matrix takes place. This, of course, is not always true, especially with low-molecular-weight substances and gels of high density. Intermolecular forces may act upon the system and may predominate over the separation mechanism based on size. As a result, small molecules are eluted before large ones and all are eluted beyond V_t, the total volume of the gel packing[1]. By a better understanding of the forces involved in the adsorption process, it should be possible to utilize these unwanted side effects and make them the basis for a new separation method. Hydrogen bonding of aromatic acids and bases in aqueous media, for instance, is already understood to a certain extent and separation based on the pK values of the solutes may occur[2].

In the Sephadex gels the cross-links (hydroxyglycerol ether bridges), and especially the hydroxypropyl groups in Sephadex LH-20, are responsible for adsorption effects[3]. A shrinkage of the gel by a rise in temperature was observed and interpreted as the result of hydrophobic interaction among the lipophilic groupings in the gel[4]. Such an interaction should affect lipophilic solutes as well. Hydrophobic interaction is what attracts two non-polar groups[5].

In the Sephadex gels the $-CH_2-$ groups in the neighborhood of the ether oxygen atom of the cross links, as well as the $-CH_3$ groups of the hydroxypropyl group in Sephadex LH-20, represent the points of interaction.

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INFLUENCE OF TEMPERATURE

Normal adsorptive forces, such as hydrogen bonding or Van der Waals' forces, are weakened by a rise in temperature because of enthalpy decrease. Hydrophobic bonding, being an entropy effect, behaves in the opposite manner, and in gel chromatography a retention of the solute should occur. Such behavior is demonstrated in Fig. 1.

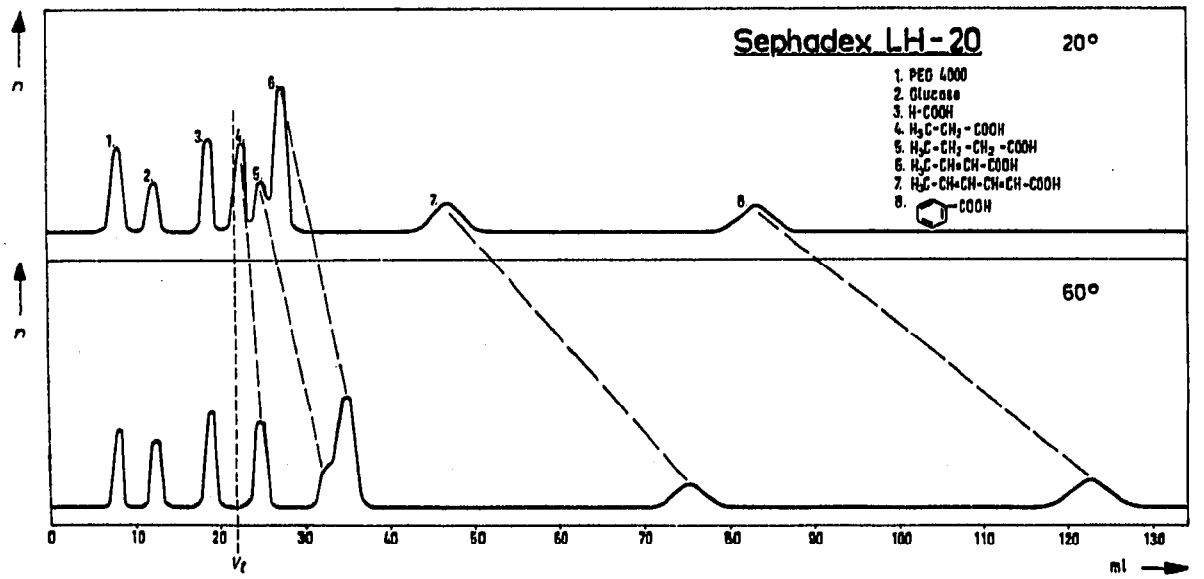

Fig. 1 Elution diagrams of a mixture of organic acids with polyethylene glycol 4000 and glucose chromatographed on Sephadex LH-20 at 20° and 60°. Ordinate: index of refraction; abscissa: elution volume. Column: 0.86 × 38 cm; V_t = 22.0 ml; eluant: 0.002 N HCl, pH 2.5; sample volume: 0.15 ml; concentration: 1 mg/ml of each substance; flow rate: 7.5 ml/h.

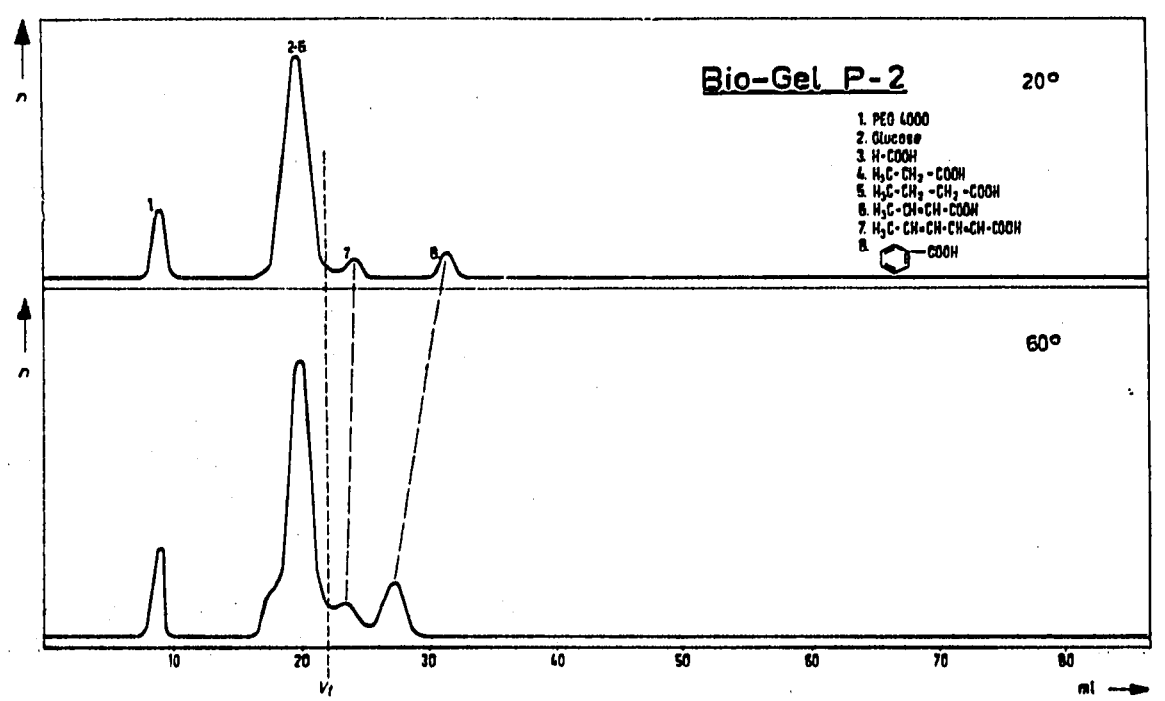

Fig. 2. Elution diagrams of the same sample as in Fig. 1 chromatographed under the same conditions but on Bio-Gel P-2.

Organic acids are retained on Sephadex LH-20 in water, and retention increases with an increase in temperature. With increasing length of the hydrocarbon chain of the organic acid, as well as with an increasing number of π-electrons in the solute, the lipophilic part of the molecule gains in importance. As a result, in gel chromatography, retention becomes stronger. MARSDEN has observed similar effects with primary alcohols and suggested that hydrophobic bonding is responsible for this unusual behavior[6].

The same sample as in Fig. 1 was chromatographed under the same conditions on a Bio-Gel P-2 column. A slight retention is observed, but here — in contradiction to the behavior of Sephadex — retention decreases as the temperature rises (Fig. 2). In Bio-Gel, a polymer network of acrylamide and N,N-methylenebisacrylamide, where lipophilic parts are absent (except for the methylene bridges), hydrophobic bonding obviously plays a minor role.

When the temperature is increased on Sephadex gels shrinking occurs and consequently the same volume will contain a greater amount of polymer. This fact may be taken into consideration by dividing the elution parameter by the weight of dry polymer. The affinity number, A, has been defined[3] as:

$$A = \frac{V_e - V_t}{g} \qquad (1)$$

where V_e is the elution volume of the retarded solute, V_t the total volume of the gel packing, and g the weight of the dry gel material. This definition roughly corresponds to the retention volume per gram of gel. It is not quite accurate because it neglects the fact that the gel matrix itself has a certain volume and a solute of low molecular weight without interaction would be eluted not exactly at V_t but, according to its size, somewhere before V_t. Nevertheless, all the parameters of A are very easily measurable and, as the effects studied are so marked, such an accuracy is not needed. Table I gives the values of A for the organic acids in Figs. 1 and 2. It can be seen that the different amount of gel in the column at different temperatures is not the cause of the unusual solute behavior.

The results of BROOK AND MUNDAY[7] fit well into our picture. They found that the retention of halogenated phenols on Sephadex gels decreases as the temperature rises, whereas for *tert.*-butylphenol the opposite was noticed. Apparently the lipophilic *tert.*-butyl group, as well as the unsubstituted aromate itself, tend to give

TABLE I

A-VALUES $[(V_e - V_t)/g]$ OF SOME ORGANIC ACIDS IN CHROMATOGRAPHIC EXPERIMENTS WITH SEPHADEX LH-20 AND BIO-GEL P-2 IN 0.002 N HCl AT 20° OR 60°

Acid	*Sephadex LH-20*		*Bio-Gel P-2*	
	$A_{20°}$	$A_{60°}$	$A_{20°}$	$A_{60°}$
Propionic acid	0.32	0.62		
Butyric acid	0.90	1.59		
Crotonic acid	1.28	1.86		
Sorbic acid	5.25	6.65	0.53	0.38
Benzoic acid	11.20	13.50	1.77	1.02

hydrophobic bonding, whilst the polar halogens, as well as the phenolic -OH group (H-bonded), participate in an enthalpy-governed adsorptive force. The measured retention is always the sum of these sometimes competing and overlapping effects.

INFLUENCE OF THE ELECTROLYTE

Increase in the ionic strength of a buffer makes the Sephadex gel shrink (Fig. 3a). Water molecules arranged in a certain order around the solvated gel chains are disturbed by the great number of ions seeking hydration. The competition for water molecules between solvated polymer chains and ions ends up in favor of the ions and results in a shrinking of the gel analogous to temperature increase. The solute behaves in a similar manner. With increasing ionic strength of the buffer, lipophilic solutes are pushed towards the gel matrix; this may be compared with the well-known salting-out effect.

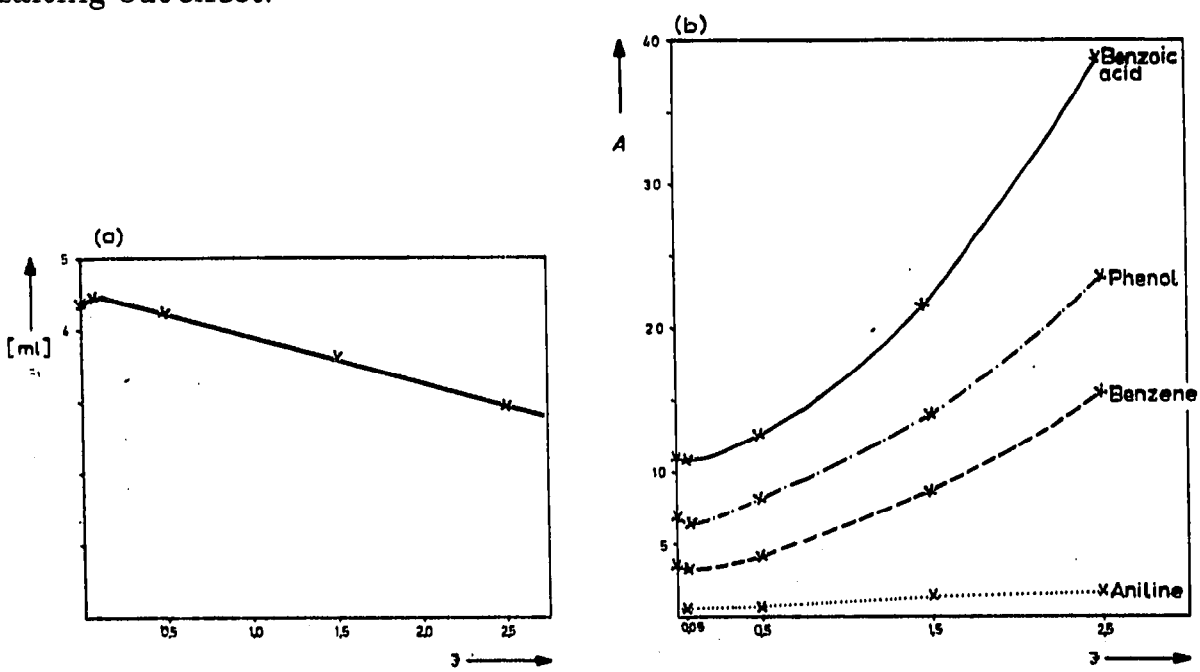

Fig. 3. (a) The swelling of Sephadex LH-20 gel (ml/g) related to the ionic strength, I, of the swelling solvent. Buffer: 0.002 N HCl + KCl, pH: 2.5. (b) Adsorption number, A, for some solutes chromatographed on Sephadex LH-20 *versus* ionic strength, I, of the eluant. Column: 0.67 × 20.5 cm; V_t = 7.2 ml; eluant: as in (a); other conditions as in Fig. 4.

Fig. 3b shows that the adsorption number A (retention (ml) per gram of dry gel) increases with increasing amount of electrolyte for a Sephadex gel.

With Bio-Gel P-2 SIMKIN observed a slight decrease in retention by electrolyte addition to the eluant[8], analogous to the effect we found when raising the temperature (Fig. 2).

INFLUENCE OF SOLVENT

Since hydrophobic bonding may only be noticed in aqueous media the solute behavior should change in an organic eluant. Fig. 4 shows such a separation of benzene, benzoic acid and some phenols chromatographed on Sephadex LH-20 in *n*-

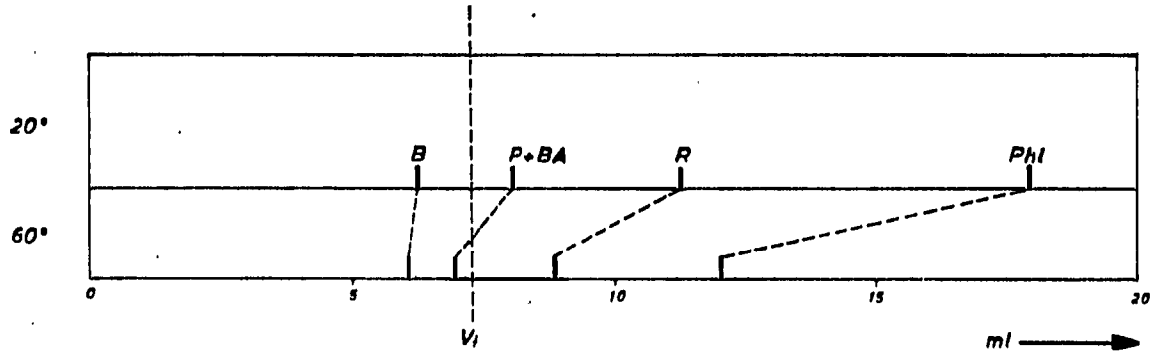

Fig. 4. Schematic elution diagram. The vertical lines represent the maxima of the elution peaks of the given substances. Benzene (B), benzoic acid (BA), phenol (P), resorcinol (R), and phloroglucinol (Phl) are chromatographed on Sephadex LH-20 at 20° and 60° with *n*-butanol as eluant. Column: 0.67 × 20.5 cm, V_t = 7.2 ml; sample volume: 0.1 ml; concentration about 0.1 mg/ml of each substance; flow rate: 5.0 ml/h; detector: LKB Uvicord (254 nm).

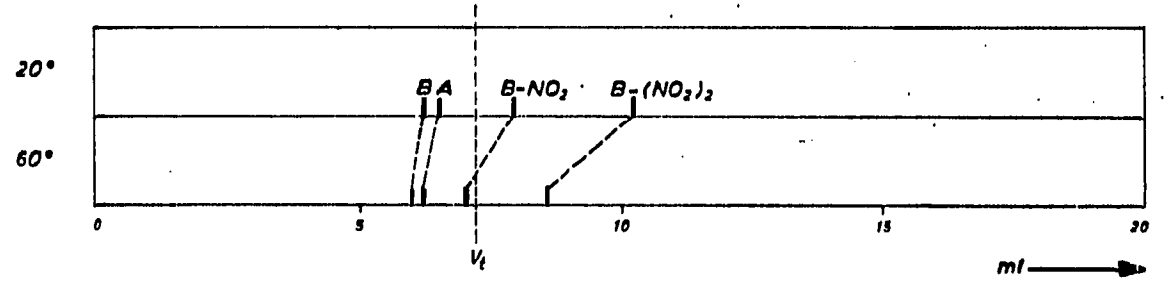

Fig. 5. Schematic elution diagram of benzene (B), anisole (A), nitrobenzene ($B–NO_2$) and *m*-dinitrobenzene ($B–(NO_2)_2$) chromatographed on Sephadex LH-20 with *n*-butanol at 20° and 60°. Conditions as in Fig. 4.

butanol at 20° and 60°. Retention is drastically decreased by a rise in temperature. Benzoic acid and phenol are eluted at the same volume. It may be pointed out that the separation of the other phenols is according to the number of their phenolic groups. The dominating force responsible for retention is in this case hydrogen bonding. These results are in good agreement with STREULI's findings[9].

STREULI[9] and JANSON[10], however, have also discussed π-electron interactions as one of the causes of adsorption in Sephadex gel chromatography. To disprove the assertation that there are no π-electrons in Sephadex gel, the following experiment was undertaken. Anisole, with its $-OCH_3$ group increasing the π-electron cloud in the aromatic ring, and nitrobenzene, where the $-NO_2$ group has the opposite effect, were both chromatographed along with benzene in *n*-butanol at 20° and 60°. For π-electron interaction the order of elution should be in the order of increasing π-electron concentration of the aromatic ring: nitrobenzene, benzene, anisole. Fig. 5 gives the experimental result, which does not support the idea of a π-electron interaction mechanism: the substituted aromates are eluted after benzene. As expected, a rise in temperature decreases adsorption. The $-NO_2$ and $-OCH_3$ groups cause these effects. As for the dominating mechanism in adsorption chromatography with alumina or silica gel, a polar interaction mechanism is suggested.

At any rate, contrary to the above mentioned ΔS-dependent hydrophobic bonding forces in aqueous media, in organic media ΔH-dependent forces are dominant.

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