

CHROM. 10,145

USE OF HYDROXYETHYL METHACRYLATE GELS IN LIQUID CHROMATOGRAPHY IN NON-AQUEOUS MEDIA*

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(Received April 19th, 1977)

SUMMARY

The chromatographic behaviour of various solutes on hydroxyethyl methacrylate gels in aprotic (tetrahydrofuran) and in protic (methanol) media has been studied. Low-molecular-weight solutes were retained due to the sorption on the amphiphilic gel surface; the sorption was dependent on the polarities of the solute and of the solvent molecules. Macromolecular solutes possessing no strongly polar groups were separated on the hydroxyethyl methacrylate gels in tetrahydrofuran by a molecular-sieving mechanism.

INTRODUCTION

Spheron, macroporous hydroxyethyl methacrylate gels, have been designated as a universal chromatographic material suitable for separations in both aqueous and organic media¹. Their universality is due to their amphiphilic character: Spheron gels contain hydrophilic hydroxyl groups, medium-polar ester groups and a hydrophobic polymeric backbone bearing methyl groups. Various solutes may interact with different sites on the gel surface, and may hence be sorbed. In the gel-permeation chromatography (GPC) of polymeric substances, sorption is undesirable, because it can lead to deviation from the molecular-sieving principle of separation. On the other hand, sorption, if weak and reversible, may improve the efficiency of the chromatographic separation of some mixtures of naturally occurring macromolecular substances^{2,3} and widen the applicability of Spheron gels also for liquid chromatography of various low-molecular-weight compounds⁴. A study of the interactions of different solutes with the gel surface is important for the successful use of the sorbent. In aqueous media, compounds with lipophilic groups are retained on Spheron gels, changes in the entropy of the system and Van der Waals forces being the main factors responsible for sorption. In organic media, sorption may be enhanced if the solute contains polar groups capable of dipolar interactions, and (especially) of hydrogen-bonding interactions. However, competition from solvent molecules must also be

* A part of the results was presented at the symposium on Advances in the Chromatographic Fractionation of Macromolecules (Birmingham, Great Britain), July 1976.

considered, and the sorption of a particular substance can be enhanced or suppressed by using different solvents.

This paper presents a study of the interactions of a series of low-molecular-weight solutes of different polarities with Spheron gel in tetrahydrofuran and methanol; the results of this study were applied to the GPC of some synthetic polymers.

EXPERIMENTAL

Materials

The Spheron 100 and 300 (bead size 25–40 μm) used were products of Lachema (Brno, Czechoslovakia). Samples of poly(isobutene), poly(vinyl chloride), poly(vinyl chloride–vinyl acetate), and poly(methyl methacrylate) were obtained from the Institute of Macromolecular Chemistry of the Czechoslovak Academy of Sciences, Prague, and samples of poly(ethylene–vinyl acetate) and poly(phenylene oxide) from the Research Institute of Macromolecular Chemistry, Chemopetrol (Brno, Czechoslovakia). Polystyrene and poly(ethylene glycol) standards were products of Waters Assoc. (Milford, Mass., U.S.A.). The tetrahydrofuran used was distilled with lithium aluminium hydride, and the methanol was purified by distillation with magnesium turnings.

Apparatus

Waters Assoc. ALC/GPC 501 liquid chromatograph, with a model 401 differential refractometer detector, connected with a stainless-steel column (40 \times 0.7 cm) packed with Spheron 100 or 300 was used. Chromatograms were recorded with an EZ 10 recorder (Laboratorní Přístroje, Prague, Czechoslovakia).

Methods

Columns were packed with Spheron 100 or 300 by a modified slurry technique⁵. Samples were applied as 5 to 10% solutions of low-molecular-weight solutes in tetrahydrofuran or in methanol (sample-injection volume 10 μl), or as 0.1 to 0.5% solutions of polymeric solutes in tetrahydrofuran (50–100 μl). The flow-rate was 0.5–0.6 ml/min.

RESULTS AND DISCUSSION

Fig. 1 shows the elution volumes of various low-molecular-weight solutes (measured for the Spheron-100 column with tetrahydrofuran as mobile phase) plotted against the solute polarities expressed by the total-solubility parameters (δ)^{6,7}. The elution volumes of most solutes increase in an approximately linear fashion with their polarity. However, the situation in the system Spheron–tetrahydrofuran–solute is more complicated than is apparent from the observed retention–polarity relationship. The total-interaction energy between the solute and the gel surface is the sum of several contributions, which, for non-ionized species, include dispersive forces, dipole–dipole and dipole–induced dipole interactions, and hydrogen-bonding. For quantification of the ability of a compound to participate in these interactions, different components of the total-solubility parameters were evaluated^{6,7}: δ_d , the

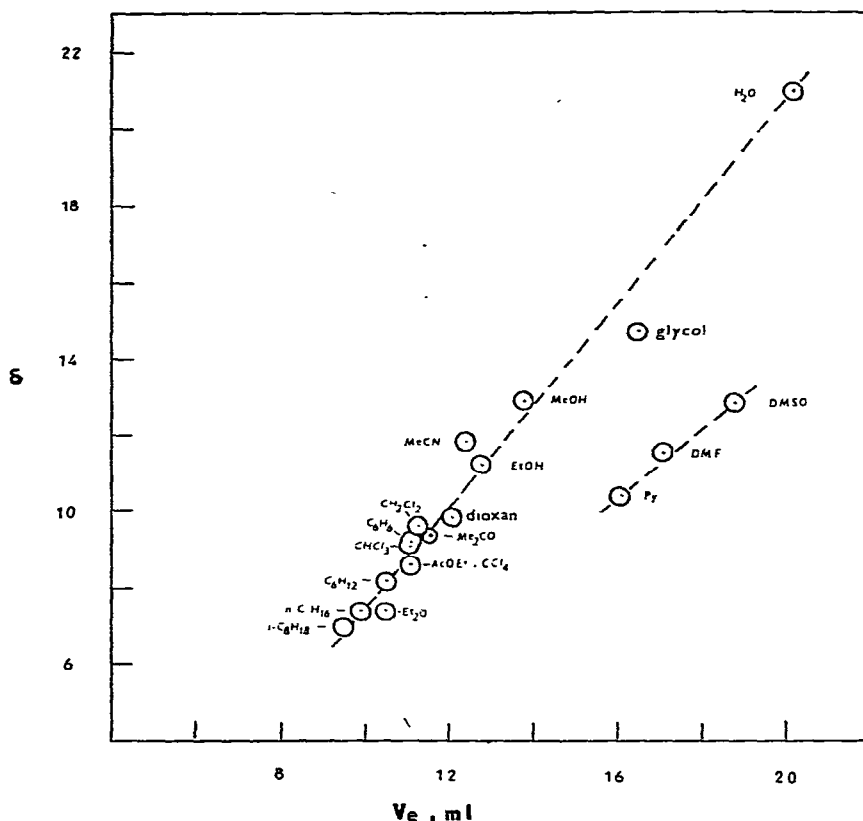


Fig. 1. Dependence of elution volumes (V_e) of various solutes on their total-solubility parameters (δ) for Spheron 100 in tetrahydrofuran. For details see "Experimental".

dispersion solubility parameter; δ_o and δ_i , the orientation and induction solubility parameters, and δ_a and δ_b , the proton-donor and proton-acceptor solubility parameters.

On the surface of Spheron gel there are groupings with different polarities, ranging from non-polar methyl groups through medium-polar ester groups to strongly polar hydroxyl groups. Tetrahydrofuran, as a strong proton-acceptor, can effectively shield the hydroxyl groups of the gel from interaction with most solutes. Such competition from the solvent molecules should be ineffective for solutes even stronger in proton-accepting character, *i.e.*, solutes for which the δ_b value is greater than that of tetrahydrofuran. These solutes may be divided into two groups (protic and aprotic). Protic solutes can probably interact with solvent molecules more strongly than with gel hydroxyl groups, so that these hydroxyl groups do not participate in the retention of protic solutes. Pyridine, dimethylformamide and dimethyl sulphoxide belong to a group of aprotic solutes having greater ability to form hydrogen bonds than has tetrahydrofuran. These solutes are sorbed more strongly than other compounds having approximately the same total polarity and show the greatest deviations from a linear retention-polarity relationship. According to published data⁷, acetonitrile and dioxan also have δ_b values greater than that of tetrahydrofuran.

However, with acetonitrile the difference is negligible, and for dioxan, the greater δ_t value reflects the dibasic character of the molecule, whereas only one ether group can interact with gel hydroxyl groups.

An undesirable feature of tetrahydrofuran is the possibility of formation of peroxides under the conditions used. Peroxides, if not removed, may attack the hydroxyethyl methacrylate gel, causing an increase in the carboxyl-group content of the gel and thereby producing certain anomalies in the chromatographic behaviour of basic solutes. In our work, after three month's use of tetrahydrofuran recycled without removing peroxides, the carboxyl-group content of Spheron 100 increased from 0.02 to 0.07 mequiv./g.

If methanol is used as mobile phase, the elution pattern is significantly changed, as shown in Fig. 2. A linear relationship between polarity and elution volume (as in

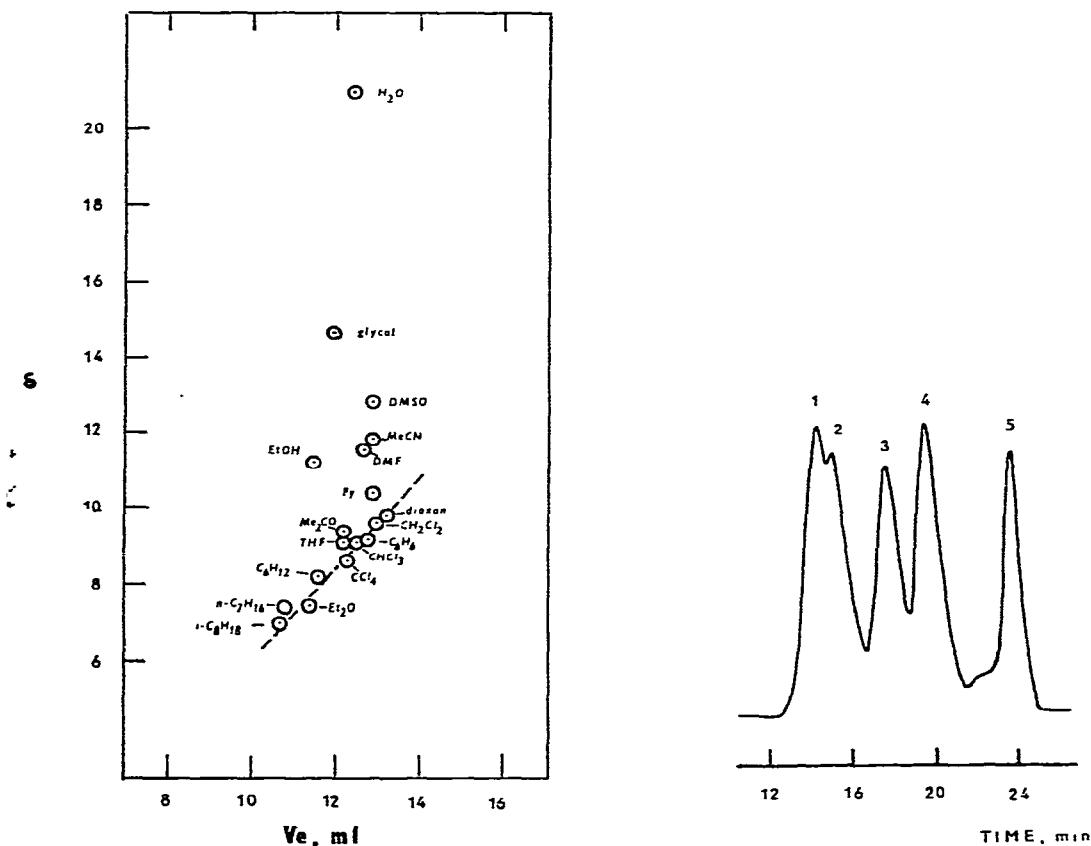


Fig. 2. Dependence of elution volumes (V_e) of various solutes on their total-solubility parameters (δ) for Spheron 100 in methanol. For details see "Experimental".

Fig. 3. Separation of a mixture of polystyrene standards on a column (40 × 0.7 cm) of Spheron 300 (25–40 μ m). Sample: 60 μ l of a mixture of 0.2% solutions of polystyrene standards (Waters Assoc.) and of benzene in tetrahydrofuran. Elution with tetrahydrofuran; flow-rate 0.5 ml/min; pressure 6 atm; detection at 254 nm. Peaks: 1–4, polystyrenes (1 = mol.wt. 402,000; 2 = mol.wt. 111,000; 3 = mol.wt. 19,750; 4 = mol.wt. 3550), 5, benzene.

tetrahydrofuran) is obtained in methanol only for non-polar and weakly polar solutes; however, the elution volumes of these solutes are increased. This may be explained by the lesser ability of methanol (in comparison with tetrahydrofuran) to compete with the solutes for the non-polar sites on the Spheron surface (for tetrahydrofuran, $\delta_d = 7.6$, for methanol⁷, $\delta_d = 6.2$). Some change in elution volumes may also be caused by differences in the swelling of Spheron in tetrahydrofuran and in methanol, and perhaps also by a difference in the quality of the column filling in the two solvents.

The elution volumes of both protic and aprotic strongly polar solutes are smaller than might be expected from a linear retention-polarity relationship. This is probably attributable to stronger solute-solvent interactions and to better shielding of polar sites on the Spheron surface (both hydroxyl and ester groups) in methanol.

Tetrahydrofuran is one of the most commonly used solvents for gel chromatography of synthetic polymers. Its use in the fractionation of polymers on Spheron gels has been mentioned in the literature¹, but no details were given. We therefore studied the chromatographic behaviour of several polymeric solutes on these gels. In tetrahydrofuran, a molecular-sieving mechanism was observed in the fractionation of poly(isobutene), poly(vinyl chloride), poly(ethylene-vinyl acetate), poly(vinyl chloride-vinyl acetate), poly(methyl methacrylate), polystyrene, poly(phenylene oxide) and poly(ethylene glycol).

This is illustrated by the chromatogram of a mixture of polystyrene standards shown in Fig. 3 and by that of two samples of poly(phenylene oxide) in Fig. 4. In Fig. 5, the calibration curve for polystyrenes on Spheron 300 is given; a similar cali-

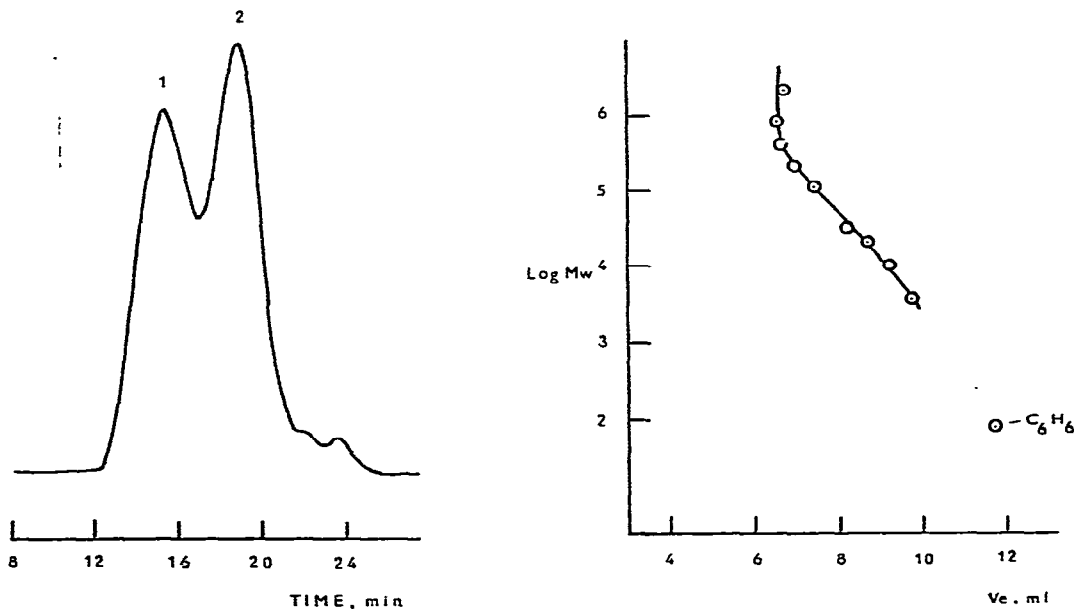


Fig. 4. Separation of two samples of poly(phenylene oxide). Column, packing and operating conditions as in Fig. 3. Sample: 100 μ l of a mixture of 0.1% solutions of poly(phenylene oxide) of intrinsic viscosity 58 (peak 1) and 10 (peak 2).

Fig. 5. Calibration curve for polystyrenes on Spheron 300. Column and operating conditions as in Fig. 3.

bration curve was also obtained for various polymers of ethylene glycol, which is in agreement with published data¹.

The separation efficiency of Spheron gels is considerable, and can be demonstrated by the number of theoretical plates for polystyrene of molecular weight 3550 (peak 4 in Fig. 3), which is 3000 per m; for benzene, the number of theoretical plates is 7500 per m, ($N_{eff} = 1100$ per m), but this is probably due to sorption of the low-molecular-weight solute.

CONCLUSIONS

In non-aqueous media, Spheron gels may be used for both the GPC of polymeric solutes and the sorption chromatography of low-molecular-weight compounds. Several polymers having no strongly polar groups can be separated according to their molecular size on Spheron gels in tetrahydrofuran. In this solvent, undesirable sorption may be expected for macromolecules with strongly polar proton-acceptor groups; for the separation of such polymeric solutes, other solvents (e.g. dimethylformamide) or acylated Spheron gels⁸ should be used.

The sorption of most low-molecular-weight solutes on Spheron is relatively small in tetrahydrofuran or in methanol, but it can lead to poor resolution of certain mixtures. Sometimes, the separation efficiency can be improved by decreasing the polarity of the mobile phase; the particular solubility parameters offer a valuable aid to the appropriate choice of solvent.

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