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Crosslinked Poly(acryloylmorpholines) as Matrices for Gel Permeation Chromatography

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Synopsis

Crosslinked poly(acryloylmorpholines) have been prepared in bead form by suspension polymerization of N-acryloylmorpholine and N,N′-methylenediacrylamide, respective molar ratios 10/1, in aqueous solution dispersed in liquid paraffin. The swelling properties of beads of two porosities, designated Enzacryl Gel K1 and Enzacryl Gel K2, were measured in a range of common solvents. Gels derived by swelling in both chloroform and tetrahydrofuran were evaluated as column packings for gel permeation chromatography. For Enzacryl Gel K1 and polystyrene solutes, molecular weight exclusion limits of approximately 4×10^3 and 6×10^2 were observed in chloroform and tetrahydrofuran, respectively. In the case of Enzacryl Gel K2, the corresponding exclusion limits were 10^4 and 5×10^3. Molecular weight fractionation ranges for both polystyrenes and polyethylene glycols are discussed in terms of gel structure.

INTRODUCTION

Organic materials generally suitable for use as column packings in nonaqueous gel permeation chromatography (GPC) are currently limited to crosslinked polystyrene,[1] dextran hydroxypropyl ethers,[2] and poly(vinyl acetates).[3] In seeking new packings of this type, we have recently investigated the gelation properties of crosslinked poly(acryloylmorpholines). These copolymers were found to swell readily in a variety of organic solvents to give mechanically stable materials of the xerogel type. (The term "xerogel" implies a highly solvated, three-dimensional space network of linear macromolecules whose movement relative to one another is constrained by covalent crosslinking or physical interaction. Removal of the solvent results in collapse of the three-dimensional space network.) These materials appeared suitable for GPC. This paper describes the preparation and chromatographic evaluation of copoly(acryloylmorpholine) beads of two porosities (Enzacryl Gel K1 and Enzacryl Gel K2), calculated to be most effective in the fractionation of solutes of relatively low molecular weight.

EXPERIMENTAL

Synthesis of Enzacryl Gel

Enzacryl Gel K1. To a 5-dm^3 glass polymerization flask, equipped with a 15-cm-diameter semicircular paddle stirrer, was added 2 dm^3 liquid

paraffin (ρ^{20} 0.85 g cm^{-3}, η^{20} 3.5–4.0 N sec m^{-2}) and a surfactant mixture, HLB 3,[4] consisting of 35 cm^3 sorbitan trioleate and 5 cm^3 polyoxyethylene-(20) sorbitan trioleate. Nitrogen was passed directly through the mixture for 1 hr, after which the gas inlet was lifted above the surface to maintain a nitrogen atmosphere in the reaction vessel. A solution of 2 g potassium persulfate in 40 cm^3 water was added rapidly, with stirring, to an oxygen-free solution of 282 g (2 mole) N-acryloylmorpholine and 30.8 g (0.2 mole) N,N′-methylenediacrylamide in 440 cm^3 water containing 150 mg quinol. The resulting mixture was immediately added to the paraffin in the polymerization flask. After a brief period (30 sec) of rapid stirring (200 rpm) to adequately disperse the aqueous phase, the stirring rate was decreased to a speed just sufficient (30 rpm) to maintain the aqueous droplets in suspension. Polymerization was complete within 1 hr.

After allowing the polymer to settle overnight, most of the paraffin was decanted off and the beads were washed with four times 800 cm^3 40–60° petroleum spirit followed by four times 800 cm^3 acetone. The beads were next equilibrated with water, transferred to a glass elutriation column, and elutriated with water at a flow rate of 50 cm^3 hr^{-1} cm^{-2} to remove fines. Finally, the remaining beads were shrunk by dropwise addition of ethanol, equilibrated with ether, and dried under reduced pressure. Beads of 72–230 dry mesh (100 g, >30% yield) were retained for column chromatography.

Enzacryl Gel K2. The preparative procedure was similar to that employed for Enzacryl Gel K1, except that the monomers were diluted with 760 cm^3 water and a surfactant mixture, HLB 2, consisting of 39 cm^3 sorbitan trioleate and 0.9 cm^3 polyoxyethylene(20) sorbitan trioleate, was employed. Beads of 72–240 dry mesh (102 g, >30% yield) were again retained for column chromatography.

Determination of Solvent Regain Values

Samples of dry beads were allowed to swell to equilibrium in a solution of polymeric, totally excluded solute in the solvent under test. The solute concentration in the solution external to the beads was then determined and the solvent regain, R_s, was calculated using the equation

$$R_s = \frac{V}{W}\left[1 - \frac{C}{C'}\right]$$

where C and C' are the respective concentrations of polymeric solute before and after bead swelling, V is the volume of solution, and W is the weight of beads. In the case of solvents not absorbing in the ultraviolet, C and C' were determined spectrophotometrically. Ultraviolet-active polymeric solutes employed were: polystyrene ($\bar{M}_n = 2 \times 10^5$), for swelling measurements in chloroform, methylene dichloride, and tetrahydrofuran; poly-(vinylpyrrolidone) ($\bar{M}_n = 7 \times 10^5$), for measurements in ethanol and methanol; and blue dextran ($\bar{M}_w = 2 \times 10^6$), for measurements in water. With solvents absorbing in the ultraviolet, C and C' were determined

TABLE I
Solvent Regain Values for Enzacryl Gel

Solvent	Enzacryl Gel K1, $cm^3\ g^{-1}$	Enzacryl Gel K2, $cm^3\ g^{-1}$
Pyridine	1.9	2.7
Chloroform	1.8	2.6
Dimethyformamide	1.5	2.6
Water	1.7	2.4
Methylene dichloride	1.7	2.3
Tetrahydrofuran	1.3	1.8
Morpholine	1.0	1.4
Methanol	0.8	1.3
Ethanol	0.7	1.1
Benzene	0.4	0.4
Petroleum ether	Nil	Nil

gravimetrically following evaporation of the solvent. Polystyrene ($\bar{M}_n = 2 \times 10^5$) was employed as the excluded solute for swelling measurements in benzene, dimethylformamide, and pyridine. Poly(vinylpyrrolidone) ($\bar{M}_n = 7 \times 10^5$) was used in the case of morpholine. The results are presented in Table I.

Chromatographic Solutes

Narrow molecular weight distribution polystyrenes ($\bar{M}_n$ = 585, 2085, 3100, 9600, and 20200) were obtained from Digby Chemicals Ltd., London, England. Polyethylene glycols were purchased from Phase Separations Ltd., Queensferry, Wales ($\bar{M}_n$ = 200, 400, 600, 750, 1000, 1500, and 4000); from British Drug Houses Ltd., Poole, England (6000); and from Micro-Bio Laboratories Ltd., London, England (18500 and 37500).

Packing and Elution of Chromatographic Columns

After swelling to equilibrium in the required solvent, the beads of Enzacryl Gel were stirred into a thick slurry and poured into glass columns 90 cm in length and 1.5 cm internal diameter. Column packing in tetrahydrofuran was completed at a flow rate of 100 $cm^3\ hr^{-1}\ cm^{-2}$. In chloroform, a somewhat higher flow rate, 150 $cm^3\ hr^{-1}\ cm^{-2}$, was necessary in order to prevent flotation of the beads. The polystyrenes and polyethylene glycols were applied to the columns in solution volumes not exceeding 3 cm^3 and at concentrations not exceeding 10 mg cm^{-3}. All chromatographic fractionations were performed at a flow rate of 25 $cm^3\ hr^{-1}\ cm^{-2}$, and at ambient temperature.

Determination of Column and Gel Parameters

For each column, the void volume, V_0, was estimated from the elution volume of a totally excluded polystyrene ($\bar{M}_n = 2 \times 10^5$) making due correction for column end fittings and tubular connections. Following the practice of Ackers[5] and Marsden,[6] the precise internal volume of the gel, V_i,

TABLE II
GPC Elution Volumes and Number of Theoretical Plates for Benzene

Column packing	Solvent	Column Dimensions: Length, cm	Column Dimensions: Diameter, cm	V_0, cm³	V_s, cm³	V_e, cm³	W, cm³	Theoretical plates, m⁻¹
Enzacryl Gel K1	Chloroform	90	1.5	62.4	132.4	111.4	10.1	2160
Enzacryl Gel K2	Chloroform	90	1.5	68.5	143.0	127.5	20.0	820
Enzacryl Gel K1	Tetrahydrofuran	90	1.0	37.5	58.3	54.1	17.6	170
Enzacryl Gel K2	Tetrahydrofuran	90	1.0	34.9	58.6	55.7	7.4	1000

available to a totally included molecule of solute or the solvent, was calculated by subtracting V_0 from the elution volume, V_s, of the deuterated solvent. In the case of partially included solutes, permeation behavior was characterized by relating elution volume, V_e, to the absolute distribution coefficient, K_d, using the Wheaton and Baumann equation[7]

$$K_d = \frac{V_e - V_0}{V_t} = \frac{V_e - V_0}{V_s - V_0}.$$

Column efficiency was quantified by measuring the number of theoretical plates per meter, n, for benzene. To calculate n, use was made of the relationship[8]

$$n = \frac{16}{L}\left[\frac{V_e}{w}\right]^2$$

where L is the column length, V_e is the elution volume, and w is the peak width as measured by the distance along the baseline between the intersections of the tangents produced through the points of inflection on the peak elution profile. The results are presented in Table II.

Analysis of Column Effluents

Column effluents were collected in 1- or 2-cm³ fractions. The concentrations of aromatic solutes in the various fractions were estimated from their ultraviolet absorption. Polyethylene glycols were determined, following evaporation of the solvent, by a spectrophotometric chromic acid assay similar to that described by Johnson and Samuelson[9] for uronic acids. Decrease in absorbance (440 nm) of the chromic acid reagent was related to solute concentration with the aid of linear calibration graphs obtained on assay of polyethylene glycol standards. Maximum solute concentration in the column effluents did not exceed 0.2% w/v for either polystyrenes or polyethylene glycols.

Deuterochloroform and perdeuterotetrahydrofuran were estimated by mass spectrometry. For deuterochloroform, peak height for the $CDCl_2^{37}+$ ion was related to deuterochloroform concentration with the aid of a linear calibration obtained on assay of standard chloroform–deuterochloroform mixtures. In the case of perdeuterotetrahydrofuran, the peak height for the molecular ion was conveniently employed for the estimation together with an appropriate calibration graph.

RESULTS AND DISCUSSION

Bead Polymerization

Copolymerization of N-acryloylmorpholine and N,N′-methylenediacrylamide in an organic solvent dispersed in water as the continuous phase is impracticable since N-acryloylmorpholine is readily miscible with water in all proportions. Consequently, a reverse procedure was adopted in which an aqueous solution of the monomers was dispersed in liquid paraffin containing suitable surfactants. The resulting copolymers, Enzacryl Gel K1 and Enzacryl Gel K2, were obtained as soft aqueous xerogels. Although these materials deformed readily under flow, the dried beads swelled readily in organic solvents to give organic xerogels of excellent mechanical stability. Packed into columns these gels proved eminently suitable for GPC.

Gelation Properties

The solvent regains of Enzacryl Gel K1 and Enzacryl Gel K2 in a number of common solvents are presented in Table I. That much more rigid xerogels were obtained in polar organic solvents than in water suggests that different gelation mechanisms may be operative in the two types of solvent. The surprising fact that the copolymers swelled poorly in hydroxylic organic solvents such as ethanol and methanol, which might be expected to have solvation properties roughly intermediate to water and chloroform, supports this hypothesis.

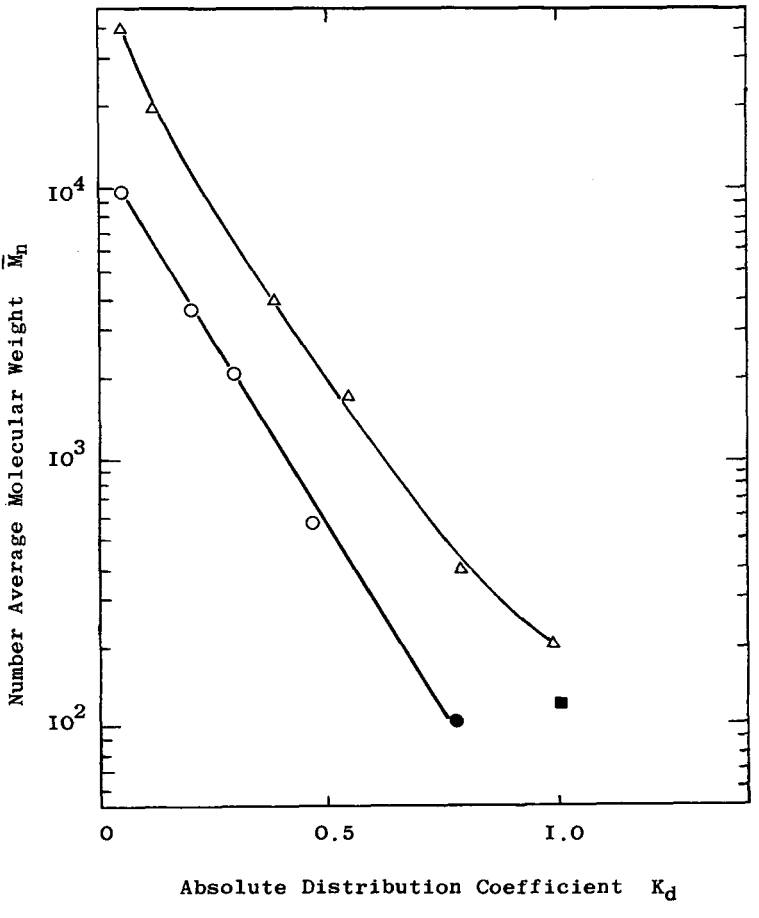
Since the swelling in chloroform, dimethylformamide, methylene dichloride and pyridine was approximately uniform, gel pore size and consequently molecular weight fractionation range are likely to be comparable in these solvents. However, in the case of tetrahydrofuran, the rather lower solvent regain should lead to a marked change in fractionation range while still affording a reasonable capacity ratio. Systematic evaluation of the column packings in one of the former solvents, chloroform, and tetrahydrofuran seemed potentially to be the most informative. Chloroform was selected for convenience of handling and ultraviolet monitoring of column effluents.

Gel Permeation Chromatography

The logarithm molecular weight–distribution coefficient relationships for a series of polystyrene and polethylene glycols on the various xerogels are

shown in Figures 1 and 2. These results suggest broadly that Enzacryl Gel K1 is likely to be most usefully applied to the fractionation of monomeric species, whereas Enzacryl Gel K2, in chloroform at least, is more suited to the fractionation of oligomers and low molecular weight polymers. Reasonable column efficiencies (see Table II) were obtained for Enzacryl Gel K2 in both chloroform and tetrahydrofuran and for Enzacryl Gel K1 in chloroform. Enzacryl Gel K1 in tetrahydrofuran gave less satisfactory results. This probably arose owing to packing difficulties caused by the rather dense nature of the particular xerogel. Plots of eluted solute concentration against elution volume gave characteristic Gaussian distribution curves in the case of all columns. In no instance could "tailing" be detected.

On GPC on Enzacryl Gel K2 in chloroform, both polystyrene and polyethylene glycols exhibited linear relationships between logarithm molecular



(a)

Fig. 1. (*continued*)

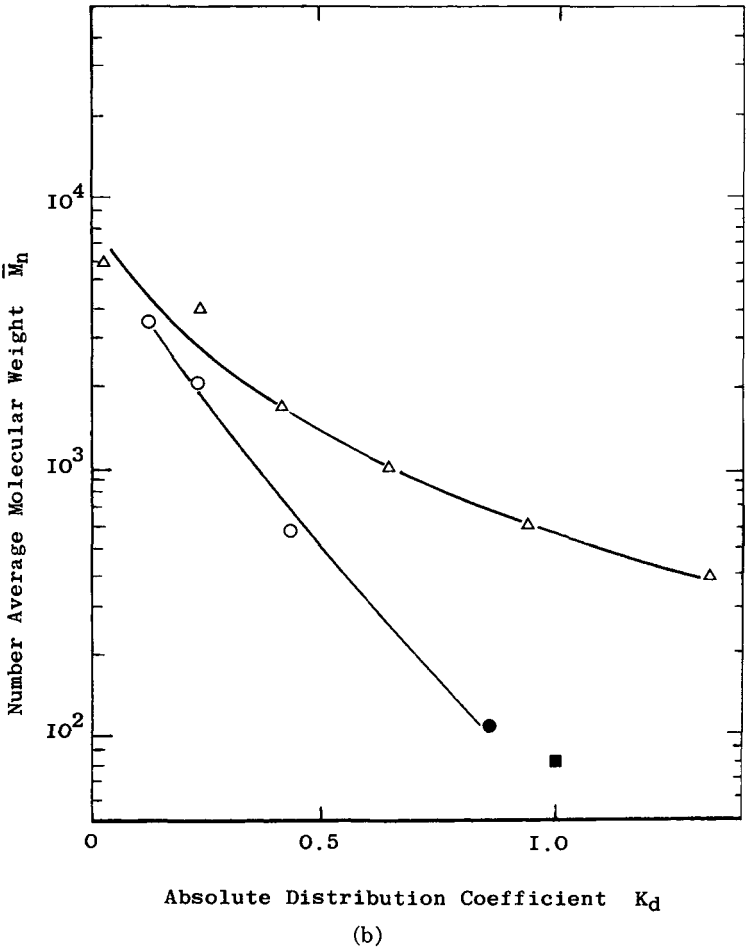


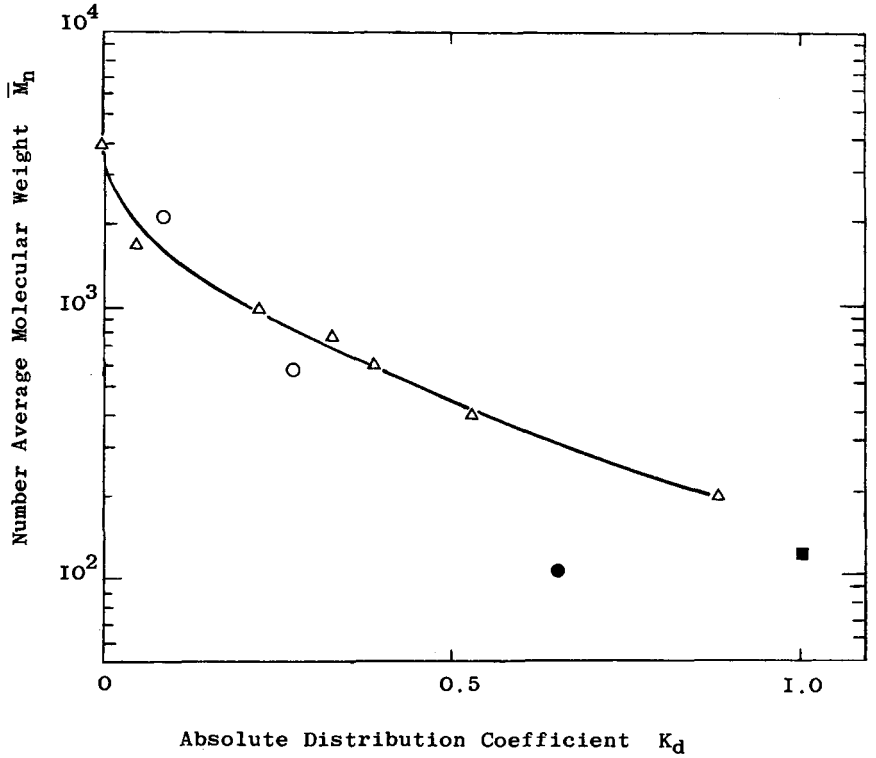
Fig. 1. Molecular weight–distribution coefficient relationships for Enzacryl Gel K2 in chloroform (a), in tetrahydrofuran (b): (△) polyethylene glycols; (○) polystyrenes; (●) styrene; (■) deuterochloroform; (b) in tetrahydrofuran, (■) perdeuterotetrahydrofuran.

weight and distribution coefficient. However, significant differences in the molecular weight fractionation range for the two types of solute were evident. This is not uncommon in GPC because retardation of a given solute is determined principally by its hydrodynamic volume in solution which is not simply related to its molecular weight.

For a given column, a universal calibration curve for all solutes, irrespective of their chemical identity, may often be obtained by applying the treatment of Grubisic and co-workers.[10] This involves plotting the logarithm of the solute viscometric hydrodynamic volume, $[\eta]M$, where η is the limiting viscosity index, against a suitable elution parameter such as distribution coefficient or elution volume. Although this treatment has found widespread use,[11–13] it has been criticized recently by Rudin and

Hoegy[14] on the grounds that it involves the assumption that the viscometric hydrodynamic volumes of solutes at infinite dilution approximate to those which pertain in GPC columns. This is often not the case with appreciably solvated polymers of relatively high molecular weight. However, since Rudin[15] has also demonstrated that at solute molecular weights and concentrations similar to those employed in the characterization of Enzacryl Gel K2, the variation between practical hydrodynamic volume and that at infinite dilution is negligible, the treatment seemed appropriate in the present studies. The logarithm viscometric hydrodynamic volume–distribution coefficient relationships for polystyrenes and polyethylene glycols on Enzacryl Gel K2 in chloroform are presented in Figure 3. Far from obtaining a universal calibration curve, the deviation in relative fractionation range for the two types of solute is emphasized.

Ideally, logarithm molecular weight–distribution coefficient plots should tend to a point approximating to the coordinates observed for the deuterated carrier phase. On chloroform chromatography on Enzacryl Gel K2, this is, in fact, the case for polyethylene glycols but not for polystyrenes. That the polystyrene plot is displaced toward lower K_d values indicates



(a)

Fig. 2. (*continued*)

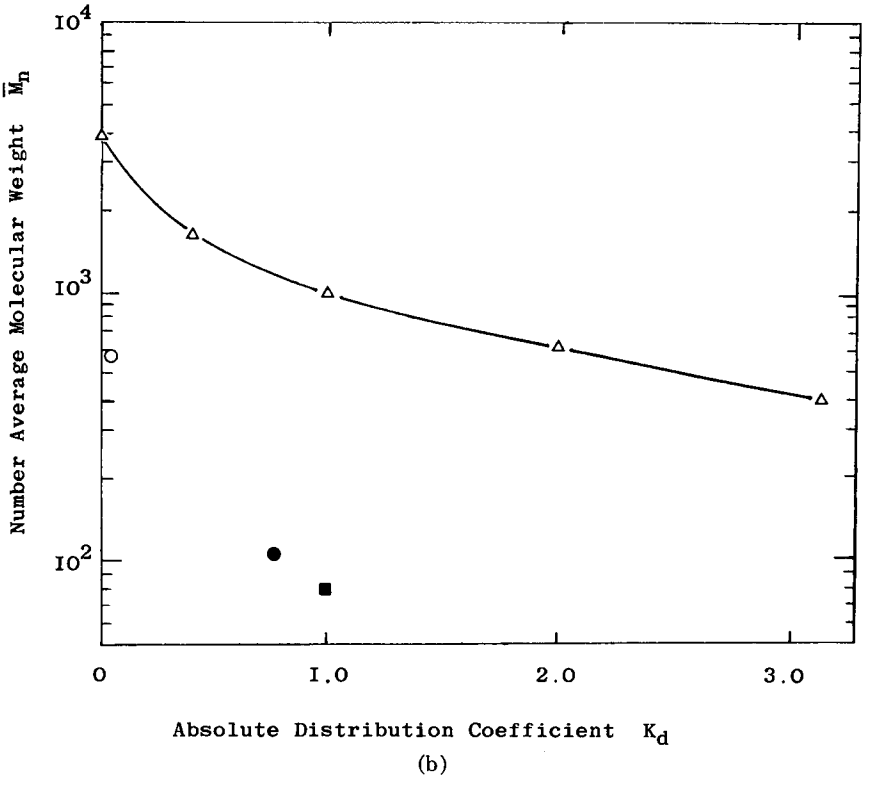


Fig. 2. Molecular weight–distribution coefficient relationship for Enzacryl Gel K1 in chloroform. Symbols same as in Fig. 1. (a) and (b).

"additional exclusion" from a certain volume of the xerogel matrix. A similar pattern of results was evident on chloroform chromatography using Enzacryl Gel K1, except that, approaching the exclusion limit, polyethylene glycols and polystyrenes of roughly equivalent molecular weight possessed similar distribution coefficients.

Surprisingly, almost ideal GPC behavior was observed when polystyrenes were chromatographed in tetrahydrofuran using Enzacryl Gel K2. Since in this case, the logarithm molecular weight–distribution coefficient plot approaches the coordinates observed for the deuterated solvent, it is apparent that "additional exclusion" is not taking place. On the other hand, retardation of the lower molecular weight polyethylene glycols by a mechanism other than GPC clearly occurred in tetrahydrofuran. The effect was most pronounced on the denser xerogel, derived from Enzacryl Gel K1, indicating that a matrix interaction was involved. In contrast, only triethylene glycol and smaller units were anomalously retarded during GPC studies in chloroform.

A tentative explanation for some of these observations may be given on the assumption that each gel bead consists partly of pure immobile solvent

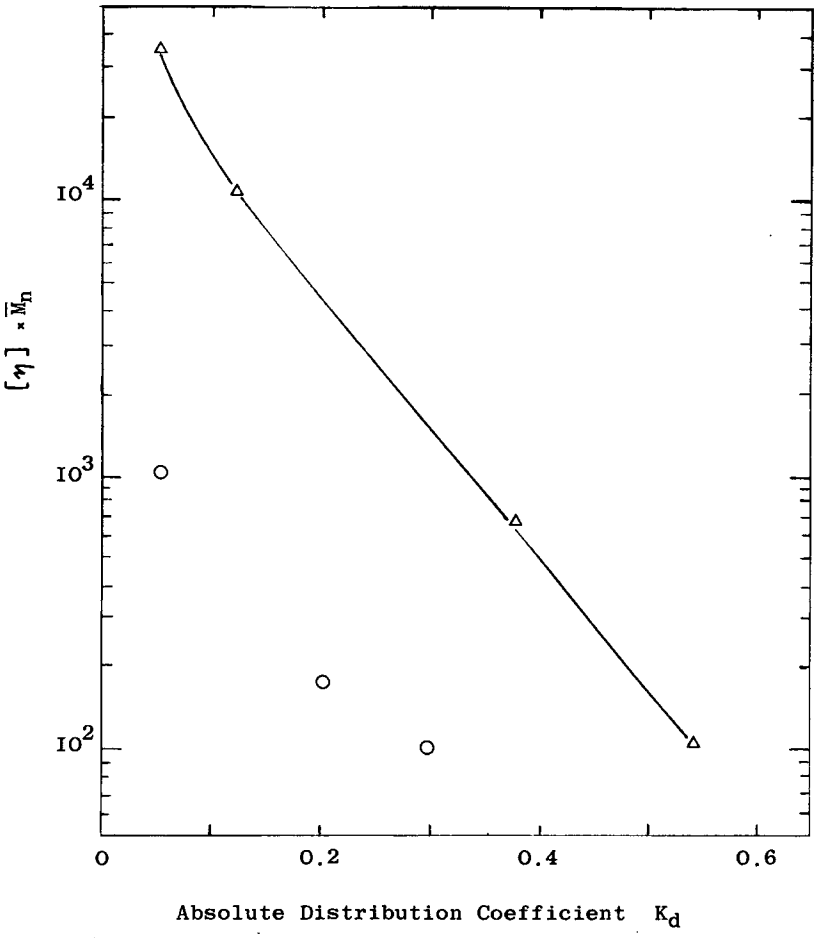


Fig. 3. Intrinsic viscosity–distribution coefficient relationships for Enzacryl Gel K2 in chloroform: (△) polyethylene glycols; (○) polystyrenes.

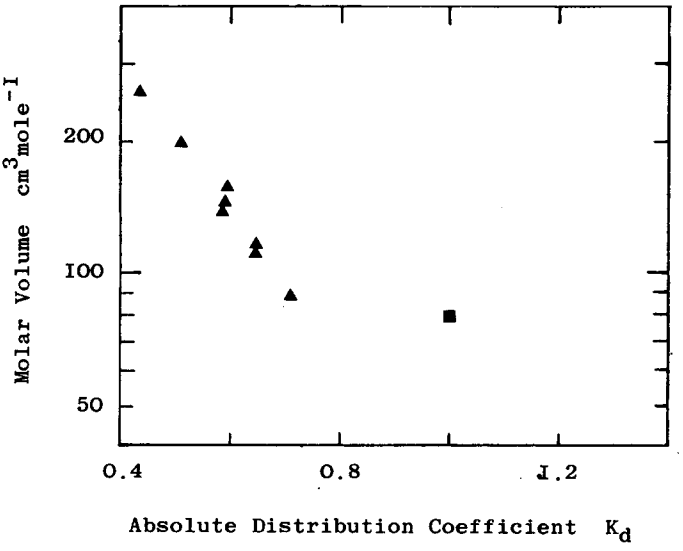
and partly of individual poly(acryloylmorpholine) chains in solution. The solvated chains occupy thread-like volumes of solution whose movement relative to one another is constrained by crosslinking. In the case of solutes having greater affinity for the immobile solvent than for the gel volume attributable to the solution of poly(acryloylmorpholine) chains, adverse micropartition may occur effectively excluding the solute from this volume within the gel bead. Poly(acryloylmorpholine) has little affinity for nonfunctionalized aromatic compounds as evidenced by its inferior swelling in these solvents. Consequently, it is not improbable that polystyrenes have greater affinity for chloroform than the internal volume of the gel corresponding to the solvated poly(acryloylmorpholine) matrix and that a relatively low molecular weight fractionation range results. During GPC in tetrahydrofuran, the structural similarity between the eluting solvent and the pendent morpholino groups of the solvated polymer matrix

leads to a similar environment for the polystyrene solutes. This may preclude disproportionate partitioning between the two microphases.

The pronounced interaction of the Enzacryl Gel matrix with polyethylene glycols, observed during GPC in tetrahydrofuran, could arise owing to less efficient solvation of both matrix and solute than is the case in chloroform. Hydrogen bonding involving the matrix and the terminal hydroxyl groups of the solute is probably very important since interaction is most serious at low solute molecular weights. Less efficient solvation of the packings is apparent from their inferior swelling in tetrahydrofuran. Lower swelling reflects the failure of the solvent to overcome physical interaction between the poly(acryloylmorpholine) chains. It is significant that, irrespective of solute type, Enzacryl Gel K2 in tetrahydrofuran exhibits a higher molecular weight exclusion limits than Enzacryl Gel K1 in chloroform even though a similar solvent regain is involved. This may indicate chain clustering in tetrahydrofuran.

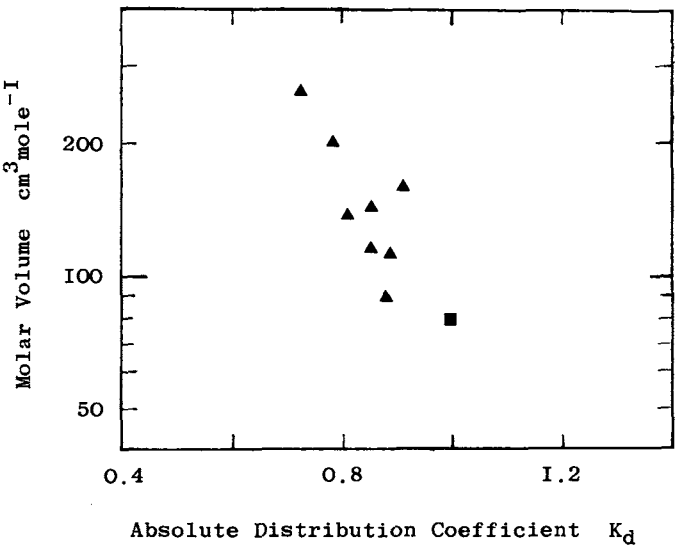
The "additional exlusion" effect has been observed by other workers.[16,17] For example, widely differing logarithm viscometric hydrodynamic volume–elution volume plots for polystyrenes and polypropylene glycols were obtained for solutes of molecular weights less than 5×10^3 on chromatography on Styragel. Significantly, in these studies the molecular weight fractionation range was highest for polystyrene solutes. Enhanced porosity to polystyrene solutes could well reflect their superior ability to penetrate solvated regions of the Styragel (crosslinked polystyrene) matrix.

Recently, Altgelt[18] has discussed the "additional exclusion" of poly(vinyl acetates) during GPC at high load on polystyrene matrices. Although high



(a)

Fig. 4. (*continued*)



(b)

Fig. 4. Molar volume–distribution coefficient relationship for Enzacryl Gel K1 in chloroform (a), in tetrahydrofuran (b): (a) (▲) aromatic compounds; (■) deuterochloroform; in tetrahydrofuran (b), (■) perdeuterotetrahydrofuran.

loads have been avoided in the present experiments, the mechanism of exclusion proposed is worth considering. It was suggested that incompatibility arose owing to the low entropies of mixing between the different macromolecular species involved, that is, between the solute and the solvated gel matrix. If the entropy term is too small to overcome a positive heat of mixing, incompatibility will result. Since a sufficiently low entropy term is only likely to arise with macromolecular solutes, incompatibility should not arise in the case of small molecules.

In this light, it is interesting to consider the logarithm molar volume–distribution coefficient relationships for monomeric aromatic solutes on Enzacryl Gel. If a micropartitioning effect is involved, "additional exclusion" should occur in much the same way as for macromolecular solutes. That this is indeed the case is apparent from Figure 4, (see also Table III), displacement of the logarithm molar volume–distribution coefficient relationship being observed for Enzacryl Gel K1 in chloroform but not for Enzacryl Gel K2 in tetrahydrofuran.

There is no obvious correlation between elution volumes and volumetric parameters in the case of very small molecules. This is readily apparent when distribution coefficients in excess of unity are observed as is the case with the solutes listed in Table IV. Micropartition of these solutes in favor of the internal xerogel volume corresponding to the solvated poly-(acryloylmorpholine) chains could well explain solute retardation in these cases.

TABLE III
Absolute Distribution Coefficients for some Aromatic Solutes

Solute	Enzacryl Gel K1 in chloroform	Enzacryl Gel K2 in tetrahydrofuran
Benzene	0.705	0.879
Diphenyl	0.583	0.813
p-Terphenyl	0.504	0.791
p-Quaterphenyl	0.432	0.725
Styrene	0.647	0.857
Naphthalene	0.640	0.890
Anthracene	0.590	0.857
Pyrene	0.590	0.912

TABLE IV
Absolute Distribution Coefficients for some Unexpectedly Retarded Solutes

Solute	Enzacryl Gel K1 in chloroform	Enzacryl Gel K1 in tetrahydrofuran	Enzacryl Gel K2 in tetrahydrofuran
Nitromethane	1.108	1.40	1.319
Nitrobenzene	0.784	0.97	1.033
1,3-Dinitrobenzene	1.036	1.25	1.231
1,3,5-Trinitrobenzene	1.583	1.60	1.462
Pyridine	0.748	1.22	1.220

CONCLUSIONS

This investigation demonstrates that organic xerogels derived from bead-polymerized, crosslinked poly(acryloylmorpholines) may be usefully employed as column packings for GPC. Specifically, the permeation characteristics of two such materials effective in fractionating monomers and low molecular weight polymers have been evaluated. Useful secondary variations in pore size may be produced by choice of appropriate eluting solvents. Excellent flow rates and column efficiencies comparable to those obtained with other column packings were obtained. Nonideal molecular weight–distribution coefficient relationships, obtained on chloroform chromatography of aromatic solutes, may be rationalized in terms of xerogel structure.

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