Poly(*N*-acryloyl-L-prolyl morpholine): a g.p.c. packing exhibiting novel temperature dependence in solute distribution coefficients

A. W. J. Brough, R. Epton, J. V. McLaren and G. Marr
Department of Physical Sciences, The Polytechnic, Wolverhampton WV1 1LY, UK
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The synthesis of poly(*N*-acryloyl-L-prolyl morpholine), a new bead packing for the g.p.c. of small molecules, is described. Characteristic g.p.c. behaviour and high column efficiencies were observed in water and benzyl alcohol. Novel, reversible temperature variations in solute Wheaton and Bauman distribution coefficients were observed. This effect, which is most marked in water, is caused by thermoreversible changes in network pore size distribution in the bead packing.

INTRODUCTION

We have described previously the synthesis and evaluation of a number of poly(acryloyl morpholine) based column packings for gel permeation chromatography (g.p.c.)[1–4]. These 'universal' g.p.c. supports are applicable in aqueous and organic solvents. Most recently, we have synthesized a cross-linked poly(acryloyl morpholine) packing (Enzacryl Gel KO), of minimal mean pore diameter, which was intended specifically for the g.p.c. of small molecules[5]. This material exhibited high column efficiences in water and performed moderately in organic solvents.

Multisolvent compatibility in an organic bead polymer destined for use as a g.p.c. packing is dependent on the subtle interplay of hydrophilic and lipophilic features incorporated in the polymer network. In this paper, we report the synthesis of a new g.p.c. column packing, poly(*N*-acryloyl-L-prolyl morpholine), intended to be similar in nature to poly(acryloyl morpholine) but of rather higher lipophilic content. The efficiency of this material as a g.p.c. packing for the separation of small molecules in water and benzyl alcohol has been investigated[6]. An unusual, reversible temperature variation in Wheaton and Bauman[7] distribution coefficient of solutes, has been observed.

EXPERIMENTAL

Synthesis of N-*benzyloxycarbonyl-L-prolyl morpholine*

N-benzyloxycarbonyl-L-proline (29.93 g, 0.1 mol) (prepared by the method of Berger *et al.*[8]) was dissolved in a mixture of dry dichloromethane (100 cm^3) and morpholine (8.71 g, 0.1 mol). A solution of *N,N'*-dicyclohexylcarbodiimide (DCCD) (21.7 g, 0.105 mol) in dichloromethane (400 cm^3) was added slowly with stirring over 5 min. Stirring was continued for 48 h after which glacial acetic acid (1 cm^3) was added. After a further 1 h the mixture was chilled to 0°C and dicyclohexyl urea was removed by filtration. The filtrate was washed successively with water (250 cm^3), hydrochloric acid (0.5 M; 250 cm^3), water (250 cm^3), aqueous sodium bicarbonate (1.0 M; 250 cm^3) and water (2 x 250 cm^3), dried over magnesium sulphate and the solvent removed under reduced pressure. The resultant crude product was redissolved in dichloromethane (50 cm^3) and the solution filtered to remove residual dicyclohexyl urea and evaporated to leave crystals. After trituration with a small volume (50 cm^3) of diethyl ether at −40°C, the product was recrystallized from acetone/petroleum ether (40°–60°C) to give *N*-benzyloxycarbonyl-L-prolyl morpholine (21.5 g, 68%); m.p. 145°–146°C; $[\alpha]_D^{22}$ = −16.8° (c 0.50% in $CHCl_3$); υ_{max} (KBr disc) 1695 (carbamic ester C=O str) and 1640 cm^{-1} (amide C=O str); ($CDCl_3$; 60 MHz) 1.7–2.2 4H, m, $CH_2(C\underline{H}_2)_2CH$, 3.2–3.7 10H, $CON(C\underline{H}_2C\underline{H}_2)_2O$ and $CH_2C\underline{H}_2N$, 4.55 1H, bs, $NC\underline{H}(CO)CH_2$, 5.00 (2H, s, $C_6H_5C\underline{H}_2O$) and 7.20 ppm 5H, $C_6\underline{H}_5$. (Found: C, 64.30%; H, 6.89%; N, 8.84%. $C_{17}H_{22}O_4N_2$ requires: C, 64.13%; H, 6.97%; N, 8.80%.)

Synthesis of N-*L-prolyl morpholine.HBr*

Hydrogen bromide in glacial acetic acid (45% w/v; 45 cm^3, 0.25 mol) was added to *N*-benzyloxycarbonyl-L-prolyl morpholine (15.92 g, 0.05 mol), and the mixture was stirred until evolution of gas had subsided (2 h). Dry diethyl ether (500 cm^3) was added and the mixture was stored at −20°C for 15 h when a mixture of crystals and an oil was deposited. The crystals and oil were washed with dry diethyl ether, triturated with dry acetone (50 cm^3) and cooled to −50°C before being collected by filtration. The product was redissolved in the minimum amount of warm dry methanol (10 cm^3) and precipitated with acetone to give N-*L-prolyl morpholine. HBr* (11.0 g, 83%), m.p. 203°–204°C, $[\alpha]_D^{22}$ = −68.9° (c 0.50% in $CHCl_3$), υ_{max} (KBr disc) 1640 cm^{-1} (amide C=O str), ($CDCl_3$; 60 MHz) 1.6–2.7 4H, m, $CH_2(C\underline{H}_2)_2CH$, 3.58 10H, $CON(C\underline{H}_2C\underline{H}_2)_2O$ and $CH_2C\underline{H}_2N$, 4.7–5.1 1H, m, $\overset{+}{N}C\underline{H}(CO)CH_2$ and 8.47 ppm 2H, bs, $CH_2\overset{+}{N}\underline{H}_2CH$. (Found: C, 40.85%; H, 6.50%; N, 10.44%. $C_9H_{17}O_2N_2$ Br requires: C, 40.77%; H, 6.47%; N, 10.56%.)

Synthesis of N-*acryloyl-L-prolyl morpholine*

A slurry of anhydrous sodium carbonate (31.8 g, 0.3 mol) and water (150 cm^3) was added to *N*-L-prolyl morpholine. HBr (7.95 g, 0.03 mol). The mixture was cooled to 0°C and acryloyl chloride (2.72 g, 0.03 mol) (Koch-Light, UK) was added with stirring over 15 min. A further aliquot of acryloyl chloride (1.36 g, 0.015 mol) was added and stirring was continued at 0°C over 1 h after which the mixture was saturated with sodium chloride and extracted with precooled (0°C) chloroform (4 x 200 cm^3). The combined, dried extracts were concentrated under reduced pressure (<30°C) to yield a syrup. Crystallization from acetone/petroleum ether (b.p. 30°–40°C) gave N-*acryloyl-L-prolyl morpholine* (4.7 g, 67%); m.p. 117.5°C; $[\alpha]_D^{22} = -42.6°$ (c 0.5% in $CHCl_3$); υ_{max} (KBr disc) 1645 (amide C=O str) and 1610 cm^{-1} (C=C str); ($CDCl_3$; 60 MHz) 1.6–2.5 4H, m, $CH_2(C\underline{H}_2)_2CH$, 3.2–3.9 10H, m, $CON(C\underline{H}_2C\underline{H}_2)_2O$ and $CH_2C\underline{H}_2N$, 4.80 1H, m, $NC\underline{H}(CO)CH_2$, 5.55 1H, m, $CH_2{=}C\underline{H}CO$ and 6.30 ppm 2H, m, $C\underline{H}_2{=}CHCO$. (Found: C, 60.33%; H, 7.58%; N, 11.58%. $C_{12}H_{18}O_3N_2$ requires: C, 60.49%; H, 7.61%; N, 11.77%.)

*Synthesis of poly(*N-*acryloyl-L-prolyl morpholine) beads*

Suspension polymerizations were carried out as described previously for poly(acryloyl morpholine) packings[3,5]. The suspending phase consisted of light liquid paraffin (ρ^{20} = 0.85 g/cm^3, η^{20} = 3.5–4.0 sec/m^2)(194 cm^3) and a surfactant mixture (6.0 cm^3) containing sorbitan trioleate and polyoxyethylene (20) sorbitan trioleate in the ratio 3:1 (v/v). The monomer solution which consisted of *N*-acryloyl-L-prolyl morpholine (11.66 g, 0.049 mol), *N,N'*-methylenediacrylamide (0.839 g, 0.0054 mol), aqueous hydroquinone (1 g/dm^3, 5.4 cm^3) and water (0.85 cm^3), was deoxygenated, initiated with aqueous potassium persulphate (25 g/dm^3; 0.625 cm^3) and dispersed in the deoxygenated suspending phase. The suspension was stirred to maintain the aqueous droplets in the range 10–50 μm (diameter). Polymerization was allowed to proceed to completion over 18 h. The polymer beads were recovered by washing with petroleum ether (b.p. 40°–60°C; 3 x 100 cm^3) followed by ethanol (3 x 100 cm^3) and were redispersed in water. Yield (dry weight) 12 g (96%)

The poly(*N*-acryloyl-L-prolyl morpholine) beads from several runs were batched and graded by wet elutriation and beads of 20–40 μm diameter were selected for g.p.c.

Gel permeation chromatography

Chromatographic standards. The standards used for g.p.c. characterization in water and in benzyl alcohol were poly(ethylene glycols) (Carbowaxes) ($\bar{M}_n$ 200, 400, 600, 750, 1000, 1500, 4000 and 20 000) (Phase Separations Ltd, UK) and ethanediol, diethylene glycol and triethylene glycol (British Drug Houses Ltd, UK). Polystyrene standards $\bar{M}_n$ 600, 2100 and 4000) (Digby Chemicals Ltd, UK), n-alkanes (pentane, octane, dodecane, hexadecane, eicosane and octacosane) (British Drug Houses Ltd) were used exclusively in benzyl alcohol. The saccharides, glucose, maltose, raffinose, stachyose, (±) -threitol and (±) -glyceraldehyde (Koch-Light, UK) were used solely for aqueous characterization.

Column preparation. The column packing was equilibrated overnight in the desired solvent and poured as a thin slurry into Jobling jacketed glass g.p.c. columns (0.4 x 100 cm) each fitted with a PTFE end-fitting, a temporary extension (0.4 x 50 cm) and loading funnel. The latter was replaced by another end-fitting and the columns were conditioned by pumping solvent at twice the projected flow rate (5–10 cm^3/h) until the gel bed volume was constant (~24 h).

Column elution and effluent analysis. Two of the columns described above were coupled in series and eluted using a positive displacement pump (Milton Roy Co., USA). The temperature of the packed columns was controlled by circulating water through the outer jackets from a thermostatically controlled bath. Samples for analysis (1.0–5.0 g/dm^3; 10–50 mm^3) were applied via a septum injection head (Jobling Ltd), under constant flow conditions and were eluted at a flow rate of 2–5 cm^3/h (pressure drop of 350–2000 kN/m^2). The column effluent was monitored using a thermostatically controlled differential refractometer (Waters model R401; Waters Associates, USA).

Gel and column parameters. The Wheaton and Bauman[7] absolute distribution coefficient, K_d, was calculated for each molecular weight standard from the relationship:

$$K_d = \frac{(V_e - V_o)}{(V_s - V_o)}$$

where V_e, V_s and V_o are the elution volumes of the solute, the solvent and a totally excluded solute, respectively. The solvent elution volumes, V_s, were determined in water and benzyl alcohol by deuterium oxide (D_2O) (Norsk Hydro,

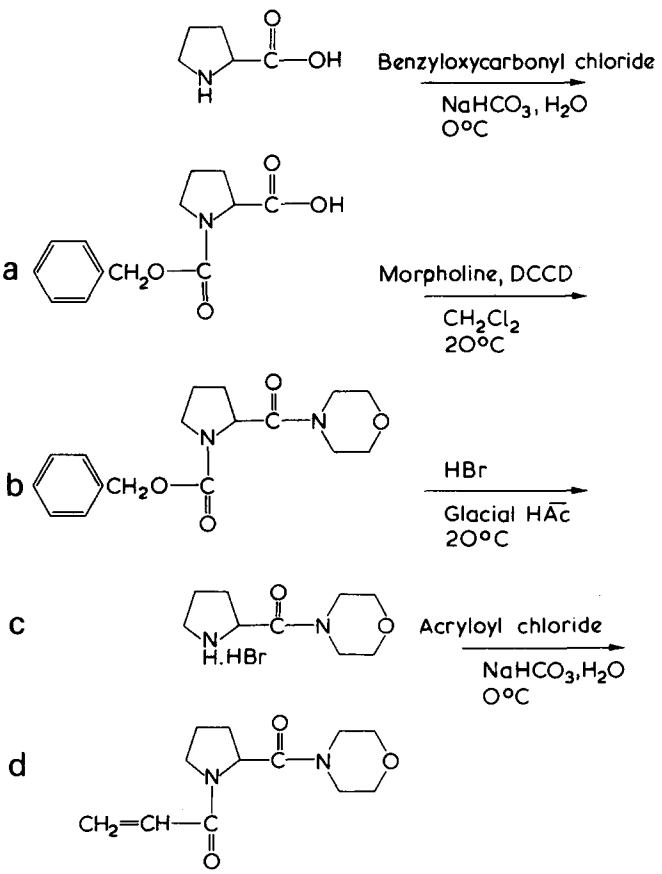

Figure 1 Synthetic route for the preparation of *N*-acryloyl-L-prolyl morpholine (d). Characterized intermediates include: benzyloxycarbonyl-L-proline (a); *N*-benzyloxycarbonyl-L-prolyl morpholine (b); *N*-L-prolyl morpholine.HBr (c)

Table 1 Gelation properties of poly(*N*-acryloyl-L-prolyl morpholine) in a variety of solvents

Solvent	Poly(*N*-acryloyl-L-prolyl morpholine)			Enzacryl Gel KO		
	Imbibition volume (cm^3/g)	Gel bed volume (cm^3/g)	Imbibition† ratio	Imbibition volume (cm^3/g)	Gel bed volume (cm^3/g)	Imbibition† ratio
m-Cresol	2.78	5.88	1.54	2.55	5.15	1.70
Acetic acid	2.44	5.30	1.36	1.81	4.08	1.21
Benzyl alcohol*	2.25	5.00	1.25	1.66	3.85	1.11
Chloroform	2.03	4.62	1.13	1.52	3.65	1.01
Ethyl lactate	1.91	4.45	1.07	1.72	3.95	1.15
Water	1.80	4.25	1.00	1.50	3.63	1.00
Pyridine*	1.80	4.25	1.00	1.21	3.20	0.81
Morpholine*	1.71	4.03	0.95	1.22	3.23	0.81
Benzonitrile*	1.59	3.90	0.88	0.83	2.65	0.55
2-Methoxyethanol	1.58	3.88	0.88	1.07	3.00	0.71
Nitromethane	1.55	3.82	0.86	0.97	2.85	0.65
Nitrobenzene*	1.53	3.80	0.85	0.76	2.55	0.51
Dimethylformamide	1.53	3.80	0.85	1.09	3.03	0.73
Dimethyl sulphoxide*	1.52	3.78	0.84	1.14	3.10	0.76
Ethanediol	1.35	3.50	0.75	0.95	2.83	0.63
Dioxane*	1.32	3.45	0.73	0.86	2.70	0.57
Cyclohexanol*	1.30	3.43	0.72	0.10	1.60	0.07
Chlorobenzene*	1.23	3.30	0.68	0.10	1.60	0.07
Cyclohexanone*	1.16	3.18	0.64	0.09	1.58	0.06
Benzene	1.13	3.12	0.63	0.00	1.45	0.00
Methanol	1.13	3.12	0.63	0.52	2.20	0.35
Acetonitrile	1.05	3.00	0.58	0.52	2.20	0.35
Tetrahydrofuran*	1.05	3.00	0.58	0.17	1.70	0.11
Ethanol	0.83	2.62	0.46	0.43	2.08	0.29
Propan-2-ol*	0.78	2.55	0.43	0.43	2.08	0.29
Acetone	0.75	2.50	0.42	0.52	2.20	0.35
Ethyl acetate*	0.60	2.25	0.33	0.26	1.83	0.17
Toluene	0.42	1.95	0.23	0.00	1.45	0.00
Carbon disulphide	0.30	1.75	0.17	0.10	1.60	0.07
Diethyl ether	0.26	1.67	0.14	0.00	1.45	0.00
Tetrachloroethylene	0.24	1.65	0.13	0.00	1.45	0.00
Carbontetrachloride	0.15	1.50	0.08	0.00	1.45	0.00
Cyclohexane	0.03	1.30	0.02	0.00	1.45	0.00
Hexane	0.03	1.30	0.02	0.00	1.45	0.00

* Both column packings swelled in these solvents only after warming either at 100°C or the boiling point of the solvent if less than 100°C, over 10 min.
† Compared with water

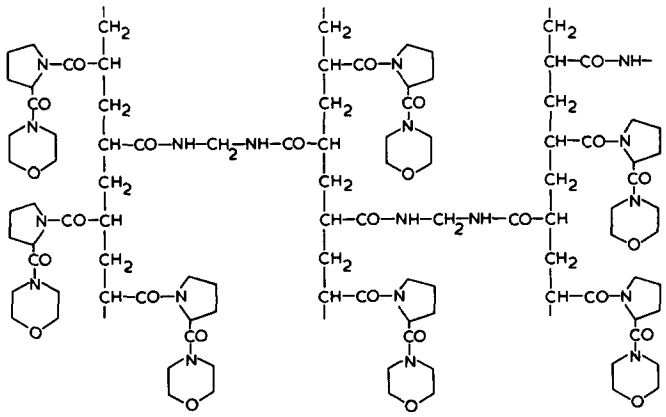

Figure 2 Schematic structure of crosslinked poly(*N*-acryloyl-L-prolyl morpholine)

Norway) and pentadeuterobenzyl alcohol ($C_6D_5CH_2OH$) (Merck AG, Germany), respectively. Similarly, the void volumes, V_o, were determined by elution of Blue Dextran 2000 ($\bar{M}_w$ 2 x 10^6) (Pharmacia Ltd, Sweden) or polystyrene ($\bar{M}_n$ 2 x 10^6) (Digby Chemicals Ltd).

Chromatographic efficiency (E) was calculated for the appropriate deuterated solvent from the relationship[9]:

$$E = \frac{16}{L}\left(\frac{V_s}{w}\right)^2$$

where L is the column length and w the peak width.

RESULTS AND DISCUSSION

The synthesis of the monomer, *N*-acryloyl-L-prolyl morpholine, was carried out using established procedures of amino-acid and peptide chemistry (*Figure 1*). Consequently, the optical activity of the L-proline residue was preserved. The monomer concentration in the polymerization mixture (65% w/v) was the maximum consistent with success in obtaining discrete beads. Our aim was to obtain a poly(*N*-acryloyl-L-prolyl morpholine) network (*Figure 2*) of minimum mean pore diameter.

Solvent imbibition studies (*Table 1*) showed that the poly(*N*-acryloyl-L-prolyl morpholine) beads swelled readily in such useful g.p.c. solvents as water, chloroform, pyridine, dimethylformamide and benzyl alcohol but were unsuitable for application in others such as diethyl ether, alkanes and fully halogenated alkanes. Overall trends in swelling proper-

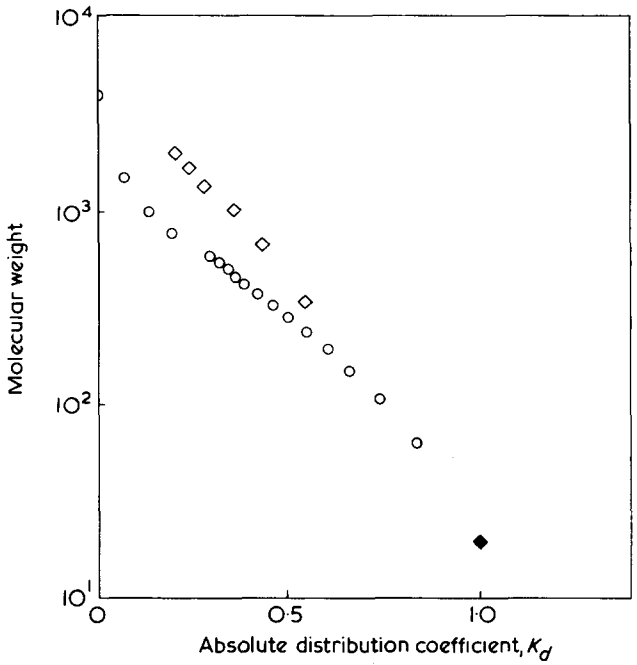

Figure 3 Logarithm molecular weight *versus* K_d relationship for oligosaccharides (◇), poly(ethylene glycols) (○) and deuterium oxide (◆) for poly(*N*-acryloyl-L-prolyl morpholine) in water

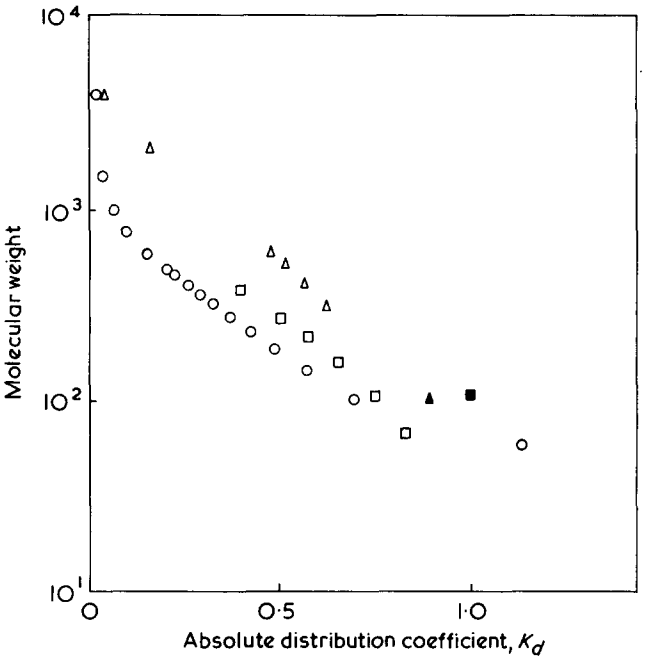

Figure 4 Logarithm molecular weight *versus* K_d relationship for polystyrenes (△), n-alkanes (□), poly(ethylene glycols) (○), ethylbenzene (▲) and pentadeuterobenzyl alcohol (■) for poly(*N*-acryloyl-L-prolyl morpholine) in benzyl alcohol

ties resemble the poly(acryloyl morpholine) networks described previously by us[1–5]. However, some significant differences do arise, notably the superior swelling of the poly(*N*-acryloyl-L-prolyl morpholine) matrix in benzene, chlorobenzene, cyclohexanol, cyclohexanone, ethanol, methanol and tetrahydrofuran. These differences reflect the relatively greater lipophilic character of poly(*N*-acryloyl-L-prolyl morpholine).

Of the less aggressive solvents, water and benzyl alcohol gave the best xerogels with poly(*N*-acryloyl-L-prolyl morpholine) and were selected for g.p.c. evaluation studies.

Typical g.p.c. behaviour was observed in that, with both solvents, most solutes were eluted from the column in a sequence of decreasing molecular weights. Characteristic Gaussian distribution curves were obtained for the eluted solutes with no evidence of 'tailing'. Ideal plots of logarithm molecular weight *versus* the Wheaton and Bauman absolute distribution coefficient, K_d, were obtained for poly(ethylene glycol) oligomers and for oligosaccharides in water (*Figure 3*) and for poly(ethylene glycol) oligomers, polystyrene oligomers and n-alkanes in benzyl alcohol (*Figure 4*).

The K_d values for some solutes which did not exhibit typical g.p.c. behaviour are recorded (*Table 2*). Benzyl alcohol was retarded when water was used as eluent and *vice versa*. This reflects the mutual incompatability of these solvents, both of which must have an obvious, high affinity for the polymer matrix. Carboxylic acids, for example benzoic acid, were anomalously retarded in both eluents presumably because the cyclic ether linkages of the packing confer weak, overall basicity. Aromatic solutes, such as benzylamine, exhibit some retardation in water.

In view of the chirality of the prolyl morpholine residues of the packing, it was of interest to investigate whether preferential retardation (resolution) of enantiomers occurred on g.p.c. of racemic mixtures. However, on chromatography of (±) glyceraldehyde, (±) threitol, (±) proline and (±) phenylalanine amide.HCl, no such resolution could be detected (*Table 2*).

It was convenient to express column efficiency in terms of the number of theoretical plates per metre for the deuterated equivalent of the mobile phase under investigation (*Table 3*). Although high column efficiency was observed overall, efficiency in benzyl alcohol was somewhat inferior to that in water at the lower temperatures studied. This divergence is likely to be caused by the relatively high viscosity of benzyl alcohol at low temperature. Similar, high column efficiencies at 60°C imply broadly similar physical

Table 2 Various solute K_d values for poly(*N*-acryloyl-L-prolyl morpholine) in water and in benzyl alcohol

Species	Molecular weight	K_d (H_2O)	K_d ($C_6H_5CH_2OH$)
Water	18	–	2.68
Benzyl alcohol	108	2.38	–
Benzylamine	107	3.0	0.64
Benzoic acid	122	1.0	1.57
Schardinger-β-dextrin	1135	1.68	–
(±) Glyceraldehyde	90	0.75	–
(±) -Threitol	122	0.72	1.70
(±) -Proline	115	–	0.54
(±) -Phenylalanine amide·HCl	201	0.58	0.77

Table 3 Dependence of g.p.c. column efficiency on temperature for poly(*N*-acryloyl-L-prolyl morpholine) packing

	Column efficiency* (plates/m)			
Solvent	0°C	20°C	40°C	60°C
Water	3980	6340	7880	11 300
Benzyl alcohol	1290	5060	9110	11 600

* Measured for the isotopically labelled solvent peaks, D_2O and pentadeuterobenzyl alcohol

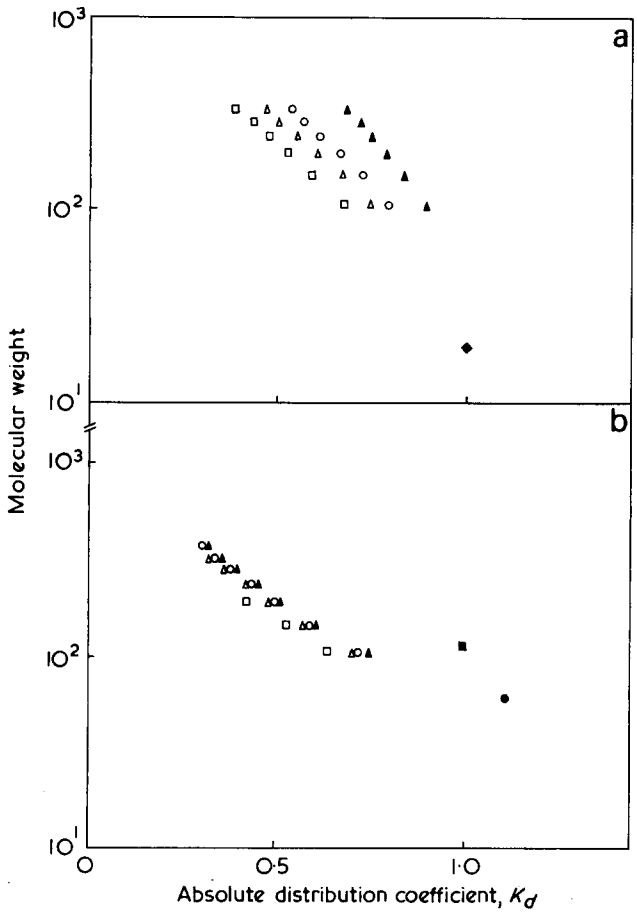

Figure 5 Variation of K_d with temperature for Carbowax 200 in water (a) and benzyl alcohol (b). Measurements were made at 0° (□), 20° (△), 40° (○) and 60°C (▲) using deuterium oxide (◆) and pentadeuterobenzylalcohol (■) as standards. Ethanediol (●) behaved similarly at all temperatures in benzylalcohol

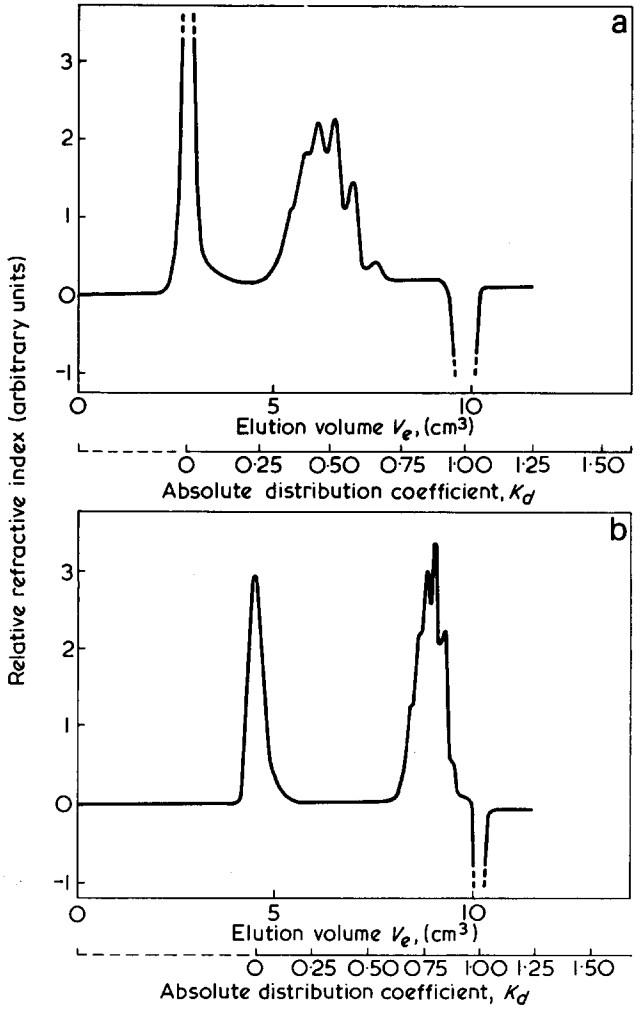

Figure 6 Elution profiles for Carbowax 200 on poly(*N*-acryloyl-L-prolyl morpholine) in water at 0° (a) and 60°C (b)

properties for the aqueous and organic xerogels derived from poly(*N*-acryloyl-L-prolyl morpholine) and a good balance between lipophilic and hydrophilic features.

During the course of our investigation of the effect of temperature on column efficiency we were surprised to observe large, thermo-reversible changes in solute K_d values (*Figures 5a* and *5b*). The effect was most marked when water was used as the eluent and was sufficient to cause major variation in the characteristic g.p.c. elution profile (*Figures 6a* and *6b*).

It is well established that K_d values in g.p.c. are independent of temperature unless temperature variation induces changes in solute hydrodynamic volume[10–12]. This is most unlikely in the case of ethylene glycol and the small poly(ethylene glycol) oligomers used in this study. Temperature elevation was accompanied by some *contraction* of the aqueous poly(*N*-acryloyl-L-prolyl morpholine) xerogel. Thus the aqueous imbibition values of the packing at 20° and 60°C are 1.80 and 1.50 cm^3/g, respectively. We conclude, therefore, that the observed, thermo-reversible *increase* in K_d values must be ascribed to major changes in polymer distribution and, consequently, pore geometry within the poly(*N*-acryloyl-L-prolyl morpholine) xerogel.

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