Polystyrene Gel Permeation Chromatography Packings Grafted with Polar Monomers—Synthesis and Use in Aqueous Organic Mobile Phases

J. G. HEFFERNAN* and D. C. SHERRINGTON, *Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, Glasgow G11XL, Scotland*

Synopsis

A number of polystyrene resins of chromatographic quality (5–10 μm) have been prepared with significant residual double bond contents (~ 2 mmol · g^{-1}). These groups have been used as sites for the grafting of eight polar macromolecules in an attempt to produce a thin uniform coating of the resin surface. Three different grafting procedures have been examined and all products characterized in terms of their toluene and water imbibition. Materials showing promise as universal column packings for gel permeation chromatography have been synthesized on a larger scale and packed in tetrahydrofuran (THF) into standard columns. The plate count of each packed column has been evaluated, and the chromatographic performance of each assessed using polystyrene standards when the eluent was THF and polyethylene oxides when water and methanol were employed. The results are discussed in terms of some simple models describing the grafted resins.

INTRODUCTION

Chromatographic separations of macromolecular substances were reported as early as 1950 and were no doubt carried out long before this. The earliest work essentially using the procedures of modern gel permeation chromatography (GPC) was referred to as gel filtration[1] and utilized column packings such as dextran gels, with water or an aqueous buffer as the eluent. In general, separation times were long because the column packings were soft and compressible, and hence operating pressures were severely restricted. Indeed, much work was completed using a simple gravity feed.

Progress in the optimization of aqueous systems was slow after the attention of commercial interest was directed to organic systems by Moore in 1964.[2] His introduction of rigid macroporous crosslinked polystyrene resins allowed for the rapid analysis of synthetic organic soluble polymers.[3,4] Since then the technology of GPC has progressed steadily as successive advances in the polystyrene column packings have been made. The latest high resolution columns employ almost monodispersed 5-μm or 10-μm polystyrene particles which allows for even faster analysis.

Over the last few years more and more attention has been directed back towards aqueous systems[5–7] in order to bring GPC technology up to the level widely available in organic media. Much of this effort has been associated with column packings. Synthetic polymers such as polyacrylamide gels[8]

* Present address: Johnson Matthey, Blounts' Court, Sonning Common, Reading, England.

Journal of Applied Polymer Science, Vol. 29, 3013–3025 (1984)
 CCC 0021-8995/84/103013-13$04.00

have been used and provide good resolution, however, in their usual form they are soft when water swollen and analysis times are long. Silica and porous glass packings[9] have been used with some success. Their rigid structures are suitable for the application of high pressure, if necessary, and short analysis times can be achieved. Unfortunately, strong adsorption of biological materials is a common problem with these packings, and many surface treatments have been developed to try to overcome this.[10–12] Other approaches involve modification of the eluent by addition of appropriate solutes, e.g., amino acids[13] and surfactants,[14] and together with variations in pH and ionic strength these have been very rewarding in particular cases.[5] The manipulation of a multitude of operating variables is still, however, an undesirable feature, and, coupled with the tendency for silica based materials to dissolve in alkaline conditions, this has made these systems far from ideal for widespread and routine application.

A few semirigid hydrophilic synthetic gels have been produced exclusively for aqueous GPC and these do function reasonably satisfactorily.[5,15] Most recently, a rigid crosslinked polyacrylamide resin has been developed[16] with a small particle size, making it suitable for application in high performance GPC. Prepacked hydrophilic columns are now available from Polymer Laboratories, Showa Denko, and Toyo Soda.[5]

In terms of operating an efficient GPC chromatograph unit, perhaps the most useful and flexible system would be one capable of utilizing all common eluents from water through to hydrocarbons. Several column packings have been described which go at least part way towards this ideal. Crosslinked poly(acryloyl morpholine)s[17] and copolymers of 2-hydroxyl-ethyl methacrylate with ethylene dimethacrylate[18] fall into this category, and have shown some promise.

Polystyrene-based resins remain the most useful ones with organic eluents, and the technology for producing these high quality column packings is the most advanced, the most flexible, and the most reproducible.

Indeed these resins may provide the bases for a universal GPC column packing. This paper describes our efforts to chemically modify polystyrene resins of chromatographic quality and to assess the GPC performance of columns packed with the resultant products using both aqueous and organic eluents. To this end, polystyrene resins with significant residual double bond contents were to be grafted with a variety of polar monomers using a number of procedures aimed at varying the likely morphology of the grafted products.

EXPERIMENTAL AND RESULTS

Materials

Monomers. acrylamide (AA) (British Drug House); *N,N*-dimethyl acrylamide (NNDMA) (Polysciences, Ltd.); *N*-vinyl pyrrolidone (NVP) (Aldrich Ltd); hydroxyethlymethacrylate (HEMA) (Aldrich, Ltd.); and glycidyl methacrylate (GMA) (Aldrich, Ltd.) were used as supplied. The oligoethyleneoxide-derived monomers I, II, and III were prepared in the laboratory.

$$CH_2=C(CH_3)C(OCH_2CH_2)_xOR$$

(I) $x = \sim 7$, $R = CH_3$

(II) $x = \sim 16$, $R = CH_3$

(III) $x = \sim 9$, $R = -CO\cdot(CH_3)C=CH_2$

Preparation of Monomers. To prepare monomer I a round-bottomed flask was charged with a mixture of polyethylene glycol 350 monomethylether (175 g 0.5 mol) and pyridine (39.5 g, 0.5 mL). The mixture was cooled in an ice bath and ice cold methacryloyl chloride (62.7 g, 0.6 mol) in sodium dried toluene (100 mL) added slowly. After the initial vigorous reaction had subsided, the mixture was heated to 30°C for 30 min before being poured into ice-cold water (200 mL). The product was extracted with ether (2 × 100 mL) and then washed successively with aqueous sodium carbonate (30%, 100 mL), aqueous HCl (10%, 100 mL), and water (100 mL). The extracts were dried over anhydrous magnesium sulphate, and the ether was removed to leave a viscous pale yellow oil.

Monomer II was prepared similarly from polyethylene glycol 750 monomethylether and monomer III from polyethylene glycol E400. In the latter case the mole ratio of methacryloyl chloride to glycol was increased to 4/1.

Polystyrene Resins. These were prepared by Polymer Laboratories, Ltd., using standard suspension polymerization techniques.[19] The resins were fractionated by an air classification technique, and the fractions supplied were always in the particle diameter range 6–12 μm. The fully cured resin, E, had an exclusion limit of 10^4, Å (polystyrene in tetrahydrofuran) and the remaining species A–D and F were prepared using identical comonomer and diluent compositions, but polymerizations were prematurely terminated in order to produce products with significant residual double bond contents. The latter functional groups were to provide sites for grafting.

Determination of Double Bond Content of Resins

Residual double bond contents were estimated by reaction with excess iodine monochloride and back titration with sodium thiosulphate. Hubl's solution was used as the source of iodine monochloride. This solution was prepared by dissolving mercuric chloride (30 g) in ethanol (96%, 500 mL) followed by addition of iodine (25 g) in ethanol (96%, 500 mL). After 2 days the solution of iodine monochloride was ready for use.

A sample of polystyrene resin (0.15–0.4 g) was suspended in chloroform (10–15 mL) to which was added Hubl's solution (25 mL). The flask was tightly stoppered and left to stand in the dark for 24 h. A blank experiment containing no polymer was similarly prepared. Prior to titration, a solution of KI (10%, 10 mL) and water (100 mL) was added, and the mixture well shaken. The liberated iodine was then titrated in the usual way using 0.1*M* thiosulphate solution and starch as the indicator. The difference in titre

between the blank and resin experiment indicates the amount of halogen added to the double bonds. The results are summarized in Table I.

Grafting of Resins

Three different techniques were examined in grafting polar polymers onto the hydrophobic polystyrene resins. The first two employed thermal fragmentation of azobisisobutyronitrile AIBN in the presence of suitable monomers, while the third used ^{60}Co γ-ray irradiation to induce graft polymerization.

First Procedure. To a suspension of polystyrene resin (1 g) in acetone (20 mL) in a 50 mL baffled flask[19] was added a polar monomer (0.1 g) and AIBN (0.01 g). The resultant slurry was agitated for 30 min to ensure thorough dissolution of the polymerization components and permeation throughout the resin matrix. The acetone was then removed by gentle vacuum evaporation *at room temperature.* The flask containing the dry impregnated resin was then immersed in an oil bath at 100°C for 5 h. The resin was removed and washed successively with acetone, water, and acetone before being extracted with acetone for 6 hs in a Soxhlet apparatus. Initially, the product was vacuum dried at 60°C for 18 h.

Second Procedure. As described above, a polar monomer was impregnated into a resin using acetone as a carrier solvent. In this case, however, the initiator, AIBN, was added subsequent to the removal of the acetone, in the form of a solution in petroleum ether (5–15 mL, 120–140°C fraction). Grafting was then carried out as before, but in the presence of the petroleum ether as a precipitant for the growing grafted polar chains. Resins were then purified and dried as before.

Third Procedure. As before, a polar monomer was impregnated into the resin using acetone as a carrier solvent. After removal of the latter no initiator was added, but instead the resin was introduced into a glass vessel which after evacuation at 10^{-5} mm Hg was sealed off. The sealed tube was then exposed to ^{60}Co γ-ray irradiation, with a dose rate of 0.21 Mrad/h, for a given time (see Table IV). Each tube was then broken open, and the grafted resin purified and dried as before.

In all cases the products showed infrared absorption bands characteristic of the grafted species (e.g., $>C=O$) and elemental microanalyses also showed changes consistent with presence of grafts. No attempt was made,

TABLE I
Double Bond Content and Solvent Imbibition Data of Precursor Polystyrene Resins

Resin	Double bond content (mmol · g^{-1})	Solvent uptake	
		H_2O (g/g)	Toluene (g/g)
A	2.13	0	3.6
B	0.63	0	2.5
C	2.74	0	3.8
D	2.04	0	3.6
E	0	0	2.1
F	1.31	0	2.5

TABLE II
Solvent Imbibition Data for Resins Grafted Using AIBN as Initiator in the Absence of Solvent

Grafted resin	Original resin	Monomer used in grafting	Wt of monomer (g/g original resin)	Solvent uptake (g/g)	
				Toluene	Water
1	A	I	0.1	0.74	0.31
2[a]	A	I	0.2	0.50	0.10
3	A	II	0.15	0.87	0.05
4	A	III	0.1	0.88	0.25
5[a]	B	III	1.0	0.47	0.55
6[a]	A	III	0.1	0.42	0.10
7[a]	A	III	1.0	0.30	0.55
8	A	AA	0.1	1.33	0.24
9[a]	A	AA	1.0	0.24	0.90
10	A	NNDMA	1.0	1.81	0.26
11[a]	A	NNDMA	1.0	0.74	1.74
12	B	NNDMA	0.5	0.80	0.96
13	B	NNDMA	0.1	0.53	~0
14	B	NNDMA	0.25	0.76	0.15
15	A	NVP	1.0	1.19	0.97
16[a]	A	NVP	1.0	0.85	1.07
17	B	NVP	0.5	1.01	~0
18	B	NVP	0.1	1.56	~0
19	A	HEMA	1.0	1.65	0.46
20[a]	A	HEMA	1.0	0.46	1.34
21[a]	F	HEMA	1.0	1.58	2.16
22	B	GMA	0.5	0.82	0.87
23	B	GMA	0.1	0.32	0.05
24[a]	B	GMA	0.5	0.37	0.46

[a] Reaction scaled up from 1 g resin to 10 g. AA = acrylamide; NNDMA = *N,N*-dimethylacrylamide; NVP = *N*-vinylpyrrolidone; HEMA = hydroxyethyl methacrylate; GMA = glycidylmethacrylate.

however, to quantify the level of grafting. The ratio of polar monomer to resin employed was varied to some extent (see Tables II–IV) and in addition, materials showing optimistic characteristics were prepared again on a larger scale (10 g).

Solvent Imbibition Measurements

In order to assign priorities to the grafted resins and, in particular, to decide which to prepare in larger quantities, some simple criterion for suitability as a universal GPC packing was required. Clearly such materials should be significantly porous to all solvents and in particular to water on one extreme and to hydrocarbons on the other. As a result, the solvent imbibition of each sample was measured using both water and toluene. The procedure adopted was a simple centrifugation method employing small glass sinter sticks to hold the wetted sample. Details of this have been given before[20] and the results are summarized in Tables II–IV. Those materials showing good uptake of both solvents and superficial physical rigidity were prepared in larger quantities, and the behavior of these is also shown in the tables.

TABLE III
Solvent Imbibition Data for Resins Grafted Using AIBN as Initiator with Petroleum Ether Present as Graft Polymer Precipitant

Grafted resin	Original resin	Monomer used in grafting	Wt of monomer (g/g original resin)	Solvent uptake (g/g)	
				Toluene	Water
25	D	NNDMA	0.1	1.71	0.54
26	D		1.0	1.55	0.40
27	D		0.1	1.32	0.65
28	D		1.0	1.03	1.61
29[a]	D		1.0	0.89	4.67
30[a]	F		0.5	1.63	1.56
31	D	AA	0.1	1.69	0.32
32	D		1.0	0.96	3.89
33	D		0.1	1.29	0.38
34	D		1.0	0.65	6.89
35	D	NVP	0.1	1.63	0.53
36	D		1.0	1.46	0.56
37	D		0.1	1.50	0.54
38	D		1.0	0.97	2.36
39[a]	D		1.0	0.99	2.18
40	D	HEMA	0.1	2.69	0.59
41	D		1.0	1.21	0.30
42	D		0.1	1.26	0.30
43	D		1.0	0.87	1.14
44[a]	D		1.0	0.73	1.85
45[a]	F		0.5	0.94	1.66
46	D	GMA	0.1	2.20	0.37
47	D		1.0	1.68	0.16
48	D		0.1	1.38	0.08
49	D		1.0	0.50	0.35

[a] Reaction scaled up from 1 g resin to 10 g. AA, NVP, HEMA, GMA, NNDMA: see footnote to Table II.

GPC Column Packing Procedure

Each resin was stirred at ~700 rpm in a large excess of tetrahydrofuran THF for 24 h and then passed through a 50-μm sieve to remove any remaining aggregrates. After similar resuspension for 24 h, the bulk of the polymer was allowed to settle, and the fines were decanted with most of the suspending solvent. If necessary, suspension and decantation were repeated.

A slurry of the resin in fresh THF (~60 mL) was poured into the top of the stainless-steel packing bomb, (A in Fig. 1), and a further measure of solvent (~30 mL) sufficient just to fill the bomb was added. The bomb was connected to the packing pump B (MCP 71C, Haskel Energy Systems, Ltd., United Kingdom) via the in-line gate valve C. The packing solvent reservoir D was connected to the inlet of the pump, and the nitrogen pressure of the latter adjusted to ~40–45 psi. The hydraulic amplification factor was ~70 so that the packing solvent was delivered at ~3000 psi. The pump was activated and ~200 mL of solvent collected at the outlet end of the column, E (0.77 cm i.d. × 25 or 30 cm length). Finally, the column was detached from the bomb, and a small amount of packing added manually before the column end was fitted.

TABLE IV
Solvent Imbibition Data for Resins Grafted Using ^{60}Co γ-Ray Irradiation[a]

Grafted resin	Original resin	Monomer used in grafting	Exposure time (h)	Wt of monomer (g/g original resin)	Solvent uptake (g/g)	
					Toluene	H_2O
50	C	NNDMA	4	0.25	0.36	0.13
51	D		4	0.25	0.83	0.03
52	E		4	0.25	0.44	0.03
53	D		4	1.0	0.22	0.41
54	D		4	0.5	0.25	0.96
55[b]	D		24	1.0	1.35	0.52
56	D	AA	4	1.0	0.71	1.63
57	D		24	1.0	0.39	2.98
58	D	NVP	4	1.0	0.81	0.13
59	D		24	1.0	0.52	0.71
60[b]	D		24	1.0	0.97	1.88
61	D	HEMA	4	1.0	0.27	0.61
62	D		24	1.0	0.23	0.58
63[b]	D		24	1.0	0.75	0.46
64	D	GMA	4	1.0	0.22	0.56
65	D		4	0.5	0.45	0.03
66	D	III	4	1.0	0.60	0.44
67	D		24	1.0	0.59	0.80
68[b]	D		24	1.0	0.79	1.26

[a] 0.21 Mrad/h.

[b] Reaction scaled up from 1 g resin to 10 g. AA, NNDMA, NVP, HEMA, GMA: see footnote to Table II.

Column Calibration and Testing

Each packed column was calibrated and tested in a standard GPC system. The pump employed was an Altex 110 (Anachem, Ltd., United States). Samples were introduced via a high pressure injection using a 200-μL loop, and the detector was a differential refractometer (Quickfit Instrumentation, United Kingdom) connected to a Philips PM 8251 Chart Recorder. All elutions were performed at ambient temperature.

Columns initially packed in THF were assessed for plate count and total penetration volume using 1,2-dichlorobenzene (0.01 mL) in THF (10 mL) as the total permeation species. Where the column was repacked using water (resins 63 and 45), the plate count was reassessed using methanol. In each case the flow rate was 1 $mL \cdot min^{-1}$. The results are shown in Table V.

The chromatographic performance of each column was then tested using polystyrene standards (MWs 1380, 14,300, 42,200, 143,200, and 960,000) in THF. Typically, sample solutions of 5mg · mL^{-1} of THF were employed with an eluent flow rate of 1 $mL \cdot min^{-1}$ and a chart speed 1 $cm \cdot min^{-1}$. With water and methanol mobile phases polyethylene oxides (200, 600, 6000, and occasionally 14,000, 20,000, 100,000) were used as the standards, with the same experimental conditions. Elutions were carried out with THF, distilled water, 25% methanol in water and methanol. In changing from THF to water as the eluent a progressive change was employed, i.e., three intermediate solutions, THF, 75%; THF, 50%; THF, 25%, were pumped at 1 $mL \cdot min^{-1}$ through the column for 1 h each, before finally using pure water.

Where sufficient data was obtained, full calibration curves were con-

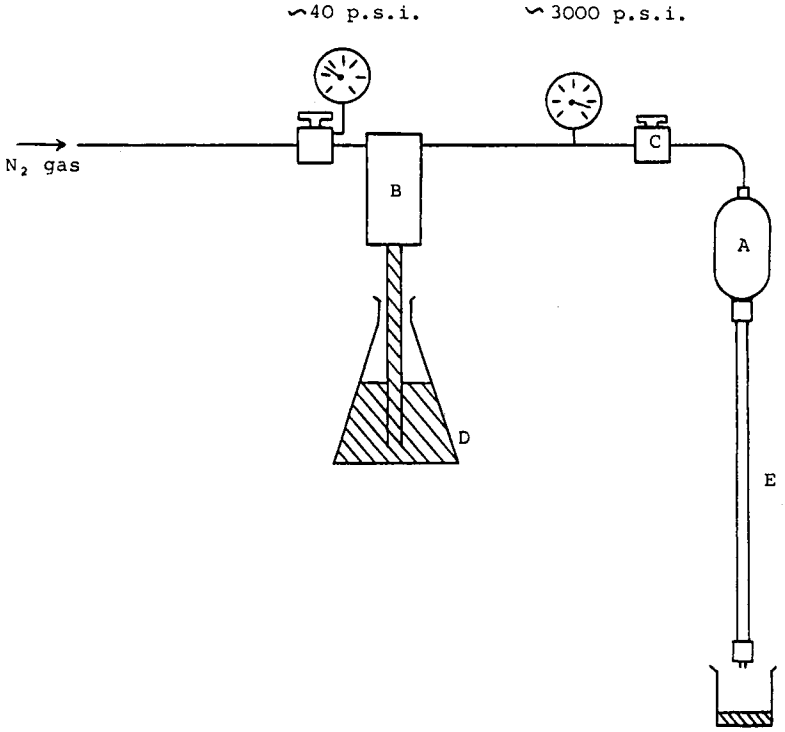

Fig. 1. Schematic representation of column packing apparatus: (A) steel packing bomb; (B) packing pump; (C) gate valve: (D) solvent reservoir; (E) GPC column.

TABLE V
Plate Count of Packed Columns[a]

Resin column packing	Monomer grafted	Plate count	Volume of total penetration (mL)
A	—	26,000	9.5
B	—	9,800	9.6
5	III	4,800	9.6
68	III	17,500	9.6
30	NNDMA	7,700	13.4
55	NNDMA	16,200	13.1
16	NVP	10,400	10.0
39	NVP	15,400	9.8
60	NVP	11,400	11.0
20	HEMA	2,800	10.1
21	HEMA	11,900	11.6
44	HEMA	5,100	15.0
45	HEMA	5,800	15.5
63	HEMA	1,000	7.4
45[b]	HEMA	5,900	13.0
63[b]	HEMA	700	11.0
24	GMA	2,500	8.3

[a] In tetrahydrofuran, with 1,2-dichlorobenzene as total permeation species. Flow rate-1 mL · min^{-1}.

[b] Repacked using water, with methanol as total permeation species. Flow rate-1 mL · min^{-1}.

structed. However, for simplicity, the results here are expressed in terms of the elution or retention ratios of the high (X) and low (Y) molecular weight standards, relative to the elution volume of the total permeation species appropriate to each eluent. Thus columns displaying simple GPC behavior for all standards would give rise to $X < Y$ and $Y < 1$. $X > 1$ and $Y > 1$ indicates an additional adsorption process. Where strong adsorption resulted samples were apparently not eluted at all, and in this case no entry appears in the summary (Table VI).

DISCUSSION

Level of Grafting

Resins A–D and F all showed significant residual double bond contents (Table I), and all of these appeared to be readily grafted using all three of the alternative procedures. The significant increase in water uptake relative to the precursor resins shows this clearly (Table II–IV) and only when monomer/resin ratios of $\sim$ 0.1/1 were employed was the water imbibition consistently lower. In general, water imbibition rises with increase in the monomer/resin grafting ratio. Although resin B had the lowest double bond content, it, nevertheless, appeared to graft as well as the others using this criterion, and this may indicate that only a small proportion of the double bonds are used as grafting sites. Significant differences from monomer to monomer did not arise as far as water uptake is concerned, except with AA, and occasionally NNDMA. For example, resins 29, 32, 34, and 57 showed imbibition $\geq$ 3.0 $mL \cdot g^{-1}$, and, perhaps significantly, HEMA, widely used in hydrogel applications, did not give rise to such excesses. Monomers I–III in contrast were disappointing. Only resin 68 was felt worthy to be examined in more detail. This was surprising since a Japanese patent[21] had suggested considerable potential, and yet, in our hands, resins grafted with these monomers even proved difficult to wet properly with water.

No consistently clear distinction arises with the different procedures for grafting. Considering a given monomer applied in a given monomer/resin ratio shows that in some cases one procedure gives a product with the highest water uptake, while in others a different procedure is more effective. Exact comparisons are difficult because often different precursor resins were employed. In addition changing the scale of the grafting reaction also gave rise to different behavior.

The results in Tables II–IV do not convey any information about the superficial mechanical properties of the grafted species, and here notable differences arise with the different monomers employed. AA, in the main, gave soft materials, likely to become compressed under pressure. The most rigid were those materials derived from NVP and GMA, with the species from HEMA and NNDMA falling somewhere in between. These observations were used along with the water imbibition data in selecting resins for scaleup and column packing.

GPC Behavior

The only real test of these materials as column packings is in their GPC behavior. In considering the results summarized in Tables V and VI it is worthwhile bearing in mind some simple models depicting the grafted res-

TABLE VI
Column Performance—Retention Ratios[a] of High (X) and Low (Y) Molecular Weight Standards Using Various Eluents

Grafted resin	Monomer grafted	THF		Water		25% CH_3OH/Water		CH_3OH		Comments[c]	
		X	Y	X	Y	X	Y	X	Y	THF	Other eluents
A	—	0.43	0.93	—	—	—	—	—	—	GPC	—
D	—	0.45	0.86	—	—	—	—	—	—	GPC	—
5	III	0.55	0.78	—	1.72	—	0.93	—	—	GPC	ADS
68	III	0.52	0.71	—	1.03	—	1.02	—	0.99	GPC	ADS
9	AA	—	—	—	—	—	—	—	—	BP	BP
11	NNDMA	—	—	—	—	—	—	—	—	BP	BP
29	NNDMA	—	—	—	—	—	—	—	—	BP	BP
30	NNDMA	0.45	0.78	—	0.70	—	0.66	0.57	0.62	GPC	ADS[d]
55	NNDMA	0.48	0.73	—	—	—	—	—	0.90	GPC	ADS
16	NVP	0.52	0.84	—	1.51	1.34	0.98	—	—	GPC	ADS
39	NVP	0.47	0.79	—	—	—	—	—	0.95	GPC	ADS
60	NVP	0.52	0.76	—	1.38	—	0.88	—	1.30	GPC	ADS
20	HEMA	0.53	0.81	—	—	—	—	—	—	GPC	ADS
21	HEMA	0.44	0.84	—	—	—	—	—	—	GPC	ADS
44	HEMA	0.53	0.80	—	—	—	—	—	—	GPC	ADS
45	HEMA	0.45	0.89	—	1.53	—	1.22	0.83	0.90	GPC	ADS[d]
63	HEMA	0.95	0.97	0.75	0.97	0.77	0.97	0.79	0.89	NR	GPC
21[b]	HEMA	—	—	—	—	—	—	—	—	BP	BP
44[b]	HEMA	0.51	0.79	—	0.92	—	—	—	—	GPC	ADS
63[b]	HEMA	0.93	0.85	0.51	0.67	0.62	0.81	0.60	0.81	NR	GPC
24	GMA	0.89	0.93	—	1.11	—	0.70	1.85	0.75	GPC	ADS

[a] Retention volumes of a high and low MW standards relative to appropriate volume of total penetration (standards: 960,000 and 1380 polystyrenes for THF eluent, and 6000 and 200 polyethyelene oxides for other eluents).

[b] Repacked under aqueous conditions.

[c] GPC = column exhibits simple gel permeation chromatographic behavior. ADS = standards substantially adsorbed onto stationary phase—either not eluted or X and $Y > 1$. BP = very high back pressures at low flow rate (> 3000 psi at 0.1 mL · min^{-1})—due to swollen packing. NR = no resolution.

[d] GPC behavior with CH_3OH eluent.

ins. Figure 2 shows schematically the possible effects of grafting a polar polymer onto an essentially hydrophobic macroreticular polystyrene resins bead, A. In case B an ideal thin surface coverage of the resin is achieved. With C the distribution of polar monomer has been nonuniform, resulting in large aggregates of grafted polymer irregularly distributed in the resin pores. D represents some intermediate distribution in which some of the original polystyrene hydrophobic surface is exposed, but with large areas substantially coated. As far as the behavior of these materials as GPC packings is concerned, the following predictions can be made with regard to the total exclusion volume V_0 and the total penetration volume V_t. (*Note*: V_0 relates to X, and V_t to Y in Table VI). With B, where the coating is uniform and thin, relatively small shifts in V_0 and V_t would be expected, hydrophobic adsorption effects would be minimal, and this indeed is the situation sought. With C significant pore volume is lost, and the entire elution profile would therefore narrow; V_t certainly would decrease, and V_0 may also decrease a little. In addition with substantial polystyrene surface remaining accessible, hydrophobic adsorption would readily occur with susceptible samples, in which case elution volumes would become larger than the original V_t, and adsorption chromatographic separation would predominate over a size exclusion mechanism. With D, again similar though less dramatic changes would be expected.

Table VI summarizes the experimental behavior of the various column packed materials. With THF as the eluent and polystyrene standards, X in general increased to some extent and Y decreased. Hence the elution profile narrows a little, and there was some loss of resolving power. The

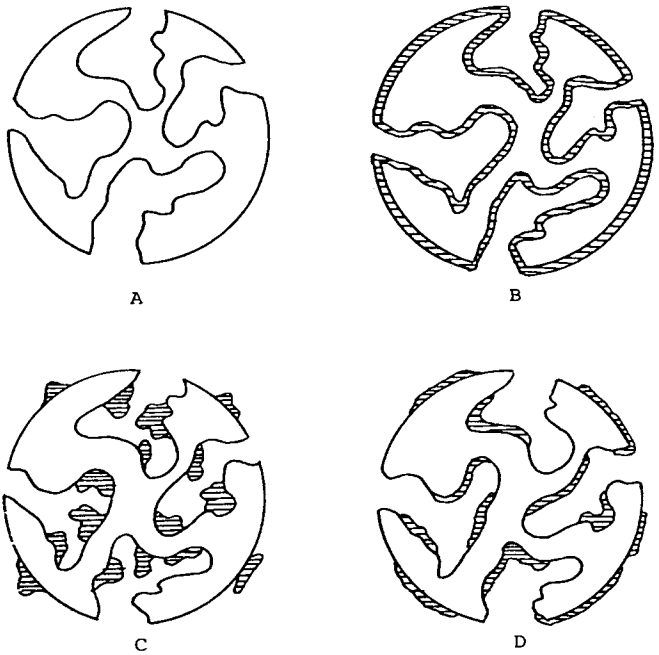

Fig. 2. Schematic representation of possible grafted macroreticular polystyrene (PS) resins: (A) uncoated PS particle; (B) ideal thinly coated PS particle; (C) aggregated and poorly distributed coating; (D) coating with some intermediate distribution.

effect, however, was not large and most of the packed materials retained their chromatographic properties under these conditions.

No consistent difference emerged with the method of grafting, and all grafted monomers behaved similarly except for AA. Resins 9, 11, 29, and 21 gave rise to excessive back pressures (BP), and no elution data are available. Resin 63, and to some extent 24, showed virtually no ability to separate the polystyrenes (NR), and, indeed, these columns displayed the lowest plate counts (Table V).

On changing to water as the eluent, polyethylene glycols were used as standards. These present a significant test for the systems because they are known to adsorb strongly to many surfaces, with the effect increasing with increasing molecular weight.[22,23] All of the materials except resin 63 exhibited strong adsorption, with X indicating no elution at all and Y emerging > 1. In general, there was little difference from monomer to monomer and from one grafting procedure to the next. With resins 30 and 45 prepared by grafting in the presence of a polymer precipitant, a size exclusion mechanism was established again (GPC) when methanol was used as the eluent.

Resin 63 was the only species which displayed GPC behavior in water, and readily chromatographed polyethylene oxides with molecular weights 200–14,000 by a simple size exclusion process. Furthermore, when this material, originally packed in THF, was repacked using water, similar behavior resulted. With methanol and aqueous methanol as eluents again simple GPC resulted. This species was prepared by ^{60}Co γ-ray irradiation grafting and certainly provides a basis for more detailed examination. Unfortunately, at the moment the material yields a column with a low plate count and no significant resolving power in organic systems. Further modification is required before a universal description can be given.

The original aim of grafting polar polymers onto polystyrene resins was to achieve a thin uniform coating [Fig. 2(B)]. It was felt that the first procedure was unlikely to achieve this, and indeed in the case of water as the eluent all the indications are that a situation closer to C or D has been produced. It was hoped, with the modification of the procedure described by Schutyser et al.[24], that the presence of the polymer precipitant might encourage the grafted species to lay down and spread more effectively. Again there is no consistent evidence that this is the case. In using ^{60}Co γ-ray irradiation it was hoped that a more effective coverage might be achieved as a result of a more random initiation of graft polymerization, involving grafting *from* the resin surface, as well as grafting *onto* and *through* surface groups. With HEMA there is some evidence that this has been achieved, but further optimization of the resulting packings is required.

We are grateful for the receipt of a CASE studentship for J. G. H. from the SERC in collaboration with Polymer Laboratories, Ltd., Church Stretton, United Kingdom. We also acknowledge the help of Mr. J. Izatt, Scottish Universities Research and Reactor Centre, National Engineering Laboratory, Fast Kilbride, United Kingdom.

References

1. J. Porath and P. Flodin, *Nature*, **183**, 1657 (1959).
2. J. C. Moore, *J. Polymer Sci., Part A*, **2**, 835 (1964).
3. J. C. Moore, *Chem. Eng. News*, (Oct), 4 (1965).

4. M. F. Vaughan, *Chem. Eng. News*, (May), 6 (1966).
5. H. G. Barth, *J. Chromatogr. Sci.*, **18**, 409 (1980).
6. A. R. Cooper and D. P. Matzinger, *Am. Lab.*, (Jan), 13 (1977).
7. A. R. Cooper and D. S. Van Derveer, *J. Liq. Chromatogr.*, **1**, 693 (1978).
8. N. Sabbagh and I. Fagerson, *J. Chromatogr.*, **11**, 83 (1976).
9. R. E. Majors, *J. Chromatogr. Sci.*, **15**, 334 (1977).
10. E. Almof and M. Larson-Razinikiewicz, *J. Appl. Polym. Sci.*, **19**, 2933 (1975).
11. S. H. Chang, K. M. Gooding, and F. E. Regmer, *J. Chromatogr.*, **125**, 103 (1976).
12. R. V. Vioukecchia, B. G. Lightbody, N. A. Thimot, and H. M. Quinn, *J. Chromatogr. Sci.*, **15**, 424 (1977).
13. T. Mizutani and A. Mizutani, *J. Chromatogr.*, **111**, 214 (1975).
14. W. Haller, *J. Chromatogr.*, **85**, 129 (1973).
15. A. R. Cooper and D. S. Van Derveer, *J. Liq. Chromatogr.*, **5**, 1 (1978).
16. J. V. Dawkins and N. P. Gabbott, *Polymer*, **22**, 291 (1981).
17. R. Epton, C. Holloway, and J. V. McLaren, *J. Appl. Polym. Sci.*, **18**, 179 (1974).
18. J. Coupek, M. Krivakova, and S. Pokoring, *J. Polym. Sci. Symp.*, **42**, 185 (1973).
19. See Appendix in *Polymer-Supported Reactions in Organic Synthesis*, P. Hodge and D. C. Sherrington, Eds., Wiley, Chichester, 1980, p. 469.
20. J. A. Greig and D. C. Sherrington, *Polymer*, **19**, 163 (1978).
21. F. Kamiyama and M. Kiramatsu, Jpn. Kokai 77,150,093 (1977).
22. A. V. Kiselev, N. V. Kovaleva, and V. Khopina, *Polym. Sci. USSR*, **16**, 1321 (1974).
23. E. Killman, *Polymer*, **17**, 864 (1976).
24. J. Schutyser, T. Buser, D. Van Aden, H. Thomas, F. Van Houdenhoven, and G. Van Declem, in *Affinity Chromatography and Related Techniques*, J. C. J. Gribnau, J. Vissen, and R. J. F. Nivard, Eds., North-Holland, Amsterdam, 1982, Chap. 2, p. 143.

Received June 15, 1983
Accepted February 2, 1984