Table 2 Thermal and radii of gyration measurements on samples of polystyrene pressured at 6 kbars and 250°C for 24 h measurements at 23°C

Deutero polystyrene matrix molecules		Protopolystyrene tagged molecules				Thermal measurements		
M_w	Dispersity	M_w	R_z (Å) at G	R_z (Å) after heating to endotherm temp.	R_z (Å) after heating above T_g	Temperature of endotherm	Endotherm (cal/g)	T_g
2800	1.09	2800	10.7	–	12.8	53	0.94	53
29 200	1.23	30 900	45.4	48.0	47.0	73	0.68	95.5
158 000	1.47	214 600	120	158	154	80	0.49	99.0

The reduction in radius of gyration of the polymer molecule on compression probably arises from the rotation of the phenyl side groups to achieve closer packing in the melt. This could result from the apparent shortening of the chain due to the coil becoming partly helical or due to the molecule occupying space closer to the centre of mass. On cooling below T_g this structure becomes frozen and persists even when the pressure is removed and the bulk material expands to give nearly the same density as the normal polystyrene. Since the macromolecules do not change their conformation this fall in density must be due to the formation of voids of molecular dimensions between chains.

The fact that the chain assumes normal conformation on heating to temperatures well below the glass transition temperature means that the molecule has freedom to move at these temperatures. Also no density change of any significance is involved implying that chains can slip by one another. The mobility at 20°C below the literature values of T_g however is of low order and not comparable in magnitude with the molecular motions occurring above this transition. Our measurements of the values of the heat absorbed agree reasonably well with those of Brown *et al.*[4].

Finally, it should be noted that since equations (1) and (2) apply at low molecular weights, this is reasonably good evidence that stems greater than 40 Å are absent from the structure. It has recently been shown[5] that in crystalline polymers where stems greater than 120 Å are known to be present, at low molecular weights $R_w \propto L_n$, (L_n is the dominant stem length) and is independent of molecular weight in this region.

References

1 Ballard, D. G. H., Schelten, J. and Wignall, G. D. *Eur. Polym. J.* 1973, **9**, 965
2 Wignall, G. D., Ballard, D. G. H. and Schelten, J. *Eur. Polym. J.* 1974, **10**, 861
3 Breuer, H. and Rehage, G. *Kolloid Z. Z. Polym.* 1967, **216**, 159
4 Brown, I. G., Wetton, R. E., Richardson, M. J. and Savill, N. G. *Polymer* 1978, **19**, 659
5 Ballard, D. G. H., Schelten, J., Longman, G. W., Crowley, T. L. and Cunningham, A. *Polymer* 2979, **20**, 402

Phenolic poly(acryloyl morpholine)-based bead matrix for solid (gel) phase peptide synthesis

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The classical Merrifield method of polymer-supported peptide synthesis has been developed mainly using supports based on crosslinked polystyrene. Such matrices undergo gelation (constrained dissolution) most readily in non-polar solvents whereas attached peptide chains are solvated best by solvents of very high polarity. Thus, the non-polar solvents which expand the matrix may cause collapse of the peptide chains and *vice versa*. It has been suggested that steric interactions, arising from this effect, are a major cause of incomplete reaction during solid (gel) phase synthesis[1].

Efforts have been made to devise alternatives to polystyrene-based supports[2–4]. Atherton *et al.*[5] have described the synthesis of a poly(*N,N*-dimethylacrylamide)-based resin which enables all protected amino-acid coupling reactions and all polymer-supported peptide *N*-terminal deprotection steps to be carried out in highly polar organic media. The chains of the copolymer matrix and of the attached peptide are solvated fully throughout. In solid (gel) phase synthesis, the resin gave results much superior to those obtained using polystyrene-based supports.

In our laboratory, we have synthesized a number of speciality copolymer networks incorporating acryloyl morpholine as the predominant monomer[6,7]. Such copolymers incorporate many exposed ether linkages and undergo gelation readily in all the polar solvents used commonly in peptide synthesis. For some time, we and others[8] have appreciated the potential of poly(acryloyl morpholine)-based matrices as supports for solid (gel) phase peptide synthesis. Our materials also bear a clear structural similarity to the poly(*N,N*-dimethylacrylamide)-based support. We describe

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here the synthesis of a bead-form, phenolic, poly(acryloyl morpholine)-based, matrix (*Figure 1*) and its preliminary application in solid (gel) phase peptide synthesis.

A major problem in producing new supports for solid (gel) phase peptide synthesis is to obtain the required copolymer in bead form. This is because the necessary diversity of polar and non-polar features in the monomers to be copolymerized causes problems in devising a suitable suspension polymerization system. An appropriate size distribution of dispersed droplets must be obtained which must not undergo irreversible agglomeration at the gel point. After numerous empirical trials we adapted a method[9] in which the desired combination of monomers was copolymerized while maintained 'salted-in' an organic phase dispersed in saturated brine. This approach proved to be much more reliable than the reverse procedure, previously used extensively by us for the preparation of acryloyl morpholine copolymers, in which the monomers are polymerized while dissolved in a dispersed aqueous phase[10].

The experimental procedure was as follows. Freshly distilled acryloyl morpholine (7.05 g, 0.05 mol), *N*-[2-(4-acetoxyphenyl)ethyl]-acrylamide (2.33 g, 0.01 mol), *N,N'*-diacryloyl piperazine (0.49 g, 0.0025 mol) and α,α'-azobisisobutyronitrile (0.75 g) were dissolved in 1,1,2,2-tetrachloroethane (50 cm^3)*. After displacement of dissolved oxygen by bubbling with tetrachloroethane-saturated nitrogen, the solution was dispersed, with mechanical stirring, in an oxygen-free continuous aqueous phase consisting of a sodium chloride-saturated mixture of distilled water (150 cm^3), aqueous 0.16% hydroxypropyl cellulose solution (6.6 cm^3) and aqueous 0.2% xanthan gum solution (33.3 cm^3). Stirring was carefully regulated to give a droplet size distribution of 50–250 μm. Polymerization was initiated by raising the temperature to 70°C. After 4 h, the temperature was allowed to fall to 50°C and stirring continued at this temperature overnight. After decanting off most of the brine, the copolymer gel was washed with distilled water and then with ethanol. Equilibration with ether and drying under reduced pressure gave the copolymer in the form of discrete beads (8.1 g, 82%). Dry beads of 45–211 μm (6.4 g, 65%) were obtained by dry sieving and used subsequently for solid (gel) phase peptide synthesis.

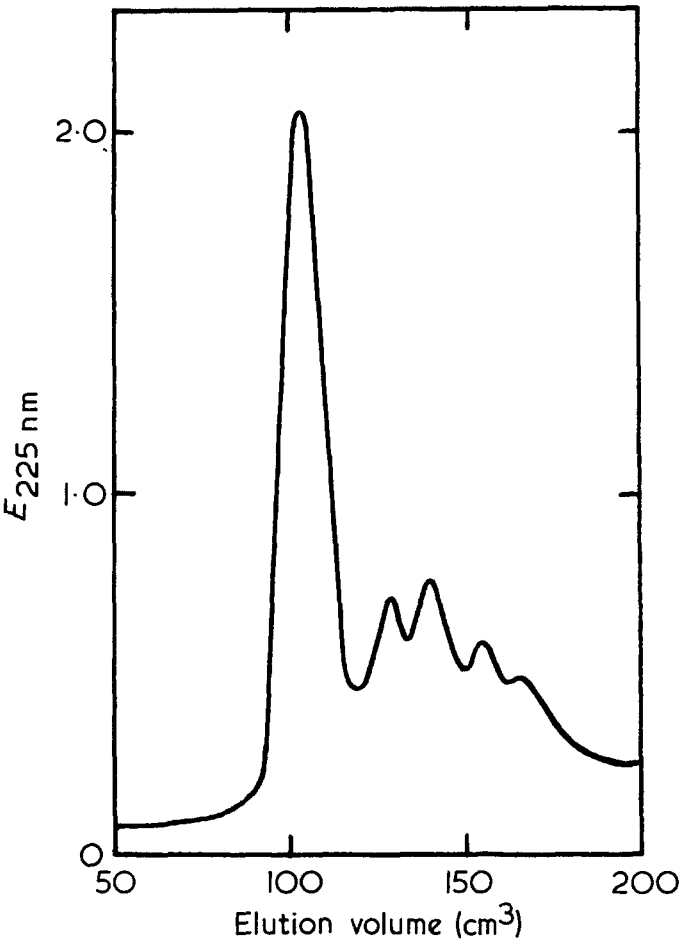

Figure 2 Gel permeation chromatography of crude Boc-Leu-Ala-Gly-Val-OH. The crude peptide (20 mg) in MeOH (1 cm^3) was applied to a Sephadex LH20 column (36 x 2.5 cm) packed in MeOH and eluted at a flow rate of 75 $cm^3\ h^{-1}$

* Attention is drawn to the relatively high toxicity of this solvent. The less toxic pentachloroethane may be used as a substitute but is less satisfactory. No other common halocarbons were found to give stable suspensions at the gel point

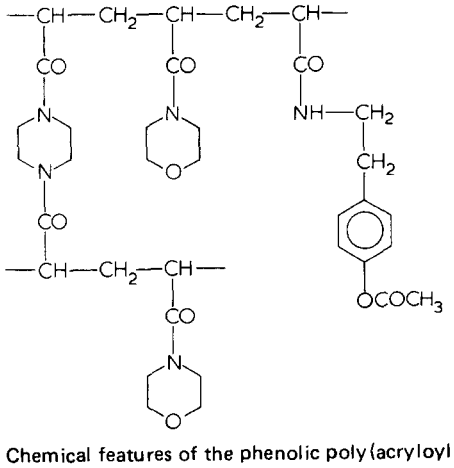

Figure 1 Chemical features of the phenolic poly(acryloyl morpholine)-based matrix in acetate form

The copolymer beads swelled readily in such polar solvents as $(CH_3)_2NCHO$ (12.8 $cm^3\ g^{-1}$), $(CH_3)_2NCOCH_3$ (12.1 $cm^3\ g^{-1}$) CH_2Cl_2 (9.3 $cm^3\ g^{-1}$), $C_6H_5CH_2OH$ (9.7 $cm^3\ g^{-1}$) and CH_3CO_2H (20.6 $cm^3\ g^{-1}$).

The comonomer *N*-[2-(4-acetoxyphenyl)ethyl]-acrylamide was selected so as to incorporate into the copolymer acetylated phenolic residues. These could later be deprotected to expose sites functionally active in binding *N*-protected amino acids *via* a labile phenyl ester linkage. Other workers have applied this strategy in the case of polystyrene-based peptide resins[11,12].

The efficacy of the bead copolymer as a solid (gel) phase peptide matrix was demonstrated by preparation of the Merrifield-Dorman standard test sequence, H–Leu–Ala–Gly–Val–OH[13,14]. The synthesis was performed in a nitrogen-stirred glass reactor similar to that described by Corley *et al.*[15]. Deacetylation of the copolymer was effected by swelling in morpholine and allowing reaction to occur for 2 h at 25°C. The Boc derivative of valine was then coupled to the matrix by the action and of DCC and a catalytic amount of 4-dimethylaminopyridine in $(CH_3)_2NCHO$ over 4 h. A five

Abbreviations: Boc, t-butyloxycarbonyl-; DCC, *N,N'*-dicyclohexylcarbodiimide

fold excess of the Boc amino-acid of DCC was employed. The matrix was then subjected to the following washing procedure (Sequence A): (1) $(CH_3)_2NCHO$, 3 x 5 min; (2) $(C_2H_5)_2O$, 2 x 5 min; (3) CH_2Cl_2, 3 x 10 min; (4) $(CH_3)_2NCHO$, 3 x 10 min; (5) $(C_2H_5)_2O$, 3 x 10 min. Blocking of residual phenolic groups was ensured by two cycles each consisting of treatment with $(CH_3CO)_2O/(C_2H_5)_3N$ in $(CH_3)_2NCHO$ for 1.5 h and washing according to Sequence A. At this stage, the valine content of the polymer was found to be 1.01 mmol g^{-1} (97% theoretical). The pattern of subsequent coupling cycles was as follows. Removal of Boc groups was effected by swelling the matrix in 1.75 M HCl in $C_6H_5CH_2OH$, standing for 5 min, equilibrating with fresh 1.75 M HCl in $C_6H_5CH_2OH$ and standing for a further 15 min. The matrix was then subjected to the following washing procedure (Sequence B): (1) $C_6H_5CH_2OH$, 2 x 10 min; (2) $(C_2H_5)_2O$, 2 x 5 min; (3) $C_6H_5CH_2OH$, 3 x 10 min; (4) $(C_2H_5)_2O$, 3 x 5 min; (5) $(CH_3)_2NCHO$, 2 x 5 min; (6) $(C_2H_5)_2O$, 2 x 5 min. Chain elongation was then effected by treatment, over 1.5 h, with a 5-fold excess of Boc amino acid and DCC in CH_2Cl_2 containing *N*-methylmorpholine followed by washing according to Sequence A.

On completion of the synthesis, the Boc-tetrapeptide (polymer-bound amino-acid ratio found: Val, 1.00; Leu, 1.00; Ala, 1.00, Gly, 1.06) was cleaved from the matrix by treatment with a mixture of 100 vol H_2O_2/dioxane (3/17) while maintaining the pH at 10.5 by titration with aqueous 0.01 M NaOH. Purification was effected by gel permeation chromatography on Sephadex LH20 using CH_3OH as eluent (*Figure 2*). Evaporation of the fractions comprising the main peak gave material which, on TLC on Merck Kieselgel 60 F_{254}, using $CH_3CO_2C_2H_5/C_5H_5N/CH_3CO_2H/H_2O$ (30/20/6/11) as eluent, followed by heat and ninhydrin spraying, gave a single spot, $R_f = 0.69$. Removal of the Boc group by treatment with 80% CF_3CO_2H in H_2O gave the Merrifield–Dorman tetrapeptide (amino-acid ratio found: Val, 1.00; Leu, 0.97; Ala, 0.89; gly, 1.09). On chromatography on Whatman No 1 paper, this gave, using C_3H_7OH/H_2O (2/1) as eluent, an $R_f = 0.71$ (literature[13], $R_f = 0.71$), and using $C_2H_5(CH_3)COH$/aq NH_4OH (3%) (5/2), as eluent, an $R_f = 0.49$ (literature[13], $R_f = 0.49$). From 96 mg of starting copolymer the yield of purified Boc-tetrapeptide was 33 mg (75% theoretical) and deprotected tetrapeptide 24 mg (65% theoretical).

The following considerations are pertinent to the use of the new phenolic, poly(acryloyl morpholine)-based copolymer as a matrix for solid (gel) phase peptide synthesis.

(1) On contact with polar solvents, the dry copolymer beads rapidly undergo gelation (constrained dissolution). On equilibration of the gel beads with a less polar solvent, such as ether, catastrophic shrinkage (reprecipitation of the copolymer) occurs. This gelation/precipitation cycle involves the phase transitions solid/solution/solid for the copolymer chains within the microenvironment of each discrete bead. Hancock *et al.*[16] have previously commented on the importance of this effect in expediting solid (gel) phase peptide synthesis. The effect may be exploited readily in the case of the new copolymer matrix.

(2) The similar solvent solubility profiles of the copolymer chains within the matrix and the attached peptide molecules permits high levels of matrix substitution when desired.

(3) Peptides and peptide fragments may be detached from the matrix in fully protected form. In addition to H_2O_2-catalysed cleavage at pH 10.5, we have shown that the method of autocatalytic cleavage with 2-dimethylaminoethanol followed by autocatalysed hydrolysis of the liberated peptide ester[17] is effective, except in the case of hindered amino-acid–resin linkages. Another cleavage possibility would be to plan a given peptide synthesis so that cleavage from the matrix is effected by reaction with a free amino group of an amino-acid which is inserted at the *C-terminal* of the peptide chain.

Work is in progress to evaluate further the above possibilities and to extend our work to the synthesis of peptide sequences of commercial importance; particularly those which are not amenable to synthesis on polystyrene-based supports. We are also investigating poly(acryloyl morpholine)-based supports with alternative functional groups as points for amino-acid attachments.

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References

1 Sheppard, R. C. 'Proceedings of the 11th European Peptide Symposium', Vienna, 1971, North Holland, Amsterdam, 1973, p 111
2 Atherton, E. and Sheppard, R. C. in 'Peptides 1974' (Ed. Y. Wolman), Wiley, New York, 1975, 123
3 Merrifield, R. B. in 'The Proteins, Vol. 2' (Ed. H. Neurath and R. L. Hill), Academic Press, New York, 1976, 278
4 Bayer, E., Jung, G., Halász, I. and Sebestian, I. *Tetrahedron Lett.* 1970, 4503
5 Atherton, E., Clive, D. L. J. and Sheppard, R. C. *J. Am. Chem. Soc.* 1975, **97**, 6584
6 Brough, A. W. J., Epton, R., Marr, G., Shackley, A. T. and Sniezko-Blocki, G. A. in 'Chromatography of Synthetic and Biological Polymers' (Ed. R. Epton), Ellis Horwood, Chichester, 1978, Ch 4; author's papers and patents cited therein
7 Epton, R., Hobson, M. E. and Marr, G. *Polymer* 1977, **18**, 1203
8 Ferruti, P. in 'Reactions on Polymers' (Ed. J. A. Moore), Reidel, Dordrecht, 1973, p 427
9 Hamann, H. C. and Stellwagen Jr, W. US Pat. 3 862 924 (1975)
10 Epton, R., Holding, S. R. and McLaren, J. V. *Polymer* 1976, **17**, 843
11 Arshady, R., Kenner, G. W. and Ledwith, A. *J. Polym. Sci.* 1974, **12**, 2017
12 Hudson, D., Kenner, G. W., Sharpe, R. and Szelke, M. *Int. J. Peptide Protein Res.* 1979, **14**, 177
13 Merrifield, R. B. *J. Am. Chem. Soc.* 1963, **85**, 2149
14 Dorman, L. C., Markley, L. D. and Mapes, D. A. *Anal. Biochem.* 1971, **39**, 429
15 Corley, L., Sachs, D. H. and Anfingsen, C. B., *Biochem. Biophys. Res. Commun.* 1972, **47**, 1353
16 Hancock, W. S., Prescott, D. J., Nalty, W. L., Weintraub, J., Vagelos, P. R. and Marshall, G. R. *J. Am. Chem. Soc.* 1971, **93**, 1799
17 Barton, M. A. ,Lemieux, R. V. and Savoie, J. Y. *J. Am. Chem. Soc.* 1973, **95**, 4501