Block copolymerization of γ-benzyl-*N*-carboxy-L-glutamate anhydride initiated by polystyrene having two terminal amino groups, fractionation and characterization of the block copolymers

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Polystyrene having terminal amino groups was synthesized by radical polymerization of styrene in the presence of a bis amino-azonitiator at 80°C. The polymeric initiators were used to synthesize block copolymers of ABA structure, containing a carbon chain and polypeptide blocks; by ring-opening polymerization of the *N*-carboxy anhydride (NCA) of γ-benzyl-L-glutamate. Fourier transform infra-red spectroscopy (*FT*i.r.) was used for the characterization of the block copolymers.

(Keywords: ABA block copolymers; poly(γ-benzyl-L-glutamate); polystyrene; fractionation)

INTRODUCTION

Block copolymers having well-characterized polypeptide and polyvinyl segments have been described in recent years by several authors. Douy and Gallot[1] employed living anionic polymerization to introduce a terminal amino group into a vinyl polymer, and used the prepolymer for the initiation of the polymerization of α-amino acid *N*-carboxy anhydride (NCA). Vlasov *et al.*[2,3] prepared amino-terminated polymeric initiators by radical polymerization of vinyl monomers with bifunctional initiators. These block copolymers can be used as polymer drug carriers[4] and as models for the study of the properties of membranes[5].

In the present investigation, we carried out the radical polymerization of styrene to produce polystyrene bearing two amino end groups. Block copolymers of γ-benzyl-L-glutamate NCA were synthesized, and the fractionation and characterization is described.

EXPERIMENTAL

Reagents

Thionyl chloride was purified by distillation. *N*,*N*-dimethylformamide was dehydrated with molecular sieves (4 Å) followed by dehydration with P_2O_5 and distilled under reduced pressure.

Monomers

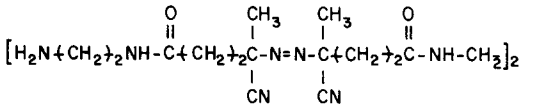
Commercial styrene was washed with alkaline solution, dried with $CaCl_2$ and distilled under reduced pressure.

γ-Benzyl-L-glutamate NCA was synthesized by suspending γ-benzyl-L-glutamate in dioxane and bubbling phosgene through the solution at 60–65°C. The crude product was recrystallized three times from ethyl acetate/cyclohexane and stored at −30°C[6].

Initiator preparation

4,4′-Azobis(4-cyanovaleric acid) was suspended in dry diethyl ether and stirred with an excess of thionyl chloride at 0°C for 24 h. The purified acid chloride was then dissolved in methylene chloride and added at 0°C to a stirred solution of a 20-fold excess of ethylene diamine in CH_2Cl_2. After 72 h the mixture was filtered and the pure initiator was obtained by repeated precipitation from a CH_2Cl_2 solution into cold diethyl ether and subsequently dried and stored at −20°C.

Titration and ^1H and ^{13}C n.m.r. analysis were used to show that the structure corresponded to a 2:3 ratio of acid and amine components. The structure can be represented by the following formula:



Synthesis of the block copolymers

Polymerization of styrene. Styrene was polymerized in *N*,*N*-dimethylformamide in the presence of the appropriate weight% of initiator at 80°C. The resulting polymer was purified by precipitating it twice in methanol.

Synthesis of block copolymers. The polymerization of γ-benzyl-L-glutamate NCA (A) with the polymeric initiator (I) was carried out in *N*,*N*-dimethylformamide at room temperature during 66 h in a mole ratio of A/I = 230. The total concentration of A and I in the solution undergoing polymerization was 5%. The block copolymer was

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Table 1 Results of the radical polymerization of styrene

Sample number[a]	Polymerization time (h)	$\bar{M}_n$ (Titration)	$\bar{M}_n$ (g.p.c.)	$\bar{M}_w/\bar{M}_n$ (g.p.c.)
PS-1	9	11800	11000[b]	1.61[b]
PS-2	9	11800	9000[c]	1.85[c]
PS-3	9	16600	16700[b]	1.82[b]
PS-4	16	25600	18800[c]	2.31[c]
PS-5	16	25600	12700[d]	2.86[d]
PS-6	9	32000	28000[b]	2.03[b]
PS-7	9	22500	18600[b]	1.86[b]

[a] PS = polystyrene
[b] G.p.c. of aminated PS modified with acetic anhydride at 85°C
[c] G.p.c. of aminated PS modified with acetic anhydride at 25°C
[d] G.p.c. of aminated PS

Table 2 Results of the polymerization of γ-benzyl-L-glutamate NCA with aminated PS

Sample[a]	A/I (mole ratio)	Prepolymer	Polymer yield (%)	$\bar{M}_w/\bar{M}_n$
PS/PBGL-a	230	PS-1	84.7	2.03
PS/PBGL-b	230	PS-7	88.3	2.33

[a] PBGL = poly(γ-benzyl-L-glutamate)

isolated by precipitation in methanol; it was stored at −30°C[1].

Characterization of the polymers

Molecular weight of the prepolymer. The molecular weight of polystyrene prepolymer was determined by gel permeation chromatography (g.p.c.) in THF at room temperature (Waters Associates) and by titration of the amino end groups with a standard perchloric acid solution in acetic acid, using crystal violet as an indicator[7].

Composition of the block copolymers. The composition of the block copolymer was determined by elemental analysis (nitrogen) and Fourier transform infra-red spectroscopy (*FT*i.r.) measurements (Bruker IFS 88 spectrometer)[8].

Conformation of the polypeptide block. The conformation of the polypeptide block in the block copolymer was analysed by i.r. spectroscopy.

RESULTS AND DISCUSSION

Synthesis of the prepolymers

The polymerization of styrene was carried out in DMF, because this was the only suitable solvent in which the azonitiator was soluble. The results of the polymerization of styrene are shown in *Table 1*. From the g.p.c. measurements the following conclusions can be drawn:

(1) aminated polystyrene was delayed on the g.p.c. columns during elution (compare 4 and 5 in *Table 1*);

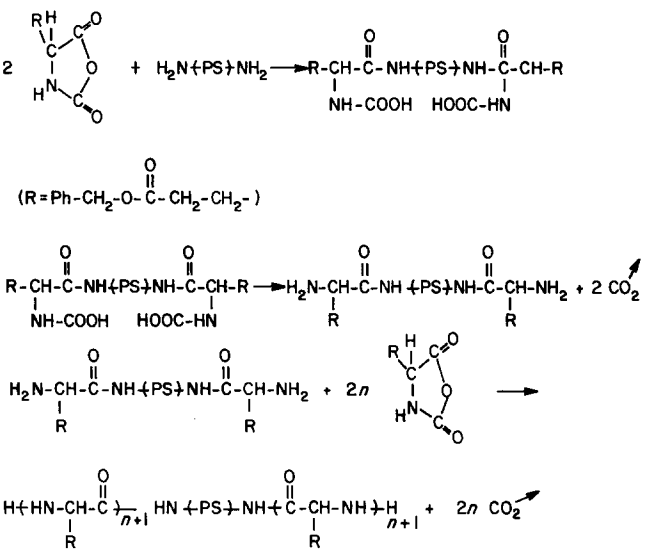
(2) the tailing observed during elution was reduced by modifying the amino end groups into amide groups by reaction with acetic anhydride at 25°C, which resulted in a lower molecular weight distribution (MWD) (compare 4 and 5 in *Table 1*). For reaction at 85°C the MWD was even better (compare 1 and 2 in *Table 1*);

(3) reducing the polymerization time resulted also in a better MWD (compare 3 and 4 in *Table 1*);

(4) the molecular weights obtained by titration (calculated for two amino end groups) and by g.p.c. measurements were in fairly good agreement for most of the obtained polystyrene prepolymers.

Synthesis of the block copolymers

The synthesis of the block copolymers is represented by the following scheme[9]:



The results of the block copolymerization with PS-1 and PS-7 are given in *Table 2*. These results show a narrow MWD for the resulting block copolymers compared with the distribution of prepolymers 1 and 7 in *Table 1*. The g.p.c. chromatogram of the block copolymer showed a considerable tailing at the high elution volume side, this could be due to a delaying effect of the amino end groups in the polymer during elution on the columns.

Conformation of the polypeptide block

The conformation of the polypeptide block in the copolymer was studied by i.r. spectroscopy. The polypeptide block showed an absorption band (amide I) at 1650 cm^{-1} and an absorption band (amide II) at 1547 cm^{-1} for both block copolymers. These findings correspond with a α-helical conformation[10].

Fractionation of the copolymer PS/PBGL-b

Before submitting the block copolymer to fractionation, homopolymers had to be eliminated by selective extraction methods. Homopolystyrene was eliminated by two successive extractions with cyclohexane. The homopolypeptide arising from traces of secondary amines in the polymerization solvent (DMF) could not easily be extracted and was eliminated during fractionation.

The block copolymer was dissolved in a mixture of chloroform and cyclohexane (1 wt% solution of polymer). The latter is a solvent of polystyrene and a non-solvent of poly(γ-benzyl-L-glutamate). Methanol was used as precipitant.

The composition of each fraction was determined by *FT*i.r.[8] and for some fractions also by nitrogen analysis. The results of the fractionation of the block copolymer are shown in *Table 3*. A plot of the cumulated weight % of precipitated polymer as a function of the γ values is given

Table 3 Results and characterization of the fractions obtained by fractionation of the block copolymer PS/PBGL-b

Fraction number	γ-factor[a]	Cumulated weight %[b]	Weight % PBGL (*FT*i.r.)	Weight % PBGL (Nitrogen analysis)
1	0.323	10.40	75.88	–
2	0.339	26.60	68.29	69.95
3	0.352	37.20	67.62	–
4	0.369	47.60	67.79	68.86
5	0.386	57.30	66.62	–
6	0.410	66.20	66.00	–
7	0.452	74.40	65.43	–
8	0.492	80.00	65.79	–
9	–	86.40	65.80	–

[a] $\gamma = \dfrac{\text{Total volume of added precipitant}}{\text{Volume precipitant} + \text{volume solvent}}$

[b] Cumulated weight % =

$$\frac{\frac{1}{2}\,\text{weight of fraction } i + \sum_{i=0}^{i-1} \textit{weight fraction } i}{\text{Initial weight of the block copolymer}} \times 100\,\%$$

in *Figure 1*. The cumulated weight % is zero up to $\gamma = 0.3$. Fraction 1 contained homopolypeptide since it contains more polypeptide than the theoretically possible content. *Table 3* shows that all the other fractions are nearly equal in polypeptide content.

The MWD of the non-fractionated block copolymer was only slightly broader than the MWD of the prepolymer; therefore the polymerization of the NCA of γ-benzyl-L-glutamate with aminated polystyrene in DMF gives rise to block copolymers with almost the same relative content of polypeptide and polystyrene.

CONCLUSION

The radical polymerization of styrene with a bis amino-azonitiator was carried out. The aminated vinyl polymer was then copolymerized with γ-benzyl-L-glutamate NCA.

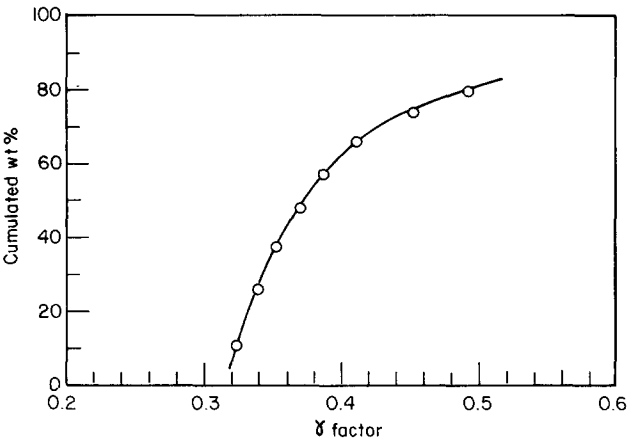


Figure 1 Fractionation of the block copolymer PS/PBGL-b. Cumulated weight per cent of precipitated polymer as a function of the γ factor

Fairly narrow MWD block copolymers were obtained in this way. The polypeptide content obtained by *FT*i.r. measurements remains constant for most of the fractions obtained by precipitation fractionation.

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