

# Copoly(styrene-divinylbenzene)-g-poly(maleic anhydride-styrene): Preparation Through Radiation Induced Grafting and Application in Organic Synthesis

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Received 21 June 1996; accepted 15 March 1997

**ABSTRACT:** Poly(styrene-divinylbenzene beads, preirradiated in air, were graft-copolymerized with mixtures of maleic anhydride and styrene. A detailed study of grafting was made under various reaction parameters. The succinic anhydride residues of a sample of copoly(styrene-divinylbenzene)-g-poly(maleic anhydride-styrene) with 111% grafting were chemically transformed into *N*-hydroxysuccinimide (Resin I) and *N*-bromosuccinimide (Resin II) residues. The esters derived from Resin I (analogous to the *N*-hydroxysuccinimide esters) were good acylating agents and their use was explored for the synthesis of peptides. Resin II was used for the bromination of benzylic substrates. The spent resins were found to be reusable without much loss to their capacity. © 1997 John Wiley & Sons, Inc. *J Appl Polym Sci* **66**: 847–852, 1997

**Key words:**  $\gamma$ -rays initiated grafting; copoly(styrene-divinylbenzene); *N*-hydroxysuccinimide; *N*-bromosuccinimide

## INTRODUCTION

Crosslinked polystyrene, poly(styrene-divinylbenzene) (PS-DVB), is prepared by free radical suspension polymerization of a mixture of styrene and 1–20 wt % divinylbenzene. The low crosslinked polymers are obtained in beaded form made up of an amorphous gel with a random network of sparsely crosslinked chains. The divinylbenzene is mainly the meta isomer. The beads are insoluble in all common solvents but swell in chlorinated and aromatic solvents, dioxane, and dimethylformamide (DMF). Chemical reactions occur throughout the matrix of the swollen gel beads, which are mechanically strong and easy to filter. These properties make PS-DVB the most suitable polymer for use as a support. Functional

groups can be introduced on the polymer for further chemical reactions.

In the present article, we describe the  $\gamma$ -radiation-induced grafting of a mixture of maleic anhydride and styrene which is known to produce chains of alternating polymers onto PS-DVB. A detailed study of the effects of changing the various reaction parameters on grafting has been made. The graft contains succinic anhydride moieties which provide the reactive functionality for the preparation of some synthetically useful polymeric reagents.

Anderson synthesized *N*-hydroxysuccinimide (NHS) esters of *N*-blocked amino acids and acyl peptides and used them for the synthesis of peptides in aqueous-organic media. These esters also resisted racemization of the concerned amino acid.<sup>1</sup> Fridkin and colleagues<sup>2</sup> were prompted to use polymeric active esters of *N*-blocked amino acids for the synthesis of the nonapeptide hormone bradykinin, with full biological activity in 39% overall yield. Laufer and coworkers<sup>3</sup> con-

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Contract grant sponsor: C.S.I.R.

verted copoly(ethylene–maleic anhydride) into a copolymer possessing NHS residues and used the hydroxy groups for esterifying *N*-blocked amino acids. The polymeric form of NHS active esters were used in peptide synthesis. Laufer and colleagues' results indicated that the use of polymeric NHS esters combined high reactivity and steric homogeneity in peptide synthesis with the advantage of a facile workup of polymeric reagent and support systems.

A mixture of styrene and maleic anhydride, when graft-copolymerized on PS–DVB, gave an alternating graft copolymer in which the maleic anhydride residues are separated by a styrene pendant. These residues were chemically transformed into NHS and *N*-bromosuccinimide (NBS) residues. The former polymer was used for the formation of peptide bonds through the polymer-bound NHS esters of *N*-blocked amino acids. The latter polymeric reagent was found to be a convenient substitute for NBS for the bromination of benzylic and allylic substrates.

## EXPERIMENTAL

Poly(styrene–2% divinylbenzene) beads, 200–400 mesh, were received from Fluka, Buchs, Switzerland. Styrene was freshly distilled and maleic anhydride was recrystallized from chloroform. Benzene and toluene used were of reagent grade. The resin was irradiated in air from a 2,100 Ci Co<sup>60</sup>  $\gamma$ -radiation source at a constant dose rate (0.080 MR/h). Analytical grade DMF was refluxed with anhydrous calcium sulfate, distilled under reduced pressure and stored over molecular sieves 4Å (Sigma, St. Louis, Missouri) before use. Dioxane was distilled and kept under nitrogen. *t*-Boc–L–leucine (Vega, Tucson, Arizona), Phe–OMe·HCl and Gly–OMe·HCl (Fluka), diisopropylethylamine (DIEA), and dicyclohexylcarbodiimide (DCC) (both from Sigma, St. Louis, Missouri) were used as supplied.

### General Procedure of Graft Copolymerization

Samples of PS–DVB, irradiated in a  $\gamma$ -chamber for different periods of time, were suspended in benzene, then heated at 105°C with mixtures of styrene and maleic anhydride for different periods of time. Similar experiments were run with toluene as the reaction medium at 105 and 140°C. After completion of reaction, the contents were filtered in a preweighed, sintered crucible and thoroughly washed with acetone and dioxane to

**Table I Percentage of Grafted Maleic Anhydride and of Styrene in Grafted Samples**

Total Grafting (%)	Grafted Maleic Anhydride (%)	Grafted Styrene (by Difference) (%)
220	110	110
378	188	190
450	224	226
380	190	190
340	170	170
410	206	204
600	300	300
302	150	152

ensure complete removal of the ungrafted material. The grafted copolymer was dried at 50°C to a constant weight. Percentage of grafting was calculated from the increase in initial weight of PS–DVB powder.

Percentage of grafting was determined as a function of total dose, reaction time, temperature, and concentration of each monomer. Effects of adding methanol and butanol to the reaction media on the percentage of grafting were also studied. Results are presented in Tables I–III and Figures 1–4, which are self-explanatory.

Grafting with maleic anhydride (0.01 mole) alone was attempted on PS–DVB (100 mg) preirradiated with a total dose ranging from 1.44 to 3.84 MR. There was essentially no grafting in any of these experiments.

### Estimation of Maleic Anhydride in the Graft Copolymer

Samples of graft copolymer (100 mg) were stirred in 2*N* KOH in dioxane (5 mL) at 40°C for 30 min. These were filtered, washed thoroughly with distilled water, and stirred with 2*N* HCl in dioxane (5 mL) for 30 min. The polymer was then filtered; washed with water, dioxane-water, dioxane, and water and ethanol; dried; and titrated with *N*/10 KOH in dioxane with phenolphthalein as indicator. A blank experiment was also run with ungrafted PS–DVB. The percentage of maleic anhydride in the graft was calculated from the amount of KOH consumed in titration. The percentage of grafted styrene was calculated by difference. Results are presented in Table I.

### Evidence of Grafting

The infrared (IR) spectrum (KBr pellet) of the grafted polymer showed peaks at 1850 and 1790

$\text{cm}^{-1}$  due to  $\text{C}=\text{O}$  stretching. This doublet appeared from coupled vibrations of two  $\text{C}=\text{O}$  groups; the high-frequency band is assigned to symmetrical vibrations and the lower-frequency band to asymmetric vibrations. These peaks confirm the grafting of maleic anhydride onto PS-DVB.

### Preparation of Resin I

The procedure of Laufer and associates<sup>3</sup> was used for the conversion of maleic anhydride residues in the graft copolymer into the residues of NHS.

Graft copolymer with 111% grafting (2.0 g) was heated on a oil bath at 90°C for 3 h with hydroxylamine hydrochloride (4 equiv. for each maleic anhydride residue) in DMF (20 mL). The resulting polymer was filtered and washed with DMF. The reaction was repeated twice to ensure complete conversion of maleic anhydride residues to NHS moieties. The resin was finally washed with water, DMF, water, dioxane, and acetone and dried at 50°C in vacuum.

The IR (KBr pellet) showed absorption at 1780 (m) and 1715  $\text{cm}^{-1}$  (s). The resin estimated a nitrogen content of 12%.

### Preparation of Boc-Leucyl Ester of Resin I (Resin III)

Resin I (2 g) was swelled in DMF (10 mL) containing Boc-leucine (2 g) for 1 h. DCC (2.2 g) dissolved in DMF (10 mL) was added and the reaction mixture was stirred for 1 h at 0°C and for 4 h at room temperature. The resin was filtered and washed with DMF. The reaction was repeated twice, and the resin was finally washed with DMF and EtOH and dried at 50°C.

A few beads of the resin after treatment with trifluoroacetic acid gave a positive test with Kaiser reagent.<sup>4</sup>

### Synthesis of Boc-Leu-Phe-OMe

Resin III (1 g) was added to a solution of Phe-OMe·HCl (0.5 g) in DMF (10 mL) containing DIEA (0.4 g). The reaction mixture was stirred at room temperature for 6 h. The resin was filtered and washed with dichloromethane, and the combined filtrate and washings were evaporated on a rotatory evaporator. The residue was taken in ethyl acetate and washed with water, 2% sodium carbonate, water, citric acid (1%, three times), and finally with water. The ethyl acetate solution was dried with anhydrous  $\text{Na}_2\text{SO}_4$  and

the solvent removed to give a solid [melting point (mp), 88–89°C] which was identical with an authentic sample of Boc-Leu-Phe-OMe on thin-layer chromatography and mixed m.p.

### Synthesis of Boc-Leu-Gly-OMe

Resin III (1 g) was similarly treated with Gly-OMe·HCl (0.35 g) and DIEA (0.4 g) in DMF (10 mL). The product melted at 131–132°C and was indistinguishable from an authentic sample of Boc-Leu-Gly-OMe.

### Preparation of Resin II

The procedure of Clarke and Dehr<sup>5</sup> was followed for the conversion of maleic anhydride residues in the graft copolymer into succinimide residues.

Resin I (4 g) was taken in distilled dioxane (30 mL) and  $\text{NH}_4\text{OH}$  (12 mL) was added to it. The reaction mixture was gently refluxed for 5 h and filtered, and the reaction was repeated 4 times. The polymer was then refluxed for 1 h in dry toluene and the toluene was removed by distillation. The process was repeated twice and the polymer was dried at 50°C.

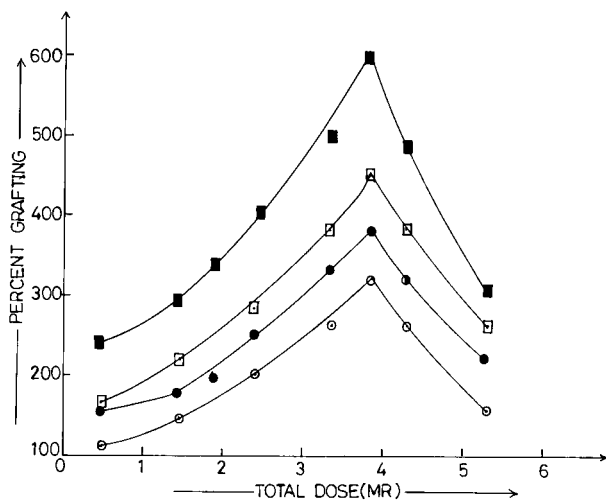
The polymer (1.0 g) was suspended in dioxane (10 mL) containing KOH (0.1 g), and bromine (0.5 mL) dissolved in carbon tetrachloride (20 mL) was slowly added with cooling. The mixture was stirred in an ice bath for 2 h, after which the polymer was filtered and washed with  $\text{CCl}_4$ , water, and dioxane. The reaction was repeated 3 times and the resulting Resin II was dried at 50°C.

### Bromination of *p*-Toluic Acid

To *p*-toluic acid (0.15 g) dissolved in ether (10 mL) was added Resin II (1 g), benzoyl peroxide (3 mg), and  $\text{CCl}_4$  (10 mL). The reaction mixture was gently refluxed for 5 h, after which the resin was filtered and the filtrate was dried to obtain the product, 4-carboxybenzyl bromide [mp, 209–210°C; IR, 3000  $\text{cm}^{-1}$ ; 1705 and 550  $\text{cm}^{-1}$  (s) (C—Br stretching) yield, 74%].

### Bromination of Acetophenone

Acetophenone (0.1 mL) was similarly treated with Resin II to yield phenacyl bromide in the form of fine needles (yield, 69%; mp 51–52°C).



**Figure 1** Effect of total dose on the percent grafting of maleic anhydride and styrene on PS-DVB (100 mg) in 3 h of reaction.

- Maleic anhydride and styrene (0.005 moles each); benzene, 3 mL; temp. 105°C.
- Maleic anhydride and styrene (0.01 moles each); benzene, 3 mL; temp. 105°C.
- Maleic anhydride and styrene (0.005 moles each); toluene, 3 mL; temp. 140°C.
- Maleic anhydride and styrene (0.01 moles each); toluene, 3 mL; temp. 140°C.

## DISCUSSION

Grafting performed in benzene (105°C) and toluene (140°C) at low (0.005 mole) or high (0.01 mole) concentration of the monomers presented similar patterns (Fig. 1) with increasing total dose of irradiation of PS-DVB and showed a max-

imum at 3.84 MR. This observation supports our earlier findings on radiation-induced grafting on PS-DVB.<sup>6</sup>

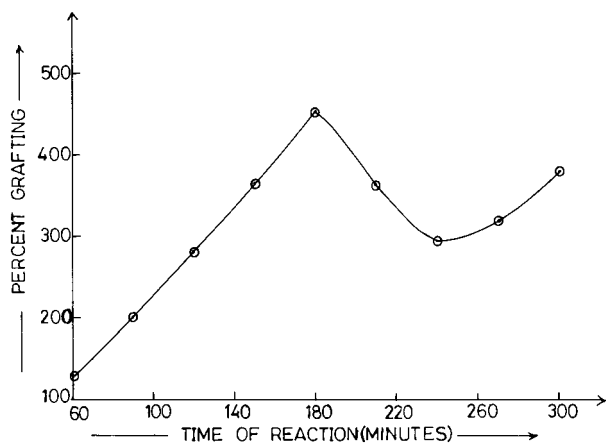
The effect of temperature on the percentage of grafting in two reaction media (benzene and toluene) with increasing total dose is presented in Table II. No grafting was observed in toluene at 105°C at any of the doses of irradiation used; whereas a familiar pattern with optimum grafting at 3.84 MR was observed when the reaction was carried out in benzene under the same conditions, although swelling and consequent diffusion of monomer took place equally well in both the solvents. This appears to be the effect of higher chain transfer constant of toluene as compared with that of benzene. Reaction done at 140°C possibly outweighs this effect, and a comparatively higher grafting was observed at all the doses used.

Increase in the temperature of reaction may be associated with several related phenomena, e.g.: (1) PS-DVB beads may swell to a greater degree, causing an increased diffusion of the monomer to the grafting sites; (2) solubility of the monomer may increase, causing a decrease in the viscosity of the medium; (3) the homolysis of the hydroperoxide may be accelerated, leading to an increase in the rate of initiation and propagation; (4) the mobility of the radicals, including the macroradicals, may increase, leading to a higher rate of termination and an increase in the formation of homopolymer.

Percentage of grafting was studied as a function of reaction time varying from 60 to 300 min (Fig. 2). It followed a familiar pattern<sup>7</sup> by first increasing linearly up to 180 min, beyond which

**Table II** Effect of Total Dose and Temperature of Reaction in Benzene and Toluene on the Percentage of Grafting of Maleic Anhydride (0.01 mole) and Styrene (0.01 mole) on PS-DVB (100 mg)

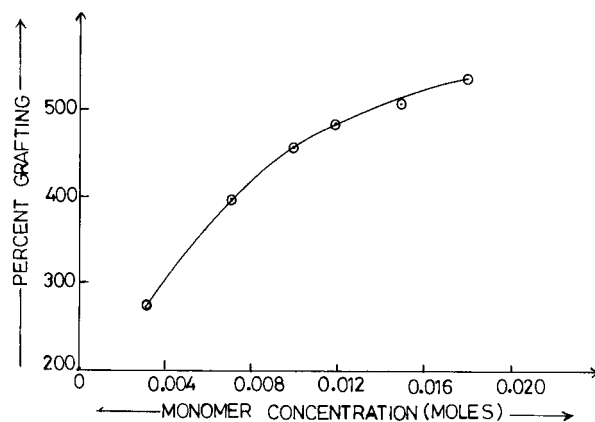
Total Dose (MR)	0.48	1.44	1.92	2.4	3.36	3.84	4.32	5.28
Medium: Benzene (3 mL) Temperature: 105°C								
Grafting	165	220	255	284	378	450	380	257
Medium: Toluene (3 mL) Temperature: 105°C								
Grafting	0	0	0	0	0	0	0	0
Medium: Toluene (3 mL) Temperature: 140°C								
Grafting	242	290	340	410	499	600	487	302



**Figure 2** Effect of time of reaction on the percent grafting of maleic anhydride and styrene on PS-DVB. PS-DVB, 100 mg; maleic anhydride and styrene (0.01 moles each); benzene, 3 mL; temp. 105°C; total dose, 3.84 MR.

it first fell to about two-thirds of its maximum value in 240 min of reaction and then rose again. Although there is no supporting evidence at hand, it may be hypothesized that the initial increase in the percentage of grafting may simply be the result of the increase in the time of reaction, during which the grafting progressed at a constant rate of 2.3 mg/min. Subsequent decrease in grafting could be the result of "backbiting" phenomenon, in which the growing grafted chains attack themselves or the neighboring chains. Increase in the percentage of grafting after the minimum is reached may be caused by the accumulation and increased concentration of homopolymer radicals, some of which are grafted back onto the polymer through chain transfer. It is interesting to note that the increase or decrease in grafting occurs at about the same rate.

Percentage of grafting increased continually with the increase in monomer concentration, but the rate of grafting gradually decreased as the concentration of the monomers was raised from 0.003 to 0.018 moles of each monomer (Fig. 3). Experiments run with a fixed total monomer concentration (0.02 moles) but with varying amounts of maleic anhydride and styrene showed an expected pattern. Since this pair of monomers give an alternating graft, maximum grafting occurred when the monomers were present in equimolar amounts (Table III). Also, styrene alone was grafted to an extent of 160% onto PS-DVB while maleic anhydride alone did not show any grafting. Increasing the total dose of irradiation did not help in the grafting of maleic anhydride on the backbone polymer.



**Figure 3** Effect of monomer concentration of maleic anhydride and styrene on grafting on PS-DVB. PS-DVB, 100 mg; benzene, 3 mL; temp. 105°C; total dose, 3.84 MR; reaction time, 3 h.

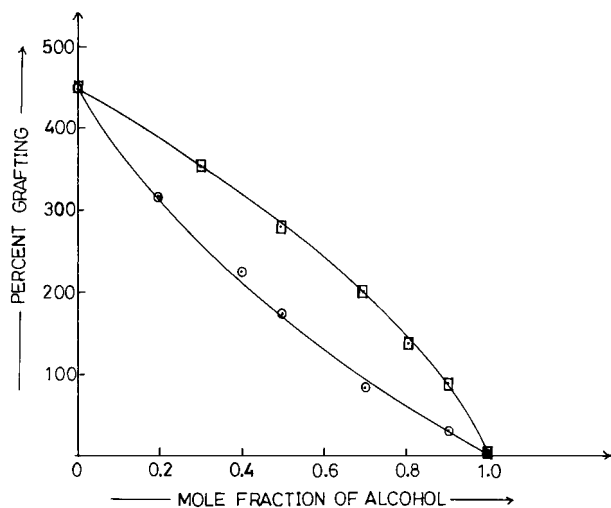
The contents of maleic anhydride in samples of PS-DVB grafted with mixtures of styrene and maleic anhydride were also estimated by first hydrolyzing the anhydride and then estimating the carboxylic groups by titration. There was a close agreement between the results from gravimetric determination and from titration (Table I).

Effect of mole fraction of alcohols in alcohol-benzene media was studied at a selected total dose. There was a steep decrease in the percentage of grafting with the increase of MeOH and of *n*-BuOH in the benzene medium (Fig. 4). This may be caused by the non-swellability of PS-DVB in these alcohols so that the diffusion of monomers to the interior of the beads is restrained. Also the chain transfer property of the alcohols causes the termination of the homopolymer radicals competing for grafting on the PS-DVB backbone. The

**Table III** Grafting with Variation in Composition of Maleic Anhydride and Styrene

Maleic Anhydride (moles)	Styrene (moles)	Grafting (%)
0.0	0.020	160
0.003	0.017	204
0.005	0.015	296
0.007	0.013	363
0.01	0.01	450
0.013	0.007	378
0.015	0.005	212
0.017	0.003	102
0.020	0.0	0

PS-DVB, 100 mg; total dose, 3.84 MR; benzene, 3 mL; temperature, 105°C; reaction time, 3 h.

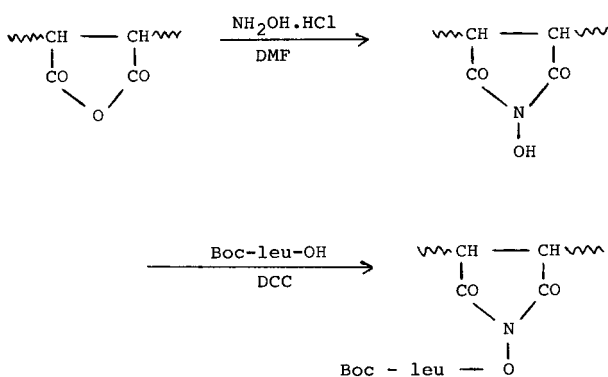


**Figure 4** Effect of mole fraction of alcohols on grafting in benzene-alcohol media. PS-DVB, 100 mg; maleic anhydride and styrene (0.01 mole each); total dose, 3.84 MR; reaction time, 3 h; ○—○ butanol; □—□ methanol.

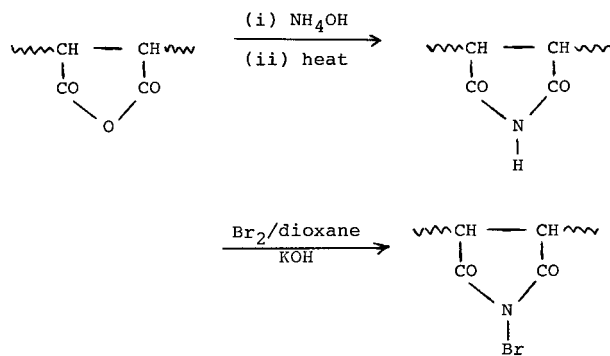
chain transfer constant of *n*-BuOH ( $C_s = 1.64 \times 10^{-4}$ ) being higher than that of MeOH ( $C_s = 0.32 \times 10^{-4}$ ) explains lower grafting in *n*-BuOH.

Maleic anhydride residues present in the graft copolymer with 111% grafting were converted into NHS functionality by treatment with hydroxylamine hydrochloride in DMF and Boc-Leu-OH was esterified to the resulting polymer with the help of DCC (see Scheme 1).

This active polymeric ester, when treated with H-Phe-OMe in DMF, formed Boc-Leu-Phe-OMe in solution. The polymer with regenerated NHS groups was filtered out and the dipeptide was isolated from the filtrate. Boc-Leu-Gly-OMe was prepared in a similar manner. Because



**Scheme 1**



**Scheme 2**

of its compatibility with the reaction parameters used, PS-DVB has been extensively used as the base polymer in its numerous modifications for the solid-phase synthesis of peptide. The present polymer is an addition to the existing repertoire. NHS-polymer with a required capacity can be fabricated by starting with a graft copolymer of desired maleic anhydride content.

Maleic anhydride residues in the same graft copolymer were transformed into NBS groups and the NBS-resin was shown to be a convenient reagent for the bromination of *p*-toluic acid and acetophenone. (See Scheme 2.) The polymer is compatible with  $\text{CCl}_4$ , the commonly used medium for carrying out bromination with NBS. The spent resin, which possesses succinimide groups, can be reconverted into the NBS-resin.

The grants of Emeritus Scientistship to one author (C.S.P.) and of Senior Research Fellowship to the other (N.G.) by C.S.I.R. is gratefully acknowledged.

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