Functionalization of Crosslinked Polyacrylamide through γ-Radiation-Induced Grafting of 4-Vinylpyridine

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ABSTRACT: Crosslinked polyacrylamide beads were irradiated in air with a Co^{60} γ-radiation source. The preirradiated beads were graft-copolymerized through heating with 4-vinylpyridine in the presence of benzoyl peroxide. Grafting was studied as a function of various reaction parameters and was determined from the increase in the weight of the original polymer and the estimation of pyridine pendants in the homopolymer-free graft copolymer. Although making the polymer basic in character, this modification retained the hydrophilic nature of polyacrylamide. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 84: 2613–2620, 2002

Key words: polyacrylamide; γ-irradiation; 4-vinylpyridine.

INTRODUCTION

Inert polyacrylamide gels are prepared by the copolymerization of acrylamide with 1–10% *N,N′*-methylenebisacrylamide. These gels are transparent and thermally irreversible. They are permeable to water, and this permeability depends on the polymer concentration but not the extent of crosslinking. The properties of these gels suggest a brush heap structure. Polyacrylamide gels are routinely used for gel electrophoresis of proteins, and for this purpose, they are prepared as slabs or blocks of the required dimensions.[1–3] In combination with sodium dodecyl sulfate, polyacrylamide gel electrophoresis is employed for the determination of molecular weights of proteins.[4] Electrophoretic separation and purification of proteins can be performed on both large and micropreparative scales. Polyacrylamide gels are useful chromatographic materials for size exclusion chromatography and desalting of water-soluble polymers.[5,6] The copolymerization of acrylamide with monomers containing reactive functional groups yields copolymers to which suitable ligands can be covalently attached. These gels are used in affinity chromatography and in the immobilization of biomolecules.

Grafting has been applied to the introduction of functional groups on crosslinked polymers,[7–24] and these polymers have been considered for different purposes. However, not much work has been done in this area. Grafting is a simple, clean, and efficient method for altering the properties of a polymer. Crosslinked acrylamide beads of various types are commercially available with grades carefully designed in terms of size and crosslinking density. With the grafting of monomers possessing acidic, basic, hydrophilic, hydrophobic, and other groups amenable to further modification for desired sizes, shapes, and chemical reactivities, a vast array of crosslinked polyacrylamides (c-PAs) can be obtained that may be useful in chromatographic applications.

This article describes the grafting of 4-vinylpyridine (4-VP) onto polyacrylamide induced by γ-radiation and benzoyl peroxide (BPO). A de-

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tailed study of various reaction parameters and their effects on grafting was conducted.

EXPERIMENTAL

c-PA in bead form (Bio-Gel P-4) was a product of Bio-Rad Laboratories. 4-VP (Fluka) was freshly distilled before use. Samples of polyacrylamide were irradiated in air at a constant dose rate of 0.3933 kGy/h from a 2100-Ci Co^{60} γ-radiation source contained in a Gamma Chamber 900 obtained from BARC (Mumbai, India).

General Procedure

c-PA (100 mg) was irradiated in air in the γ-chamber for 24–168 h. A predetermined amount of 4-VP was added to the irradiated polymer in water, along with a known amount of BPO, and the mixture was gently refluxed. The poly(4-vinylpyridine) homopolymer formed during the reaction was completely removed by thorough washing with a warm water/methanol (1/1) mixture in a weighed sintered crucible that was then dried at 50°C to a constant weight. The percentage of grafting was calculated from the increase in the initial weight of c-PA and was expressed as follows:

$$\text{Percentage of Grafting} = \frac{W_2 \times W_1}{W_1} \times 100$$

where W_1 is the weight of the original c-PA and W_2 is the weight of the grafted polymer after complete removal of the homopolymer.

The percentage of grafting was determined as a function of the total dose, amount of BPO used, molar concentration of the monomer, refluxing temperature, composition of the reaction medium, and refluxing time. The percentage of grafting was determined in selected samples from titrations by determination of the uptake of HCl by the grafted polymer. Changes in the swelling properties of c-PA as a result of grafting were determined in selected solvents for an idea of any unintentional crosslinking accompanying the course of the reaction.

Characterization of the Graft Copolymer

The infrared spectrum (KBr) of the grafted polymer showed absorption at 1580 (—C═N) and 2940 cm^{-1} (C—H stretching in —CH═ of pyridine), indicating the presence of pyridine rings introduced through grafting. The grafted polymer also took up HCl equivalent to the pyridine rings introduced (discussed later).

Effect of the Amount of BPO

Samples of c-PA (100 mg) irradiated for 120 h (total dose = 47.2 kGy) were refluxed with 4-VP (1 mL, 9.24 mmol) and various amounts of BPO in water (3 mL) for 3 h. Figure 1 presents the results.

Effect of the Total Dose on Grafting

Samples of c-PA (100 mg) were irradiated for 24–168 h in air. The irradiated samples were gently refluxed for 3 h at 120°C with 4-VP (9.24 mmol) and BPO (0.1 mmol) in water (3 mL). The results are presented in Figure 2.

Effect of the Monomer Concentration on Grafting

Samples of c-PA (100 mL) irradiated for a total dose of 47.2 kGy were refluxed for 3 h with 4-VP (9.24 mmol) and BPO (0.1 mmol) in various amounts of water. Figure 3 presents the results.

Effect of the Reaction Time

Samples of c-PA (100 mg) preirradiated in air for a total dose of 47.2 kGy were refluxed in water (3 mL) along with 4-VP (1 mL) and BPO (0.1 mmol) for 1–5 h. The results are presented in Figure 4.

Effect of the Reaction Temperature

Samples of c-PA (100 mg) were irradiated to a total dose of 47.2 kGy. These were subsequently heated with 4-VP (9.24 mmol) and BPO (0.1 mmol) in water (3 mL) in sealed tubes immersed in oil baths maintained at fixed temperatures. Cooled tubes were cut, and the grafted c-PA was freed from the homopolymer as usual. The results of grafting in each case were determined and are recorded in Table I.

Effect of the Total Dose on the Hydroperoxidation of c-PA

Samples of c-PA (100 mg) were irradiated in air for a total dose of 9.4–66.1 kGy. Irradiated sam-

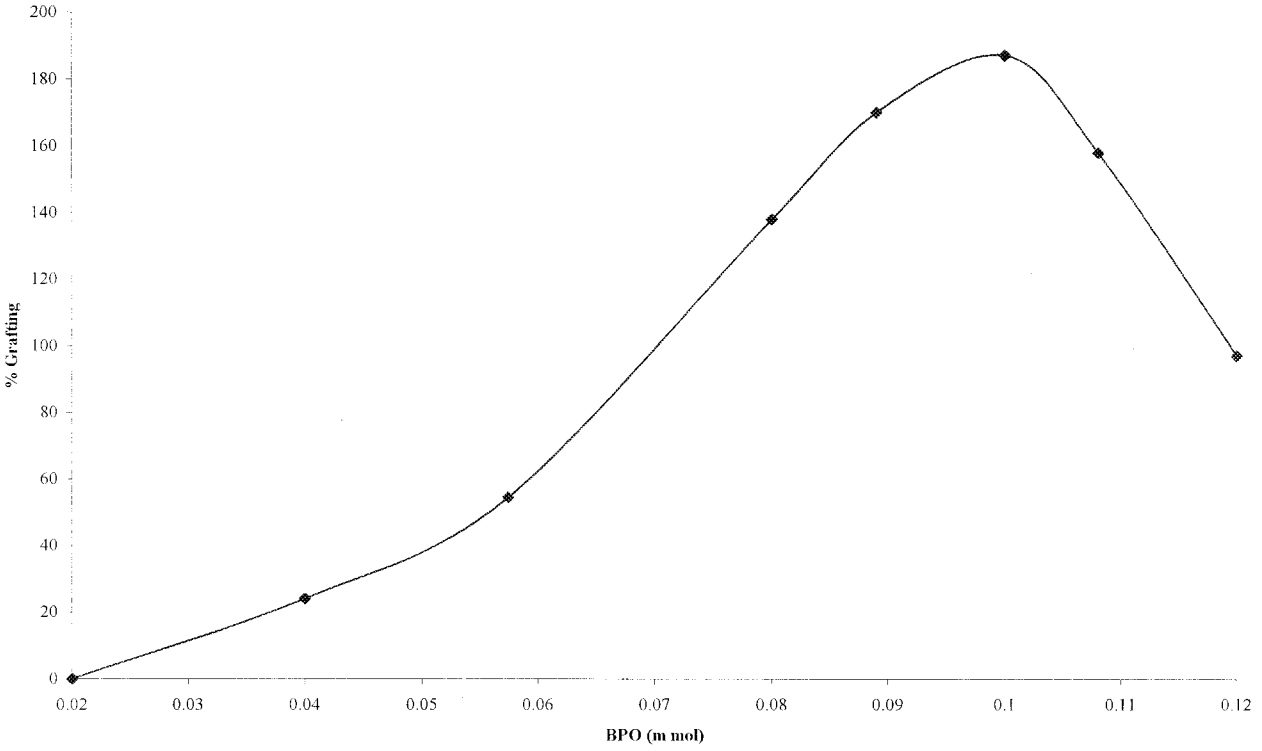


Figure 1 Effect of the amount of BPO on grafting (c-PA = 100 mg; 4-VP = 9.24 mmol; water = 3 mL; heating time = 120°C; total dose = 47.2 kGy).

ples were taken in deaerated water (5.0 mL), KI (0.01*M*, 5.0 mL) and H_2SO_4 (0.1*N*, 1.0 mL) were added, and the samples were kept in the dark for 2 h. The liberated iodine was determined by titration with sodium thiosulfate (0.01*N*). The results are presented in Figure 2.

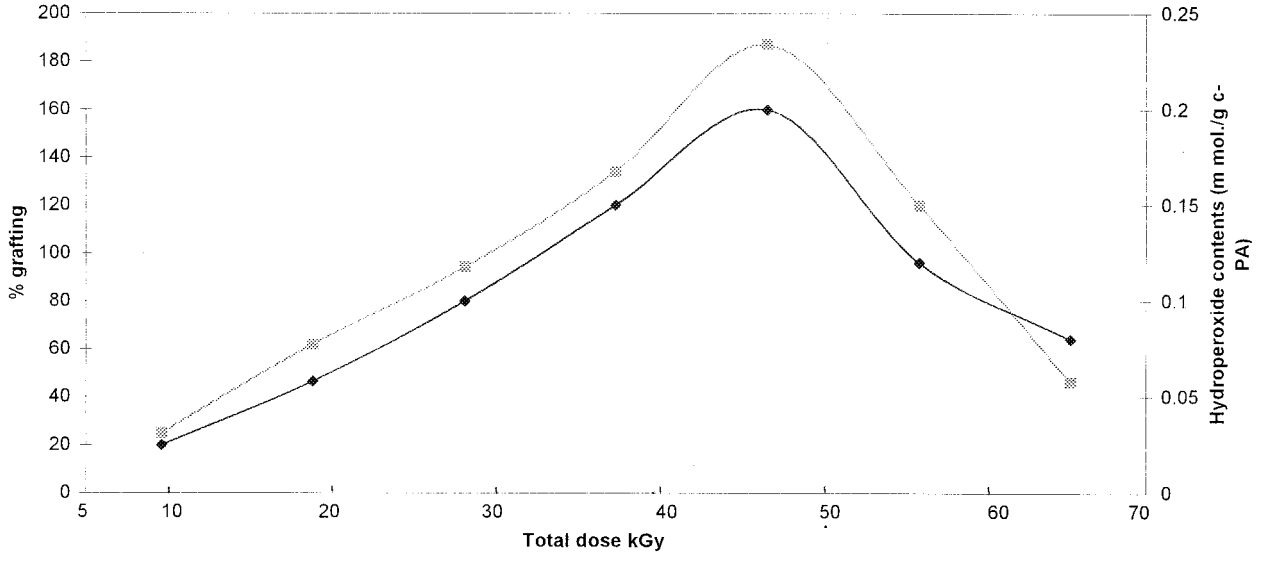


Figure 2 Effect of the total dose on the hydroperoxide contents of c-PA and grafting: (■) the total dose versus the percentage of grafting (c-PA = 100 mg; 4-VP = 9.24 mmol; BPO = 0.1 mol; water = 3 mL; heating time = 3 h; temperature = 120°C) and (♦) the total dose versus the hydroperoxide content (c-PA = 100 mg).

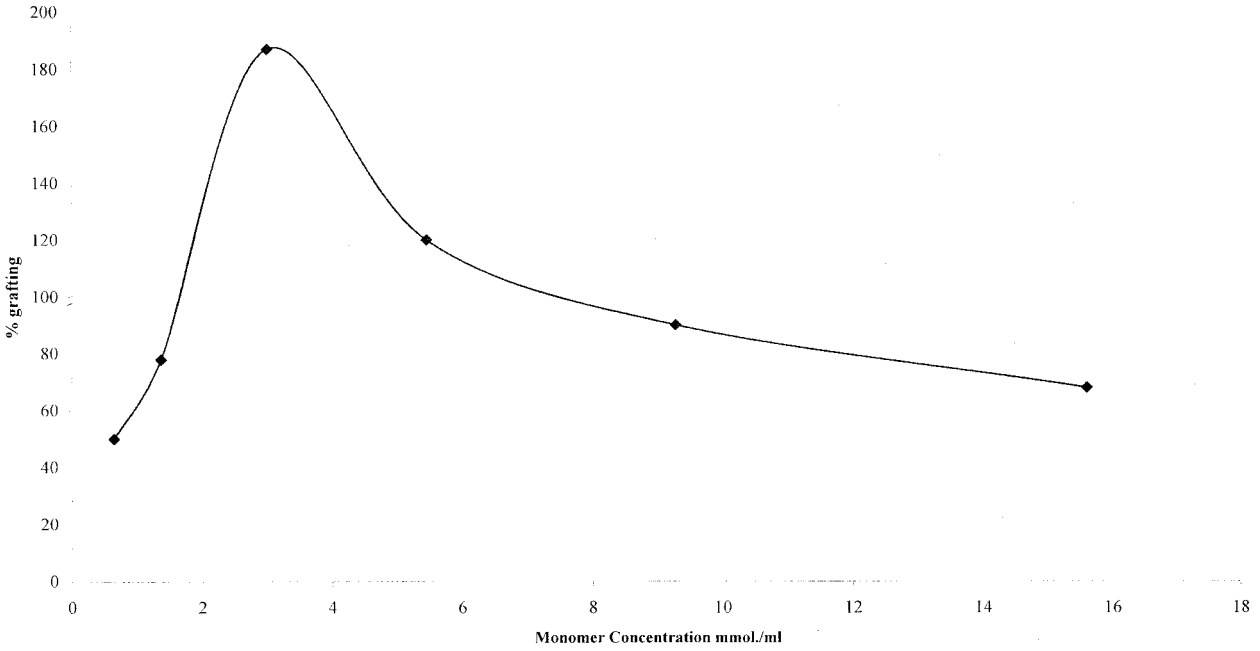


Figure 3 Effect of the monomer concentration on grafting (c-PA = 100 mg; BPO = 0.1 mmol; total dose = 47.2 kGy; reaction time = 3 h; temperature = 120°C).

Effect of the Composition of the Reaction Medium

Preirradiated samples (100 mg) of c-PA were refluxed in mixtures (3 mL) of water and methanol of different compositions, BPO (0.1 mmol), and 4-VP (9.24 mmol) for 3 h at 120°C in closed tubes. The percentage of grafting is recorded in Table II.

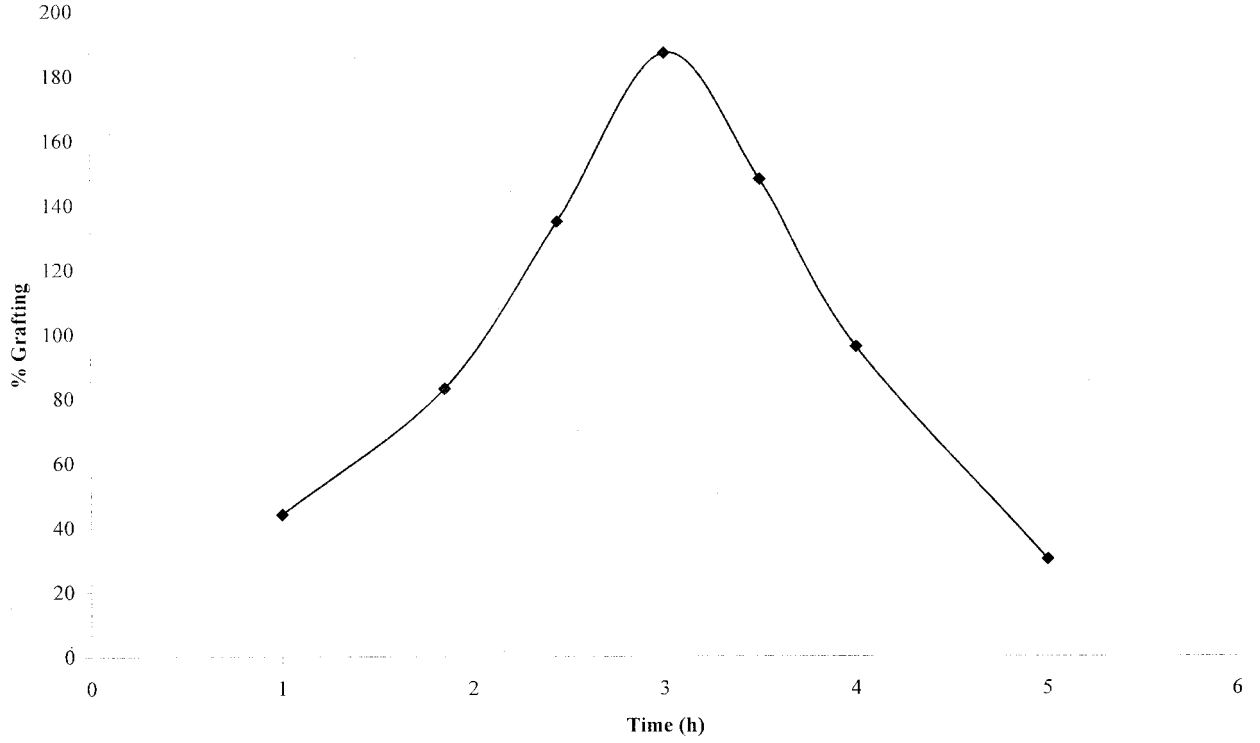


Figure 4 Effect of the reaction time on grafting (c-PA = 100 mg; 4-VP = 9.24 mmol; total dose = 47.2 kGy; BPO = 0.1 mol; water = 3 mL; temperature = 120°C).

Table I Effect of the Temperature of Reaction on Grafting on c-PA

Temperature (°C)	Percentage of Grafting
90	15.0
100	50.0
110	114.0
120	187.0
130	120.0
140	70.0

Determination of the Percentage of Grafting by Titration

Samples (100 mg) of c-PA-*g*-poly(4-vinylpyridine) with different grafting percentages were taken in 0.1*N* HCl and swirled occasionally for 1.5 h. The polymer was filtered off, and the filtrate was titrated against 0.1*N* Na_2CO_3. The percentage of grafting was calculated from the amount of HCl taken up by the graft. The results are compared in Table III.

Determination of the Swelling Properties of the Polymers

One hundred milligrams each of c-PA, c-PA irradiated to a total dose of 47.2 kGy, c-PA irradiated and heated for 3 h in H_2O, and c-PA-*g*-poly(4-vinylpyridine) was lowered to the bottom of a dry and narrow graduated tube marked in hundredths of a milliliter. It was fabricated from a graduated pipette, the lower end of which was sealed and the upper end of which was shaped as a tiny funnel. Water or dioxane was introduced, and the polymer was allowed to swell for 20 h at room temperature. The bed volumes of the swollen gels were determined. The results are presented in Table IV.

Table II Effect of the Reaction Medium on Grafting

Composition of Reaction Medium		Percentage of Grafting
Methanol (mL)	Water (mL)	
0.0	3.0	187.0
0.5	2.5	90.0
1.0	2.0	40.0
1.5	1.5	12.0
2.0	1.0	12.0
2.5	0.5	4.0
3.0	0.0	0.0

Table III Percentage of Grafting

Percentage of Grafting Determined	
By Weight	By Titration
30.0	28.0
62.0	63.0
90.0	89.2
120.0	118.8
130.0	131.2
187.0	187.8

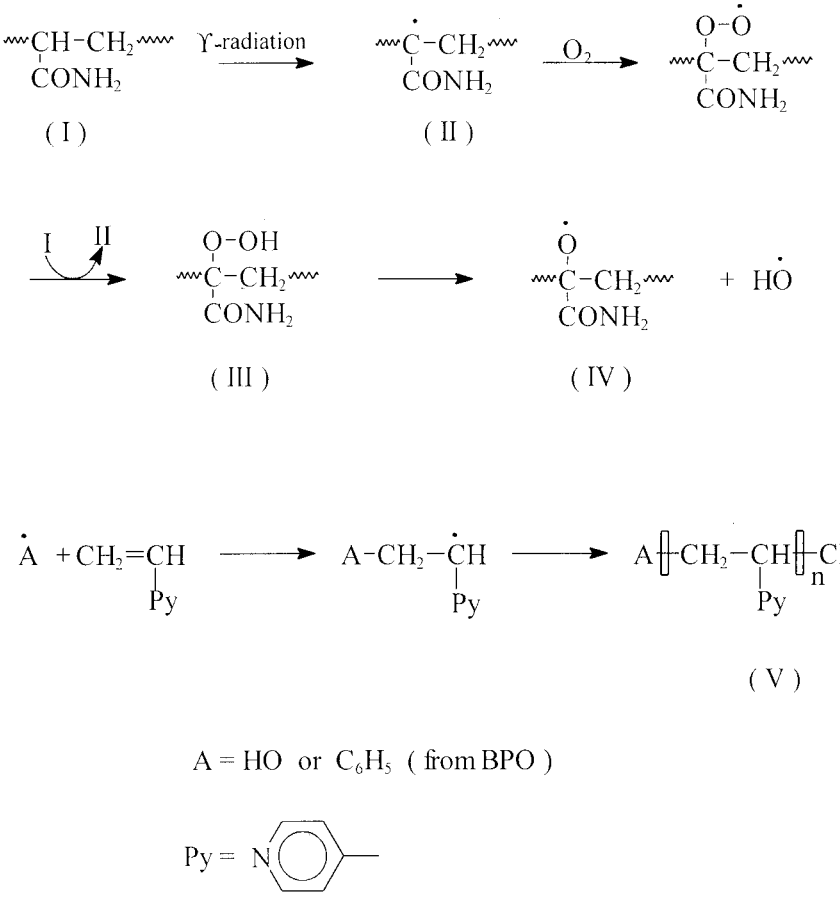
RESULTS AND DISCUSSION

Preirradiated c-PA that was heated with 4-VP in water showed poor grafting. Attempts to graft 4-VP in the presence of BPO also resulted in poor grafting. However, if, when preirradiated, c-PA was refluxed in water in the presence of 4-VP and BPO, sufficient grafting was noticed. The optimum conditions for maximum grafting were 0.1 mmol of BPO/100 mg of c-PA and a total preirradiation (in air) dose of 47.2 kGy.

The percentage of grafting showed a regular increase up to a maximum dose of 47.2 kGy of preirradiation and 0.1 mmol of BPO/100 mg of c-PA. This was likely due to the effect of the generation of an increasing number of free-radical sites on the polymer. As expected, the grafting occurred (i) with the initiation of polymerization of the monomer by the radical sites on the backbone polymer both on the surface and in the interior of the beads and (ii) by chain termination onto the polymer, as presented in Scheme 1. Re-

Table IV Bed Volumes of Swollen Polymers in Water and Dioxane

	Bed volume (mL)	
Polymer (100 mg)	In H_2O	In Dioxane
c-PA	0.50	0.20
c-PA (irradiated, total dose-47.2 kGy)	0.45	0.16
c-PA (irradiated and heated)	0.30	0.12
c-PA-*g*-poly(4-vinylpyridine) (187% grafting)	0.62	0.40



Scheme 1

actions such as (ii) were restricted to the c-PA exterior because a macromolecular radical such as **V** could not diffuse through the pores of the beads. Bio-Gel P-4, which was used in this work, excludes molecules with molecular weights greater than 4000.

A high concentration of **V** radicals with high molecular weights was caused when (1) the total dose of irradiation exceeded the optimum value (Fig. 2), (2) the amount of BPO was excessive (Fig. 1), and (3) the monomer concentration was high (Fig. 3). Under these conditions, most of the grafting occurred through route (i). The concentration of the **V** macroradicals outside the beads became high, and the viscosity of the solution increased, affecting the mobility of **V**. The macroradicals then combined together, leading to the formation of homopolymer molecules with a consequent drop in the grafting. An examination of Figures 1–3 shows that the grafting, with respect to the variables studied, increased up to an optimum value because, as expected, it occurred through

both routes. Smaller macroradicals at low concentrations could diffuse into the interior of the c-PA beads.

The effect of the reaction time on the percentage of grafting is presented in Figure 4. The reaction rate gradually rose to 3 h of heating. The increase in the reaction rate might have been caused by autoacceleration or grafting of homopolymer radicals of intermediate size by route (ii). On further heating, the percentage of grafting decreased. Such a fall in grafting is possible if segments of grafted chains are released from the grafted polymer. This may occur if a growing grafted chain is sufficiently long and has enough flexibility to attack itself or a neighboring chain through a backbiting mechanism.[25]

The reaction was carried out at different temperatures with the other parameters kept constant. Grafting increased up to a temperature of 120°C, after which it fell. A rise in temperature enhanced the solubility and so reduced the viscosity of the reaction medium containing macromolecular radicals. There was also an increase in the kinetic energy of the reacting species, which promoted grafting. As the temperature was raised further, degradation reactions such as backbiting also became prominent, and this may be attributed to low grafting at high temperatures.

The effect of increasing the ratio of methanol to H_2O in the binary reaction medium on the grafting of 4-VP onto c-PA is presented in Table II. Under the same set of conditions, the percentage of grafting fell with an increase of methanol in the reaction medium. In pure methanol, it was zero. Two factors may be contributing to these results. One is the reduced compatibility of c-PA with media containing more than 20% methanol, and the other is its high chain-transfer constant. That the addition of methanol increased the solubility of BPO was not sufficient to counter the more dominant factors. Also, BPO had sufficient solubility in a mixture of 4-VP and water.

The percentage of grafting was also determined by titration of the pyridine moieties in selected samples of the graft copolymer. Table III shows that there is a close correspondence between the figures obtained from the increase in weight on grafting and the titration. This also excludes any possibility of the graft copolymer containing free c-PA.

The swelling behavior of the beads of a crosslinked polymer in an appropriate medium gives a qualitative indication of any unintentional crosslinking in the course of a chemical reaction performed on it. Exposure to high-energy radiation, heating of the irradiated polymer, and grafting are reactions that have potential for introducing crosslinks. The results presented in Table IV show that irradiation caused a little shrinking, whereas irradiation followed by heating caused significant crosslinking. Polymer chains are known to crosslink under the influence of high-energy radiation. Polyacrylamide is a crosslinking type of polymer. In a crosslinked polymer such as c-PA, the flexibility of the chains is reduced, and further crosslinking is not a highly favored reaction. However, the different pathways through which radiolysis can proceed in the presence of air are highly complex, and one cannot exclude the formation of additional crosslinks. The grafted c-PA swelled more than the original polymer in both water and dioxane. It was difficult to determine if any crosslinking occurred in this process. The excess swelling might well have been the result of grafted poly(4-vinylpyridine) chains that were compatible with water and dioxane.

c-PA in beaded form (Bio-Gel P) is available in various sizes and crosslinking densities and with exclusion limits of 1800–100,000 Da. Unlike carbohydrate-based chromatographic materials, these gels do not support microbial growth and can be operated at pH 2–10 and room temperature. The beads are extremely hydrophilic and can tolerate up to 20% ethanol without significantly alteration of their chromatographic characteristics. Various functional groups can be introduced into the c-PA through grafting that alter its properties. The functional groups could serve as ligands for binding substrates through which enzymes can be immobilized. The modified support may be used for chromatography, affinity chromatography, or immunoaffinity chromatography.[26]

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REFERENCES

1. Janas, V. F.; Rodriguez, F.; Cohen, C. Macromolecules 1980, 13, 977.
2. Chrambach, A.; Rodbared, D. Science 1971, 172, 440.
3. Masrer, H. R. Disc Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis, 2nd ed.; de Gruyter: Berlin, 1971.

4. Andrews, A. T. Electrophoresis: Theory, Techniques, and Biochemical and Clinical Applications; Clarendon: Oxford, 1981.
5. Hjerten, S.; Mosbach, R. Anal Biochem 1962, 3, 109.
6. Lathe, G. H.; Ruthven, C. R. Biochem J 1957, 62, 665.
7. Schutten, J. H.; Van-Hastenberg, C. H.; Piet, P.; German, A. L. Angew Macromol Chem 1980, 89, 201.
8. Guyot, A.; Bartholin, M. Prog Polym Sci 1982, 8, 277.
9. Brunelet, T.; Bartholin, M.; Guyot, A. Angew Macromol Chem 1982, 106, 79.
10. Soldatov, V. S.; Pokrovskaya, A. I.; Martsinkevich. Zh Prikl Khim (in Russian) 1984, 5, 2030.
11. Soldatov, V. S.; Ivannikova, V. M.; Elinson, I. S.; Isygankov, V. I. Akad Navuk BSSR Ser Khim Navuk (in Russian) 1986, 5, 17.
12. Shunkevich, A. A.; Papova, O. P.; Belotserkovskaya, T. N.; Soldatov, V. S. Zh Prikl Khim (in Russian) 1986, 59, 2708.
13. Soldatov, V. S.; Papova, O. P.; Shunkevich, A. A.; Krul, L. P.; Zonov, Yu. G. Inst Fiz-Org Khim Vysokomol Soedin Ser B (in Russian) 1988, 30, 602.
14. Battaerd, H. A. J.; Tregear, G. W. Graft Copolymers; Wiley-Interscience: New York, 1967.
15. Potts, J. T., Jr.; Tregear, G. W.; Keutmann, H. T.; Niall, H. D.; Sauer, R.; Deftos, L. J.; Dawson, B. F.; Hogan, M. L.; Aurbach, G. D. Proc Natl Acad Sci USA 1971, 68, 63.
16. Tregear, G. W.; Van Rietschoten, J.; Greene, E.; Keutmann, H. T.; Niall, H. D.; Reit, B.; Parsons, J. A.; Potts, J. T., Jr. Endocrinology 1973, 93, 1349.
17. Tregear, G. W.; Van Rietschoten, J.; Greene, E.; Niall, H. D.; Keutmann, H. T.; Parsons, J. A.; O'Riordan, J. L. H.; Potts, J. T., Jr. Hoppe-Seyler's Z Physiol Chem 1974, 355, 415.
18. Tregear, G. W. In Chemistry and Biology of Peptides; Meienhofer, J., Ed.; Ann Arbor Science: Ann Arbor, MI, 1972; p 175.
19. Poulsen, K.; Burton, J.; Haber, E. Biochemistry 1973, 12, 3877.
20. Pande, C. S.; Gupta, N. J Appl Polym Sci 1996, 62, 1793.
21. Pande, C. S.; Gupta, N. J Appl Polym Sci 1997, 66, 847.
22. Pande, C. S.; Gupta, N. J Appl Polym Sci 1999, 71, 2163.
23. Pande, C. S.; Gupta, N. J Int Acad Phys Sci 1997, 1, 19.
24. Pande, C. S.; Mehta, I. K.; Ambasta, B. K.; Archna, M. K. J Appl Polym Sci 2000, 77, 475.
25. Pande, C. S.; Single, S.; Gupta, N. J Appl Polym Sci 1995, 58, 1735.
26. Mueller-Schulte, D. Ger Pat Derwent Biotech Abstr 1993, 05954.