GRAFT COPOLYMERIZATION OF VINYL MONOMERS ONTO COLLAGEN—PART I: PHLOXINE SENSITIZED GRAFT COPOLYMERIZATION IN THE PRESENCE OF VISIBLE LIGHT

T. NAGABHUSHANAM, K. PANDURANGA RAO, K. THOMAS JOSEPH & M. SANTAPPA*

Central Leather Research Institute, Madras

Received on April 24, 1973

Dye-sensitized photochemical graft copolymerization of vinyl monomers has been studied in aqueous medium using monochromatic light of λ = 546 nm. Oxygen was found to reduce the degree of grafting. Depending upon the experimental conditions 18-40% of grafting was achieved. Poly(methyl methacrylate) gave higher % of grafting when compared with poly(methylacrylate) demonstrating that grafting reaction depends on the type of the vinyl monomer used. Proof of grafting was obtained by infra-red spectra of collagen-vinyl graft copolymers after enzymatic digestion. It is suggested that the excitation of the dye to the singlet excited state followed by the transition to the triplet state by intersystem crossing may result in the abstraction of hydrogen from the collagen, creating active centres for the initiation of graft copolymerization of vinyl monomers.

INTRODUCTION

In the last decade dyes have been successfully used as the photosensitizers for homopolymerization reactions by various workers. [1-4]. In recent years, grafting of vinyl monomers on the biopolymers e.g. collagen, cellulose and wool in the presence of dyes [5-7] and redox systems [8-15] has gained much impetus. In all these studies the presence of oxygen depended on the photosensitizer used. The present investigation consists of the photo-graft copolymerization of acrylic monomers onto collagen dispersed in aqueous medium, using visible light. Treatment of proteins with high energy radiation leads to rupture of the polypeptide backbone. However, in dyesensitized systems using low energy radiation, the degradation of the backbone is almost negligible;[16] hence visible light may be advantageously used for grafting vinyl monomers to proteins such as collagen.

The aim of the present investigation was to graft-copolymerize vinyl monomers onto collagen using low energy radiation. An attempt has also been made to provide proof of grafting by infra-red spectroscopy.

EXPERIMENTAL

1. *Optical arrangements*

The light source used was a 250-W high pressure mercury vapour lamp (Mazada box type, B.T.H. Co., U.K.) fitted with a glass window. The lamp gave a steady light intensity when connected through a stabilizing choke. With the help of a quartz condenser lens, it was possible to produce a nearly parallel beam. The lens was mounted on an optical bench along with cells containing filter solutions to isolate the required wavelength. The filter solutions necessary

* Department of Physical Chemistry, University of Madras, Madras-25. Present address: Director, CLRI.

to isolate the required wavelength used in this investigation were prepared according to Bowen.[17]

For $\lambda = 546$ *mm*

A 10 ml. portion of $CuCl_2 \cdot 2H_2O$ (100 g. in 1 litre of water) mixed with 90 ml. calcium chloride (33 g. anhydrous salt in 1000 ml. of water) taken in one cell and neodimium nitrate (3 g. in 100 ml. of water) in the other cell (1 cm.) were put in the light path to isolate the light of $\lambda = 546$ nm.

A parallel beam of light consisting of monochromatic radiation is allowed to fall on the quartz window of a metal thermostat, inside which the reaction cell was mounted. A cylindrical cell 4.6 cm. long (in the direction of the beam) and 5 cm. in diameter, with outlet tubes of standard B-14 taper was used as the reaction vessel.

2. *Reagents*

(a) *Collagen*: Collagen prepared [7a] from the middle corium of buffalo hide was used as the source of insoluble collagen.

(b) *Monomers*: Methyl methacrylate (MMA) and methyl acrylate (MA) obtained from Rohm & Haas, USA, were purified by standard methods as previously described.[1]

(c) *Dye*: The dye Phloxine (BDH) was microscopically pure and was used in this investigation without further purification.

(d) *Enzyme*: Pronase B grade (Cal Biochem; USA) was used without further purification.

All solutions were prepared using water which had been distilled twice over alkaline permanganate (in an all-glass apparatus) and passed through Biodeminrolite resin. (Permutit, U.K.)

3. *Preparation of collagen-vinyl graft copolymers*

An aqueous dispersion of collagen (100 ml.) containing dye (1×10^{-5} M), 2% acrylic monomer and 2-3 g. collagen (hide powder) was placed in the reaction vessel into which oxygen-free[18] nitrogen was passed for 30 minutes. The reaction vessel was then mounted inside a thermostat maintained at 30 ± 0.1°C by toluene regulator and a hot wire vacuum switch relay (Gallenkamp, U.K.) Nitrogen gas was bubbled through the reaction mixture during irradiation. Monochromatic light of $\lambda = 546$ nm (dye $\lambda_{max} = 530$ nm) was used for irradiation. After irradiation the resulting products were separated by filtration, washed with distilled water and extracted with appropriate organic solvents to remove loosely bound homopolymer. In all experiments, care was taken to prevent stray light falling on the contents of the reaction vessel.

4. *Analytical methods*

The determination of % grafting and isolation of the grafts by pronase digestion were carried out as described in previous papers.[8-15] The infra-red spectra of the grafts isolated by enzymatic hydrolysis and the corresponding homopolymers were measured with a Perkin-Elmer Model 337 grating infra-red spectrophotometer in the form of potassium bromide (KBr) pellets (500 mg.) containing 2-6 mg. powdered polymers.[16a]

RESULTS AND DISCUSSION

Photo-excited dyes were reported to be effective in photo-tendering reactions of biopolymers, producing active centres on the biopolymer itself.[6] These active centres can be utilized for the grafting of vinyl

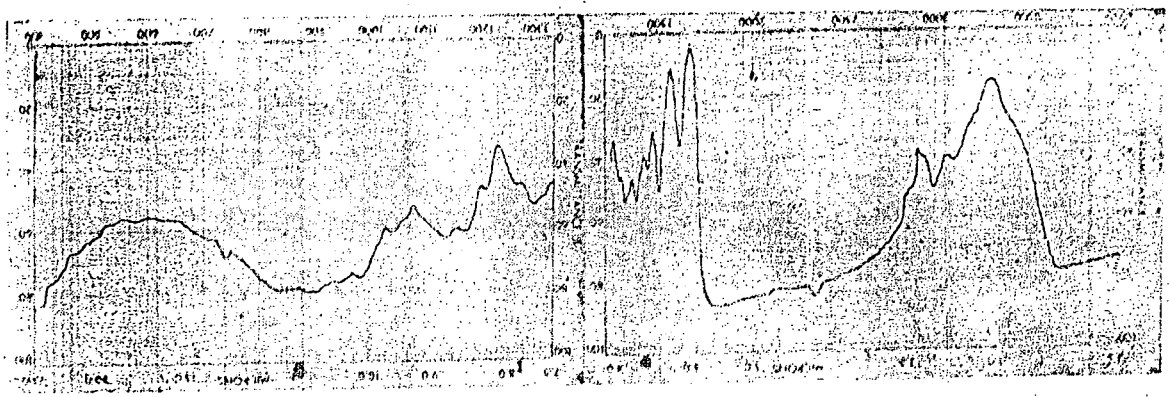

Fig. 1. Infrared spectrum of untreated collagen film.

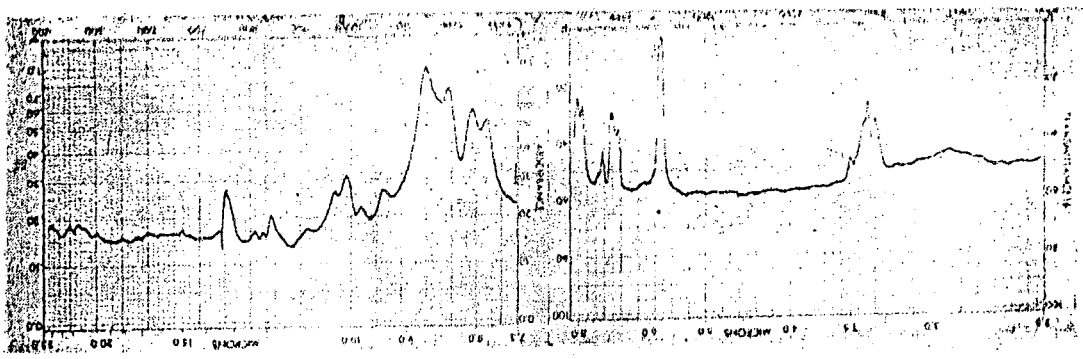

Fig. 2. Infrared spectrum of PMMA Homopolymer prepared by the ceric ion method.

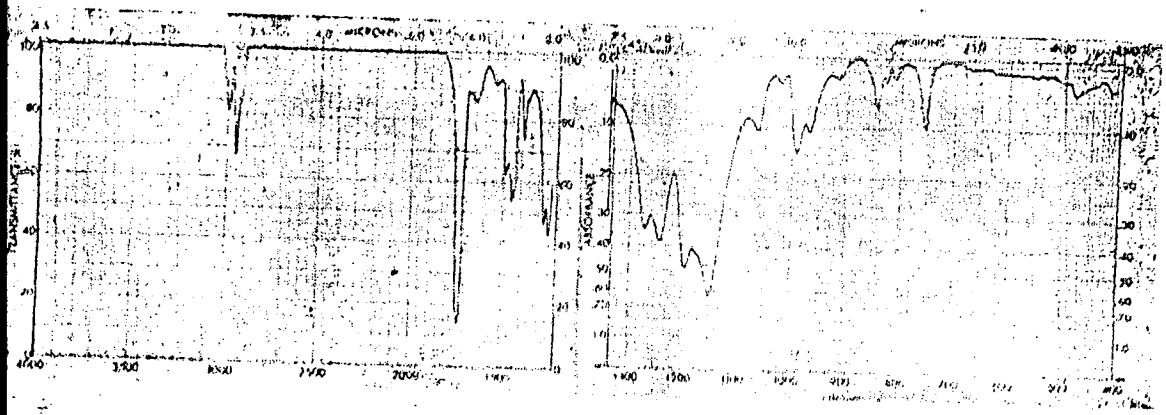

Fig. 3. Infrared spectrum of PMMA grafts isolated from collagen — PMMA graft copolymer by pronase disgestion. (Ferric ion method).

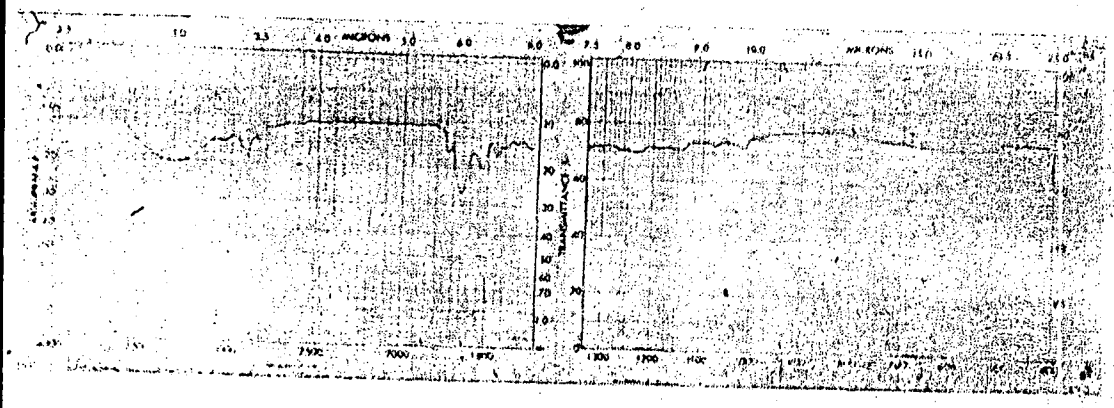

Fig. 4. Infrared spectrum of PMMA grafts isolated from collagen — PMMA graft copolymer by pronase digestion. (Photo-induced method).

monomers. In the present investigation, phloxine, a fluorescein dye was found to be effective as a photosensitizer for the graft copolymerization of methyl methacrylate and methylacrylate. Both in the presence of oxygen and in its absence, graft copolymerization took place. In the latter case, the % grafting was higher (Table 1). The

TABLE 1

COMPOSITION OF COLLAGEN-GRAFT COPOLYMERS PREPARED BY VISIBLE LIGHT IRRADIATION IN THE PRESENCE OF DYE (PHLOXINE)

Sample	Time of irradiation in hours	% Nitrogen	% Collagen	% Polymer in grafted product	% Grafting
Control	2½	18.47	—	—	—
HP_2-1	2	14.96	84.07	15.93	18.95
HP_2-2	2½	12.39	69.55	30.45	43.82
HP_2^1-1	2	14.85	83.41	16.59	19.89
HP_2^1-2	2½	12.70	71.34	28.66	40.17
HP_2^2-3*	2½	15.34	86.20	13.80	16.02
HP_2^2-4**	2½	17.34	97.40	2.60	2.70

*in the presence of oxygen.
**vinyl monomer used is methyl acrylate. In all other experiments methyl methacrylate was used.
HP_2 Hide powder sample 1.
HP_2^1 Hide powder sample 2.
Control contains no monomer.

lower value of % grafting in the presence of oxygen may be attributed to the retarding effect of oxygen on the graft copolymerization. Increase in the time of irradiation leads to higher % grafting in the two different preparations of collagen (HP_2^1, HP_2^2) used in this investigation (Table 1). Similar results were reported for the grafting of vinyl monomers to collagen photosensitized by iodocosine.[7] The % grafting was also found to be higher (Table 1) in the case of methyl methacrylate than for methyl acrylate, showing that graft copolymerization depends upon monomer type.[6]

The infra-red spectra of polyvinyl side chains isolated from graft copolymers by pronase digestion were expected to give additional proof of grafting. When the products are true graft copolymers, the infra-red spectra of the isolated side chains will give typical bands of amino acid residues and those of grafted polymer chains. The infra-red spectra of unreacted collagen and that of homopolymer (PMMA) are given in Figures 1 and 2. In the grafted side chains isolated by pronase digestion the characteristic absorption bands for the amide groups can be seen (1550 & 1660 cm^{-1}) along with absorption bands for PMMA (1730 cm^{-1}). It was reported earlier[15] that enzymatic digestion of the collagen-vinyl graft copolymers produces longer fragments of the collagen main chain attached to the end of the isolated grafts. Hence, the absorption bands for the peptide amide bands will be more prominent in these cases. The extent of hydrolysis of denatured collagen also depends on the nature of the enzyme. Pronase was used in the present investigation to digest the collagen since it has a much broader reaction than pepsin or trypsin.

Comparison of the infra-red spectra of isolated graft copolymers prepared by thermal methods (initiated by the ceric ion) and photochemical methods (initiated by

phloxine) shows that the amide absorption bands are more prominent in the latter case (Figs. 3 & 4).

This may be due to the fact that the molecular weights of the polyvinyl-grafted chains were smaller and the concentration of the attached amino acid residues were consequently more in this case.

Further, we have observed no reaction when dye and monomer were irradiated. However, when ascorbic acid was present, polymerization occurred showing that the dye was effective in the presence of such a reducing agent. Similarly, when ascorbic acid was substituted by collagen (Table 1, Fig 3) graft copolymerization took place showing that collagen functioned both as a reducing agent and an active site for graft copolymerization. From the control experiment, it may be concluded that no degradation of the collagen backbone takes place when it is irradiated by visible light and in the presence of dye. From these observations, we propose a mechanism similar to that suggested by earlier workers[6], consisting of excitation of the dye to the singlet excited state, followed by transition to the triplet state by intersystem crossing. This in turn abstracts hydrogen from the collagen backbone so creating active sites for the initiation of graft copolymerization. Further work to elucidate the reaction mechanism using ESR techniques is in progress.

REFERENCES

1. Nagabhushanam, T. & Santappa, M., *J. Polym. Sci.*, A-1, **10**, 1511 (1972).

2. Anwaruddin, Q. & Santappa, M., *J. Polym. Sci.*, A-1, **7**, 1315 (1969).

3. Sheriff, A. I. Md. & Santappa, M., *Proc. Indian Acad. Sci.*, **62**, 56 (1965).

4. Toppet, S., Delzenne, G. & Smets, G., *J. Polym. Sci.*, A-1, **2**, 1539 (1964).

5. Geacintov, N., Stannett, V., Abrahams, E. W. & Hermans, J. J., *J. Appl. Polym. Sci.*, **3**, 54 (1960).

6a. Needles, H. L. & Wasley, W. L., *Text. Res. J.*, **39**, 97 (1969).

6b. Needles, H. L., *J. Appl. Polym. Sci.*, **12**, 1557 (1968).

7. Kudaba, J., Cizicenaite, E. & Jakubenaite, V., *Vysokomolek Soedin*, **12**, 818 (1970).

8. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *Leath. Sci.*, **16**, 401 (1969).

9. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *J. Polym. Sci.*, A-1, **9**, 3199 (1971).

10. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *Leath. Sci.*, **19**, 27 (1972).

11. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *Leder*, **19**, 77 (1968).

12. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *Kozarstvi*, **22**, 36 (1972).

13. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *Leath. Sci.*, **14**, 73 (1967).

14. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., Under publication.

15. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *J. Appl. Polym. Sci.*, **16**, 975 (1972).

15a. McCall, E. R., Miles, S. H. & O'Connor, R. T., *Amer. Dyestuff Rptr.*, **56**, 13 (1967).

16. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *J. Sci. Ind. Res.*, **29**, 559 (1970).

17. Bowen, E. J., *Chemical Aspects of Light*, Oxford Univ. Press, London, 1946, p. 279.

18. Fieser, L. F., *J. Am. Chem. Soc.*, **46**, 2639 (1924).