GRAFT COPOLYMERIZATION OF VINYL MONOMERS ONTO COLLAGEN PART III – CHARACTERIZATION OF GRAFT COPOLYMERS AND THE MECHANISM OF GRAFT COPOLYMERIZATION

T. NAGABHUSHANAM, K. T. JOSEPH & M. SANTAPPA

Central Leather Research Institute, Madras.

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Molecular weight and grafting sites of the poly (methyl methacrylate) side chains isolated from collagen-g-poly (methyl methacrylate) samples by acid hydrolysis are determined. Molecular weights of the polymers were in the order of $10.^5$ The number of grafting sites did not show any significant change with the time of exposure.

Introduction

In comparison with cellulose and starch, proteins have been largely neglected as substrates for the chemically initiated grafting of vinyl polymers. Investigations of graft copolymerization of hide collagen with a variety of vinyl monomers using different initiating systems have been going on in our laboratory for the past decade.[1-3] In the last few years several papers have been published reporting various graft copolymers obtained by employing several techniques for generating free radicals on the collagen molecule for initiation of polymerization. In two previous communications[4,5] the grafting of vinyl polymers onto the collagen backbone in the presence of light, using fluorescein dyes has been reported. In these studies (involving different fluorescein dyes), erythrosine was found to be the best sensitizer. In the present investigation an attempt has been made to determine the molecular weights of the grafted poly (methyl methacrylate) side chains isolated by acid hydrolysis from collagen grafted with vinyl polymers using three different sensitizers. The number of grafting sites on the collagen backbone has also been determined and the possible mechanisms of the initiation are discussed.

Experimental

(a) *Grafting procedure*: The collagen-g-poly (methyl methacrylate) samples were prepared as described in earlier papers.[5]

(b) *Hydrolysis*: The hydrolysis of the graft copolymers were carried out using hydrochloric acid for molecular weight determination. Pronase digestion was carried out for the poly (methyl methacrylate) side chains for infrared spectra.

(c) *Molecular weight*: The molecular weight of the poly (methyl methacrylate) side chain obtained from collagen-g-poly (methyl methacrylate) samples was determined using a PCL suspended dilution viscometer.[2] The number average molecular

weight, $\bar{M}n$, was calculated using the equation

$[\eta] = 8.69 \times 10^{-6} \bar{M}n^{-70}$ in benzene for poly (methyl methacrylate)

(d) *Grafting sites*: The number of grafting sites on the collagen backbone was calculated by the equation reported earlier.[1]

(e) *Infra-red spectra*: To provide proof of grafting the infrared spectra of the grafts isolated by enzymatic (pronase) hydrolysis were taken with a Perkin Elmer Model 337 grating infra-red spectrophotometer in the form of potassium bromide pellets (500 mg.) containing 2-6 mg. powdered protein.[5]

Results and Discussion

In Table I are given the molecular weights of poly (methyl methacrylate) grafts isolated from collagen-g-poly (methyl methacrylate) samples prepared by erythrosine, rosebengal and phloxine sensitizers. In general, the magnitude of the order of molecular weights of the side chain poly (methyl methacrylate) is the same (about 10^5) (Table I) for all the graft copolymers prepared by the three sensitizers. These results show that the type of the sensitizer has no significant effect on the length of the poly (methyl methacrylate) chain grafted onto the collagen backbone. Similar results were also obtained by Huang *et al*[6] in the case of grafting of poly (methyl methacrylate) on cellulose. The molecular weight data obtained in the present study are in agreement with the values reported by investigators which are mostly of the order of 10^5. The results (Table I) also indicate that in the initial stages of graft-copolymerization, the sensitizers initiated a large number of growing poly methyl methacrylate branches, in agreement with Huang *et al*.[6] However, independent of the time of grafting, the number of grafting sites remains more or less the same. This indicates that initially the available active centres will be more and the grafting of poly (methyl methacrylate) will thus be more. But on further increase in time of exposure, the available active sites on collagen backbone being the same, the molecular weight and the percent grafting of poly (methyl methacrylate) will be increased (Table I). The infra-red spectra of the poly (methyl methacrylate) grafted side chains obtained by the pronase digestion are given in Figures 1 to 3. The characteristic peak for the poly (methyl methacrylate) side chains centered at (1730 cm^{-1}) can be seen along with the amide absorption (1550 cm^{-1}, 1660 cm^{-1}) which provide the proof of grafting.

In the light of the experimental results three mechanisms can be discussed. (1) The dye becomes excited to the higher energy level followed by transition to the triplet state by inter system crossing, which in turn activates the substrate collagen. (2) A hypothetical complex between the dye and collagen may be formed in the dark, which upon excitation decomposes to give rise to the activated collagen. (3) The excited dye may transfer its excitation energy to the collagen which in turn may decompose to give rise to the active substrate. The fact that the concentration of the dye has decreased after exposure, in the total system or when the dye and collagen only are exposed, indicates that the dye is consumed in the reaction. Hence the first mechanism may be operative. Evidence in support of the second mechanism could not be obtained due to the heterogeneity of the system. The third mechanism does not become possible because, if the energy is transferred from the excited dye to the collagen, there should be no decrease in concentration of the dye, which is contrary to our observations. Hence, probably the first mechanism of initiation may be operative, in agreement with the work of Needles *et al*.[7]

TABLE 1

The number of grafting sites and the molecular weights of the grafted side chains of poly(methyl methacrylate) on collagen

Sample No.	Time of exposure Noon — p.m.	NATURE OF THE DYE USED								
		Erythrosine			Rosebengal			Phloxine		
		% grafting	Mol. wt. x 10^{-5}	No. of grafting sites	% grafting	Mol. wt. x 10^{-5}	No. of grafting sites	% grafting	Mol. wt. x 10^{-5}	No. of grafting sites
1	12 —	34.50	1.148	0.9228	2.6	1.472	0.0530	0.95	4.276	0.0067
2	12 — 3	34.53	4.770	0.2735	0.84	0.4634	0.0485	2.01	5.861	0.0103
3	12 — 4	53.55	6.607	0.2375	3.70	2.203	0.05174	2.21	8.126	0.0083
4	2 p.m. — 4 p.m.	51.07	5.140	0.2990	10.50	3.428	0.09183	5.10	6.330	0.0024

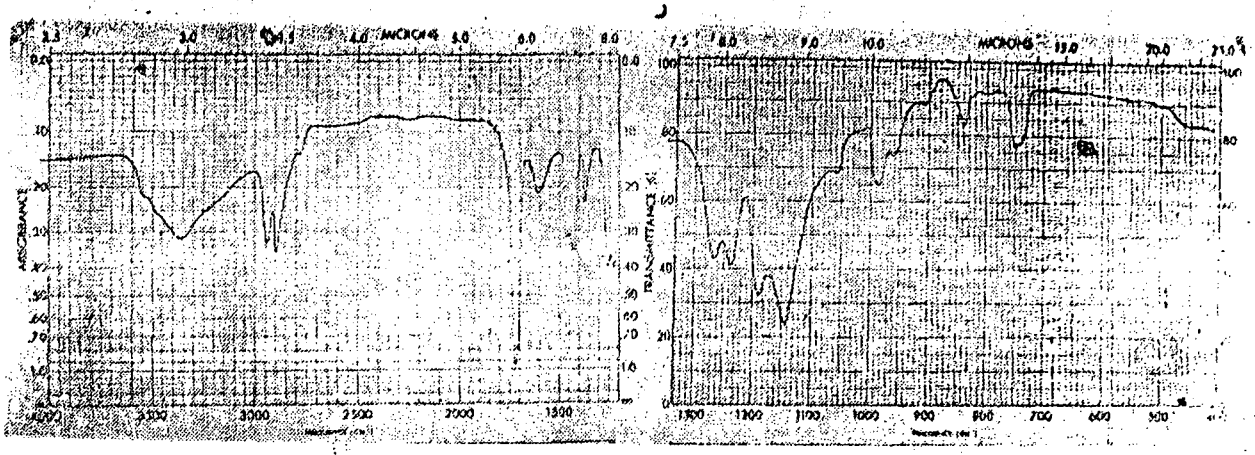

Fig. 1. Infra-red spectrum of poly (methyl methacrylate) side chains prepared by using erythrosine as the *initiator*.

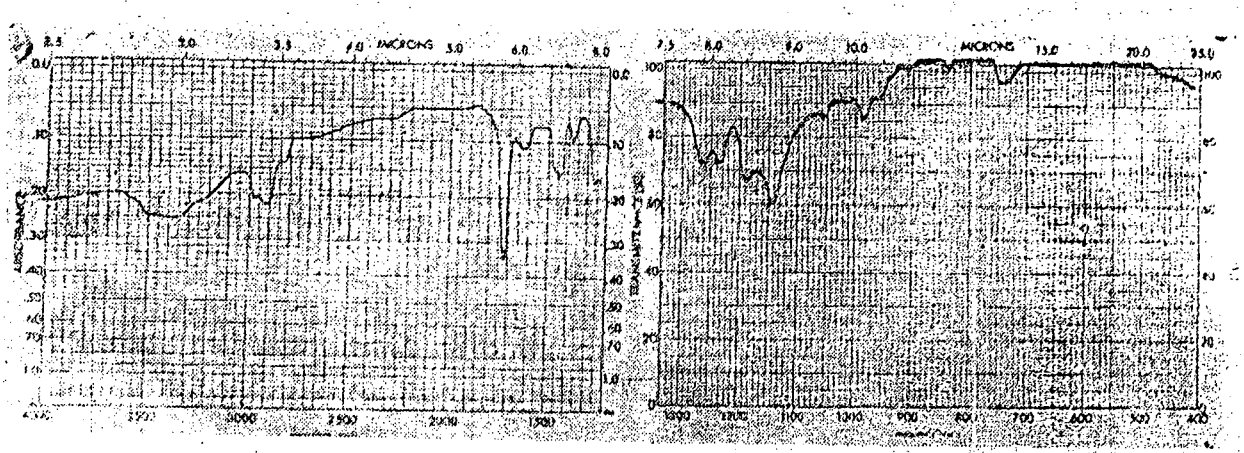

Fig. 2. Infra-red spectrum of poly (methyl methacryate) side chains prepared by using rosebengal as the *initiator*.

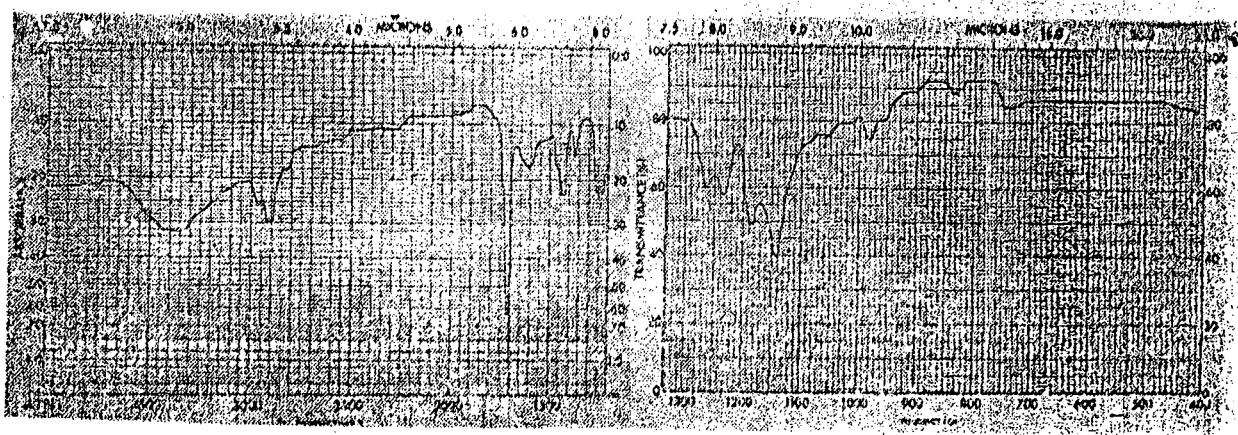

Fig. 3. Infra-red spectrum of poly (methyl methacrylate) side chains prepared by using phloxine as *initiator*.

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