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PHOTO-INDUCED GRAFT POLYMERIZATION OF METHYL METHACRYLATE ONTO COLLAGEN IN THE ABSENCE OF PHOTSENSITIZERS USING SUNLIGHT AS A LIGHT SOURCE

K. SATISH BABU, K. PANDURANGA RAO, K. THOMAS JOSEPH, M. SANTAPPA & Y. NAYUDAMMA*

Central Leather Research Institute, Madras.

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The photo-induced graft polymerization of methyl methacrylate (MMA) to collagen was carried out in aqueous methanol without photosensitizer in the presence of air using direct sunlight as a light source. Direct sunlight of wavelengths longer than 300 nm was effective as light source and grafting proceeded very rapidly and efficiently after an induction period of an hour. The percent grafting, efficiency of grafting and the average molecular weights of grafted branches were found to be highest at the lower methanol concentrations. However, grafting frequency values were almost identical after a particular interval of time and increased with polymerization time.

The successful grafting of polymers onto collagen involves a free radical mechanism by means of which activated sites are produced on the peptide chains of collagen. Free radicals are electron-deficient systems and because of their extreme reactivity initiate polymerization of vinyl monomers. They may be produced on the peptide backbone in a variety of ways such as chemical-redox systems and radiation methods. The properties of graft copolymers depend on such factors as the monomer used, the type of initiator used and the molecular weight of grafted polymers. The initiation of free-radicals by means of oxidising agents such as Ce⁴⁺ and Mn²⁺ which are capable of attacking collagen directly has been studied in

detail.¹⁻³ Other chemical-redox methods and photo-induced irradiation methods^{10,11} have also been investigated in order to compare the effect of the different initiating systems on the composition of collagen-vinyl graft copolymers.

Sunlight-induced graft copolymerization of vinyl monomers onto wool and nylon fibres has been reported by some investigators.^{12,13} Since sunlight is abundantly available in India, it was thought pertinent to study the photo-induced graft copolymerization of vinyl monomers onto the collagen backbone in relation to chemical-redox methods and irradiation methods. In this paper, the preparation of collagen-methyl methacrylate (MMA) graft copolymers and the effects of various experimental factors, such as time and methanol concen-

*Present Address: Director-General, Council of Scientific & Industrial Research, New Delhi.

tration on the composition of the graft copolymers have been investigated.

Experimental

Materials

Collagen : Collagen prepared from the middle corium of buffalo hide was used as the source of insoluble fibre substrate.

Monomer : Methyl methacrylate (MMA) obtained from Rohm & Haas, USA was purified by standard methods as described earlier.^{2,3}

Enzyme : Pronase, B-grade (Cal-Biochem, USA) was used without further purification. Other chemicals used were reagent grade.

Grafting procedure

The graft copolymerization reactions were carried out in round bottomed single-necked flasks of one litre capacity and fitted with a glass stopper, in the presence of air. The collagen powder was dispersed in aqueous methanol solution to which a known quantity of monomer (methyl methacrylate) was added. Sunlight irradiation was carried out intermittently on the roof at Central Leather Research Institute during the daytime of fine days in July. The reaction flasks were occasionally stirred and the reaction was allowed to proceed for definite intervals of time. The temperature of the reaction mixture was occasionally noted. After the irradiation, the products were separated by filtration, washed with distilled water and extracted with appropriate organic solvents to remove loosely bound homopolymer. The homopolymer which could not be extracted was considered as the grafted polymer.

Calculations

The percent grafting, the efficiency of grafting, rate of grafting and the number of grafting sites were calculated according to:

$$\text{Percent grafting} = \frac{\text{Total weight of grafted copolymer} - \text{weight of collagen}}{\text{Weight of collagen}} \times 100$$

$$\text{Efficiency of grafting} = \frac{\text{Weight of PMMA grafted}}{\text{weights of PMMA grafted and homo-polymer}} \times 100$$

Rate of grafting in %/hr. was converted to R_p by use of the equation

$$R_p = \frac{\%/\text{hr} \times a \times \text{mole}}{\text{wt. of monomer}} \times \frac{1}{3600} \text{ mole/l/sec.}$$

where 'a' is g. of collagen per litre of monomer — water — collagen mixture.

$$\text{No. of grafting sites} = \frac{\text{Weight of PMMA/Mol. wt. of PMMA}}{\text{Weight of used collagen/Mol. wt. of collagen}}$$

Isolation of poly (methyl methacrylate) side chains

The grafted PMMA side chains were isolated from collagen trunk by acid hydrolysis with hydrochloric acid and also by digestion with pronase according to the procedures given in our earlier papers.^{2,3}

Molecular weight determinations

The intrinsic viscosity of the PMMA grafts isolated by acid hydrolysis was determined in benzene at 30°C using a PCL suspended level dilution viscometer. The

number average molecular weight was calculated from the equation¹⁴
 $[\eta] = 8.69 \times 10^{-5} \bar{M}_n^{0.76}$

Number average degree of polymerization (P_n) of PMMA was calculated from the intrinsic viscosity measured in benzene at 30°C by using the following equation¹⁵
 $\log P_n = 3.346 + 1.32 \log [\eta]$

Spectral measurements

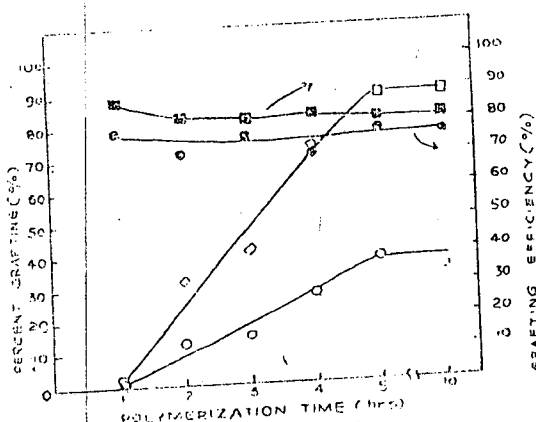
The infrared spectra were recorded on a Perkin-Elmer Model 337 grating infrared spectrophotometer in the form of potassium bromide (KBr) pellets as reported earlier.⁶

Results & Discussion

Solvent effects:

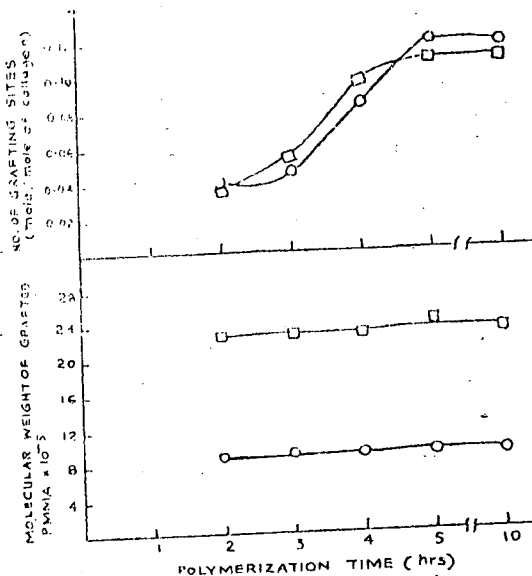
The results of photo-induced graft copolymerization of MMA on collagen in aqueous solutions containing 50 and 20 volume percent MeOH as medium are shown in Tables 1-3 and Figs. 1 & 2.

Fig. 1



Relation of polymerization time to percent grafting [higher methanol concentration (O), lower methanol concentration (□)] and grafting efficiency [higher methanol concentration (O) lower methanol concentration (□)]

Fig. 2



Plots of molecular weight of grafted PMMA and number of grafting sites vs. grafting time for grafting of MMA onto collagen. Higher methanol concentration (O), lower methanol concentration (□).

As can be seen from Table 1 and Fig. 1, with increase in the amount of water the monomer conversion to polymer was much higher unlike in the case of more methanol and less water. It is likely that the presence of water in the system leads to a strong dipole association and dispersed water molecules increase the hydroxyl and peroxide centers thereby increasing grafting.

Percent grafting and grafting efficiency

The percent grafting and the efficiency of grafting were observed to be lower with the higher concentration of methanol (Fig. 1, Tables 1 & 2). Although the efficiency of grafting was found to remain constant the percent grafting increased with grafting time for both the sets of experiments and reached a limiting value after 5 hrs. In both the cases, only traces

TABLE I
Composition of collagen—MMA graft copolymers prepared by sunlight—
induced grafting

Sample No.	Volume solvent (ml.)		Irradiation time (hr.)	Composition of graft		
	Water	CH ₃ OII		Collagen (%)	PMMA (%)	Grafting (%)
1S ₁	250	250	1	98.24	1.76	1.825
2S ₁	"	"	2	88.88	11.12	12.47
3S ₁	"	"	3	87.12	12.88	14.82
4S ₁	"	"	4	79.02	20.98	26.54
5S ₁	"	"	5	72.23	27.77	38.49
6S ₁	"	"	10	74.49	25.51	34.25
1S ₂	400	100	1	99.65	0.35	0.684
2S ₂	"	"	2	75.48	24.52	32.48
3S ₂	"	"	3	71.42	28.58	40.15
4S ₂	"	"	4	57.37	42.63	73.89
5S ₂	"	"	5	53.35	46.65	87.42
6S ₂	"	"	10	53.36	46.64	86.72

TABLE 2
Effect of irradiation time on the rate of grafting and efficiency of grafting

Sample No.	Irradiation time (hr.)	Grafting (%)	Efficiency of grafting (%)	Rate of grafting $R_p \times 10^2$
1S ₁	1	1.825	78.36	2.535
2S ₁	2	12.47	71.12	8.660
3S ₁	3	14.82	76.63	6.863
4S ₁	4	26.54	71.03	9.237
5S ₁	5	38.49	77.91	10.690
6S ₁	10	34.25	75.90	4.756
1S ₂	1	0.684	87.56	0.95
2S ₂	2	32.48	82.12	22.56
3S ₂	3	40.15	81.85	18.59
4S ₂	4	73.89	83.30	25.65
5S ₂	5	87.42	80.48	24.29
6S ₂	10	86.72	81.10	12.04

TABLE 3

Effect of Irradiation time on the Molecular weights of grafted Poly (MMA) branches and on the number of grafting sites

Sample No	Irradiation time (hr)	Grafting (%)	Intrinsic Viscosity (η)	Mol. wt. of grafted PMMA chains $M \times 10^{-3}$	Degree of Polymerization $P_n \times 10^{-3}$	No. of grafting sites mole/mole
1S ₁	1	1.825	—	—	—	—
2S ₁	2	12.47	2.8	8.933	9.042	0.04187
3S ₁	3	14.82	3.0	9.354	9.458	0.04753
4S ₁	4	26.54	3.0	9.354	9.458	0.08511
5S ₁	5	38.49	3.0	9.354	9.458	0.1234
6S ₁	10	34.25	2.8	8.933	9.042	0.1153
1S ₂	1	0.684	—	—	—	—
2S ₂	2	32.48	6.0	23.07	23.66	0.04224
3S ₂	3	40.15	6.0	23.07	23.66	0.05220
4S ₂	4	73.89	6.0	23.07	23.66	0.09610
5S ₂	5	87.42	6.1	23.71	24.15	0.1106
6S ₂	10	86.72	6.0	23.07	23.66	0.1128

of PMMA homopolymer were found as a milky suspension in solution; however, part of the polymer formed inside the collagen fibres could be extracted with the solvent for the homopolymer. With increasing percent grafted polymer, the formation of homopolymer also was found to be increased. The graft copolymerisation reaction was accompanied by an induction period of one hour and proceeded gradually to attain approximately 35% and 85% grafting at 50% and 20% methanol concentrations respectively.

Molecular weights of grafted branches

PMMA side chains were isolated for molecular weight determination from the collagen trunk polymer by digesting the collagen fibres with 6N hydrochloric acid. The number average molecular weights of

the grafted chains were of the order of 10^5 and remained constant surprisingly through different intervals of grafting (Table 3). As compared with the system containing 50% MeOH, 20% MeOH gave higher molecular weights (Table 3 and Fig. 2). It is therefore apparent that methanol influences the propagation reactions of grafted polymer radicals. Methanol is hydrophilic and so able to dissolve MMA. Therefore it is reasonable to suppose that monomer solubilities increase in methanol-water solutions to stimulate monomer supply for the growing radicals and finally to increase the molecular weight. On the other hand, at the higher methanol concentration (50%) acceleration of the chain-transfer reactions take place from the radicals and the molecular weight drops owing to the activated termination react-

ions. Many investigators¹⁷ have also reported that graft copolymerization of vinyl monomers onto cellulose using irradiation methods was impossible at 100% concentration methanol. It was also suggested that in some cases the organic solvents themselves inhibit photo-induced reactions. The degree of polymerization also followed the same trend (Table 3).

Number of grafting sites

It can be seen (Table 3 and Fig. 2) that the calculated values for grafting frequency remain constant for the two methanol concentrations whereas, the molecular weights of the grafted PMMA chains showed wide variation. This indicates that the actual number of PMMA branches per mole of collagen chain remains unchanged but grafted chains of shorter length has been found in the case of higher methanol concentrations. The wide variation of the molecular weights of the grafted chains can be seen from Table 3 and Fig. 2. The decrease in the molecular weights of grafted PMMA chains with an increase in the volume of methanol used could be due to an increased dilution of the monomer as well as to an increased chain-terminating ability of the solvent system at higher methanol concentrations. However the number of grafting sites increased with grafting time in both the cases and then level off after 5 hrs.

Rate of grafting (R_p)

The rate of grafting shows (Table 2) generally an increase with time in both the concentrations of methanol up to 5 hrs. and then falls off. The rate of grafting decreases with methanol concentration. The variations in R_p values at different time intervals and methanol concentrations indicate that R_p depends, to a certain extent, on time and also on methanol concentration. The lower rate of grafting

at higher methanol concentration may be due to dilution effect of monomer in addition to chain termination reactions. Although the rate of formation of grafts was varying in each system, almost the same number of grafts was ultimately obtained for both the systems after a specific period of time.

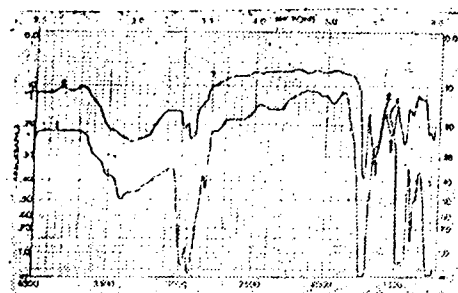
Effective wavelength

Photo-induced graft-polymerization of monomer is not governed by the irradiated light to the reaction system but by the absorbed light. Many constituents in the reaction system absorb light of different wavelengths and therefore reaction is affected by the wavelength of the absorbed light. The shorter the wavelength the longer the energy of the photon, thus ultraviolet light with a shorter wavelength has high reactivity. Therefore, it has an ability to accelerate monomer polymerization and photodegradation. Using low energy, the degradation of the backbone is almost negligible and hence visible light may advantageously be used for grafting vinyl monomers to proteins such as collagen. Earlier studies^{16,17} of photochemical graft polymerization in the absence of photosensitizers on wool, nylon and cellulose indicated that visible light of wavelength between 300 and 380 nm is very effective for grafting, compared with light of shorter wavelengths below 300 nm which showed very small percent grafting. Hence in the present investigation the photo-induced graft polymerization of MMA to collagen fibres was carried out without photosensitizers by using direct sunlight which emitted mainly the light of wavelength longer than 300 nm.

Infrared spectra

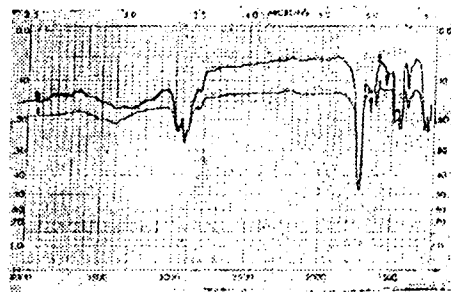
Proof of grafting has been provided by infrared spectra (Fig.3). The infrared

Figure - 3
(a)



Infrared spectrum of PMMA grafted chains isolated from collagen - PMMA graft copolymer [at higher methanol concentration]. 1) by acid hydrolysis (2) by pronase digestion

(b)



Infrared spectrum of PMMA grafted chains isolated from collagen - PMMA graft copolymer [at lower methanol concentration.] 1) by acid hydrolysis 2) by pronase digestion.

spectra of PMMA side chains isolated from graft copolymers by pronase digestion were expected to give proof of grafting. When the products are true graft copolymers, the infrared spectra of the isolated PMMA side chains will give typical bands of amino acid residues in addition to those of grafted PMMA side chains. In the grafted PMMA side chains isolated by pronase digestion the characteristic absorption bands for amide groups can be seen (1550 and 1660 cm^{-1}) along with absorption bands for PMMA (1730 , 1450 , 1150 and 790 cm^{-1}). Grafted side chains isolated by acid hydrolysis did

not show prominent amide absorption bands for reasons explained in earlier papers.⁴

Grafting mechanism

Graft polymerization without the use of a sensitizer differs from sensitized graft polymerization. In the former case grafting may be initiated by the excitation of collagen backbone, monomer (MMA) or both, and also by subsequent interaction between excited entities. Accordingly the combination of backbone substrate, monomer and the wavelength of exciting light plays an important role in the grafting reaction. Oxygen also plays a major role in the grafting mechanism in the case of nonsensitized graft polymerization of MMA to collagen. It was reported¹⁴ particularly in the regions of longer wavelength (sunlight, tungsten lamp) that oxygen was indispensable for the graft polymerization. For these reasons the present grafting experiments were all carried out in the oxygen atmosphere.

REFERENCES

1. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *Leder*, 19, 77 (1968)
2. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *Leath. Sci.*, 14, 73 (1967)
3. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *Leath. Sci.*, 16, 401 (1969)
4. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *Leath. Sci.*, 19, 27 (1972)
5. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *J. Poly. Sci. A-1*, 9, 3199 (1971)
6. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *J. Appl. Poly. Sci.*, 16, 975 (1972)
7. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *Makromol. Chem.*, 175, 729 (1974)
8. Panduranga Rao, K., Thomas Joseph, K. & Nayudamma, Y., *J. Sci. Ind. Res.*, 29, 559 (1970)
9. Satish Babu, K., Panduranga Rao, K., Thomas Joseph, K., Santappa, M. & Nayudamma, Y., *Leath. Sci.*, 21, 261 (1974)

10. Nagabhushanam, T., Panduranga Rao, K., Thomas Joseph, K. & Santappa, M., *Leath. Sci.*, 20, 301 (1973).
11. Nagabhushanam, T., Thomas Joseph, K. & Santappa, M., *Leath. Sci.*, 20, 403, (1973).
12. Ishibashi, H., *Kobunshi Kagaku.*, 23, 620 (1966).
13. Ishibashi, H. & Oku, M., *Proc. Int. Wool Text. Res. Conf.*, Vol. 3 (Sect. III) 385, (1965).
14. Fox, T. G., Kinsinger, J. B., Mason, H. L. & Schluete, E. M., *Polymer*, 13, 71 (1962)
15. Welch, F. J., *J. Poly. Sci.*, 61, 243 (1962)
16. Ishibashi, H & Tamaki, H., *Kobunshi Kagaku.* 24, 1 (1967)
17. Kubota, H. & Ogiwara, Y., *J. Appl. Poly. Sci.*, 15, 2767 (1971)
18. Ishibashi, H., *Kobunshi Kagaku.* 26, 331 (1968).