

Grafting onto Soluble Protein: Part III—Effect of Amines in Ceric Ion Initiated Graft Copolymerization of Ethyl Acrylate onto Gelatin†

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The effect of various amines in ceric ion initiated grafting of ethyl acrylate (EA) onto gelatin has been studied in aqueous medium. A small amount of nitric acid is found to catalyze the graft copolymerization. The amines used are ammonia, methylamine, dimethylamine, isopropylamine, *n*-butylamine, triethylamine, triethanolamine and pyridine. Optimum conditions for affording maximum grafting in the presence of Ce^{4+} -amine system as redox initiator have been evaluated. Not all amines are equally effective in promoting graft copolymerization. The reactivity of different amines towards graft copolymerization has been explained in terms of basicity, nucleophilicity and steric requirements of different amines.

Modification of fibrous proteins by graft copolymerization technique has been extensively studied¹⁻³. It has been possible to effect grafting of a number of acceptor and donor vinyl monomers as well as mixed monomers onto wool fibre using radical initiators⁴, various redox systems^{2,3}, metal chelates⁴ and gamma-irradiation⁵. Very little work has been reported on the modification of soluble proteins by graft copolymerization technique. Japanese workers have reported⁶ grafting of methyl methacrylate (MMA) onto blood protein in the presence of Bu_3B . Santappa *et al.*⁷ have been successful in effecting grafting of ethyl acrylate (EA) onto gelatin using persulphate as radical initiator.

Amines are known to promote ceric ion initiated polymerization of acrylonitrile (AN)⁸. In earlier publications from our laboratory grafting of methyl acrylate (MA)⁹ and methyl methacrylate (MMA)¹⁰ onto gelatin in the presence of ceric ion was reported. We also reported the grafting of vinyl monomers onto wool fibre in the presence of Ce^{4+} -amine system and observed that certain amines enhance percentage of grafting. Effect of amines on ceric ion initiated grafting of vinyl monomers onto soluble protein (gelatin) has not been studied. As an extension of our earlier work¹⁰ on ceric ion-initiated grafting of ethyl acrylate (EA) onto gelatin, we report in the present paper graft copolymerization of EA onto gelatin in the presence of Ce^{4+} -amines system as a redox initiator.

Materials and Methods

Gelatin (Oxoid, England) in the form of granules was used as received. Ethyl acrylate (EA) was purified as

described earlier¹¹. Ceric ammonium nitrate (BDH) was used as such. Nitric acid of known strength was used.

Graft copolymerization

Gelatin (1 g) was dissolved in hot deaerated distilled water (100 ml). The reaction mixture was brought to the desired temperature and flushed with oxygen-free N_2 gas for 20 min. A definite amount of ceric ammonium nitrate (CAN) dissolved in a definite volume of nitric acid was added to the reaction flask, followed by a known volume of amine. A slow stream of oxygen-free N_2 gas was passed through the reaction mixture and copolymerization was carried out under oxygen-free N_2 blanket with constant stirring at various temperatures. After the reaction was over, the reaction mixture was poured into acetone (400 ml) and kept for 48 hr. The grafted gelatin was filtered, extracted with acetone and treated with boiling water (150 ml) to remove unreacted gelatin, dried and finally weighed. Percentage of grafting was calculated as follows:

$$\% \text{ Grafting} = \frac{\text{Wt of grafted material}}{\text{Wt of gelatin}} \times 100$$

Evidence of grafting

(i) Grafted gelatin was hydrolyzed with 6-7.5 N HCl at refluxing temperature for 6-8 hr. All the gelatin went into solution, leaving behind a resinous mass which was identified by IR spectroscopy as poly(ethyl acrylate) (PEA).

A mixture of gelatin (1 g) and PEA (0.5 g) was prepared by stirring gelatin in an acetone solution of PEA for about 6 hr. The mixture was filtered and the residue extracted with acetone for 48 hr, when all the

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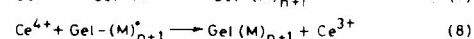
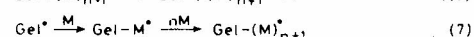
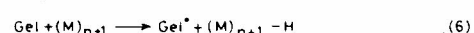
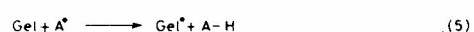
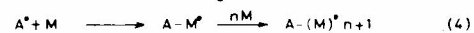
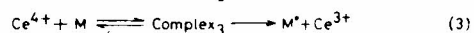
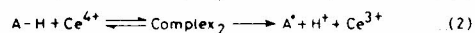
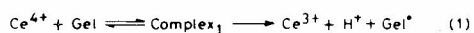
homopolymer was completely removed and the gelatin (0.9 g) recovered. The amount of gelatin recovered indicated that solvent extraction completely removed the homopolymer from the mixture of gelatin and PEA. Isolation of PEA after acid hydrolysis of grafted gelatin constituted evidence of grafting.

(ii) Infra red spectra of gelatin-g-PEA was recorded on a Perkin-Elmer infracord model 337 by swelling the graft samples in CH_2Cl_2 and then pressing the swollen material between two sodium chloride plates. The IR spectrum of this sample exhibited an intense band at 1735 cm^{-1} assigned to $\nu\text{C}=\text{O}$ of grafted PEA. No such band was observed in the IR spectrum of pure gelatin.

Results and Discussion

Ceric ion is known to effect grafting of vinyl monomers onto polyhydric alcohols such as starch^{12,13}, cellulose¹⁴ and polyvinyl alcohol¹⁵ without homopolymer formation. A variety of vinyl monomers have been successfully grafted to collagen¹⁶ and wool fibre^{2,3} in the presence of ceric ion. Gelatin primarily contains $-\text{NH}_2$ and $-\text{OH}$ groups and is soluble in water. Owing to the absence of $-\text{SS}-$ group, gelatin behaves as a linear polymer. Because of the greater accessibility of functional groups of gelatin, percentage of grafting in aqueous medium initiated by ceric ion is much higher as compared to that obtained in the case of keratin.

Amines are known to form complexes with ceric ion and such complexes have been found to promote polymerization of acrylonitrile (AN)⁸. We have observed that certain amines promote¹¹ grafting of vinyl monomers onto wool protein in the presence of ceric ion. However, not all amines are equally effective in promoting ceric ion initiated grafting. In fact certain amines have retarding effect on graft copolymerization of monomers onto wool protein. In order to explain the effect of different amines on ceric ion initiated graft copolymerization of ethyl acrylate (EA) onto gelatin, the mechanism given in Scheme 1 is proposed for grafting.



where Gel = gelatin, A-H = Amines & M = vinyl monomer.

Scheme 1

It is apparent from Scheme 1 that initiation of grafting can occur by processes (1), (5) and (6). The initiation of grafting by process (6) involves abstraction of hydrogen atom from gelatin by the growing polymeric radical. The growing polymeric chains are formed by polymerization of M by either A^* or M^* arising from the complexes between amine and Ce^{4+} and between Ce^{4+} and vinyl monomer. EA is a poor complex forming monomer with Ce^{4+} and therefore process (3) involving formation of complex₃, particularly in the presence of strong complex forming agents such as gelatin and amines may be assumed to have negligible effect. Thus initiation of grafting by hydrogen abstraction from the gelatin may be exclusively due to the growing polymeric chains that arise from the polymerization of monomer by A^* . This indicates that amines should influence grafting of EA onto gelatin. Amines may influence initiation of grafting also by process (5) which involves abstraction of hydrogen from gelatin by A^* formed by decomposition of the $\text{Ce}^{4+} - \text{AH}$ complex. In a previous paper, it has been reported that in the presence of ceric ion alone EA can be successfully grafted onto gelatin to afford maximum grafting of 420.6% under optimum conditions.

Effect of concentration and nature of amines

The ability of amines to influence grafting depends upon the ease with which the amines can enter into complex formation with electrophilic Ce^{4+} . The facility with which amines can form complexes with Ce^{4+} is dependent upon several factors, such as basicity, nucleophilicity, steric requirements and concentration of amines.

It is evident from Table 1 that there exists an optimum concentration of amines below or above which the percentage of grafting decreases. This indicates that at lower amine concentration, Ce^{4+} may interact with vinyl monomer to give homopolymer at the expense of graft. At higher concentration amines act as chain transfer agents and decrease the percentage of grafting. Besides concentration of amines, the nature of amine is also found to exert profound influence on percentage of grafting. The effect of basicity and nucleophilicity of different amines on grafting ratio has been studied and the results are presented in Table 2. A perusal of the relative grafting ratio values (Table 2) shows that different amines fall into three categories. This is expected, since in accordance with the mechanism of grafting postulated in Scheme 1, the facility with which different amines form complexes with Ce^{4+} will influence the initiation of grafting. In the first category are included isopropylamine, dimethylamine, methylamine and *n*-butylamine. All these amines enhance percentage of

Table 1—Effect of Varying Concentration of Amines on Percentage of Grafting of PEA onto Gelatin^a

Sl. No.	[Amine] × 10 ² mol dm ⁻³	% Grafting
Ammonia		
1	8.0	50.0
2	16.0	440.6
3	21.4	396.6
4	26.7	77.0
5	34.6	74.6
Methylamine		
6	1.5	150.8
7	3.0	289.0
8	4.5	482.8
9	6.0	58.2
Isopropylamine		
10	11.8	393.4
11	19.0	526.4
12	23.7	512.4
13	35.5	464.2
<i>n</i> -Butylamine		
14	1.25	428.0
15	2.05	488.4
16	4.0	334.0
17	8.1	89.4
18	12.1	157.6
Dimethylamine		
19	8.9	438.0
20	17.8	433.0
21	35.6	515.0
Trimethylamine		
22	1.4	437.0
23	2.8	421.4
24	7.1	299.0
25	14.3	38.6
26	17.2	59.6
Triethanolamine		
27	0.49	38.4
28	0.98	44.8
29	1.98	70.2
30	3.9	225.2
31	7.9	109.6
32	16.7	158.6
Pyridine		
33	9.9	194.2
34	19.8	281.2
35	29.7	406.8
36	39.6	31.8

^aGelatin = 1 g; H₂O = 100 ml; [CAN] = 10.9 × 10⁻³ mol dm⁻³; time = 90 min; [EA] = 46.0 × 10⁻² mol dm⁻³; [HNO₃] = 4.2 × 10⁻² mol dm⁻³.

grafting. The second group of amines comprising triethylamine and ammonia has marginal effect on percentage of grafting while pyridine and triethanolamine belonging to the third category exert retarding effect on percentage of grafting. It is apparent from Table 2 that highly basic amines promote

Table 2—Effect of Nature of Amines on Percentage of Grafting onto Gelatin

Sl No.	Amine	pK _b	Nucleophilic constants (n)	Maximum grafting %	Relative† grafting ratio
	None	—	—	420.6	—
1	Ammonia	4.75	4.23	440.6	1.05
2	Methylamine	3.36		482.8	1.15
3	Isopropylamine	3.37	4.68	526.4	1.25
4	<i>n</i> -Butylamine	3.39		488.4	1.16
5	Dimethylamine	3.30		515.0	1.21
6	Triethylamine	3.28	4.09	449.8	1.07
7	Triethanolamine	6.23		225.2	0.53
8	Pyridine	8.85		406.8	0.97

†Taken from references 18-20.

‡Relative grafting ratio =

Maximum percentage of grafting in presence of amine

Maximum percentage of grafting in absence of amine

grafting. This tends to indicate that more basic amines are capable of forming complexes with Ce⁴⁺ with greater facility and lead to enhancement in grafting. If such an explanation is correct then triethylamine (pK_b = 3.28) should have afforded highest relative grafting ratio. Actually maximum relative grafting ratio (1.25) is obtained with isopropylamine. This indicates that besides basicity, nucleophilicity (n) of amines assumes importance in influencing grafting. Relative grafting ratios as function of nucleophilic constants (n) of those amines available from the literature¹⁷ are also presented in Table 2. It is observed that isopropylamine with n = 4.68 affords higher percentage of grafting than triethylamine with n = 4.09, supporting the view that nucleophilicity of amines is more important than basicity in promoting grafting. Both triethanolamine and pyridine are weakly basic and therefore percentage of grafting is low. However, triethanolamine is more basic (pK_b = 6.23) than pyridine (pK_b = 8.85) and yet the percentage of grafting in the case of triethanolamine is less than that in the case of pyridine. This can be explained as follows: the oxygen atoms in the β-position considerably reduce the electron density on nitrogen atom and hence complex formation between Ce⁴⁺ and triethanolamine is not favoured. Besides, because of greater steric requirement, complex formation between triethanolamine and Ce⁴⁺ is difficult.

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