# Graft Copolymerization of Collagen with *N,N,N',N'*-Tetra(2-Hydroxypropyl) Ethylene Diamine Methacrylate by Use of Potassium Persulfate as an Initiator

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## Synopsis

Graft copolymerization of collagen with *N,N,N',N'*-tetra(2-hydroxypropyl) ethylene diamine methacrylate (THPEDM) was carried out in an aqueous medium using potassium persulfate as the radical initiator. Collagen-*g*-poly(THPEDM) was characterized by the percent yield and the copper chloride test. The percentage of grafting was determined as functions of concentration of monomer, concentration initiator, reaction time, and temperature.

## INTRODUCTION

Modification of the natural polymers like cellulose, starch, and lignin by graft copolymerization has been extensively studied.[1-4] Attempts have been made to study the graft copolymerization of collagen and gelatin with various monomers[5-9] mainly because of practical importance and interest. On the other hand several monomers that have biomedical applications have been grafted on the previously mentioned polymers. The monomer, *N,N,N',N'*-tetra(2-hydroxypropyl) ethylene diamine methacrylate[10] has shown to possess immunological activity. Since this material was tested for the immunological activity by injecting this monomer internally, the grafting of such a monomer to collagen, a biopolymer, would increase the strength of material and could be used externally in the form of bandage.

In the present investigation, we report studies on the grafting of *N,N,N',N'*-tetra(2-hydroxypropyl) ethylene diamine methacrylate,[10] a biologically active compound, on collagen in the presence of potassium persulfate as, the radical initiator. The percentage of grafting was reported as a function of various reaction variables.

## EXPERIMENTAL

### Materials

Insoluble collagen was used as received without further purification. All reagents and solvents used were purified before use. *N,N,N',N'*-tetra(2-hydrox-

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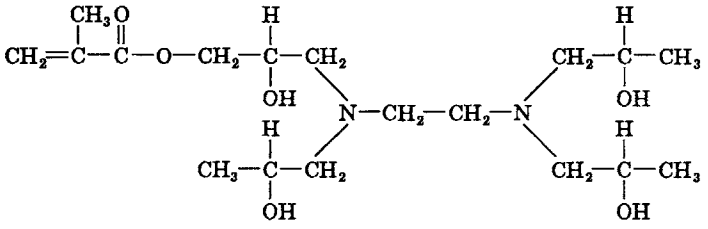
Journal of Applied Polymer Science, Vol. 40, 1541–1545 (1990)
 CCC 0021-8995/90/9-101541-05$04.00

ypropyl) ethylene diamine methacrylate [1] was prepared and purified by the method as reported in ref. 10.

Poly([*N*,*N*,*N*′,*N*′-tetra(2-hydroxypropyl) ethylene diamine methacrylate] used in this study was prepared as described in ref. 11.

Deionized distilled water was used for the polymerization reactions.



[1]

## Synthesis

Collagen was dispersed in water under nitrogen in a three-necked round-bottomed flask equipped with condenser, thermometer, and nitrogen inlet for 20 min under stirring. A known amount of potassium persulfate was added to this solution and kept stirring for 5 min. A definite amount of THPEDM was added to the reaction mixture, and the graft copolymerization was carried out at a particular temperature for specific period of time.

After completion of the reaction, the mixture was filtered and the product was extracted with water for 24 h. After complete removal of the homopolymer, the polymer was dried under vacuum at 50°C to constant weight.

The percentage grafting and conversion were calculated as follows.

$$\% \text{ grafting} = \frac{\text{wt grafted THPEDM (g)}}{\text{wt collagen used (g)}} \times 100$$

and

$$\% \text{ conversion} = \frac{\text{wt grafted THPEDM (g)}}{\text{wt THPEDM used (g)}} \times 100$$

## Purification of Graft Copolymer

The graft copolymer was purified by dispersing the graft copolymer in water to remove water-soluble homopolymer, poly[*N*,*N*,*N*′,*N*′-tetra(2-hydroxypropyl) ethylene diamine methacrylate]. The whole mixture was stirred for 24 h and then the polymer was filtered. The graft copolymer was washed with water and dried under vacuum at 50°C to constant weight.

The complete removal of the homopolymer was confirmed by extracting the physical mixture of collagen (0.2 g) and the homopolymer (1.0 g) using the same method as used for the graft copolymer purification and recovered almost all the collagen used in this experiment (99.9%). This indicates that this purification method removes all homopolymer associated with the graft copolymer.

TABLE I
Effect of Mode of Monomer Addition on Graft Copolymerization[a]

| | Mode of monomer addition | Initiator concn (mol/L) × $10^4$ | Monomer concn (mol/L) | % Grafting | % Conversion |
|---|---|---|---|---|---|
| Method 1 | Before initiator | 21.6 | 0.133 | 18.0 | 3.0 |
| Method 2 | After initiator | 21.6 | 0.133 | 18.0 | 3.0 |

[a] Reaction conditions: collagen = 0.2 g, water = 20 mL, temperature = 30°C, time = 24 h.

### Proof of Grafting

The formation of the graft copolymers was confirmed by the percentage yield of the polymer and the copper chloride test.

1. The yield of the graft copolymers was found to be higher than the original collagen used. This increase in the weight must be brought by the grafting of poly[*N*,*N*,*N*′,*N*′-tetra(2-hydroxypropyl) ethylene diamine methacrylate] to collagen in the heterogeneous medium.
2. The presence of poly[*N*,*N*,*N*′,*N*′-tetra(2-hydroxypropyl) ethylene diamine methacrylate] in the graft copolymer was confirmed by the copper chloride test, which develops blue coloration with poly(THPEDM) through complex formation.[10] Since all homopolymer was removed by the water extraction, the blue color developed by the graft copolymer must be due to the graft copolymer containing poly(THPEDM). On the other hand collagen does not develop the blue color with copper chloride solution.

## RESULTS AND DISCUSSION

A number of experiments was conducted in order to understand the nature of graft copolymerization of THPEDM on collagen.

Table I describes the effect of mode of the monomer addition on the graft copolymerization. Since there was no effect of mode of monomer addition on the graft copolymerization, the polymerization reactions were carried out by Method 2 (Table I) to minimize homopolymer formation. The formation of the graft copolymer was confirmed by the copper chloride test, which detects the presence of THPEDM. Since the yield of the graft copolymer was the same

TABLE II
Blank Test[a]

| | Collagen (g) | Water (mL) | Initiator concentration (mol/L) × $10^4$ | Temp (°C) | Yield (g) |
|---|---|---|---|---|---|
| 1 | 0.2 | 20 | 21.6 | 30 | 0.17 |
| 2 | 0.2 | 20 | 21.6 | 50 | 0.13 |

[a] Reaction time = 24 h.

TABLE III
Effect of Concentration of Initiator and Monomer, Time, and Temperature upon Potassium Persulfate Initiated Graft Copolymerization[a]

| | Initiator concentration (mol/L) × $10^4$ | Monomer concentration (mol/L) | Temperature (°C) | Time (h) | % Grafting | % Conversion |
|---|---|---|---|---|---|---|
| 1 | 21.6 | 0.133 | 30 | 24 | 18.0 | 3.0 |
| 2 | 21.6 | 0.133 | 50 | 24 | 154.0 | 20.0 |
| 3 | 21.6 | 0.133 | 60 | 24 | 135.0 | 18.0 |
| 4 | 21.6 | 0.133 | 70 | 24 | 125.0 | 17.0 |
| 5 | 21.6 | 0.133 | 80 | 24 | 119.0 | 16.0 |
| 6 | 21.6 | 0.133 | 50 | 1 | 123.0 | 16.0 |
| 7 | 21.6 | 0.133 | 50 | 3 | 127.0 | 16.5 |
| 8 | 21.6 | 0.133 | 50 | 5 | 131.0 | 17.0 |
| 9 | 21.6 | 0.133 | 50 | 8 | 131.0 | 17.0 |
| 10 | 21.6 | 0.133 | 50 | 48 | 123.0 | 16.0 |
| 11 | 10.8 | 0.133 | 50 | 24 | 131.0 | 17.0 |
| 12 | 32.45 | 0.133 | 50 | 24 | 131.0 | 17.0 |
| 13 | 43.25 | 0.133 | 50 | 24 | 85.0 | 11.0 |
| 14 | 21.6 | 0.0665 | 50 | 24 | 39.0 | 10.0 |
| 15 | 21.6 | 0.266 | 50 | 24 | 323.0 | 21.0 |
| 16 | 21.6 | 0.399 | 50 | 24 | 515.0 | 33.5 |

[a] Reaction conditions: collagen = 0.2 g, water = 20 mL.

as the original collagen used, it compelled us to run the blank test on collagen. Collagen was treated under the similar conditions that were used for the graft copolymer synthesis with no monomer. The results are tabulated in Table II. These results suggest that the collagen degrades under these conditions, and hence these quantities (Table II) were used as the original weight of collagen for the percent grafting and the percent conversion calculations. However, a blank test was carried out only at 30 and 50°C.

Table III describes the effect of initiator concentration, monomer concentration, temperature, and reaction time on the percent grafting.

It is well known that in a chain transfer process, a free-radical species such as growing chain or radical fragments arising from the decomposition of the initiator can abstract a labile hydrogen to generate active sites onto polymeric backbone where grafting of vinyl monomer can occur. Scheme 1 is suggested

$$I_2(S_2O_8^{2-}) \rightarrow 2I^{\bullet}(2SO_4^{\bullet-}) \quad [1]$$

$$I^{\bullet} + M \rightarrow IM^{\bullet} \rightarrow I(M)_m^{\bullet} \quad [2]$$

$$COLL{-}H + I(M)_m^{\bullet} \rightarrow COLL^{\bullet} + I(M_m)H \quad [3]$$

$$I^{\bullet} + COLL{-}H \rightarrow IH + COLL^{\bullet} \quad [4]$$

$$COLL^{\bullet} + M \rightarrow COLL - (M)_n^{\bullet} \quad [5]$$

$$COLL^{\bullet} + (M)_m^{\bullet} - I \rightarrow COLL - Mm - I \quad [6]$$

Scheme 1

for the grafting of THPEDM to collagen in the presence of potassium persulfate as the radical initiator. Thus in the postulated mechanism the generation of active sites can occur in two ways: the initiator radical [I•] arising from the decomposition of the initiator may abstract the hydrogen atom from collagen by process [4] or the growing polymeric radical may abstract a hydrogen atom from collagen to give free-radical sites by process [3]. However, the mechanism shown by process [4] is more favored over process [3].

It is apparent from Table III that as the monomer concentration increases, the percent grafting increases. At lower monomer concentration the percent grafting is low.

Table III shows that the percent grafting increases with an increase in the initiator concentration, reaches maximum, and then decreases. It seems that initiator radicals and radicals formed on substrate are wasted by recombination or other termination processes.

The effect of the temperature on the percent grafting was studied. From Table III, it is seen that a lower reaction temperature favored higher percent grafting. At higher temperature the radicals initiate the homopolymerization over the graft copolymerization, thereby showing a decrease in percent grafting.

The effect of time on the grafting percentage shows that it increases with time. This agrees with earlier observation with free-radical initiated polymerization.[12]

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Received February 16, 1989
Accepted December 14, 1989