Hydrogels Based on Graft Copolymers of Collagen Synthesis

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Synopsis

Graft copolymerization of 2-hydroxyethyl methacrylate in combination with hydrophobic monomers onto soluble collagen was employed in the synthesis of hydrogels. The hydrogels were formed by simultaneous graft copolymerization and crosslinking. In order to study the effect of various crosslinking agents on the water retention character of the hydrogels, three different crosslinking agents, namely, *N,N'*-methylene bis acrylamide, 1,4-butanediol dimethacrylate, and hexamethylene urethanediacrylate were used. Hexamethylene urethanediacrylate crosslinked systems require a minimum amount of crosslinking agent to attain maximum water content in comparison to the other systems.

INTRODUCTION

Polymeric hydrogels are of considerable importance due to their potential applications.[1-5] It is of interest to synthesize hydrogels from synthetic macromolecular moieties and study their utility in various applications especially in biomedical aspects. One of the methods of approach in the synthesis of hydrogels is to graft hydrophilic monomers in combination with hydrophobic monomers onto natural polymers such as starch, cellulose, collagen, etc.[6-12]

Hydrophilicity is an important factor for hydrogels for medical use. Homogeneous poly(2-hydroxyethyl methacrylate) (PHEMA) gels have maximum water content of approximately 40%. In the present study, the hydrogels were prepared by simultaneous graft copolymerization of 2-hydroxyethyl methacrylate in combination with methyl methacrylate/glycidyl methacrylate and crosslinking using different crosslinking agents. The gels so obtained were characterized for their water retention character which is an important parameter for a given hydrogel apart from its tolerance and biocompatibility.

EXPERIMENTAL

Soluble collagen was extracted from rat tail tendon by the method given by Dumitru and Garrett.[13] 2-Hydroxyethyl methacrylate (HEMA) (Fluka), glycidyl methacrylate (GDMA) (Fluka), and hydroxyethyl acrylate (HEA) (Fluka) were vacuum-distilled before use. Methyl methacrylate (MMA) (BDH) was distilled as per standard procedure.[14] The middle fraction of the monomers were used for the grafting reactions.

N,N'-methylene bis acrylamide (MBAM) (Fluka) and 1,4-butanediol dimethacrylate (BDDM) (Polysciences, Inc.) were used as such without further purification. Hexamethylene diisocyanate (HMDI) (Fluka) and dibutyltin

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dilaurate (Fluka) were used without further purification.

Ceric ammonium nitrate (CAN) (Baker, U.S.A) was used as such without further purification. The initiator solution was prepared by dissolving in 1*N* nitric acid to give appropriate concentration of ceric ion.

Preparation of Hexamethylene Urethane Diacrylate (HMUDA)

One mole of HMDI was reacted with 2 mol of hydroxyethyl acrylate at 40 ± 0.01°C for about 3 h under nitrogen atmosphere using dibutyltin dilaurate (0.01 wt% with respect to total solids) as catalyst. The resulting product was dissolved in methanol, reprecipitated in ice cold water, filtered, and then dried under vacuum:

$$OCN(CH_2)_6NCO + 2CH_2{=}CHCOOCH_2CH_2OH \rightarrow$$

$$CH_2{=}CHCOOCH_2CH_2OOCNH(CH_2)_6NHCOOCH_2CH_2OOCCH{=}CH_2$$

Grafting Procedure

The grafting reaction was carried out in double-walled glass reaction tubes. The reaction was maintained at 25°C by circulating water through the reaction tubes from a thermostat maintained at constant temperature. In all grafting reactions, 5 mL of 1% collagen solution was used. After oxygen-free nitrogen was bubbled through the solution for 15 min, the required amount of monomers and crosslinking agent were added, followed by initiator. The concentration of initiator used was 4.2×10^{-3} mol/L. The total volume of the solution was adjusted to 20 mL in all cases. The flask was tightly sealed. The contents were stirred by means of a magnetic stirrer and the reaction was allowed to proceed for 3 h. The resulting product was poured onto a glass plate to form a gel.

Grafting of HEMA and MMA (3 : 2 mole ratio) onto soluble collagen was carried out using different crosslinking agents, viz., *N,N'*-methylene bis acrylamide, 1,4-butanediol dimethacrylate, and hexamethylene urethane-diacrylate. Grafting of HEMA and GDMA onto soluble collagen was also carried out. The percent GDMA content was varied with a constant amount of about 2 g of crosslinking agent and the water contents determined. The composition of GDMA for which maximum water content was obtained was kept constant, and the amounts of crosslinking agents were varied.

The water content of a hydrogel is important for the physical characterization of a given gel. There are several conventional ways of expressing the amount of water in hydrogels. In the present study, the percent water content of the hydrogels prepared was expressed as

$$\%\ \text{Water Content} = \frac{\text{wet wt gel-dry wt gel}}{\text{wet wt gel}} \times 100$$

The gel was blotted between sheets of filter paper and then weighed immediately. Repeated water contents are the average of at least 3–4 determinations.

RESULTS AND DISCUSSION

The crosslinking agent was varied for a fixed amount of collagen, HEMA, MMA, and initiator concentration at constant temperature, time, and total volume. Tables I–III show the effect of variation of crosslinking agents in the water retention capacity of the hydrogels. The influence of structure of the crosslinking agent on the resulting product can be shown in the percent water content of the hydrogels prepared from collagen-*g*-HEMA-co-MMA. When about 0.4 mol/L of the crosslinking agents were used, it was observed that the percent water content is more in HMUDA crosslinked hydrogels, intermediate in BDDM crosslinked gels, and minimum in MBAM crosslinked materials. From the structural formulae of MBAM, HMUDA, and BDDM, it is noticed that HMUDA has maximum chain length, BDDM has intermediate chain length, and MBAM has minimum chain length in between two vinyl groups. In addition to this, in a unit volume of hydrogel, the number of crosslinks

TABLE I
Graft Copolymerization of HEMA and MMA (3 : 2 Mole Ratio) onto Soluble Collagen Using MBAM as Crosslinking Agent

Sample no.	Amount of crosslinking agent (mol/L)	Percent water content 1	2	3	4	Average
1	0.62	60	58	62	61	60.3
2	1.20	65	62	63	66	64.0
3	1.82	58	61	62	63	61.0
4	2.47	55	62	59	58	58.5

TABLE II
Graft Copolymerization of HEMA and MMA (3 : 2 Mole Ratio) onto Soluble Collagen Using HMUDA as Crosslinking Agent

Sample no.	Amount of crosslinking agent (mol/L)	Percent water content 1	2	3	4	Average
1	0.11	75	72	74	74	73.8
2	0.24	78	80	78	77	78.3
3	0.46	74	75	73	76	74.5
4	0.70	73	72	71	71	71.8

TABLE III
Graft Copolymerization of HEMA and MMA (3 : 2 Mole Ratio) onto Soluble Collagen Using BDDM as Crosslinking Agent

Sample no.	Amount of crosslinking agent (mol/L)	Percent water content 1	2	3	4	Average
1	0.42	68	69	67	69	68.3
2	0.82	72	70	74	73	72.3
3	1.24	68	70	72	69	69.8
4	1.68	64	66	67	66	65.8

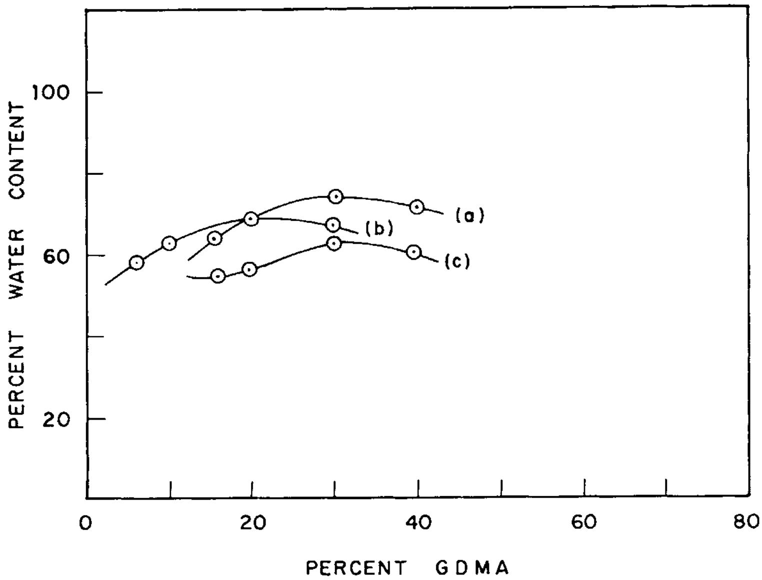

Fig. 1. Effect of variation of GDMA in the monomer mixture on water content using (a) HMUDA, (b) MBAM, and (c) BDDM as crosslinking agents.

varies with the molecular weight of the crosslinking agent. These two factors, i.e., the difference in number of crosslinks and the difference in chain length in between two crosslinking points may be operative in retaining the amount of water content in the hydrogel.

MMA was replaced with GDMA in the graft copolymerization onto soluble collagen with a view to understand microenvironmental effect of the monomer. The graft copolymerization was carried out by keeping the synthetic parameters such as concentration of backbone and initiator, time, temperature, total volume of the reaction, and amount of crosslinking agents constant. For MBAM crosslinked gels, as percent glycidyl methacrylate in the monomer mixture is increased, the percent water content increases and attains a maximum at about 20%. Further increase of GDMA shows almost no change in the percent water content of the hydrogel (Fig. 1). But in the case of HMUDA and BDDM crosslinked systems, it was observed that at about 30% GDMA the hydrogels obtained exhibit maximum water content.

TABLE IV
Graft Copolymerization of HEMA and GDMA onto Soluble Collagen Using MBAM as Crosslinking Agent[a]

Sample no.	Amount of crosslinking agent (mol/L)	Percent water content				
		1	2	3	4	Average
1	0.65	62	64	64	65	63.8
2	1.33	66	68	67	65	66.5
3	2.01	63	64	62	61	62.5
4	2.66	56	57	58	60	57.8

[a][HEMA]: 1.569 mol L^{-1}, GDMA: 20%.

TABLE V
Graft Copolymerization of HEMA and GDMA onto Soluble Collagen Using HMUDA as Crosslinking Agent[a]

Sample no.	Amount of crosslinking agent (mol/L)	Percent water content				
		1	2	3	4	Average
1	0.13	70	69	69	68	69.0
2	0.25	72	74	75	73	73.5
3	0.51	72	69	73	72	71.5
4	0.78	69	69	69	—	69.0
5	1.03	66	67	69	66	67.0

[a] [HEMA]: 1.793 mol L^{-1}, GDMA: 30%.

TABLE VI
Graft Copolymerization of HEMA and GDMA onto Soluble Collagen Using BDDM as Crosslinking Agent[a]

Sample no.	Amount of crosslinking agent (mol/L)	Percent water content				
		1	2	3	4	Average
1	0.22	57	60	61	60	59.5
2	0.44	63	66	67	66	65.5
3	0.91	69	71	70	70	70.0
4	1.37	64	65	66	65	65.0
5	1.81	60	59	61	62	60.5

[a] [HEMA]: 1.793 mol L^{-1}, GDMA: 30%.

After determining the required GDMA concentration, the amount of crosslinking agents was varied by maintaining the concentration of GDMA constant. The observed percent water contents are given in Tables IV–VI. The concentration of MBAM, BDDM, and HMUDA required to obtain hydrogels with maximum water retention capacity are 1.33, 0.91, and 0.25 mol/L, respectively. As in the case of HEMA–MMA-based system, the retention of water content obtained is maximum with HMUDA, intermediate with BDDM, and minimum with MBAM for HEMA–GDMA-based hydrogels.

References

1. L. Sprincl, J. Kopecek, and D. Lim, *J. Biomed. Mater. Res.*, **5**, 447 (1971).
2. D. G. Murray and J. A. S. Dow, *J. Biomed. Mater. Res.*, **9**, 699 (1975).
3. K. Kliment, M. Stoll, K. Fahoun, and B. Stockar, *J. Biomed. Mater. Res.*, **2**, 237 (1968).
4. M. F. Refojo and F. L. Leong, *J. Biomed. Mater. Res.*, **15**, 497 (1981).
5. B. D. Ratner and A. S. Hoffman, *ACS Symposium Series No. 31*, J. D. Andrade, Ed., American Chemical Society, Washington, DC, 1976.
6. G. M. Brauer and D. J. Termini, *J. Biomed. Mater. Res.*, **8**, 451 (1974).
7. M. Chvapil, R. Holusa, K. Kliment, and M. Stoll, *J. Biomed. Mater. Res.*, **3**, 315 (1969).
8. L. Sprincl, J. Vacik, J. Kopecek, and D. Lim, *J. Biomed. Mater. Res.*, **5**, 197 (1971).
9. W. M. Muir, R. A. Gray, J. M. Courtney, and P. D. Ritchie, *J. Biomed. Mater. Res.*, **5**, 415 (1971).
10. J. Drobnik, P. Spacek, and O. Wichterle, *J. Biomed. Mater. Res.*, **8**, 45 (1974).

11. K. J. Muzykewicz, E. B. Crowell, Jr., A. P. Hart, M. Schutts, C. J. Hill, Jr., and S. L. Cooper, *J. Biomed. Mater. Res.*, **9**, 487 (1975).

12. S. Amudeswari, C. Rami Reddy, and K. Thomas Joseph, *J. Macromol. Sci.–Chem.*, to appear.

13. E. T. Dumitru and R. R. Garrett, *Arch. Biochem. Biophys.*, **66**, 245 (1957).

14. C. H. Bamford and E. F. S. White, *Trans. Faraday Soc.*, **52**, 716 (1956).

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