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RESEARCH PAPER

Organic Redox-Initiated Polymerization Process for the Fabrication of Hydrogels for Colon-Specific Drug Delivery

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ABSTRACT

Organic-redox initiated polymerization technique based on the co-initiators system comprising benzoyl peroxide and *N*-phenyldiethanolamine was used at ambient temperature to fabricate pH-responsive hydrogels. The effects of changes in the concentration of the co-initiators system, the ratio in which the co-initiators combined, the type of the polymerization solvent, the pH of the hydrating medium, the concentration of the cross-linking agent based on azo-bond and the pH-sensitive cross-linking agent on the properties of the hydrogels were investigated. Increasing the concentration of the co-initiators system, decreasing the concentration of the two types of cross-linking agents, and replacing DMSO by ethanol as the polymerization solvent resulted in hydrogels with increased equilibrium swelling ratio and increased molecular weight between cross-links at pH 7.4. Increasing the concentration of *N*-phenyldiethanolamine while keeping the concentration of benzoyl peroxide constant gave hydrogels with increased equilibrium swelling ratios. The equilibrium swelling ratios of the hydrogels at pH 2.0 were not affected by the factors investigated. The polymerization technique may be suitable for the design of drug delivery systems containing thermolabile bioactive agents like peptides and proteins.

Key Words: Organic-redox initiated polymerization; Hydrogels; Benzoyl peroxide; *N*-Phenyldiethanolamine.

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INTRODUCTION

The design of drug delivery systems which have the property of selective release of drug in the colon has received a lot of attention. The colon-specific drug delivery systems should be capable of protecting the drug en route to the colon (i.e., drug release and absorption should not occur in the stomach and the small intestine and the bioactive agent should not be degraded). Over the years, efforts have been made to deliver drugs to the colon, and the two major therapeutic applications which have been considered for oral colon-specific drug targeting are the treatment of local disorders in the colon and the systemic administration of drugs, especially peptide and protein drugs.[1] The colon is believed to be suitable as an absorption site for peptides and proteins for the following reasons: less diversity and intensity of digestive enzymes; the colon has a long retention time and is highly responsive to absorption enhancers. Moreover, further impetus for colon-specific drug delivery includes the opportunity to explore the pH gradient between the stomach and the small intestine for drug delivery. Though there is no pH gradient between the small intestine and the colon, the short residence time in the small intestine can be manipulated [i.e., using systems with controlled kinetics of swelling[2]] so as to prevent premature release of drugs in the small intestine as a result of extensive swelling. Another impetus is the gradient in bacterial counts between the ileum, the cecum, and the colon, which can be used to advantage in the design of colon-specific drug delivery systems.

Several approaches have been used for site-specific delivery of bioactive agents to the colon[1,3]: (a) prodrug (low molecular weight and macromolecular prodrugs); (b) polymeric physical carriers of drugs (sustained release; pH-sensitive polymer coatings; time-released system; pH-sensitive and bacterial degradable polymers and hydrogels; bacterial degradable matrix). Two major technological problems to be solved in the use of hydrogels for colon-specific drug delivery, especially for peptide and proteins drugs, are as follows: protection of the drug both during preparation and en route to the colon; enhancement of penetration through the intestinal epithelium.[2] These two factors are important for successful oral delivery of peptide and protein drugs and may be responsible for the lack of oral drug products containing peptides and proteins available commercially to patients. Hydrogels that are responsive to pH and degradable in the colon can solve part of the first problem; however, the fabrication process should also ensure the integrity of the drugs, especially thermolabile compounds.

Rationale for This Work

It is often stated that, although hydrogels can be degraded in vivo, they suffer from the disadvantage that the amount of drug that can be incorporated is limited. The belief is that drug loading in hydrogel devices can only be effected in one of two ways: (a) soaking of the dry gel in the solution of the drug; (b) adding the drug to the monomer feed composition prior to polymerization.[4] However, the new processes of preparing hydrogels reported by Kopecek and his coworkers [cross-linking of polymeric precursors and polymer–polymer reaction[3]] can be used to coat pharmaceutical dosage forms, like capsules, which can accommodate a large amount of drugs. To extend the possibility of dosage form coating with hydrogels to copolymerization processes, we decided to investigate organic redox-initiated copolymerization. The process can be carried out at an ambient temperature (suitable for thermolabile bioactive agents like peptide and protein drugs). Further, the copolymerization process has been used for the fabrication of biomaterials for human applications: bone cements and dental biomaterials.

Background Information on the Applications of Organic-Redox Initiator Systems

Methylmethacrylate monomer and polymer slurries are used extensively as the major components of bone cements for hip prostheses in orthopedic surgery and to form replacements for skull defects in neurosurgery. The polymerizable compositions for bone cements are similar to those used in the room-temperature-curing dental biomaterials: a liquid made from a methylmethacrylate monomer containing an inhibitor, a powder consisting of poly(methyl methacrylate) (PMMA), and organic redox-initiator system (benzoyl peroxide/amine system).[5] In the case of dental biomaterials, photopolymerizable compositions that can be cured at room temperature using visible light has been described.[6] In fact, the technique involving *N*,*N*-dimethylethanolamine and *dl*-camphoroquinone using visible light has been employed to fabricate poly(HEMA) hydrogels.[7–9] Though the *N*,*N*-dimethylethanolamine and *dl*-camphoroquinone photoinitiator system can be

used for azo-bond-containing hydrogels, the reducing agent (*N*,*N*-dimethylethanolamine) can only reduce the photosensitizer (*dl*-camphoroquinone) when the photosensitizer is in the excited state. Thus in addition to the photoinitiators, a light source is needed, which may be contraindicated for light-sensitive bioactive agents.

A complex series of reactions occurs between benzoyl peroxide and amine in an organic redox-initiator system, producing radicals which initiate polymerization. The initiation of free radical polymerization by the organic redox-initiator system is known to be significantly different from the initiation by the thermal decomposition of benzoyl peroxide with respect to the practical use of the process as well as to the chemical kinetics of polymerization.[10] Low energy of activation is associated with the oxidation/reduction system. Consequently, an amine/benzoyl peroxide initiator system can initiate free radical polymerization at a lower temperature (commonly at room temperature) compared to the typical decomposition temperature of benzoyl peroxide, which is 70°C or above. This condition makes it suitable for curing of bone cement and dental biomaterials. Further, it involves only one step. Consequently, the technique appears to be useful for the fabrication of azo-containing hydrogels for coating drug delivery devices containing thermolabile bioactive agents like peptides and proteins.

The classical amine used in the amine/benzoyl peroxide initiator system is *N*,*N*-dimethyl-4-toluidine (DMT). Studies have been carried out to improve the biocompatibility of the system by using other tertiary amines. Tertiary aromatic amines are the most popular.[11] Addition of benzoic acid and methacrylic acid to polymerizable compositions containing peroxide-amine systems has been reported to increase the rate of polymerization: the setting time was lowered, but the peak temperature reached on curing increased. Methacrylic acid was found to be slightly more effective than benzoic acid in lowering the setting time.[5] This report, indicating that additives will not adversely affect the polymerization initiated by amine/peroxide redox system, further encouraged us to investigate the system for the fabrication of hydrogels containing a cross-linking agent with azo-group.

EXPERIMENTAL

Materials

N,*N*-Dimethylacrylamide (DMMA) and acrylic acid (AA) (Aldrich) were distilled under vacuum before use. 4,4′-Diaminoazobenzene, 2,2′-azobisisobutyronitrile (AIBN), and *N*-*tert*-butylacrylamide (BuAA) (Polysciences) were recrystallized from ethanol, methanol, and acetone, respectively. Hydroxylamine hydrochloride and dimethyl sulfoxide (DMSO) were obtained from Aldrich and used as received. Chloroform, pyridine, and diethyl ether were obtained from Fisher Scientific, Fluka, and J. T. Baker, respectively. *N*-Phenyldiethanolamine (PDEA) and benzoyl peroxide were obtained from Aldrich.

Methods

Synthesis of 4,4′-Di(Methacryloylamino)-azobenzene and *N*,*O*-Dimethacryloyl-hydroxylamine

4,4′-Di(methacryloylamino)azobenzene (DMAAB) was synthesized as previously reported.[12] We modified the method for the synthesis of *N*,*O*-dimethacryloylhydroxylamine (MANHOMA) reported in the literature.[2,13] To a solution of hydroxylamine hydrochloride (9.0 g) in 72 mL of pyridine, methacryllol chloride (27 mL) was added dropwise with stirring. The temperature of the mixture was kept below 30°C. The reaction mixture was left at room temperature for 10 h. It was dissolved in 150 mL chloroform and then neutralized with 48 mL HCl. The product was extracted with distilled water (4×150 mL), dried over magnesium sulfate, and then dissolved in ether, followed by filtration, drying, and recrystallization. Yield: 28%, mp: 55 ± 1°C, ^{1}H-NMR (300 MHz, $CDCl_3$): ($C_8H_{11}NO_3$) $\delta = 2.01$ [s, 3H, -CO-C(C<u>H</u>$_3$)=CH_2], 2.04 [s, 3H, -O-CO-C(C<u>H</u>$_3$)=CH_2], 5.6, 5.85 [s, 2H, -O-CO-C(CH_3)=CH_2], 6.0, 6.4 [s, 2H, -N-CO-C(CH_3)=C<u>H</u>$_2$], 9.55 [s, 1H, -CO-N<u>H</u>-O-]. FTIR (KBr pellet): 1770 cm^{-1} [-C=O, ester], 1665 cm^{-1} [-C=O, amide], 1095 cm^{-1} [C-O].

Fabrication of Hydrogels

The fabrication of hydrogels was by radical cross-linking copolymerization of the monomers and the cross-linking agents in 70% v/v DMSO or DMSO:ethanol (1:4) using *N*-phenyldiethanolamine and benzoyl peroxide as organic redox-initiator system at 25°C for 15 h. Various concentrations of the redox-initiator system were investigated; also, the molar ratios in which the benzoyl peroxide combined with *N*-phenyldiethanolamine were varied. The hydrogels were based on biocompatible

N,*N*′-dimethylacrylamide. Acrylic acid and BuAA were also incorporated to impart pH sensitivity and mechanical strength to the hydrogels, respectively, following the model earlier reported by Kopecek and his coworkers.[2,12] Two cross-linking agents were also incorporated: DMAAB and MANHOMA. The monomer solution was bubbled with nitrogen for 5 min and transferred to a Teflon® mold containing a silicone rubber spacer to obtain gels of desired thickness. The gels were washed in ethanol for 4 days (the ethanol was changed daily), then the gels were transferred gradually into an aqueous solution [pH 2.0 (0.01 M HCl)]. Various formulations of the hydrogels are shown in Tables 1–3.

Characterization of Hydrogels

Kinetics of Swelling and Equilibrium Swelling Ratio

Each hydrogel disc (three discs per sample) was equilibrated in 20 mL buffer (pH 2.0; ionic strength of 0.16) at 37°C. The gel was placed on a copper wire-gauze to allow excess surface water to be removed by

Table 1. Hydrogel formulations showing monomer feed compositions with varying concentrations of the coinitiator system.

Formulation	PDEA:benzoyl peroxide (molar ratio)	Coinitiator concentration (% w/v)	DMAAB (mol%)	DMAA (mol%)
D1	1:1	0.2	0.1	49.9
D2	1:1	0.3	0.1	49.9
D3	1:1	0.5	0.1	49.9
D4	1:1	1.0	0.1	49.9
E5	1:1	0.2	0.1	49.9
E6	1:1	0.3	0.1	49.9
E7	1:1	0.5	0.1	49.9
E8	1:1	1.0	0.1	49.9
D9	1:1	0.5	0.1	49.9
D10	1:1	1.0	0.1	49.9
D11	1:1	1.5	0.1	49.9
ED12	1:1	0.5	0.1	49.9

Note: D (DMSO) is the polymerization solvent; E (ethanol) is the polymerization solvent; ED [ethanol:DMSO (4:1)] is the polymerization solvent; all samples contained 10 mol% of *N*-*tert*-butylacrylamide (BuAA) and 40 mol% acrylic acid (AA); DMAA and DMAAB are *N*,*N*-dimethylacrylamide and 4,4′-di(methacryloylamino)azobenzene, respectively; *N*-phenyldiethanolamine (PDEA).

Table 2. Hydrogel formulations showing monomer feed compositions with varying concentrations of the cross-linking agent (DMAAB).

Formulation	PDEA:benzoyl peroxide (molar ratio)	Coinitiator concentration (%w/v)	DMAAB (mol%)	DMAA (mol%)
D13	1:1	1	0.05	49.95
D14	1:1	1	0.10	49.90
D15	1:1	1	0.15	49.85
D16	1:1	1	0.20	49.80
ED17	1:1	1	0.15	49.85
ED18	1:1	1	0.20	49.80

Note: D (DMSO) is the polymerization solvent; ED [ethanol:DMSO (4:1)] is the polymerization solvent; all samples contained 10 mol% of *N*-*tert*-butylacrylamide (BuAA) and 40 mol% of acrylic acid (AA); DMAA and DMAAB are *N*,*N*-dimethylacrylamide and 4,4′-di(methacryloylamino)azobenzene, respectively; *N*-phenyldiethanolamine (PDEA).

Table 3. Hydrogel formulations showing monomer feed compositions with varying concentrations of the hydrolyzable cross-linking agent (MANHOMA) and molar ratio of the coinitiators.

Formulation	PDEA:benzoyl peroxide (molar ratio)	AA (mol%)	MANHOMA (mol%)	DMAA (mol%)
D19	1:2	40.0	0.0	49.85
D20	1:2	39.5	0.5	49.85
D21	1:2	39.0	1.0	49.85
D22	1:2	38.0	2.0	49.85
D23	1:2	37.0	3.0	49.85
D24*	1:5	40.0	0.0	49.85
D25*	1:2	40.0	0.0	49.85
D26*	1:1	40.0	0.0	49.85
D27*	2:1	40.0	0.0	49.85
D28*	5:1	40.0	0.0	49.85

Note: D (DMSO) is the polymerization solvent; all samples contained 10 mol% of *N-tert*-butylacrylamide (BuAA), 0.1 mol% 4,4′-di(methacryloylamino)azobenzene (DMAAB), and 1%w/v of the coinitiator, except the formulations marked * which contained 1.5%w/v of the coinitiator system and 0.15 mol% DMAAB; *N*-phenyldiethanolamine (PDEA).

blotting with a laboratory tissue. The pH of the hydrating solution was checked with a pH meter and found unchanged. After being weighed, the gel was transferred to a vial containing 20 mL of 0.05 M phosphate buffer (ionic strength 0.16; pH 7.4) already equilibrated at 37°C in a water bath. Changes in weight were monitored at time intervals until equilibrium swelling was reached. The pH of the hydrating solution remained unchanged during the experiment. The swelling of the hydrogels was expressed as swelling ratio (Q: weight of wet gel/weight of dry gel).

RESULTS AND DISCUSSION

Figure 1 details the preliminary data to determine the appropriate concentration of the coinitiator system for the fabrication of hydrogels based on poly(DMAA-co-AA-co-BuAA) cross-linked with DMAA. Time to reach gel-point decreased with increase in the concentration of the coinitiator system (gel-point is defined as the time for polymerization solution to become solid enough to be unpourable from the vial). The hydrogels were homogeneous and optically transparent on visual inspection. Polymerization proceeded faster in DMSO than ethanol. DMSO was found appropriate as the polymerization solvent for this type of hydrogel in previous studies.[2,12,14] Ethanol is used to wash the hydrogels after polymerization. Consequently, it was considered reasonable to investigate ethanol as a polymerization solvent. Time to reach gel-point was

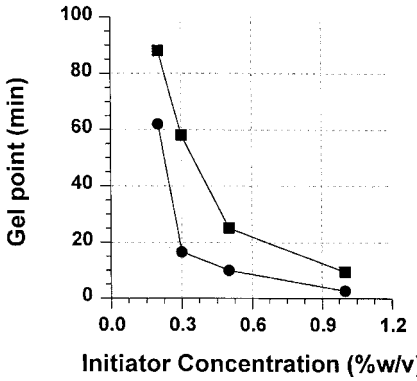

Figure 1. Influence of coinitiator [*N*-phenyldiethanolamine/benzoyl peroxide (1:1 molar ratio)] concentration on gel-points of hydrogels. Table 1: formulations D1 to D4 and E5 to E8 with DMSO (●) and ethanol (■), respectively, as polymerization solvents.

shorter for hydrogel prepared with DMSO than corresponding gels prepared with ethanol. Probably the more viscous nature of DMSO prevented the diffusion of unpolymerized monomer solution in the gels; thereby facilitating more rapid polymerization. Further, ethanol was probably not resistant to attack by free radicals, resulting in a retarding effect on polymerization in a manner reminiscent of the interaction between free radicals from benzoyl peroxide and eugenol causing the inhibition of the polymerization of methylmethacrylate monomers.[15]

Figure 2 shows that the equilibrium swelling ratio (Q) of the hydrogels, after an abrupt change

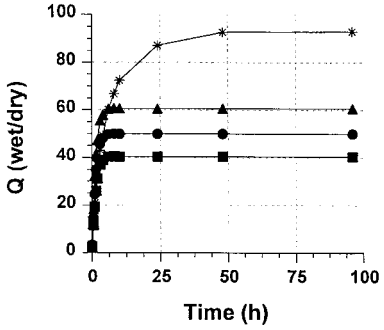

Figure 2. Swelling isotherms (pH 7.4; 37°C) of poly(DMAA-co-AA-co-BuAA) hydrogels polymerized in DMSO (initiator concentration, %w/v): ■, 0.5; ●, 1.0; ▲, 1.5; polymerized in ethanol:DMSO (4:1): ✳, 0.5%w/v. Standard deviation ε (1.56; 2.12). Table 1: formulations D9 to D11 and ED 12.

from pH 2.0 to 7.4, increased with increase in the concentration of the coinitiator system (Table 1: D9 to D11 and ED12). Hydrogels, as the name implies, have a high liquid content; they are given form by the network of polymer strands. It is known that the liquid within the hydrogel network is held by a balance of forces. The swelling of hydrogels is often due to the osmotic driving force for water uptake which causes an elongation of polymer chains and the resistive force exerted by the polymer chains as a result of the elastic contractility of the polymer chains to counteract the swelling osmotic force. Some of the factors that may affect the swelling of ionic hydrogels, such as the type studied in this work, include degree of ionization, the charge of the ionic monomer, the pKa of the ionizable groups, the concentration of the ionizable monomer in the network, the nature of the counter-ion, the nature of the swelling solution in terms of pH, ionic strength and composition (i.e., buffer), the cross-linking density, and the network structure and hydrophilicity of the polymer. In this experiment, the only variables which might affect the polymer network structure are the pH of the hydrating medium, the type of polymerization solvent, and concentration of the coinitiator system. Consequently, as the pH of the swelling medium increased from 2 to 7.4, fast ionization of the carboxylic groups due to acrylic acid in the network would occur. The pKa of carboxylic acid groups in the polymer network is lower than pH 7.4. This condition would favor ionization, increase in charge density, charge hydration, electrostatic repulsion between adjacent carboxylic acid groups, and expansion of the polymer network. The increase in equilibrium swelling ratio or degree of swelling (Q) at pH 7.4 with increase in the concentration of the coinitiator system might be due to a reduction in the elastic retractive force of the hydrogel network. The equilibrium degree of swelling (Q) of the hydrogels was insensitive to the graded changes in the concentration of the coinitiator system in an acidic buffer (pH 2.0; ionic strength of 0.16) at 37°C (data not shown). This result could be attributed to a favorable microenvironment for polymer–polymer interactions due to intermolecular hydrogen bonding, which decreased the openness of the polymer network and hence reduced absorption of water. The type of polymerization solvent also affected the equilibrium swelling ratio (Q) of the hydrogels as shown in the swelling isotherms (Fig. 2). At 0.5% w/v level of the coinitiator system, hydrogels prepared with ethanol:DMSO (4:1) showed over two-fold equilibrium degree of swelling compared to hydrogels of similar composition prepared with DMSO. This result correlates with the data shown in Fig. 1 and may be due to ethanol not being resistant to attack by free radicals, resulting in a retarding effect on polymerization and hence cross-linking density of the hydrogels.

An empirical equation, which represents second-order kinetics (for modeling swelling data obtained from hydrogels) and which is applicable to the entire swelling period, was developed recently by Schott.[16] It is shown in Eq. (1). At relatively high degrees of swelling, first-order swelling kinetics would not give a good approximation to the swelling behavior of hydrogels. Schott[16] demonstrated that the weight, W, of aqueous buffer solution absorbed per gram of dry polymer can be expressed as a linear function of time (t), as shown in Eq. (1).

$$t/W = A + Bt \tag{1}$$

A and B are constants. It is known that the equilibrium swelling, W_∞, is reached when the swelling osmotic force equals the elastic contractility of the gel network. At long times of swelling, $W \to W_\infty$, and $Bt \gg A$ and the slope $B = 1/W_\infty$ (i.e., B is the reciprocal of the equilibrium swelling).

At short times, $A \gg Bt$, then in the limit, Eq. (1) becomes Eq. (2)

$$\lim_{t \to 0}(dW/dt) = 1/A \tag{2}$$

Thus intercept A in Eq. 1 represents the reciprocal of the initial swelling rate.[16]

The equilibrium swelling ratio or the degree of swelling, Q, earlier defined as the weight of wet gel

divided by the weight of dry gel is often used to express the swelling behavior of hydrogels. Q is related to W in Eq. (1) (W is the difference between the weight of wet gel and the dry gel divided by the weight of the dry gel). Consequently, in our analysis, Q (the equilibrium swelling ratio) was substituted for W in Eq. (1). Figure 3 shows the result obtained with modeling the swelling data using Eq. (1). Straight lines with correlation coefficients grater than 0.99998 were obtained, indicating that the swelling behavior of the hydrogels at high degrees of swelling could be modeled with the second-order kinetics. The values of reciprocal of B (equilibrium swelling) calculated from Eq. (1) for all concentrations of the coinitiator system were exactly the same as those obtained experimentally, indicating that Eq. (1) is very useful in predicting equilibrium swelling of this class of ionizable hydrogels. Furthermore, the value of A in Eq. (1) decreased with decrease in the concentration of the coinitiator system (D9 to D11 in Table 1: data not shown). Thus the initial rate of swelling, defined as the reciprocal of A in Eq. (1), increased with decrease in the concentration of the coinitiator system. Hydrogels prepared with ethanol:DMSO (4:1) showed the fastest initial rate of swelling (ED 12 in Table 1).

Brannon-Peppas and Peppas[17] developed an equilibrium swelling model for charged polymeric networks. The model has been applied to predict the equilibrium swelling of pH-sensitive hydrogels.[18] The model is shown in Eq. (3). The original equation was for homopolymers containing one ionizable group. The ionic contribution [the first term or the term on the left-hand side of Eq. (3)] to the equilibrium swelling equation has been modified to analyze the swelling behavior of a copolymer containing one ionizable component as well as one or more non-ionizable components.[19] The modification is the introduction of f' (the mole fraction of the ionizable group in the polymer, which is 0.4 in the hydrogels studied in this work). Equation 3 was used in this work to estimate the number average molecular weight between cross-links, M_c.

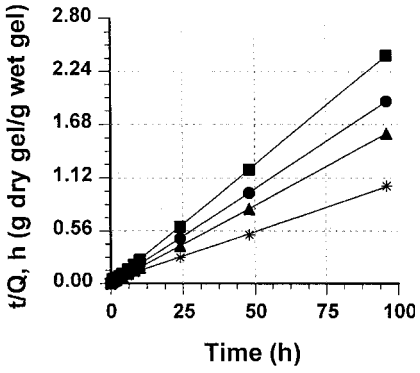

Figure 3. Linearization of swelling isotherms in Fig. 2 by second-order equation. Polymerized in DMSO (initiator concentration, %w/v): ■, 0.5; ●, 1.0; ▲, 1.5; polymerized in ethanol:DMSO (4:1): ∗, 0.5%w/v.

$$\frac{V_1}{4I}\left(f'\frac{v_{2,s}}{\overline{V}}\right)^2\left(\frac{K_a}{10^{-\mathrm{pH}}}+K_a\right)^2 = \ln(1-v_{2,s})+v_{2,s}+\chi(v_{2,s})^2 + \left(\frac{V_1}{\overline{V}\,\overline{M}_c}\right)\left(1-\frac{2\overline{M}_c}{\overline{M}_n}\right)v_{2,r} \times\left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3}-1/2\left(\frac{v_{2,s}}{v_{2,r}}\right)\right] \quad (3)$$

In Eq. (3), M_n is the number average molecular weight of the polymer chains before cross-linking. Since the copolymerization occurred simultaneously with cross-linking, it was not possible to determine M_n experimentally to obtain values that would actually approximate M_n in the hydrogel network. In an analytical work on the structure of hydrogels reported by Peppas et al.,[20] it was shown that a value of 75,000 was adequate since the calculated M_c was independent of M_n if it exceeded 10,000. The same value was used recently to analyze the structure of pH-sensitive hydrogels at equilibrium swelling.[18] Thus the value of M_n was taken to be 75,000 (g/mol). V is the specific volume of the polymer which is expressed as the reciprocal of the density of the dry polymer (1.22 g/cm^3) reported for similar gels.[12] V_1 is the molar volume of the swelling agent which is equal to 18 cm^3/mol; I is the ionic strength of the hydrating solution, which is 0.16.

$v_{2,r}$ and $v_{2,s}$ are respectively the polymer volume fraction in the relaxed polymer network (the value was calculated at pH 2) and polymer volume fraction in the swollen polymer network (the value was calculated at pH 7.4). The calculation was as described earlier.[12] The dissociation constant (K_a) for the ionizable group in the polymer was calculated from the pK_a of polyacrylic acid, which is equal to 4.75.[17] The equation for the estimation of polymer–water interaction parameter (χ) at pH 7.4 and ionic strength of 0.16 for poly(DMAA-co-AA-co-BuAA) cross-linked with N,N'-(ω-aminocaproyl)-4,4′-diaminoazobenzene) has been reported as follows[21]:

$$\chi = 0.091 + 1.318v_{2,s} \quad (4)$$

Given the similarity of the polymer network composition of the hydrogels investigated in this work and the swelling medium compared to those investigated earlier,[21] χ values were estimated using Eq. 4.

Table 4 shows the values of equilibrium swelling ratio together with the corresponding M_c. It should be emphasized that the calculated M_c are only a rough estimation because of the assumptions made in the application of Eq. 3. However, the rough estimates provide an insight into the influence of the structure of the hydrogel on the swelling behavior. It is believed that one of the main parameters that describe the basic structure of hydrogel is the molecular weight between cross-links.[19] As shown in Table 1 for polymerizable compositions and Table 4 for Q and M_c, Q (the equilibrium swelling ratio) increased with increase in the concentration of the coinitiator system, and this trend is associated with increase in M_c (gels D9 to D11 swollen at pH 7.4). At pH 2, the equilibrium swelling ratio is virtually the same for all the gels (data not shown). Similar reports have been given previously[2,12,14] and can be attributed to greater interactions among polymer chains (hydrophobic interactions and hydrogen bonding) at a low pH. It is known that the cross-linking density of a polymeric network is inversely proportional to the molecular weight between cross-links, M_c.[19,22] The higher cross-linking density in hydrogels of low M_c resulted in greater network elastic retractive force which restricted the swelling of the hydrogels, since the equilibrium swelling ratio of a hydrogel network is a function of the balance between elastic forces and the swelling forces.

Figure 4 shows the linearization of the swelling isotherms obtained for hydrogels prepared with varying concentrations of DMAAB but a constant concentration (1% w/v) and a constant molar ratio (1:1) of the coinitiator system (Table 2). The equilibrium swelling ratios obtained experimentally were predicted perfectly by Eq. (1) [the reciprocal

Table 4. Relationship between equilibrium swelling ratio (Q) and molecular weight between cross-links (M_c) for different hydrogel formulations.

Formulation	Q (equilibrium swelling ratio)	M_c (g/mol)
D9	40.38 ± 2.08	957.71 ± 57.70
D10	49.99 ± 1.56	$1{,}081.65 \pm 48.97$
D11	60.62 ± 1.68	$1{,}785.77 \pm 53.41$
ED12	92.84 ± 2.12	$2{,}783.03 \pm 56.07$
D13	71.37 ± 2.52	$1{,}583.93 \pm 59.16$
D14	49.99 ± 1.56	$1{,}081.65 \pm 48.97$
D15	33.90 ± 0.58	810.40 ± 5.82
D16	30.25 ± 1.48	672.16 ± 22.28
ED17	59.62 ± 3.23	$1{,}767.66 \pm 69.22$
ED18	57.12 ± 1.36	$1{,}596.01 \pm 83.34$
D19	45.33 ± 0.08	994.32 ± 12.03
D20	42.26 ± 1.02	828.34 ± 23.50
D21	31.18 ± 0.60	625.44 ± 79.65
D22	25.12 ± 0.85	490.71 ± 32.95
D23	20.07 ± 1.41	414.85 ± 39.58
D24*	35.99 ± 0.76	720.01 ± 21.87
D25*	42.26 ± 0.98	842.97 ± 19.95
D26*	44.80 ± 0.70	900.94 ± 44.90
D27*	52.44 ± 1.04	$1{,}139.96 \pm 10.67$
D28*	53.50 ± 0.85	$1{,}159.28 \pm 21.87$

Note: D (DMSO) is the polymerization solvent; ED [ethanol: DMSO (4:1)]; all samples contained 10 mol% of *N-tert*-butylacrylamide (BuAA), 0.1 mol% 4,4′-di(methacryloylamino)azobenzene (DMAAB), and 1% w/v of the coinitiator, except the formulations marked * which contained 1.5% w/v of the coinitiator system and 0.15 mol% DMAAB; *N*-phenyldiethanolamine (PDEA).

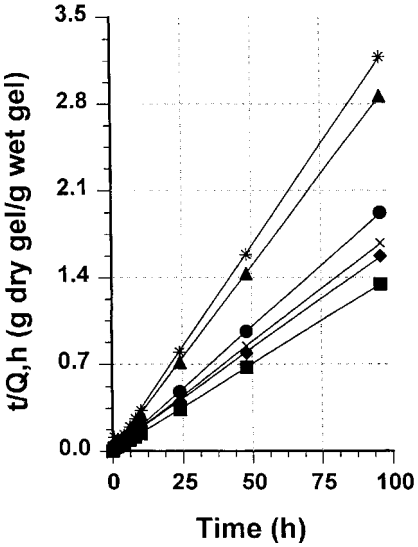

Figure 4. Linearization of the swelling isotherms by second-order equation for hydrogels prepared using different concentrations of the cross-linking agent (DMAAB): polymerized in DMSO; DMAAB concentration, mol%; ■, 0.05; ●, 0.1; ▲, 0.15; ✳, 0.2; polymerized in ethanol:DMSO (4:1): ◆, 0.15; ×, 0.2.

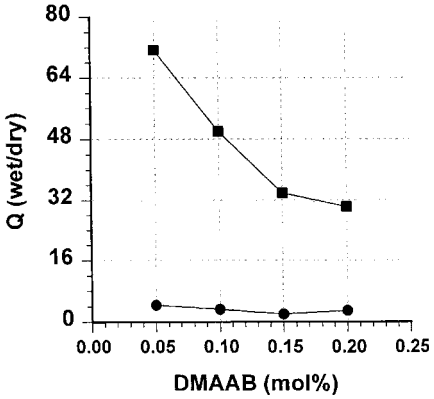

Figure 5. Dependence of equilibrium swelling ratio on the content of cross-linking agent DMAAB: (■, pH 7.4; ●, pH 2.0). pH 2.0: standars deviation ε (0.09; 0.41); pH 7.4: standard deviation ε (0.58; 2.53).

of B in Eq. (1)] for all the hydrogels and were found to decrease with increase in the concentration of the cross-linking agent. Data (not shown) also showed that the initial swelling rate, expressed as the reciprocal of A in Eq. (1), decreased with increase in the concentration of the cross-linking agent for hydrogels prepared with DMSO. The initial swelling rate is less for the hydrogels prepared with 0.15 mol% of DMAAB than those prepared with 0.20 mol% of DMAAB in hydrogels prepared with ethanol:DMSO (4:1). This observation might be due to the fact that the gels were under a very high stress as observed during the experiment (the initial circular shape of the gel was not assumed until about 4 to 6 h after transfer to the hydrating medium at pH 7.4). However, the equilibrium swelling ratio, Q, decreased with increase in the concentration of DMAAB, and this was perfectly predicted by the reciprocal of B in Eq. (1).

Figure 5 shows the dependence of the equilibrium swelling ratio, (Q) at pH 2 and 7.4, on the concentration of the cross-linking agent (DMAAB): Table 2, D13 to D16 and ED 17 and ED 18. The equilibrium swelling ratio is not sensitive to changes in the degree of cross-linking at pH 2 because of poor thermodynamic quality (often measured as the polymer–solvent interaction parameter) of the hydrating medium, which should favor intramolecular and intermolecular interactions among chains of the polymer network as reported previously.[2,12,23,24] The effect of the graded changes in the concentration of the cross-linking agent on the equilibrium swelling ratio (Q) becomes more discernible at pH 7.4, where there is ionization of the acrylic acid moieties along the polymer chains, which promotes swelling: Q decreased with increase in the concentration of DMAAB. As shown in Table 4, M_c correlates with Q. Thus the increase in cross-linking density with decrease in the value of M_c caused restriction to the expansion of the hydrogel network.

The hydrogels studied in this work were based on the model earlier reported by Kopecek and his coworkers:[2,12,14] the hydrogel systems have a low equilibrium degree of swelling in the low pH of the stomach and are able to protect the drugs in the acidic condition of the stomach. But the hydrogels swell in the high pH of the small intestine. To achieve a controllable kinetics of swelling during the transit time in the small intestine en route to the colon such that a high equilibrium degree of swelling necessary for biodegradation of the hydrogels could be achieved, *N*,*O*-disubstituted hydroxylamine moieties were introduced to the side chains or cross-links of the hydrogels.[2] It was of interest to us to see whether or not monomer solutions containing DMMA, AA, BuAA, DMAAB, and graded concentrations of the hydrolyzable cross-linking agent (MANHOMA) (Table 3) could be polymerized by the redox coinitiator system studied in this work.

Figure 6 shows the swelling isotherms of the hydrogels containing different amounts of hydrolyzable cross-linking agent: the initial burst in the

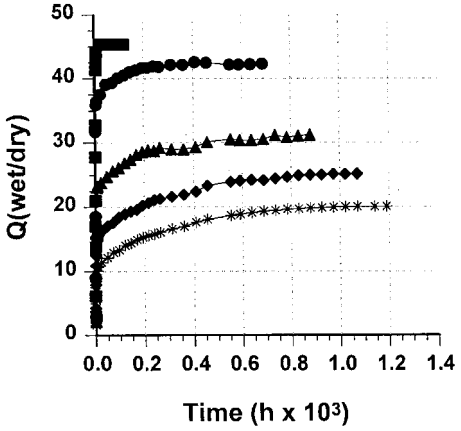

Figure 6. Effect of concentration (mol%) of hydrolyzable monomer (MANHOMA) on the swelling isotherms (pH 7.4; 37°C) of poly(DMAA-co-AA-co-BuAA) hydrogels doubly cross-linked with DMAAB and MANHOMA (Table 3, D19 to D23): ■, 0; ●, 0.5; ▲, 1.0; ◆, 2.0; ✳, 3.0. Standard deviation ε (0.08; 1.41).

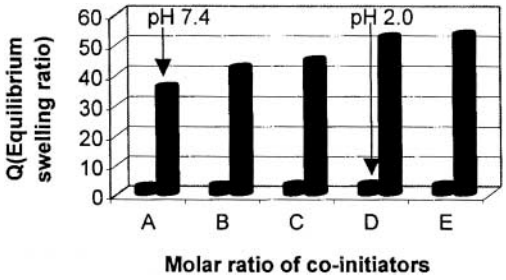

Figure 7. Effect of molar ratios of coinitiator (*N*-phenyldiethanolamine:benzoyl peroxide) on equilibrium swelling ratio (Q) of hydrogels (A 1:5; B 1:2; C 1:1; D 2:1; E 5:1). Table 3: D24* to D28*.

swelling of the hydrogels decreased with increase in the concentration of the hydrolyzable cross-linking agent (MANHOMA). The rate of swelling, consequent upon the hydrolysis of the -COONHCO- group in the hydrolyzable cross-linker, decreased with increase in the concentration of the cross-linking agent. It is possible that the two types of cross-linkers (one based on azo-bond and the other based on hydrolyzable -COONHCO- group) have a synergistic effect on the total cross-linking density of the hydrogels. The spaces between the neighboring polymer strands would be severely reduced due to the presence of two types of cross-links. Then the attractive forces holding the polymer lattice together such as van der Waals or hydrogen bonding forces, in addition to the cross-links, would be greatly increased. The increase in attractive forces coupled with polymer–polymer overlap would increase tremendously the contribution of chain entanglements to the cross-linking density. Table 4 shows that M_c and Q decreased with increase in the concentration of MANHOMA, which reflects an increase in the cross-linking density of the hydrogels. The concentration of either of the cross-linking agents can be manipulated to achieve hydrogels with desirable physical properties and which are degradable in the colon.

Tertiary amines have been used for a number of years as coinitiators for the peroxide-catalyzed polymerization of methyl methacrylate. Such systems have been widely used in the curing process of dental acrylic resins and bone cements. The amine and the peroxide molecules interact to produce free radicals that react with the methacrylate groups, bringing about polymerization. In the dental field, it is believed that at the time of formulation, it is important to know the appropriate concentration of peroxide for one portion and the concentration of amine for the other portion so that when the components are mixed together in specified amounts, the peroxide and the amine will have the proper stoichiometry.[25] Further, though the amine coinitiators comprise only a minor ingredient of the formulation of dental composite, their presence greatly influences such important properties as molecular weight, mechanical strength, shade, and color stability of cured material.[26] Figure 7 shows the influence of the molar ratio in which benzoyl peroxide and *N*-phenyldiethanolamine combined on the equilibrium swelling ratio of the hydrogels at pH 2.0 and 7.4. The molar ratios in which the coinitiators combined did not affect the swelling of the hydrogels at pH 2.0, due to a favorable microenvironment for polymer–polymer interactions. When the hydrogels were equilibrated at pH 7.4, then the molar ratios in which the coinitiators combined became significant. Decreasing the molar concentration of benzoyl peroxide in the presence of a constant amount of the amine (D24* to D26*, Table 3) gave hydrogels with higher equilibrium water content. Increasing the molar concentration of the amine in the presence of a constant amount of benzoyl peroxide (D26* to D28*, Table 3) increased the equilibrium water content, but the effect became reduced at higher concentration of the amine.

The relationship between the rate of polymerization of vinyl monomers and the initial concentrations of tertiary aromatic amine and benzoyl peroxide (coinitiator system) has attracted some interest.

The overall rate of polymerization, R, has been expressed as shown in Eq. (5)[25]

$$R = k([A][BP])^m \quad (5)$$

R is the overall rate of polymerization, k is an empirical constant, and [A] and [BP] are the initial concentrations (mol/L) of amine and benzoyl peroxide, respectively, in the monomer solution and the exponent, m, may depend on the experiment. This equation suggests that the rate of polymerization depends only upon the product [A] [BP] and not on the individual concentrations of the coinitiators. However, Bowen and Argentar[25] have revealed that as the polymerization proceeds, some benzoyl peroxide is wasted by a form of radical-induced decomposition that does not produce additional free radicals and that some of the certain derivatives of the amine formed after the original reaction might be capable of decomposing additional peroxide molecules. Their data showed that when the product of the concentrations of the coinitiators were held constant, the rate of polymerization was not constant as the ratio of amine and peroxide was varied from 1:40 to 40:1. They found the optimal ratio of the molar concentrations of peroxide to amine to be approximately 1.5:1.[25] Other workers have reported similar results. An excess amount of *N*,*N*-dimethyl-*p*-toluidine with respect to benzoyl peroxide was found to inhibit the polymerization process of methyl methacrylate.[27] Nitroxide radicals were detected when excess amine was used, which are capable of reacting rapidly with carbon-centered free radicals, thereby making them efficient inhibitors of the polymerization process. The inhibitory effect of excess amine in amine/peroxide coinitiator system has also been reported by Vazquez et al.[28] The data shown in Table 4 indicating an increase in Q as well as an increase in M_c with increase in the molar amount of the amine may be due to the effect of excess amine on the overall rate of polymerization. This reasoning is supported by the decrease in Q with increase in the concentration of benzoyl peroxide. More data appear to be needed in order to make a general conclusion on the effect of the molar ratio of the coinitiator system on the properties of the hydrogels.

CONCLUSIONS

Organic redox-initiated copolymerization process can be used to fabricate *N*,*N*′-dimethylacrylamide-based hydrogels at ambient temperature. The graded changes in the concentration of the organic-redox coinitiator system, the ratio in which coinitiators (benzoyl peroxide/*N*-phenyldiethanolamine) combined, the type of polymerization solvents, graded changes in the amount of cross-linking agent based on the aromatic azo-bond and that based on pH-sensitive moieties all affected the physical properties of the hydrogels. The polymerization technique appears suitable for the fabrication of drug delivery systems containing thermolabile bioactive agents like peptides and proteins.

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