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Hydrogels based on graft copolymerization of HEMA/BMA mixtures onto soluble gelatin: swelling behaviour

B. Vázquez*, M. Gurruchaga and I. Goñi
Dpto. Ciencia y Tecnología de Polímeros, Facultad de Química de San Sebastián, Apartado 1072, San Sebastian 20080, Spain
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Graft copolymerization of mixtures of 2-hydroxyethyl methacrylate (HEMA) with n-butyl methacrylate (BMA) onto soluble gelatin was attempted using ceric ammonium nitrate as initiator, with the aim of obtaining hydrogels based on two different components: a natural polymer and a synthetic polymer. Other hydrogels were formed by simultaneous graft copolymerization and crosslinking using ethylene glycol dimethacrylate (EGDMA) as crosslinking agent. Per cent grafting (%*G*), per cent grafting efficiency (%*GE*) and per cent total conversion (%C_t) were calculated for various feed compositions and for various EGDMA concentrations. Equilibrium water content of the hydrogels was investigated as a function of the hydrophilicity of the polymers and the degree of crosslinking agent, and the influence of pH on the swelling behaviour of the hydrogels was determined. It was observed that the equilibrium water content increased as the percentage of hydrophilic monomer in the copolymer increased, and the swelling values increased with pH changes from the acid to the alkaline range.

(Keywords: graft copolymerization; hydrogels; swelling behaviour)

Introduction

Polymeric hydrogels are of considerable importance due to their potential applications as biomaterials[1,2]. Thus, it is of interest to synthesize hydrogels from synthetic macromolecular moieties and to study their utility in various applications, especially in biomedical areas. Since the incorporation of a biological macromolecule results in good tissue tolerance both *in vitro* and *in vivo*, it was assumed that a combination of synthetic macromolecules with natural macromolecules might yield composites whose properties would combine the advantages of both materials: mechanical stability and biological acceptability by the organism. One method of approaching this synthesis of hydrogels is to graft hydrophilic monomers in combination with hydrophobic monomers onto natural polymers such as starch, cellulose or collagen[3–6]. In this sense, composites of gelatin offer a good prospect for success in the field of biomaterials[7,8], and it was presumed that copolymeric hydrogels of gelatin and 2-hydroxyethyl methacrylate (HEMA) with n-butyl methacrylate (BMA) may combine some of the advantageous properties of the three constituents. On the other hand, the biocompatibility of HEMA–n alkyl methacrylate copolymers has been largely demonstrated[9,10].

In the present study, hydrogels were prepared by simultaneous graft copolymerization of HEMA in combination with BMA onto gelatin. The relative amounts of the comonomers were changed in order to create polymeric networks with different swelling characteristics, and the effect of ethylene glycol dimethacrylate as crosslinking agent on the grafting reaction was studied. The gels so obtained were characterized for their water retention, which is an important parameter for a given hydrogel.

* To whom correspondence should be addressed

Experimental

Materials. Gelatin was in the form of granules and was used as received. HEMA, BMA and ethylene glycol dimethacrylate (EGDMA; from Merck) were purified by washing twice with dilute alkali and then several times with distilled water to remove the inhibitor before distillation under nitrogen reduced pressure.

A solution of 0.1 N ceric ammonium nitrate (Fluka) in 1 N nitric acid was used as initiator.

Preparation of hydrogels. Grafting of HEMA and BMA onto gelatin was first carried out from different feed compositions without using a crosslinking agent. In all preparations, 1 wt% gelatin solution in water was used with varying concentrations of HEMA and BMA monomers, and the reaction was initiated by ceric ammonium nitrate[11,12]. Comonomer feed solutions contained HEMA of 0, 20, 40, 50, 60, 80 and 100 mol%, and the remainder of the mixture was BMA. In each case, the gelatin solution (300 cm^3) was purged by passing purified nitrogen for 30 min. Appropriate quantities of both monomers (0.047 mol total) were added to the gelatin solution, then 5 min later 10 cm^3 of the initiator solution, performing the grafting reaction under magnetic stirring. The polymerization reaction was allowed to proceed for 4 h at 30°C under nitrogen atmosphere and was terminated by addition of hydroquinone[13]. The reaction was carried out under a constant light source because the oxidative capability of the Ce(IV) ions varies considerably under light[14].

Another series of grafted samples, with equal amounts of HEMA and BMA (50/50 mol ratio) and with different amounts of EGDMA added to the monomer mixture as a crosslinking agent, were prepared in the same way. The EGDMA concentration was varied from 0 to 11.3 mol% with respect to total monomer concentration in the feed.

EGDMA was added to the gelatin solution with the monomer mixture.

At the end of the reaction, the reaction medium was neutralized at pH 7 with dilute alkali and then centrifuged at 12 500 rev min^{-1} for 30 min. The resulting products were then exhaustively washed with 1 N nitric acid and distilled water to remove residual monomer and initiator. This was followed by Soxhlet extraction with tetrahydrofuran and ethanol to remove the homopolymer. The amount of apparent homopolymer was obtained from the difference in weight between extracted and original specimens, each weighed in a moisture-free state.

The percentage grafting (%G) was calculated as follows:

$$\%G = \frac{\text{weight of graft copolymer} - \text{weight of gelatin}}{\text{weight of gelatin}} \times 100$$

The grafting efficiency (%GE) was calculated using:

$$\%GE = \frac{\text{weight of grafted polymers}}{\text{weight of grafted polymers} + \text{weight of unbound homopolymer}} \times 100$$

The per cent total conversion (%C_t) was calculated as follows:

$$\%C_t = \frac{\text{weight of total acrylic polymer}}{\text{weight of initial monomer}} \times 100$$

Characterization. Grafted acrylic copolymer composition. The composition of the grafted acrylic copolymers was determined by ^{13}C n.m.r. spectroscopy. The spectra were recorded with 20% (w/v) perdeuterated dimethylsulfoxide and pyridine at 80°C, a pulse width of 13 μs, a relaxation delay of 5 s and inverse gated decoupling in the acquisition and spectral width of 16 k data points. These conditions ensure the complete relaxation of all the ^{13}C nuclei analysed. The relaxation times (T_1) for carbon nuclei were determined from the inversion/recovery curves in order to select adequate peaks for integration. Relative peak intensities were measured from peak areas calculated by means of an electronic integrator.

Swelling behaviour. The xerogel was obtained by drying the hydrogel in a vacuum oven at 60°C over $CaCl_2$. The water content of the rehydrated xerogels was determined after adequate equilibration time in water. The water content was calculated using the relationship[5,15]:

$$\text{Water content } (\%) = \frac{W_{eq} - W_{dry}}{W_{eq}} \times 100$$

where W_{dry} = initial dry weight of the sample and W_{eq} = wet weight after equilibration in water.

The influence of pH on the swelling behaviour of the hydrogels was determined by placing the hydrogels in buffers with pH values ranging from 2.0 to 10.0 at room temperature until equilibrium was attained. Buffers used for the swelling studies were: pH 2.0, 0.012 N hydrochloric acid; pH 10.0, boric acid/potassium chloride sodium hydroxide; pH 7.4, 0.01 M phosphate buffer.

Results and discussion

Grafted acrylic copolymer composition. Analysis of the composition of the grafted copolymers was carried out from their proton decoupled ^{13}C n.m.r. spectra. The spectra were recorded in conditions of quantitative relationship, i.e. a long delay time and minimum nuclear Overhauser enhancement effect as well as a high number of scans. So, to establish any comparison among the different peaks, the relaxation times must be taken into account. For a 13 μs pulse width (as in our case) the pulse spacing must be five times the spin-lattice relaxation time (T_1) to ensure 99% relaxation[16], since the spin-lattice relaxation time (T_1) defines the rate of the equilibrium process. Thus, in order to calculate the copolymer composition we have measured the T_1 of the carbons of poly(HEMA) and poly(BMA) (*Table 1*). In view of these values, and taking into account that we used a delay between pulses of 5.0 s, signals adequate for quantitative study are as follows: poly(HEMA): C^{β}, OCH_2, CH_2OH, $(CH_3)_{\alpha}$; poly(BMA): C^{β}, C^1, C^2, $(CH_3)_{\alpha}$. The copolymer composition was calculated by comparing the integrated intensities of the signal at δ 66.31, which was assigned to the resonance of OCH_2 carbon of poly(HEMA) units with that of the signals at δ 30.15 assigned to the resonance of C^2 of poly(BMA) units.

The composition of the grafted branches obtained from HEMA/BMA molar fractions in the feed of 80/20, 60/40, 50/50, 40/60 and 20/80, were 60/40, 58/42, 38/62, 13/87 and 10/90 respectively, which means that BMA comonomer presented higher reactivity than HEMA in the graft copolymerization carried out under the experimental conditions described above.

Grafting parameters. The variations of grafting parameters with feed composition for the first series of experiments are shown in *Figure 1*. It can be seen that the %GE is high in all cases, ranging from 90 to 100%. Taking into consideration that a grafting efficiency of 100% means no homopolymer formation, the high values obtained for this parameter in all cases indicate the trend of acrylic molecules to react with the radicals formed on the gelatin macromolecules.

On the other hand, the values of %G obtained from each feed are higher than those obtained when only one monomer took part in the grafting reaction. This parameter yields a maximum value at HEMA/BMA 60/40 mol ratio in the feed. For the rest of the feed

Table 1 Spin-lattice relaxation times (T_1) for different carbons of poly(HEMA) and poly(BMA)

Polymer	Carbon	T_1 (s)
poly(HEMA)	C^{β}	0.154
	C^{α}	1.940
	OCH_2	0.250
	CH_2OH	0.210
	$(CH_3)_{\alpha}$	0.007
poly(BMA)	C^{β}	0.100
	C^{α}	1.400
	C^1	0.261
	C^2	0.483
	C^3	1.066
	C^4	1.076
	$(CH_3)_{\alpha}$	0.081

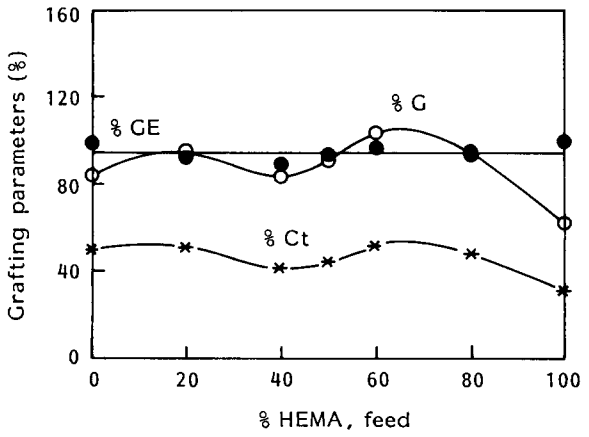

Figure 1 Variation of grafting parameters with feed composition for the graft copolymerization of HEMA/BMA mixtures onto soluble gelatin

Table 2 Graft copolymerization of HEMA and BMA (50/50 mol ratio) onto soluble gelatin using EGDMA as crosslinking agent

EGDMA (mol%)	G (%)	C_t (%)
11.30	163	64
5.65	139	59
2.77	116	52
1.13	110	50
0.55	112	51
0.28	91	41
0.00	85	38

compositions, $\%G$ remained nearly the same. The values of the per cent total conversion ($\%C_t$) varied with feed composition in a similar way as $\%G$ did. Yields ranging between 40 and 50% were obtained when both monomers were grafted onto gelatin.

Table 2 gives the results from a second series of graft copolymerizations with equal amounts of both monomers and different amounts of crosslinker added to the grafting reactions. The grafting efficiency for all cases was 100%. Taking into consideration the large values of this parameter obtained when grafted without crosslinking agent, this seems to indicate that the crosslinking agent molecules cause all the acrylic copolymer formed in the reaction to graft onto gelatin[4]. Both $\%G$ and $\%C_t$ increased with EGDMA concentration in the whole range of EGDMA concentrations. This can be explained by the fact that an increase in EGDMA concentration means an increase in monomer concentration.

Swelling behaviour. The swelling behaviour of the grafts with different copolymer composition was determined gravimetrically by measuring the weight gain with time when the gels were placed in the corresponding buffered solution at room temperature. Measurements were taken until the equilibrium was reached, which was considered to be when three consecutive determinations gave the same weight. The time to reach swelling equilibrium was a week for all the samples. The swelling behaviour for these gels based on gelatin was first explored using water, and the results obtained are shown in *Figure 2B*. All the gels investigated contained both a hydrophilic and a hydrophobic component. Pure gelatin was soluble in water. Pure poly(HEMA) showed only a 40% equilibrium swelling value[17]. Gelatin-*g*-poly(HEMA) hydrogel swelled in water to a maximum of 60%. This appeared to be a very interesting observation, since a natural macromolecule of high molecular weight such as gelatin could influence the swelling behaviour of poly(HEMA) to a significant extent. It has been observed that the equilibrium water content of the graft copolymers increases as the percentage of hydrophilic monomer in the copolymer increases.

The influence of pH on the swelling behaviour of the hydrogels (*Figure 2*) was determined by placing the hydrogels in buffers of pH 2.0 (the stomach pH), pH 7.4 (the colon pH), and pH 10.0, since all of these values are important in biomedical applications. Results of equilibrium water content for samples equilibrated in buffered solution of pH 7.4 were similar to those obtained for samples equilibrated in water. It can be observed that the equilibrium swelling values increased with increasing hydrophilicity of the hydrogels for any pH. The more hydrophilic the gels, the lower the pH sensitivity and therefore the smaller the difference between swelling at high and low pH. This behaviour is clearly observed for the gelatin-*g*-poly(HEMA) copolymer, which showed a degree of swelling of 53% at pH 2, 58% at pH 7.4, and 62% at pH 10.0. In the rest of the compositions it was observed that the equilibrium swelling values increased with pH changes from the acidic (A) to neutral (B) or alkaline (C) range.

The water contents of a second series of samples, synthesized at HEMA/BMA 50/50 mol ratio in the feed in the presence of different crosslinker (EGDMA) amounts, are shown in *Figure 3* as a function of pH. The water content of these hydrogels (*Figure 3B*) showed a slight increase for low levels of crosslinking agent. Further increases in crosslinking agent concentration gave rise to a decrease in the equilibrium water content, which means that high crosslinking density restricts the swelling. This may be due to the fact that an increase in the crosslink density decreases both mobility and hydrophilicity within the network and, therefore, the availability of the

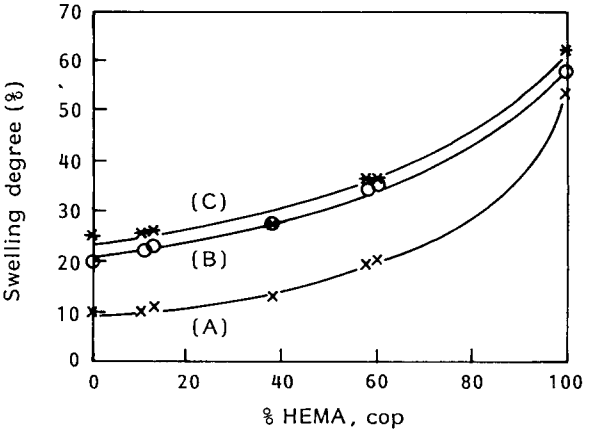

Figure 2 Variation of equilibrium water content (%) as a function of HEMA concentration in the grafted copolymer at different pH values: (A) pH 2.0; (B) pH 7.4; (C) pH 10.0

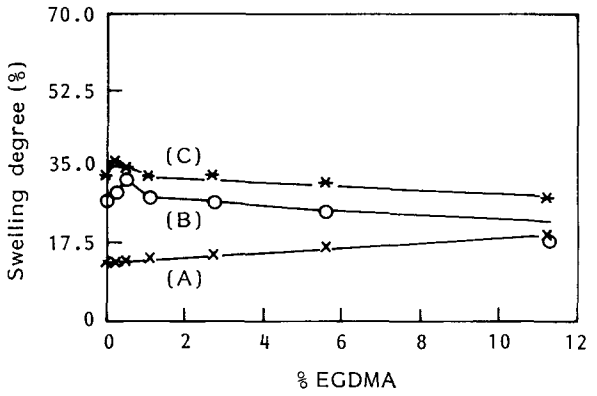

Figure 3 Variation of equilibrium water content (%) as a function of EGDMA concentration in the feed at different pH values: (A) pH 2.0; (B) pH 7.4; (C) pH 10.0

hydrophilic binding sites due to increased steric occlusion, as reported by Corkhill *et al.*[18] in their water binding studies in hydroxyalkyl acrylate and methacrylate copolymers. These results are also consistent with those for swelling of crosslinked poly(HEMA) found by other investigators[19,20]. Regarding the swelling behaviour at different pH values, we again obtained lower equilibrium water contents at acidic pH.

It is clear from *Figures 2* and *3* that the structure of the grafted copolymers, crosslinked or not, influenced the pH dependent swelling. As is well known, these pH transitions are characteristic of specimens based on polyelectrolyte gels of polyacrylic acid or copolymers bearing carboxylic groups as side substituents[21,22]. Thus, in acidic media the hydrophobic nature of the non-ionized acidic groups affects the conformational arrangement of the random coil and the macromolecular segments tend to collapse, with the resulting exclusion of the buffered solution. In this sense, we point out that the lower equilibrium water contents obtained at low pH for our copolymers might be due to the presence of hydrophobic interactions and/or intermolecular interactions through hydrogen bonds with the hydrophilic residues, which would lead to a compact conformation of the macromolecular random coil with a corresponding decrease of the polymer chain mobility[23–25]. However, we want to stress here that this explanation should be considered only as a possible or partial explanation since a deeper investigation by other techniques, e.g. differential scanning calorimetry, is needed to advance a convincing reason for this effect.

Conclusions

We have shown that by combining different amounts of both hydrophobic and hydrophilic monomers with a natural substrate such as gelatin, in the presence or absence of crosslinking agent, we have obtained pH sensitive hydrogels with different degrees of swelling. These characteristics, together with the demonstrated biocompatibility of the three constituents[9,10,26], offer an excellent system to be used as support for controlled drug delivery formulations[27–29].

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