Swelling of Crosslinked Poly(Methylmethacrylate-Acrylic Acid) Copolymers in Serum and Saline Solutions

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ABSTRACT: In recent articles, our research group explored the use of crosslinked Poly(methylmethacrylate-acrylic acid) and composites based on this copolymer for bone implant applications such as suture anchors. The swelling response of this system was studied first *in vitro*, using a 0.85 g/100-mL saline solution (chosen because it simulates well the *in vivo* environment), and later *in vivo* by using samples implanted for various time periods in the lateral femoral condyles of New Zealand white rabbits. It was found that the swelling response of the crosslinked copolymer *in vivo* was much greater than that in the saline solution. The present investigation was conducted to determine the mechanism of excessive swelling in the *in vivo* tests. The approach used was to establish the changes occurring in the chemical structure of the copolymer due to immersion in serum. A number of hypotheses that can potentially explain the observed excessive swelling in serum were investigated and are discussed in this article. The results of this study indicate that the mechanism of excessive swelling in serum was the neutralization of —COOH groups in the copolymer to produce salts of acrylic acid, which are known to result in greater swell due to their higher degree of dissociation compared to free acid. It was also found that, for compositions containing the acrylic salts (produced by preswelling in high pH solutions and drying), the swelling behavior in serum was similar to that in saline solution, and more importantly, equilibrium swelling was reached in a relatively shorter time period, which has several practical advantages for bioimplant applications. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 79: 1653–1664, 2001

Key words: crosslinked polymethylmethacrylate-acrylic acid copolymers; *in vivo* entironment

INTRODUCTION

The use of swelling materials in the body for contact lenses and drug delivery applications is already well established.[1–6] In particular, polymeric and copolymeric materials based on poly(hydroxyethylmethacrylate) (HEMA), polyvinylpyrrolidone (PVP), and polyacrylic acid (PAA) have been explored aggressively for these applications. Attempts at developing swelling polymeric composites for bone implant applications have, however, been relatively unsuccessful. A stringent requirement for bone implant applications is obtaining good fixation strengths, which can only be achieved by controlled swelling of the implant. Excessive swelling of the implant causes a signif-

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kicant deterioration of the mechanical stiffness and strength of the implant, and adversely affects the fixation strength. The most promising attempt at using swelling polymers for bone implant applications was by Kamel and coworkers in the late 1970s, who developed composites comprised of a PAA matrix reinforced initially with alumina particles and later with graphite fiber braids.[7–10] Their aim was to provide implants that swell by absorption of body fluids, and thereby achieve bone fixation, superior to conventional metal implants, via an expansion fit mechanism. *In vivo* implantations using a canine model showed good compatibility and implant anchorage, without inflammatory response. However, the implants achieved excessive swelling (40–80%).

Recently, our research group explored the use of crosslinked Poly(methylmethacrylate-acrylic acid), hereafter denoted as P(MMA-AA), and composites based on this copolymer for bone implant applications such as suture anchors.[11–13] The degree of swelling of this system is controlled not only by the amount of acrylic acid (AA), but also by the amount of crosslinker, allylmethacrylate (AMA). Higher AA content results in higher swell, whereas higher AMA content results in lower swell.

Various compositions of this system were recently studied *in vitro* using a 0.85 g/100 mL saline solution, and *in vivo* by using samples implanted for various time periods in the lateral femoral condyles of New Zealand white rabbits. It was expected that the swelling behavior in saline solution would be similar to that *in vivo*. Instead, we found that the swelling *in vivo* was much greater than that in saline solution. This was confirmed by *in vitro* swelling studies of the same crosslinked system in serum. The present investigation was conducted to determine the mechanism of excessive swelling in the *in vivo* tests. The approach we used was to establish the changes occurring in the chemical structure of the copolymer due to immersion in serum. Once this was established, these changes were related to the composition of serum, and the swelling results were rationalized based on the chemical modifications that had been affected. The final conclusions were verified by chemically modifying the original copolymer to the same structure postulated after immersion in serum and checking the effect on swelling behavior.

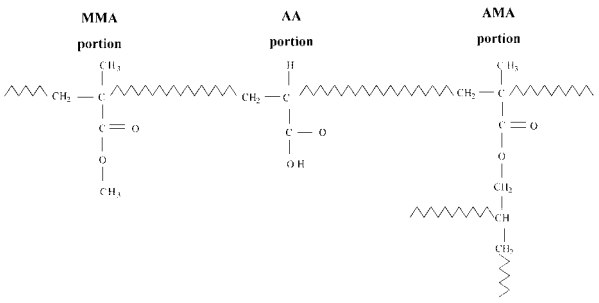


Figure 1 Chemical structure of the crosslinked P(MMA-AA) copolymer used in this study.

MATERIALS AND METHODS

Polymer Preparation

The P(MMA-AA) copolymer used in this study was made from the monomers methylmethacrylate (MMA) and acrylic acid (AA), and the crosslinking agent allylmethacrylate (AMA). All chemicals were purchased from Aldrich Chemical Co., Milwaukee, WI, and used as received without any further purification. The structure of the polymer made from these monomers is depicted in Figure 1.

Polymerization was carried out in bulk using a free radical mechanism with the initiator 2,2′-azobis 2,4-Dimethylvaleronitrile obtained from E.I. Dupont de Nemours & Co., Wilmington, DE. Varying ratios of MMA/AA monomers were mixed thoroughly with desired amounts of the crosslinker and the initiator. The amount of the initiator used was fixed, in all cases, to 0.4 g/100 mL of the total mixture. Table I lists the different compositions used in this study, along with the labeling system used that was used in our previous studies.[11–13] In this labeling system, the material is referred by a set of three numbers as *x*/100-*x*/*y*, and denotes that *x* mL of MMA, 100-*x* mL of AA, and *y* mL of AMA were used for producing that sample. The components were mixed and then allowed to sit for several hours before polymerization to ensure complete degassing. Polymerization was conducted in glass test tubes, which were tightly sealed and placed upright in a temperature controlled water bath. The course of the polymerization was chosen so as to prevent the formation of gas bubbles. The temperature was gradually raised (3°C/h) over several days to 65°C and kept at that temperature for 2 days. The glass tubes were removed from the bath, allowed to cool, and the polymer samples retrieved after

Table I Compositions of the Samples Used in This Study

Sample ID	% Volume		
	MMA	AA	AMA
100/0/0	100	0	0
100/0/10	90.91	0	9.09
95/5/10	86.36	4.55	9.09
90/10/10	81.82	9.09	9.09
85/15/10	72.27	16.64	9.09
80/20/0	80	20	0
80/20/5	76.19	19.05	4.76
80/20/10	72.73	18.18	9.09
80/20/15	69.57	17.39	13.04
80/20/20	66.67	16.67	16.67
70/30/10	63.64	27.27	9.09
60/40/10	54.55	36.36	9.09

breaking the tubes. After this primary polymerization stage was completed, the samples were postcured to complete the crosslinking reaction and to ensure that no free monomers still remained. This was accomplished by placing them in a temperature-controlled oven where the temperature was raised slowly (1°C/min) to 150°C. The samples were left at 150°C for a period of at least 5 h, followed by overnight cooling. After the postcuring stage, the samples were either machined to shape using a lathe or cut to shape using a low-speed diamond saw, depending upon the desired shape.

Swelling Measurements

Swelling measurements (described here as percentage weight changes) were carried out on samples containing MMA/AA/AMA in various ratios. (Percentage weight changes can be realted to percentage volume changes in this system through the densities of the solid and the fluid absorbed.[11]) Thin circular disks of the polymer, of approximately 1 mm thickness and 13 mm in diameter, were cut from the tube-shaped material using a diamond saw and dried in an oven for 6 h at 80°C. Subsequently, groups of each sample type were fully immersed in different media at 25°C. The media used in this study included: (1) distilled water; (2) 0.85% NaCl solution obtained from Fisher Scientific, Fair Lawn, NJ; (3) fetal bovine serum obtained from Atlanta Biologicals, Norcross, GA; and (4) buffered pH solutions (from pH = 6 to 9), also obtained from Fisher Scientific.

At various time intervals the swollen discs were weighed, after being surface dried, and the percentage swell at any given time (% weight gain, %W) was calculated as follows:

$$\%W = [(W_t - W_o)/W_o)] \times 100 \quad (1)$$

where W_t is the weight of the disk at time t, and W_o is the weight of the disk in the dry state at time zero. At least two samples were used in each swelling study reported in this article. The measurements were always very consistent between the samples used for the same swelling study (typically the differences are less than 1%), and in the plots presented in this article, the average value has been used.

It should be noted that, although the control material (100% polymethylmethacrylate) is referred to as nonswelling, it does achieve a small percentage of swelling (1.7%), inherent to many polymers.

Titration Measurements

Titration techniques were used to measure the carboxyl content in the dry and swollen copolymers. Because titration requires the polymer samples to be fully dissolved, this necessitated that only uncrosslinked samples were used. For this purpose, we chose two compositions: 80/20/0 and 100/0/0 (MMA/AA/AMA). After swelling for various periods of time, the samples were dissolved in appropriate solvents and then titrated with 0.1 *M* NaOH solution using phenolphthalein, alcoholic 0.5%, as an indicator.

Toluene/acetone solvent in a ratio of 4 : 1 was used to dissolve the pure PMMA (100/0/0) samples. Methylene chloride/ethanol solvent in a ratio of 3 : 1 was used to dissolve the 80/20/0 copolymer before and after swelling in saline solution. However, the same copolymer after swelling in serum required the use of dimethyl sulfoxide for dissolution.

The carboxyl content in each sample was quantified based on the original dry weight of sample before swelling and the amount of NaOH required to reach an end point in the titration.

RESULTS AND DISCUSSION

Effect of Polymer Composition on Swelling Behavior

Figures 2–4 show the effect of polymer composition on swelling behavior in water, saline solu-

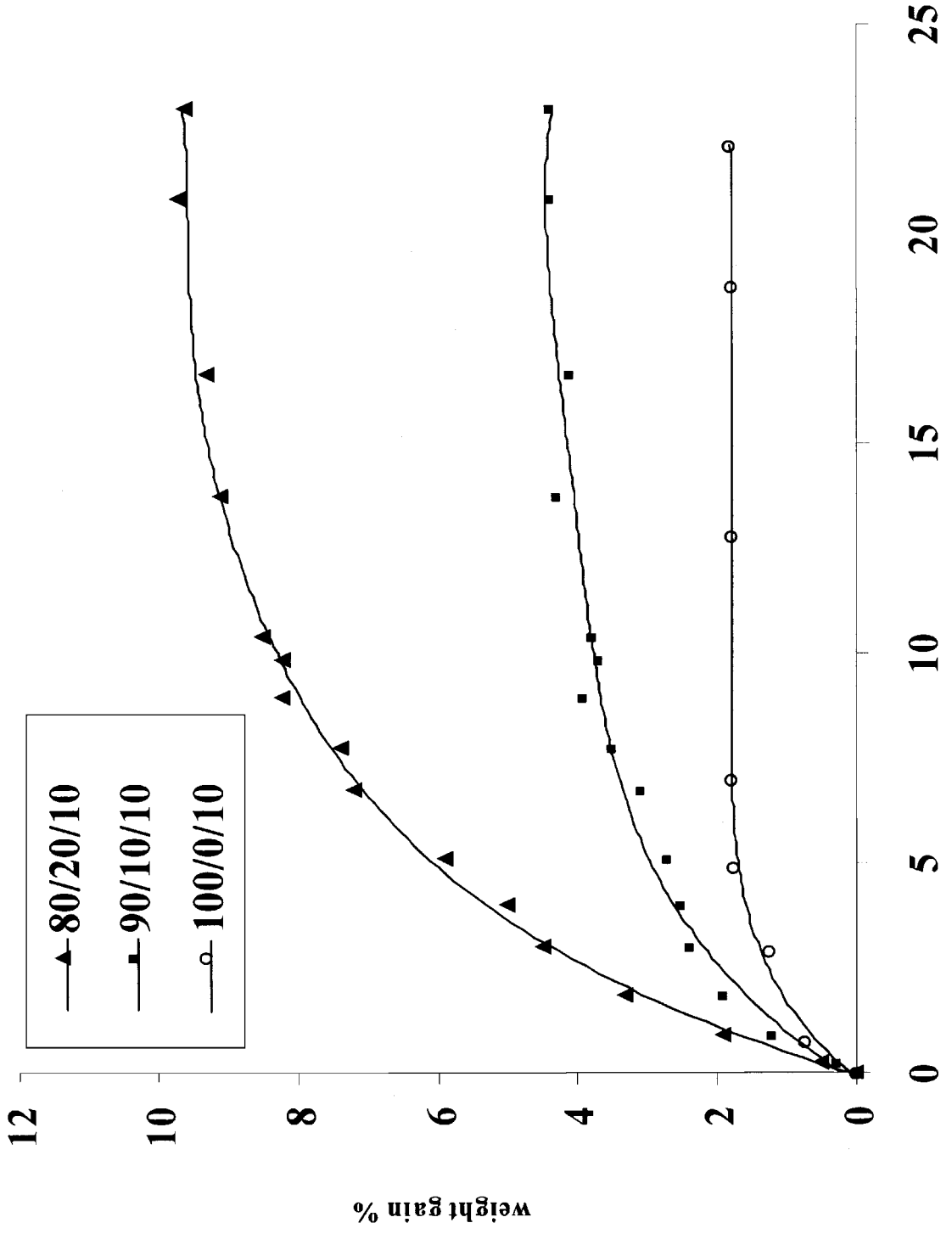
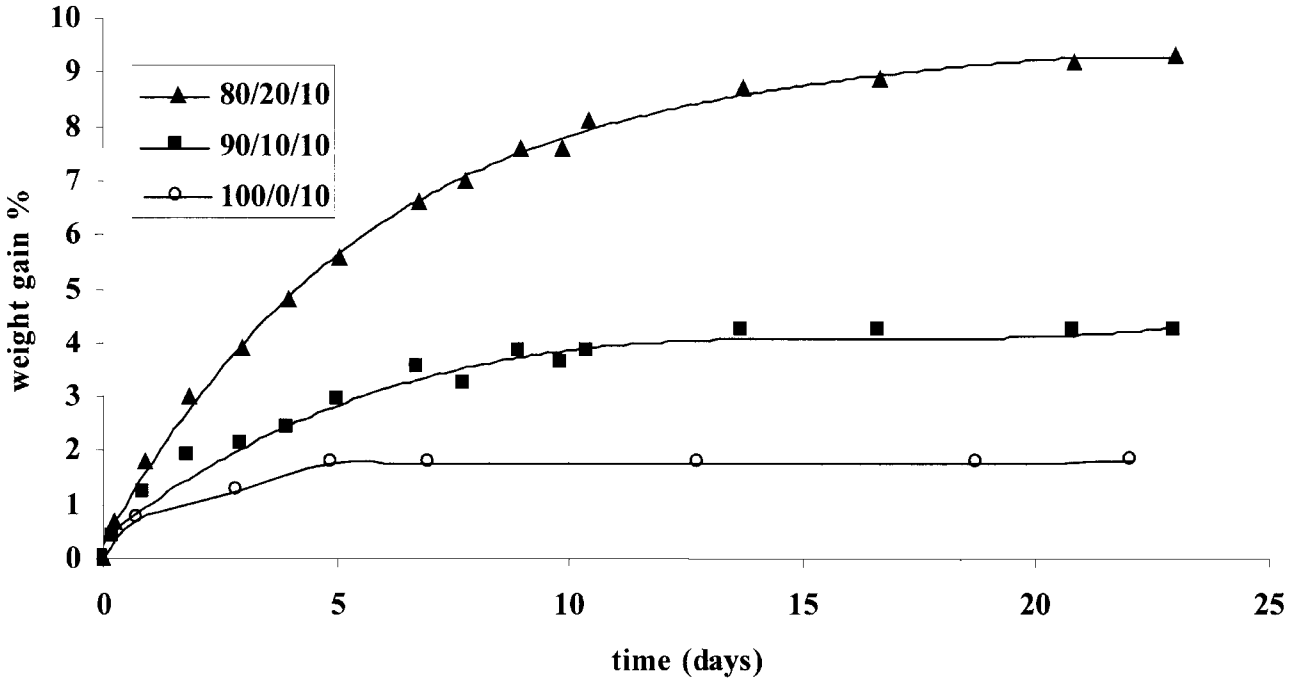
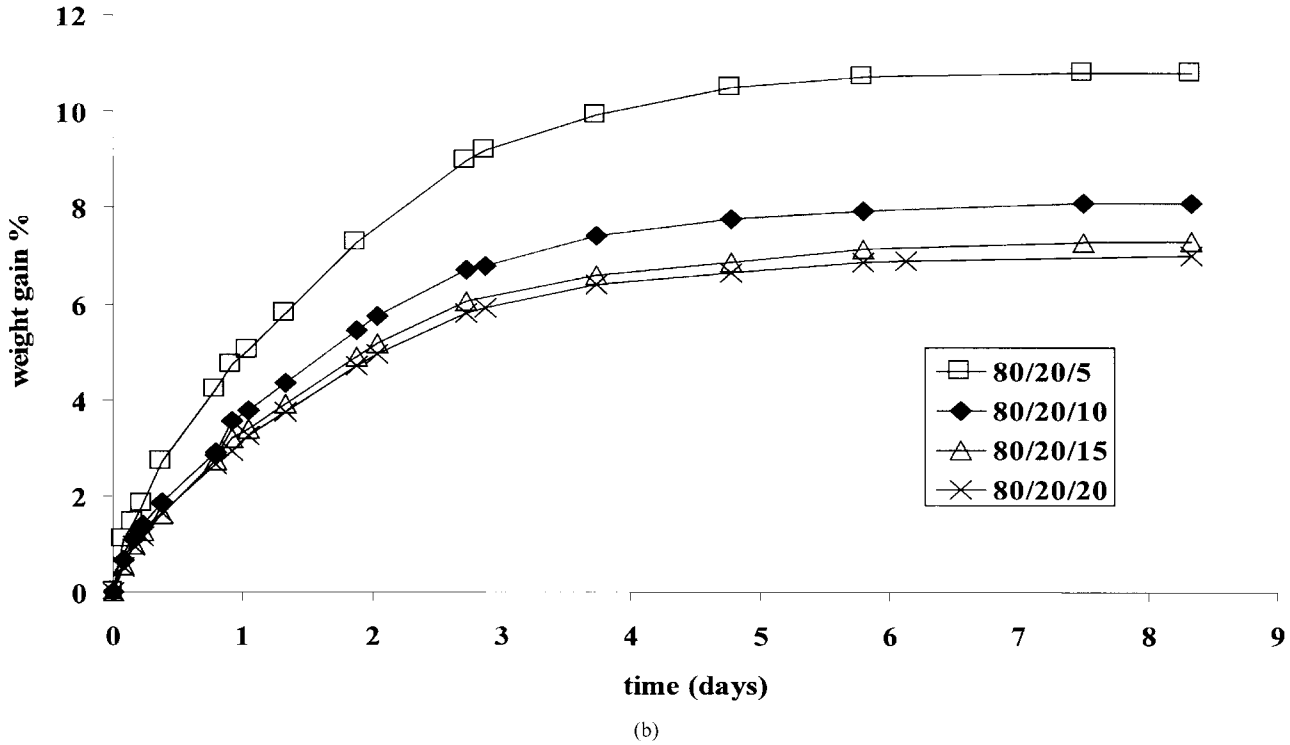


Figure 2 The swelling behavior of the copolymer MMA/AA/AMA system in distilled water for different ratios of AA at fixed AMA content.



(a)



(b)

Figure 3 (a) The swelling behavior of the copolymer MMA/AA/AMA system in 0.85% saline solution for different ratios of AA at fixed AMA content. (b) The swelling behavior of the copolymer MMA/AA/AMA system in 0.85% saline solution for different ratios of AMA at fixed AA content.

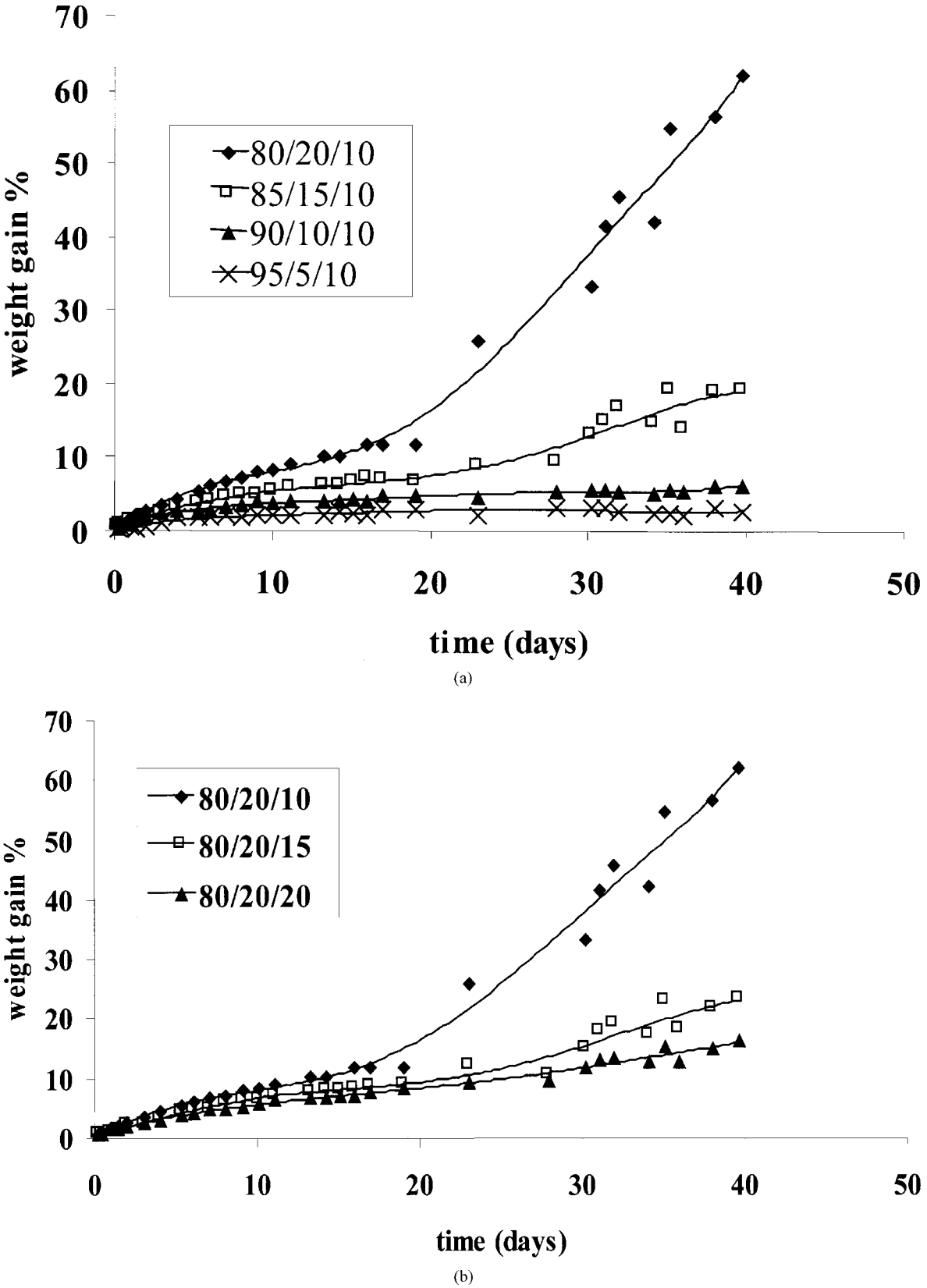


Figure 4 (a) The swelling behavior of the copolymer MMA/AA/AMA system for different ratios of AA at fixed AMA content swelled in bovine serum. (b) The swelling behavior of the copolymer MMA/AA/AMA system for different ratios of AMA at fixed AA content swelled in bovine serum.

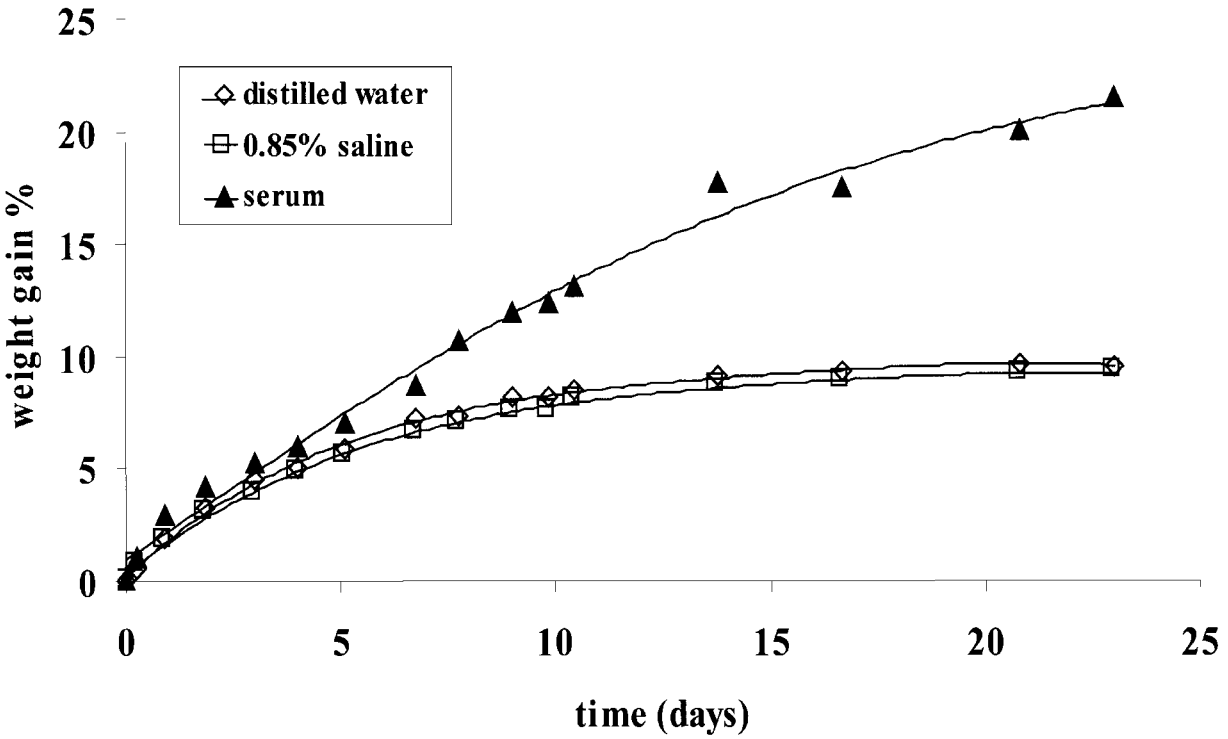


Figure 5 Comparison of the swelling behavior of the 80/20/10 (MMA/AA/AMA) copolymer in saline solution, serum, and distilled water.

tion, and serum. In all media, the % swell consistently increased as the concentration of acrylic acid increased at constant crosslinked density (represented by the AMA concentration); and decreased as the crosslink density increased at constant acrylic acid content.

Effect of Swelling Medium on Swelling Behavior

Figure 5 shows the effect of medium on the swelling behavior of an 80 MMA/20 AA/10 AMA composition. The % swell in saline solution was roughly the same as the % swell in distilled water. This behavior is unlike the established and known trends of the swelling behavior of superabsorbent polymers in the same solutions. It is well known that the swellability of superabsorbent polymers in saline solution is significantly lower than that in distilled water,[14–18] Figure 5 also shows that swelling in serum is substantially higher than swelling in saline solution. This is despite the fact that both saline solution and serum contain similar concentrations of sodium salt (145 mEq/L in saline and 132 mEq/L in serum). This finding was particularly surprising because it was expected that the swelling behavior of the material in saline solution would be similar to the swelling behavior in the *in vivo* fluid. To determine the mechanism of excessive swelling in serum, three hypotheses were formulated and investigated: (1) Hypothesis I—the crosslink density of the copolymer network decreased when exposed to serum. (2) Hypothesis II—some of the ester groups of the MMA and AMA components were hydrolyzed resulting in an increase in the —COOH groups of the system. Hydrolysis of the ester groups of AMA would additionally lead to a decrease in crosslinking, and, thus, increase swelling. (3) Hypothesis III—The —COOH groups in the copolymer were neutralized to give salts of acrylic acid, which are known to result in greater swell due to their higher degree of dissociation compared to free acid.

Hypothesis I was based on previously established results that showed an increase in swelling with a decrease in crosslink density [Fig. 3(b)]. However, for this to take place, a cleavage of C—C bonds in the polymer network or hydrolysis of some the ester groups of AMA would have to take place. Cleavage of C—C bonds was considered to be very unlikely under the mild swelling conditions used for these measurements. Further, if hydrolysis of the ester groups of AMA was taking place, the same should have happened to the

Table II Summary Table of Titration Results

Sample Swelling Medium	Average Amt. of Swelling Achieved (%W)	Solvent	Average Expected % COO— (wt %)	Average Actual % COO— (wt %)
80/20/0 dry control	0	3 : 1 methylene chloride/ethanol	13.18	14.57 (n = 6)
80/20/0 saline solution	17.94	3 : 1 methylene chloride ethanol	13.18	11.32 (n = 2)
80/20/0 serum	48.31	DMSO	13.18	6.85 (n = 3)
	300	DMSO	13.18	3.52 (n = 1)
100/0/0 dry control	0	4 : 1 toluene/acetone	0	0
100/0/0 saline solution	1.80	4 : 1 toluene/acetone	0	0
100/0/0 serum	2.22	4 : 1 toluene/acetone	0	0

more abundant ester groups of MMA. In both cases, one would expect an increase in the carboxyl group content, which is the premise of the second hypothesis.

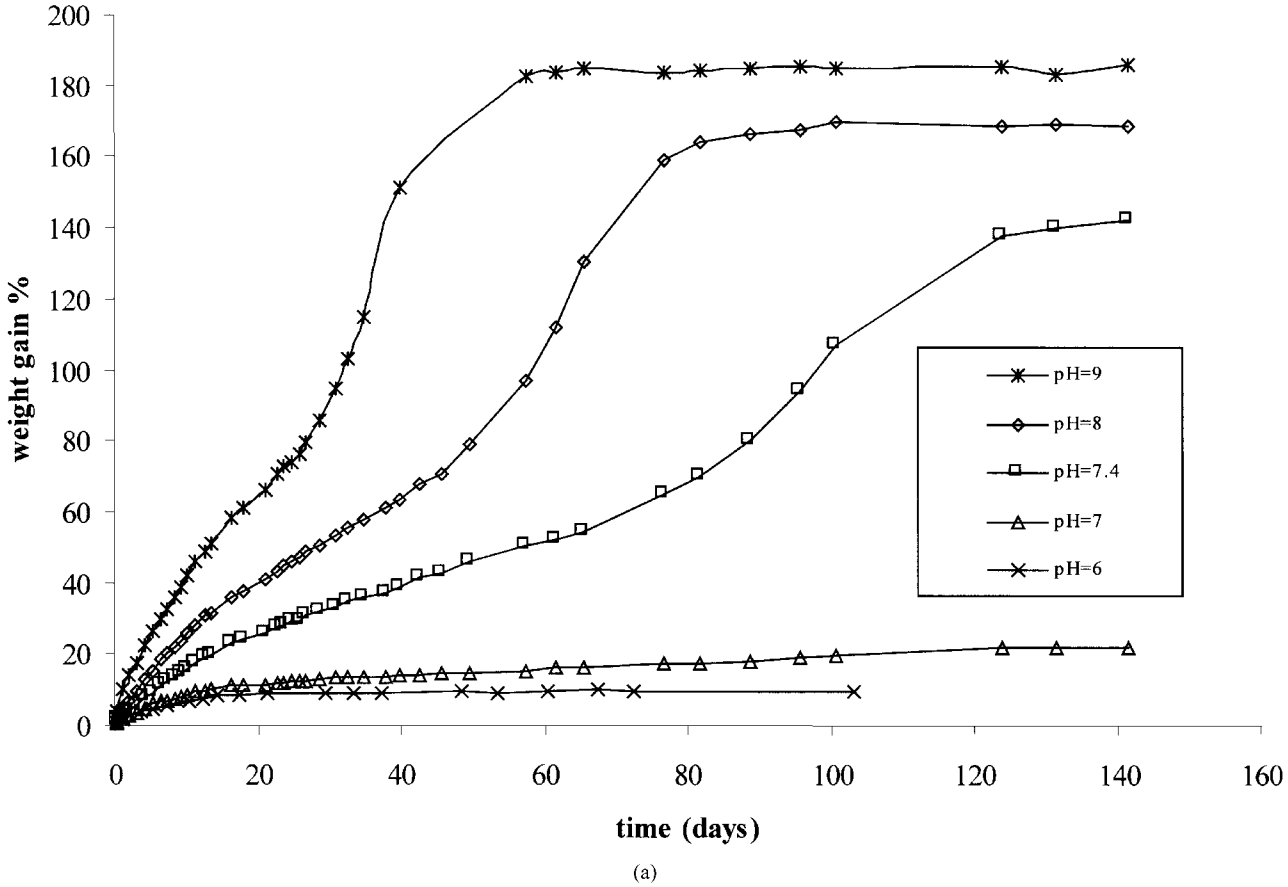
According to Hypothesis II, hydrolysis of some of the ester groups during immersion in serum could result due to the presence of certain enzymes acting as catalysts. This hypothesis was motivated by the observation that an increase in carboxyl content has been shown to result in increased swelling [Fig. 3(a)]. To check the validity of this hypothesis, changes in carboxyl content of the polymers were followed by titration studies before and after swelling according to the procedures described in the Materials and Methods section. Both the 100/0/0 dry and swollen samples, and 80/20/0 dry samples were used as test controls to establish the titration technique. The 100/0/0 control sample solutions consistently turned red upon the addition of the first drop of sodium hydroxide solution, indicating no carboxyl groups, as expected. The 80/20/0 dry and saline swollen sample solutions showed the presence of 14.6 and 11.32%, respectively, which closely corresponded to the acrylic acid charge in the polymerization (Table II). However, the titration of 80/20/0 serum swollen sample solutions resulted in an unexpected decrease in carboxyl groups, depending upon the degree of swelling. At the higher percentages of swell, the number of —COOH groups was substantially reduced.

If Hypothesis II was the likely explanation of increased swelling in serum, then we would have expected an increase in the carboxyl content of the polymer. However, based on the fact that we observed a decrease in carboxyl content, Hypothesis III was the most likely explanation for the increased swelling in serum and the observed decrease in carboxyl groups. According to this hypothesis, the carboxyl groups in the copolymer were neutralized by a monovalent cation in the serum to form a salt, such as —COONa. It has been well established in the polyacrylic acid superabsorbent technology that, when the carboxyl groups are converted to monovalent salts, this results in greater swell due to their higher degree of dissociation compared to the free acid.[14–18]

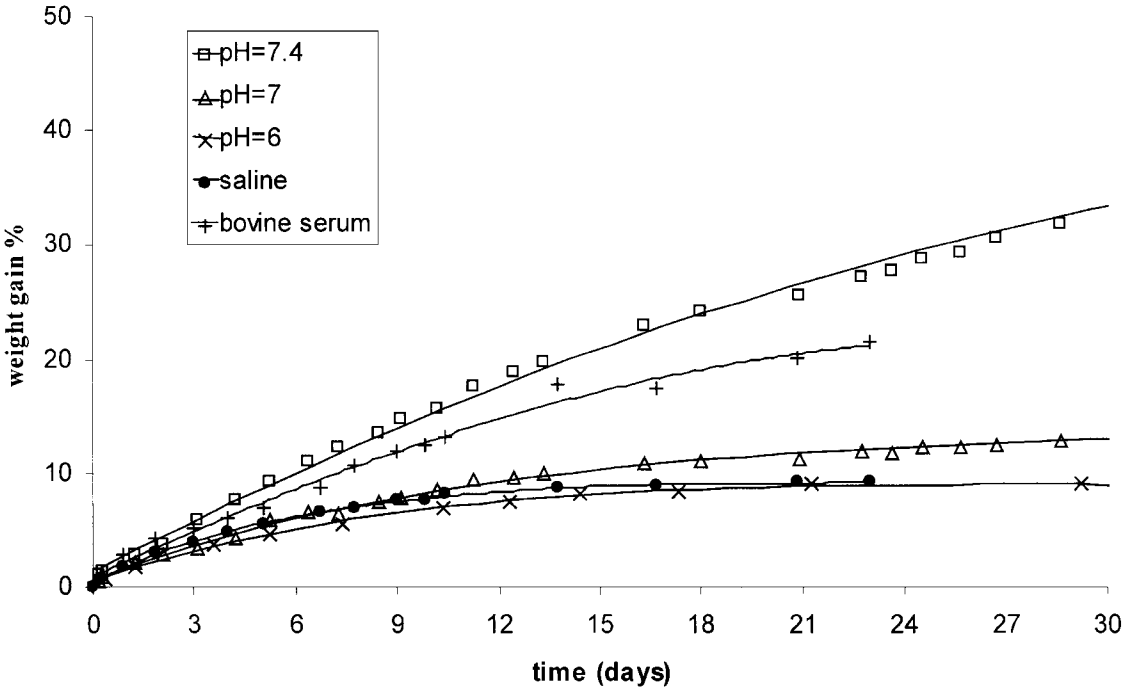
The swelling behavior of the 80/20/10 MMA/AA/AMA system was also studied in buffered solutions at varying pH values. The % swell increased dramatically as the pH was increased from 6 to 9 [Fig. 6(a)]. This result is consistent with the hypothesis described above. It is expected that a higher pH level promotes the dissociation of carboxyl groups and their conversion to the monovalent salts, and therefore, results in a higher % swell. It is also worth noting that the pH of saline solution used in this study is about 6.5, and that the swelling behavior in the saline solution falls between the swelling responses in buffered solutions at pH levels of 6 and 7 [Fig. 6(b)]. Further, the pH of the serum is about 7.3, and the swelling response in serum is quite close to the swelling response measured in the buffered solution at a pH of 7.4 [Fig. 6(b)].

Reswelling Behavior of Converted MMA/AA/AMA Systems

Hypothesis III implies that if the —COOH groups in copolymer (80/20/10 MMA/AA/AMA) are converted to salts (i.e., —COONa), this *converted* copolymer (80/20/10 MMA/salts of AA/AMA) will have higher equilibrium swelling in saline than the *unconverted* copolymer (80/20/10 MMA/AA/AMA). To confirm the above, copolymers (80/20/10 MMA/AA/AMA) were preswollen in NaOH solution (0.1 M) to equilibrium swelling levels (to en-



(a)



(b)

Figure 6 (a) The swelling behavior of the 80/20/10 (MMA/AA/AMA) copolymer in different pH buffered solutions. (b) Comparison of the swelling behavior of the 80/20/10 (MMA/AA/AMA) copolymer in saline solution, serum, and pH buffered solutions.

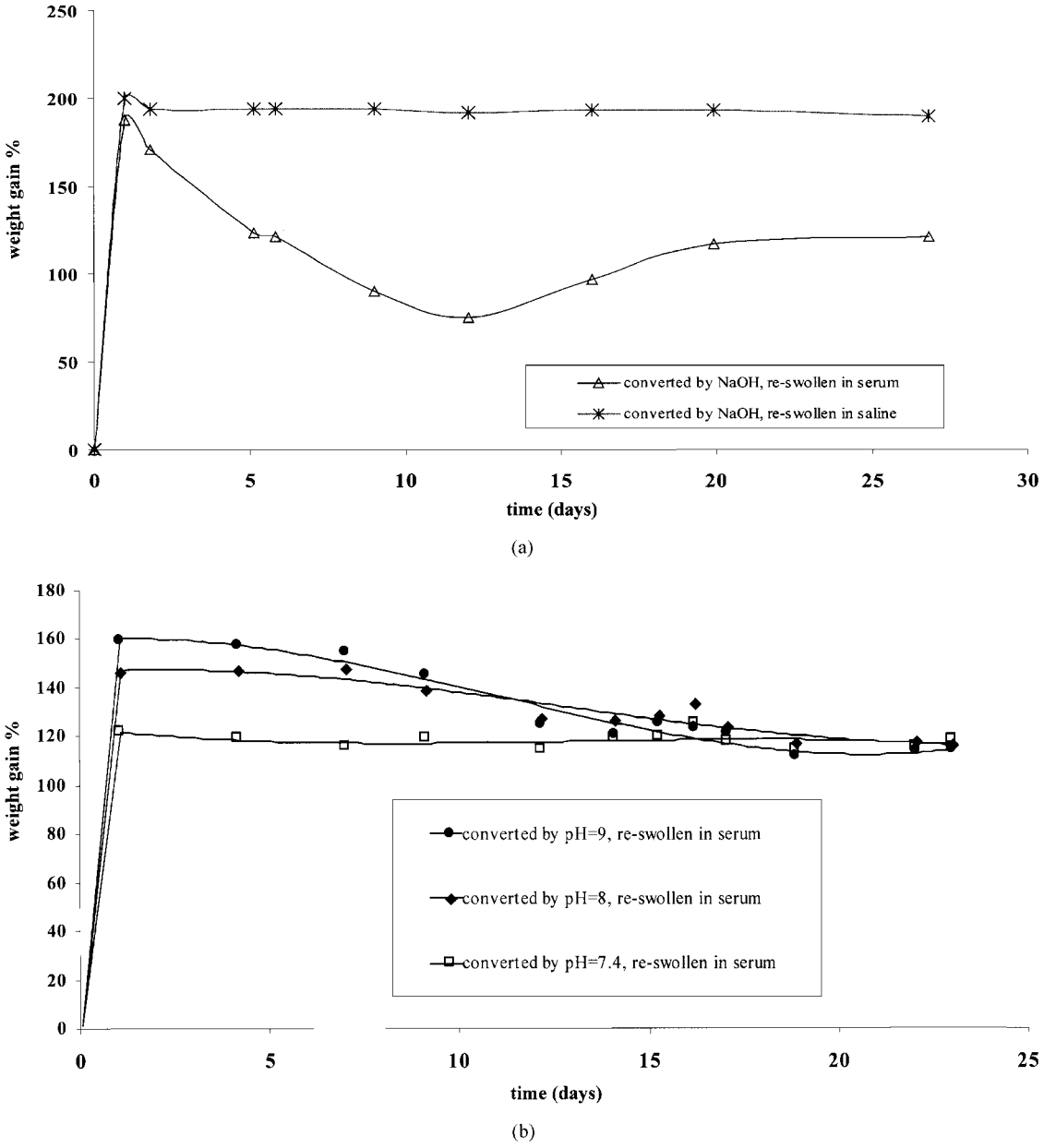


Figure 7 (a) Reswelling behavior of converted (80/20/10) samples (by preswelling in NaOH solution) in saline solution and serum. (b) Reswelling behavior of converted (80/20/10) samples (by preswelling in different pH buffered solutions) in serum.

sure conversion to acrylic salts), dried out completely, and reswollen in saline. The swelling behavior of converted copolymer (80/20/10 MMA/salts of AA/AMA) in saline is shown in Figure 7(a) and is denoted as "converted by NaOH, reswollen in saline."

It is seen that the equilibrium swelling level of converted copolymer (80/20/10 MMA/salts of AA/AMA) swollen in saline is about 190% [Fig. 7(a)], while the equilibrium swelling of the unconverted copolymer (80/20/10 MMA/AA/AMA) in saline was only about 9% [Fig. 3(a)]. Thus, the conversion of acrylic acid groups to acrylic salts did have a strong influence on the equilibrium swelling in saline. As discussed earlier, this is expected as a consequence of Hypothesis III, and provides additional corroboration to this hypothesis.

It is also seen in Figure 7(b) that the converted samples reached equilibrium swelling levels in about a day during reswelling, whereas the un-

converted samples took about 60 days or more to reach equilibrium swelling levels [see Fig. 6(a)]. The faster kinetics of the swelling behavior of the converted samples is also consistent with the assumed changes in the chemistry and polymer structure brought on by the dissociation and conversion of the carboxylate groups. This is particularly useful for the intended application, because it means that it is possible to speed up the swelling and the fixation process in the *in vivo* conditions by using a sample that is already preswollen in a high pH medium (to facilitate the conversion of the acrylic acid groups to salts).

For the bone implant applications we are exploring, it was therefore important to investigate the influence of the media used during preswelling on the reswelling behavior in both saline and serum, and note especially any differences in the reswelling behaviors in saline and serum. For this purpose, the reswelling measurement shown in Figure 7(a) in saline was repeated in serum (i.e., the samples were preswollen in 0.1 *M* NaOH solution to their equilibrium swelling levels as before, dried completely, and reswollen in serum). The results are also presented in Figure 7(a). Comparing the two plots in Figure 7(a), it is seen that the medium used in reswelling has a strong influence even though the samples were initially converted in the same solution to the same equilibrium level.

An interesting feature of the sample reswollen in serum is a decrease in the % swell from about 180 to 115%, following the initial abrupt increase in % swell from the dry condition. Because serum had a lower pH of about 7.3 compared to the high pH of the initial NaOH swelling medium, this could result in a reversion of the carboxylate ions to carboxyl groups (i.e., —COONa + H^+ → —COOH + Na^+), and thus helps explain the decrease in the equilibrium swelling observed.

Based on this theory, it should be expected that the transient observed in the reswelling in serum is dependent on the difference in the pH level of the medium used for initial preswelling (i.e., conversion to acrylic salts) and the pH of the medium used for reswelling. This is validated in the measurements shown in Figure 7(b), where the pH level of the medium used for preswelling was changed from 7.4 to 9.0. Indeed, when the pH level of the medium used for preswelling was close to the pH of serum (pH = 7.3), the transient in the reswelling behavior disappeared.

The results presented in Figure 7(a) also show that reswelling of the converted samples in saline solution did not show the transient behavior shown in serum, despite the fact that the pH of saline solution is about 6.5. This observation indicates that the reversion (i.e., —COONa + H^+ → —COOH + Na^+) of converted copolymer was restricted in saline due to the lack of a source of H^+ ions. The bovine serum, however, acts as a buffer in which the H^+ ions can be produced again. This notion was confirmed by measuring the behavior of a sample initially converted in NaOH solution and then reswollen in a pH = 6 buffered solution. In this case, the % swell showed a decrease after the initial abrupt increase from the dry condition similar to the transient response seen for a similarly converted sample that was reswollen in serum.

CONCLUSIONS

In the present study, swelling and titration analysis were performed to determine the mechanism of excessive swelling of the crosslinked P(MMA-AA) copolymer in serum. Based on titration studies, which showed a decrease in the number of —COOH groups, the excessive swelling phenomenon was attributed to the conversion of the carboxyl groups to a salt of acrylic acid. This result was confirmed by performing swelling studies on MMA/AA/AMA samples in which the —COOH groups were neutralized to —COONa groups. This study suggests new approaches to achieving similar swelling in serum and saline solutions with significantly faster kinetics compared to those that were obtained in previous studies.

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