

Swelling and Mechanical Properties of Modified HEMA-based Superporous Hydrogels

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ABSTRACT: Superporous hydrogels (SPHs), based on poly(2-hydroxyethyl methacrylate) (PHEMA), were prepared by adding minute amounts of an ion-complexable hydrophilic acrylic acid. PHEMA SPHs are generally strong, but their swelling is minimal. To improve the swelling, different poly(HEMA-co-acrylic acid) hydrogels were polymerized and crosslinked, then physically treated with divalent calcium and trivalent aluminum cations. The incorporation of acrylic acid copolymer into the SPH, followed by crosslinking of the copolymer with calcium or aluminum ions produced SPHs with improved swelling and strength. Cells in the presence of hydrogel showed high viability indicating the absence of cytotoxicity and stimulatory effect.

KEY WORDS: superporous hydrogel, physical crosslinking, 2-hydroxyethyl methacrylate, ionotropic gelation, toxicity.

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INTRODUCTION

Superporous hydrogels (SPHs) are hydrogels that swell to equilibrium within minutes in aqueous media. They were initially developed for applications where a large surface area and fast transfer of mass are beneficial, such as gastric retention devices [1,2]. SPHs are generally prepared based on very hydrophilic monomers, such as acrylic acid, methacrylic acid, acrylamide, and acrylic salts. These offer unique swelling properties as significant amount of water is absorbed by these structures in a very short period. Moreover, due to their interconnected pore structure, the maximum absorption or swelling capacity of these hydrogels is independent of their size. However, the SPH polymers inherently have very weak mechanical properties. Also, due to high swelling capacity, and lower solid content, these hydrogels have little resistance to mechanical or physical forces in the swollen state.

There are many applications where SPHs have to serve under severe conditions. Therefore, a stable swelling over a broad pH range and to serve under static or dynamic mechanical loading are needed. Some SPHs are required to have specific volumes in the swollen state, as in gastric retention applications. For this application, a swollen hydrogel with a diameter larger than 15 mm and mechanical resistance to pressure as low as 9–12.5 kPa is required [3,4]. Many SPHs are of high swelling ratio but low strength, including those based entirely upon acrylic acid. Acrylamide-based SPHs [5] tend to be stronger, although studied in drug delivery, acrylamide-based hydrogels may not be acceptable for certain pharmaceutical and biomedical applications [6,7].

Hydrogels based on poly(2-hydroxyethyl methacrylate) (PHEMA) have low swelling ratio, strong, and durable with biomedical and pharmaceutical applications such as implants, contact lens, cell immobilizer [8–10] as well as drug delivery carriers [11–13]. Due to a good structural hydrophilic and lipophilic balance, a low-swelling PHEMA-based SPH can potentially offer stable swelling and mechanical properties. The most common method to enhance the hydrogel strength is to increase the hydrogel crosslink density. Enhancing the hydrophobic nature of the hydrogel can also improve its strength. For example, the addition of dimethacrylamide or N-isopropylacrylamide into an acrylamide-based hydrogel [14], or addition of polycaprolactone into a PHEMA-based hydrogel [15]. Chemicals with the potential to increase the hydrogel molecular weight can also enhance the hydrogel modulus [16]. Hydrogel annealing [17], grafting a hydrogel onto nonwoven hydrophobic fabrics [18], using a mix of hydrophobic (methyl methacrylate) and hydrophilic monomers (2-HEMA) [19], multiple hydrogel systems

(such as chitosan–polyacrylic acid interpenetrating networks) [20], grafting an anionic monomer onto a hydrogel substrate in aqueous medium of a cationic monomer [21], interpenetrating an amphoteric substrate with a nonionic synthetic monomer [22], crosslinking of natural polymers with an aldehyde [23–25], and addition of clay [26–29] are among some approaches to make a stronger high swelling hydrogel.

PHEMA, itself, has been used to enhance mechanical properties of thermosensitive hydrogels based on poly(*N*-isopropylacrylamide) [30]. In the area of SPHs, combined chemical crosslinking and ionotropic gelation [31,32], ion equilibration [33], freeze–thawing and drying [34], acidification [35] as well as increased monomer concentration [36] have all been attempted to enhance hydrogel strength. Although swelling and mechanical property of hydrogels are dependent on many factors including solvent composition during the synthesis [37], one can generally find an inverse correlation between the swelling and mechanical properties in almost any research on hydrogels.

This research was aimed at balancing the swelling and mechanical properties of PHEMA-based hydrogel compositions, which are prepared in aqueous solutions. For this purpose, different copolymer hydrogel compositions of 2-PHEMA and acrylic acid were synthesized and subjected to special ion treatment using divalent calcium and trivalent aluminum.

EXPERIMENTAL

Materials

2-Hydroxyethyl methacrylate, poly(ethylene glycol) diacrylate, glacial acrylic acid, Lutrol F127 (BASF), *N,N,N',N'*-tetramethyl ethylenediamine (TEMED), ammonium persulfate (APS), sodium bicarbonate (NaHCO_3), hydrated calcium chloride ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$), hydrated aluminum chloride ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$). All solutions were freshly made using distilled water at room temperature before use.

General Synthesis for Superporous Hydrogels

To make SPHs, a monomer, crosslinker, water, foam stabilizer, acid, polymerization initiator, initiation catalyst and foaming agent were added sequentially to a glass test tube (25 mm outer diameter \times 150 mm height). HEMA (800 μL) was placed into a test tube; to this, 15 μL of

PEGDA, 50 v/v% aqueous acetic acid solution, 30, 60, and 90 μL of an aqueous acrylic acid solution (50 v/v%), and 100 μL of 10 w/v% aqueous Lutrol F127 solution were added under mild shaking. After some vigorous shaking of the complete mixture, 25 μL of TEMED (40 v/v%) was added with shaking for 30 s, then 25 μL of distilled water was added. This was followed by the addition of 50 μL APS solution (20 w/v%). The contents of the test tube were mixed for 90 s. Sodium bicarbonate (170 mg) was immediately added and carefully dispersed using a spatula and the reaction mixture placed into a 65°C water bath. The hydrogels obtained were thoroughly washed in distilled water and their swelling sizes and strengths were determined. All concentrations of acrylic acid were expressed based on 800 μL of the primary HEMA monomer.

Ionotropic gelation of hydrogels: The hydrogel samples synthesized above were placed in separate solutions including distilled water, 10 wt% calcium chloride solution, and 10 wt% aluminum chloride solution for 1 h. All hydrogel samples were thoroughly washed in water and were subjected to further evaluations. Digital caliper was used to measure the dimensions of the cylindrical hydrogels in their swollen state. A Chatillon TCD-200 test stand with a DFGS force gage was used for each strength measurement. The fully swollen hydrogels were loaded under the probe of the mechanical tester at a constant rate.

Cell Viability

The effect of the hydrogel on cell viability and growth was assessed by MTT-reduction assay. A 30×10^4 of HeLa and human mesenchymal stem cells (hMSc) were suspended in growth medium containing DMEM (Invitrogen, Carlsbad, CA), 10% fetal calf serum (Hyclone, Waltham, MA) and 1% antibiotic and antimicotic solution (Sigma, St. Louis, MO), and plated in a flat-bottom 24-well plate. The cells were allowed to adhere to the plate for 4 h, and then sterilized hydrogels, 50 and 100 mg wet weight, were introduced into separate wells and incubated at 37°C in a 5% CO₂ containing incubator for 48 h. A 100 μL of 2 mg/mL solution of MTT in phosphate buffered saline (PBS) was added to the wells after the designated incubation times and further incubated for 4 h at 37°C in a CO₂ incubator. The medium containing the free dye was removed, the cell layer was briefly rinsed with PBS and the formazan crystals were dissolved in 200 μL of DMSO and the absorbance was measured at 570 nm. The values for each of the treatments represent the average from duplicate wells.

RESULTS AND DISCUSSION

HEMA polymerization in an aqueous solution using water-soluble ingredients is usually used where the purity of the final product as well as a green synthesis is desirable. This, however, causes a few challenges. First, HEMA itself, as opposed to very hydrophilic monomers, is less soluble in water. Because of its poor solubility, the amount of water within the formulation should be minimized in order to conduct the polymerization successfully. More importantly, the foaming process in preparing SPHs is due to the interaction of a foaming agent (sodium bicarbonate) and a foaming aid (acetic acid), which occurs in an aqueous medium. Due to low concentration of water, this interaction is kinetically not feasible at room temperature, which requires the polymerization to be conducted at higher temperatures. Thirdly, the preparation of SPH is generally very fast in the presence of air. This requires all ingredients to be dissolved or dispersed in 15–20 v/v% water. Usually, hydrogels prepared by this method may have a heterogeneous physical structure due to heterogeneous foaming when the foaming and gelling reactions are not well synchronized.

HEMA does not produce a high swelling hydrogel unless it is combined with a more water soluble monomer. However, the addition of another monomer with a different solubility nature can produce undesirable structural heterogeneity in the final product. Therefore, the formulation should contain very little of the comonomer; moreover, high concentration of a more water soluble monomer can offset the expected mechanical properties of HEMA. Therefore, factors such as simultaneous foaming and gelling reactions, a balance of hydrophilicity and hydrophobicity of the reacting mixture, a balance of swelling and mechanical properties, and other considerations need to be taken into account.

In this study, a novel and feasible method was developed to achieve a good balance of swelling and mechanical properties. Two techniques for the hydrophilic addition and subsequent ionotropic gelation were simultaneously utilized; a very water soluble monomer, acrylic acid, was added into the hydrogel formulation at different concentrations and the prepared hydrogel was treated in solutions containing ions of different natures [31,38].

Swelling Properties

Three PHEMA hydrogels containing 1.87, 3.75, and 5.62 v/v% of acrylic acid were prepared and compared with the control, the polyHEMA hydrogel with no acrylic acid. Samples from the same batch were then treated with

calcium chloride and aluminum chloride in an aqueous solution. Swelling behavior of hydrogels, after washing and drying, is shown in Table 1. For pure PHEMA hydrogel with no acrylic acid and no ion-treatment, swelling was $\sim 11.5 \text{ cm}^3/\text{cm}^3$. Acrylic acid in the HEMA copolymer formulation increased the hydrogel swelling to 18.8, 40.2, and $82.4 \text{ cm}^3/\text{cm}^3$ for hydrogels containing 1.87, 3.75, and 5.62 v/v% of acrylic acid, respectively. The swelling trend versus acid addition followed an exponential behavior of $10.7 \times e^{4.45 (\text{acid content})}$. Within the same hydrogel series treated in calcium solution, the pure polyHEMA hydrogel displayed swelling capacity of $7.23 \text{ cm}^3 \cdot \text{cm}^{-3}$. The swelling was increased for hydrogels containing 1.87, 3.75, and 5.62 v/v% of acrylic acid to 13.4, 18.8, and $33.5 \text{ cm}^3/\text{cm}^3$, respectively. Again, an exponential trend was observed; $7.5 \times e^{3.3 (\text{acid content})}$. Lastly, for the same hydrogels treated in aluminum solution, the swelling values were found to be 5.2, 9.2, 13.44, and $17.15 \text{ cm}^3/\text{cm}^3$ for hydrogels containing 0, 1.87, 3.75, and 5.62 v/v% acid, respectively.

A similar exponential trend of $5.6 \times e^{2.64 (\text{acid content})}$ was observed for the aluminum series. The swelling behavior of aluminum-treated polyHEMA hydrogels also fitted a linear trend ($5.2 + 26.7 (\text{acid content})$) with the change in acid content of the copolymer hydrogel.

Pure polyHEMA hydrogels containing no acrylic acid displayed different swelling behavior under different treatments. Despite no acid addition, the hydrogel still reacted to the cation, presumably due to interaction of hydroxyl groups and cations. With increases in the cation valence, the magnitude of the interaction was also increased which caused less swelling. The cation valence had its most profound effect on hydrogels containing high acid concentrations. The swelling data shows that a small addition of acid to a more hydrophobic network can dramatically change the swelling behavior of the hydrogel. On the other hand, the acid addition makes the hydrogel sensitive to the cation present in the solution (especially di- and tri-valent cations), which offsets the hydrogel swelling.

Mechanical Properties

The mechanical properties of the hydrogels with different compositions and treatments are shown in Table 2 and Figure 1. When the

Table 1. Swelling capacities of the hydrogel compositions.

Swelling (cm^3/cm^3)	0 v/v% AA	1.87 v/v% AA	3.75 v/v% AA	5.62 v/v% AA
No treatment	11.4	18.8	40.2	82.4
Calcium treated	7.2	13.4	18.8	33.5
Aluminum treated	5.2	9.2	13.4	17.1

hydrogel was loaded under constant rate, the hydrogel deformed to the applied load. The hydrogel modulus was measured from the slope of the load–extension data before the hydrogel failed. The stronger the hydrogel, the sharper the slope of the load–extension trend was.

The load–extension data for nontreated hydrogels is shown in Figure 2. The pure polyHEMA hydrogel broke apart at 5.46 N. Acrylic acid in amounts of 1.87, 3.75, and 5.62 v/v% resulted in hydrogels with weaker mechanical properties, which failed, at 0.75, 0.58, and 0.31 N, respectively. Comparing load–extension data for pure polyHEMA and HEMA copolymer containing 1.87 v/v% of acrylic acid indicated different fracture modes for these hydrogels. With the former (a rigid hydrogel), fracture occurred in a brittle mode with the slope of 1.17×10^{-3} kN/mm, while the latter (a soft hydrogel) failed under a ductile mode with the load–extension slope of 1.57×10^{-4} kN/mm.

Table 2. Mechanical strength of the hydrogel compositions.

Breaking point (kN)	0 v/v% AA	1.87 v/v% AA	3.75 v/v% AA	5.62 v/v% AA
No treatment	5.46	0.75	0.58	0.31
Calcium treated	No failure	No failure	No failure	1.9
Aluminum treated	No failure	No failure	No failure	No failure

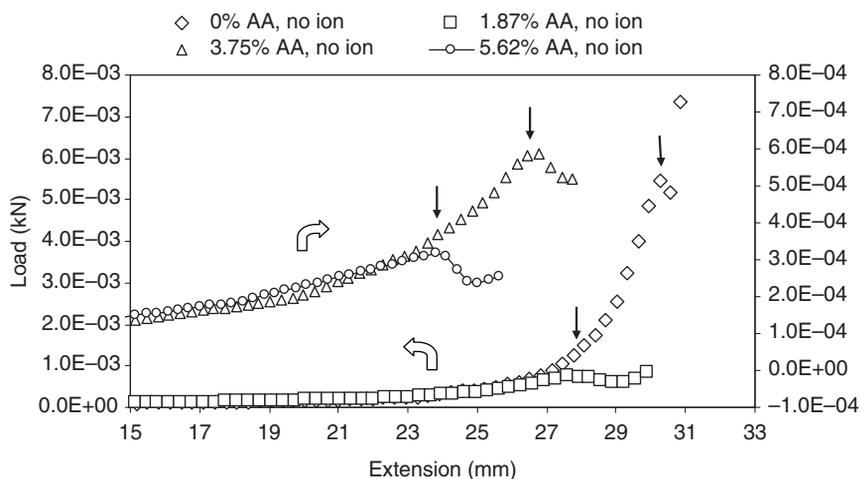


Figure 1. Load–extension data for nontreated hydrogel compositions containing different amounts of acrylic acid.

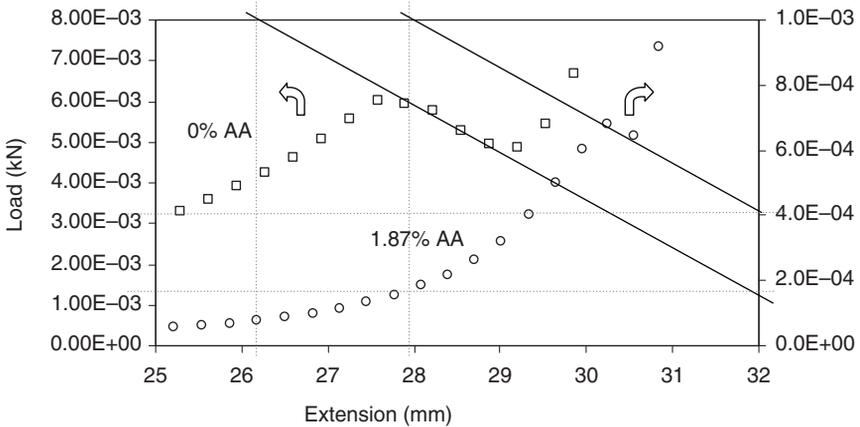


Figure 2. Fracture mode of nontreated hydrogel compositions; pure polyHEMA vs poly(HEMA-co-acrylic acid) containing 1.87 v/v% AA.

The strength data for hydrogels with the same compositions treated in calcium chloride solutions indicated that only HEMA-based hydrogels containing the highest acid concentration failed at about 1.9 N. These data along with the swelling data show that hydrogels with a good balance of swelling and mechanical properties can be obtained if acrylic acid is lower than 5.62 v/v% and if hydrogel is treated with calcium.

The same hydrogel compositions did not fail when they were treated with aluminum (Table 2). All hydrogel compositions resisted loading pressure of at least 10 N. The hydrogel containing 5.62 v/v% acrylic acid treated with aluminum showed excellent balance of swelling ($17.15 \text{ cm}^3/\text{cm}^3$) and mechanical strength without failure.

Load–extension data for the pure (with no acrylic acid) nontreated and treated HEMA-based hydrogels are shown in Figure 3. Although acrylic acid is not included into the hydrogel structure, the hydrogels treated with calcium, and iron showed improved mechanical properties. The Al-treated polyHEMA hydrogel was two times stronger than the Ca-treated counterpart, based on the slope of the initial modulus of both hydrogels ($4.7 \times 10^{-4} \text{ kN/mm}$ vs. $2.71 \times 10^{-4} \text{ kN/mm}$). The hydrogels containing 1.87 v/v% of acrylic acid displayed improved mechanical property when treated in calcium and aluminum solutions. The hydrogel containing no acrylic acid failed under 0.75 N. Similar behavior was observed for hydrogels containing 3.75 v/v% of acrylic acid, except that the hydrogel with no cation treatment displayed a weaker strength with a failure at around 0.58 N. When the acrylic acid content of the copolymer was increased to 5.62 v/v%, both nontreated and

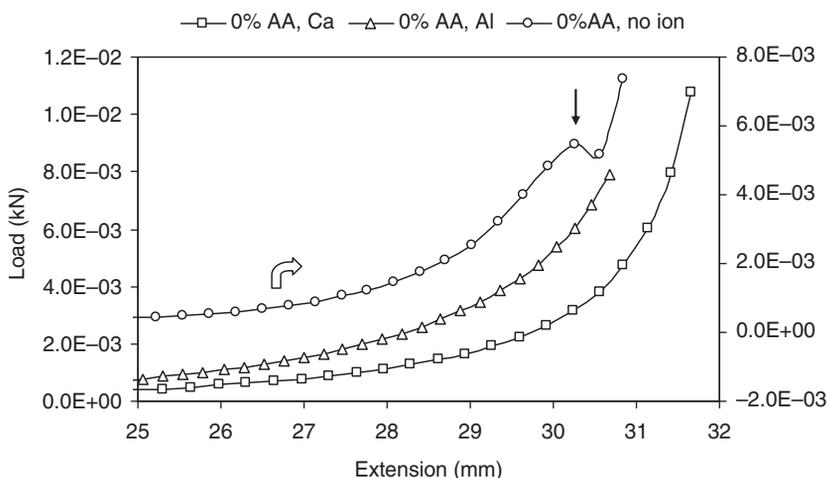


Figure 3. Load–extension data for pure polyHEMA hydrogel compositions; nontreated vs ion-treated.

calcium-treated copolymers failed under 0.31 and 1.9 N, respectively. The aluminum-treated hydrogel appeared to be stronger as it survived loadings of at least 13 N.

Combined Swelling and Mechanical Properties

The term hydrogel weakening is the reciprocal of the hydrogel breaking point, and simply reflects the mechanical weakness of the hydrogel. A correlation between the swelling and weakening properties of the nontreated copolymer hydrogels with increase in the acid content of the hydrogel is shown in Figure 4. While both properties were increased with the acid, the swelling showed a linear behavior and the hydrogel weakening showed an exponential behavior. It appears that the acid addition is more severe on the hydrogel weakening than on the hydrogel swelling for acid concentrations over the maximum limit in this study, that is, >5.62 v/v%.

Two sets of hydrogels with similar swelling capacities can be identified in Table 1. One set are hydrogels based on nontreated HEMA, calcium-treated HEMA-co-acrylic acid (AA content of 1.87 v/v%) and Al-treated HEMA-co-acrylic acid (AA content of 3.75 v/v%). These displayed swelling capacities of 11.5, 13.4, and 13.4 cm^3/cm^3 , respectively. The second group are hydrogels based on nontreated HEMA-co-acrylic acid (AA content of 1.87 v/v%), calcium-treated HEMA-co-acrylic acid (AA content of 3.75 v/v%), and aluminum-treated HEMA-co-acrylic acid

(AA content of 5.62 v/v%). These displayed swelling capacities of 18.8, 18.8, and 17.1 cm^3/cm^3 , respectively. The load-extension behaviors of these two hydrogel sets are shown in Figures 5 and 6. With the same chemical crosslinking, and assuming that the swelling capacity is a critical function of both chemical and physical crosslinks, one can

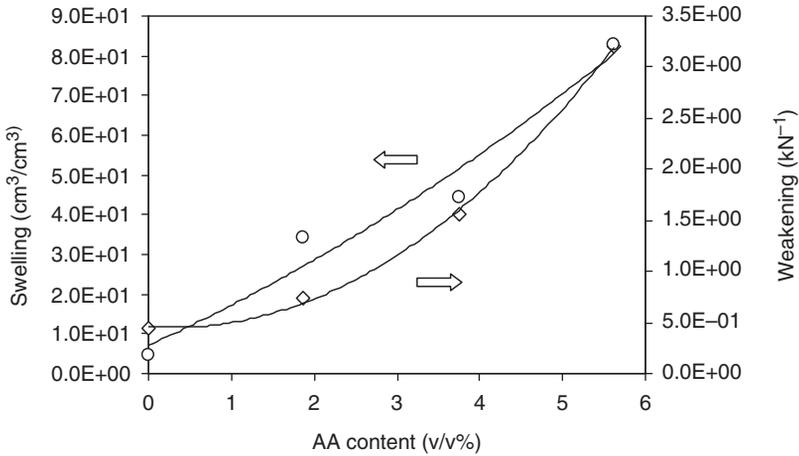


Figure 4. Hydrogel swelling and weakening vs acid content of the hydrogel.

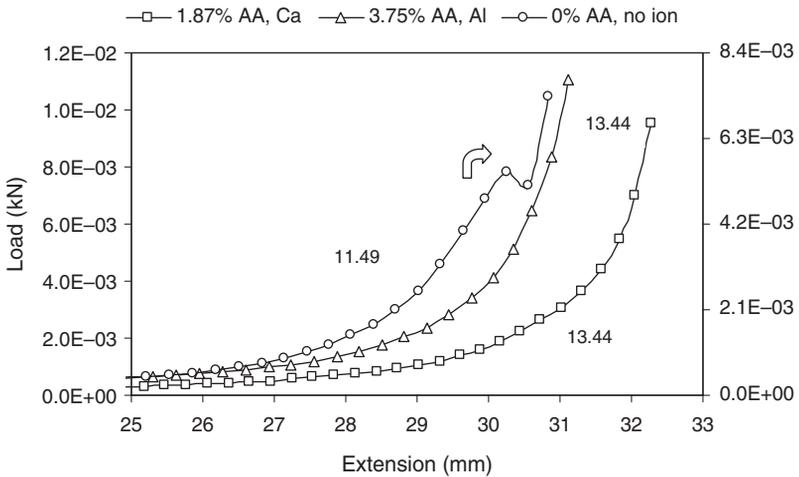


Figure 5. Load-extension data for nontreated and ion-treated hydrogels possessing swelling capacity in the range of 11.5–13.4 cm^3/cm^3 .

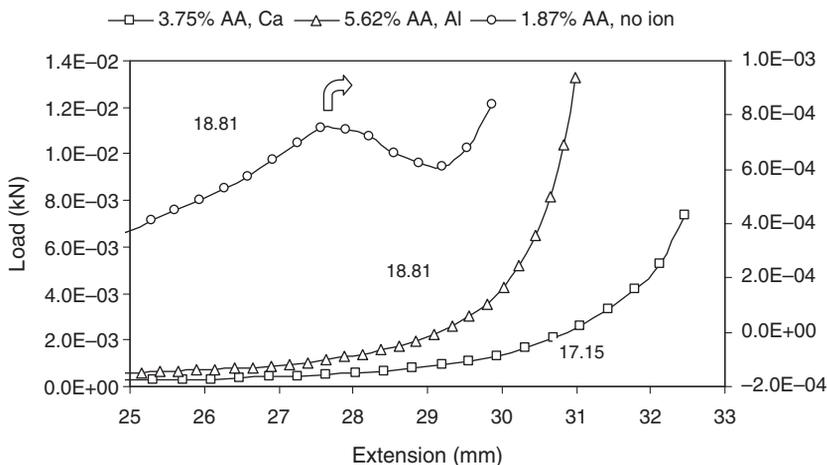


Figure 6. Load–extension data for nontreated and ion-treated hydrogels possessing swelling capacity in the range of 17.1–18.8 cm³/cm³.

conclude that the physical crosslink density should be identical for all members of each set. One can also conclude that the strength of the physical crosslinks should be different for each hydrogel set as they display different mechanical strengths. Based on the data, the hydrogel mechanical strength increased in the hydrogels containing high acid concentrations with the high valence cations.

Cell viability assays were performed using MTT assay as described by Mosmann [39]. The results indicated no significant variation in cell viability and hence hydrogels apparently have no toxicity or stimulatory effect on the selected cells.

CONCLUSION

HEMA-based SPHs were prepared with better swelling and mechanical properties. The strongest SPH was made without acrylic acid, but they did not swell much. Attempts to improve the swelling ratio of the SPH were made by the addition of acrylic acid to the formulation. The swelling was improved, but at the cost of strength. Calcium and aluminum were used as a tool to strengthen the SPH while balancing the hydrogel swelling and mechanical properties; the aluminum produced a stronger crosslinking effect than calcium. The hydrogels with similar swollen sizes were compared in terms of their strength; the aluminum, followed by calcium, produced hydrogels with stronger

structures than nontreated hydrogels. Although any approach to increase mechanical properties of hydrogel offsets the hydrogel swelling property, the technique of hydrophilic addition followed by ionotropic gelation was found to improve both. Swelling and strength data also suggests that a HEMA-based hydrogel with a good balance of swelling and mechanical property can be achieved with more acrylic acid and high ion valence. These improved hydrogels, which have no apparent cytotoxicity or stimulatory effects, have potential applications in biomedical and pharmaceutical area.

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