Preparation, Characterization and Controlled Release Investigation of Biocompatible pH-Sensitive PVA/PAA Hydrogels

Iman Manavi-Tehrani, Mohammad Rabiee, Maryam Parviz, Mohammad Reza Tahriri, Zahra Fahimi*

Summary: In the present research hydrogel films based on polyvinyl alcohol (PVA) and polyacrylic acid (PAA) blend, with various crosslink densities, have been prepared through different thermal treatment. The results of FTIR and DSC confirmed quality and quantity of conclusion on miscibility of PVA/PAA blends, respectively. Besides, biocompatibility of the samples has been proved in cytotoxicity tests using L929 cells, according to ISO10993–5. Water uptake of the hydrogel blends is measured. pH sensitivity properties of blends are studied with and without boiling in NaOH solutions where the effect of swelling in water before boiling has also been investigated. Preswellings in water and NaOH concentration have been found to be mainly effective on pH sensitivity of PVA/PAA blends. Biocompatibility and pH sensitivity behavior make these hydrogels appropriate candidates to orally deliver drugs such as insulin and peptides that can be released in basic pH of intestine. The stability of these films in acidic solutions and its expansion and also the consequent release of drugs in basic solutions have been studied by using Teofilin as a model drug by UV-spectrophotometeric measurements.

Keywords: cytocompatibility; drug delivery systems; hydrogels; PVA/PAA blends

Introduction

Hydrogels are polymeric networks which can absorb a large amount of solvent in the aqueous medium and swell without being dissolved. When a hydrogel is in the contraction mode, polymer chains are highly entangled that limit molecular diffusion due to the cramped space available. On the other hand, when a hydrogel reaches its maximum swelling rate, the swelling pressure will be balanced out by the forces holding the chains together and they are referred to as cross linking forces. Ionic hydrogels contain an ionic side chain and gel is immersed in an electrolyte fluid. These polymers are known as active because they expand (swell) or contract (shrink) in response to environmental stimuli which include changes in temperature,[1] electric voltage,[2,3] pH[4–10] and surrounding fluid.[11–15] Their expansibility and contractile to a considerable extent have made them suitable candidates to control drug release systems,[16] biosensors[17] and other biomedical applications.

PVA and PAA are hydrophilic polymers having been used in many biomedical researches as active hydrogels recently.[18] There are several ways of crosslinking PVA, such as gamma radiation, chemical crosslinking agents, and heat treatment.[18–25] PAA chains can be trapped in PVA network while crosslinking and also forming new bonds.[26] Preparing polymer blends is a useful method of simply controlling hydrogel behavior. Crosslinking PVA/PAA blends by heat treatment leads to the stable hydrogels that can be used to

Biomaterial Group, Faculty of Biomedical Engineering, Amirkabir University of Technology, P. O. Box: 15875-4413, Tehran, Iran
Fax: (+98-21) 66468186; E-mail: mparviz@aut.ac.ir

deliver drugs such as insulin indentured through acidic media of stomach.[19] The swelling attribute of pH-sensitive hydrogels depends on the functional acidic or basic groups at the polymer backbone such as acidic groups in PAA.

One of the specified concerning active hydrogels is their low response time to the surrounding stimuli; preparing thin and light hydrogel films is an appropriate method of reducing the response time to seconds which does not change polymer's mechanical properties. Used as biomaterial, these smart hydrogels must not stimulate the immune system response and show inert behavior. Therefore, preparing smart biocompatible hydrogels with low response time is advantageous to most of biological applications.

We used different thermal crosslinking conditions for preparation of PVA/PAA pH sensitive hydrogel films in order to have stable materials not having leaching crosslinking agents remained appropriate to use in biomedical applications. The miscibility of PVA/PAA blends; physical properties such as swelling in water and responding to pH value change, besides the in vitro cytotoxicity have been examined to prove their functionality as biomaterials. In this work, attempts have been made to strengthen the pH sensitivity of PVA/PAA blends by boiling gels in NaOH solutions with different concentrations; besides, samples are swelled in water before boiling in NaOH solutions to investigate pre swelling effect on their pH sensitivity behavior. The hysteresis behavior of these hydrogels in response to increasing and decreasing the pH values has also been investigated. Finally, the release of Teofilin as a model drug in different hydrogels at various pH values has been evaluated.

Experimental Part

Preparation of Hydrogel

First, 1 gr polyvinyl alcohol (Mw = 72000, 98% hydrolyzed from Merck) was dissolved in 40 ml deionized water for 2h at 98°C using reflux technique. 1 gr Polyacrylic acid (Mw = 240000, supplied by Aldrich) was also dissolved in deionized water for 1h, the final concentration was 1/40 gr/ml, and then this solution was added to the prepared PVA solution and mixed for 30 min. The combined solution is afterwards poured into a glass pan and placed in an electronically controlled oven at 25 ± 0.5 °C (Heraeus, Germany) for 3 days, to *evaporate the whole water*. At this stage, a transparent thin film was easily pealed up the glass. In order to gel crosslinking (Table 1), thermal treatment was performed; variations in temperature and time during thermal treatment significantly affects the prepared gel.

To increase the pH-sensitivity of gels, boiling in NaOH solution is an effective way whereby Na^+ causes COOH group dissociation and so COO^- pendent groups form in hydrogel network.[27] The PVA/PAA hydrogel blends were boiled in 1M and 2M NaOH solutions for 30 seconds to consider the effect of NaOH concentration on magnifying pH sensitivity property (sample 2 and 3, respectively). To study the effect of water inserted into the hydrogels, on the activity of Na+ groups, gel was immersed in deionized water until it achieved equilibrium state at 25 °C and after removing surface moisture, it was boiled in 1M NaOH solution for 30 sec (sample 1). In summary, samples were prepared under particular conditions as specified in Table 2 and their elongation ratios (%) in different pH values were calculated by following expression (1). Where L_0 is the initial length of the sample and L_t is its length in desired pH value at

Table 1.
Thermal treatment of PVA/PAA hydrogel.

Temperature (°C)	Time (min)
110	20,30,45,60,120,180,240
120	20,30,45,60,120,180,240
130	20,30,45,60,120,180,240

Table 2.
Preparing pH-sensitive samples.

Sample	Preparation condition
Sample 1	Swelled in water and boiled in 1 M NaOH solution
Sample2	boiled in 1M NaOH solution
Sample3	boiled in 2M NaOH solution

equilibrium state.

$$\text{Swelling elongation ratio}\,(\%) = [(L_t - L_0)/L_0] \times 100 \quad (1)$$

For characterization tests, gels that were treated for 60 min at 130 °C were used because they remain physically constant in swollen state for 4 months.

Cytotoxicity Test

In vitro cytotoxicity of the samples has been assessed as per ISO-10993–5. The mouse L929 fibroblast cells were used as a test model in this study. The cells were maintained in Roswell Park Memorial Institute (RPMI)-1640 growth medium, supplemented with 100 IU/mL penicillin, 100 g/ml streptomycin, and 10% fetal calf serum. A routine subculture was used to maintain the cell line. The cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C. After 1-week incubation, then the monolayer was harvested by trypsinization. The cell suspension of 4×105 cells/ml was prepared before seeding. The hydrogel samples were washed with PBS and rinsed with distilled water and then sterilized in an autoclave at 120 °C. The sterilized hydrogel films placed in a multiwell tissue culture polystyrene plate (Nunc, Denmark), which was contained 5 ml cell suspension, with another cell suspension well kept as a negative control. They were maintained in the incubator for one week. After incubation, the cells were observed under optical microscopy (Nicon E200).

Drug Loading

Teofilin was used as a model drug to evaluate the ability of PVA/PAA hydrogel films for drug release application. Gel was placed in Teofilin solution for 3 days to load drug into polymeric network via diffusion, while the ratio of hydrogel to drug was 5:1(%wt). Then the gel was completely washed in distilled water and drug release was measured in different pH values by UV-spectrophotometer (Milton Roy 601, USA). The pH value of environmental solution was monitored with a pH/Ion meter (Metrohm 692, Switzerland). Hydrochloric acid (HCl), sodium hydroxide (NaOH) and sodium hydrogen carbonate which were purchased from Merck were added to maintain the desired pH value. This combination is the appropriate environment to model stomach acidic mucus.

Physical and Chemical Characterization

Circularly cut (surface area = 5 cm^2) disk-shaped membranes were kept in a desiccators over anhydrous calcium chloride for 48 h before use. The initial mass of samples was taken on a single-pan digital microbalance (CAS, South Korea). The samples were placed inside the air-tight test bottles containing water. Test bottles were placed in an oven at 25 °C. After 24 h (i.e., to reaching the equilibrium state), samples were picked up and surface adhered solvent drops were removed by means of soft filter papers and weighed immediately. Water uptakes of hydrogels have been calculated as follows (2):

$$\text{Water uptake}\,(\%) = [(W_t - W_d)/W_t] * 100 \quad (2)$$

Where W_t is the weight of hydrogel sample in the swollen equilibrium state at different time duration (t) and W_d is the weight of the dry gel.

To study the swelling behavior of PVA/PAA blend in response to increasing and decreasing pH values, gel sample 2 has been chosen and the elongation ratio (%) is measured according to expression 1. pH keeps rising until no increase in gel size observed. The results of this assessment are shown in Figure 5.

FTIR spectra have been measured on a Shimadzu (Kyoto, Japan) FTIR-8300 for pure PVA, PVA/PAA blend and pure PAA films. In the case of PAA, potassium

bromide (KBr) was mixed with PAA to make a thin disk for the IR measurement because the prepared PAA film is quite brittle. The samples were analyzed in the range of 500 to 1200 cm^{-1} at the scan speed of 23 scan /min with 4 cm^{-1} resolution. The DSC curve was obtained in a Shimadzu TGA-50 apparatus which proved the conclusion on miscibility of PVA/PAA blend. For this purpose, 30 mg of dry samples were scanned from room temperature up to 210 °C with the heating rate of 5 °C/min under a nitrogen atmosphere.

Results and Discussion

As shown in Figure 1, water uptake percentage increases as the heating temperature is decreased but maybe there is no general rule for the heating duration time. Water uptake increases to a maximum value at 120 °C and 130 °C, after that growth of crystalline regions in polymeric gel causes a decrease in water uptake by increasing the time of thermal treatment. Additionally, gel becomes rigid and brittle by decreasing the elasticity of polymer network. Gel behavior at 110 °C seems to be totally different, maybe this temperature is not enough for crosslinking PVA/PAA blends because the these blends physical stability observed to be weaken.

The IR spectra of the samples are shown in Figure 2. The band of O—H stretching vibration at 3288 cm^{-1} from pure PVA sample, when compare with that of PVA/PAA hydrogel sample has gradually shifted to higher wave number, weakened, and broadened with addition of PAA content in PVA/PAA hydrogel sample. This band, lastly, become so broad and weak as to almost overlap with the broad band of 2400–3500 cm^{-1} of COOH with addition of PAA and then it is not evident in PVA/PAA hydrogel. This observation indicates that hydrogen bonds in PVA gradually has been replaced by hydrogen bonds interactions between PVA and PAA.

The C—O stretching vibration at 1269 cm^{-1} from pure PVA became stronger and broader with addition of PAA because of new intermolecular hydrogen bonds of PVA/PAA hydrogel. The dissymmetry stretching vibration of C—O at 1094 cm^{-1} from pure PVA shifts to higher wave-number with addition of PAA. As it can be observed in this figure, the wave-number of the peak from 1094 cm^{-1} for pure PVA has shifted to 1098 at PVA/PAA hydrogel. Besides, the peak intensity apparently became weak in the structure of PVA/PAA hydrogel. These observations indicate formation of new hydrogen bonds in PVA/PAA hydrogel.

The dissymmetry stretching vibration of C=O of COOH from pure PAA shifts to

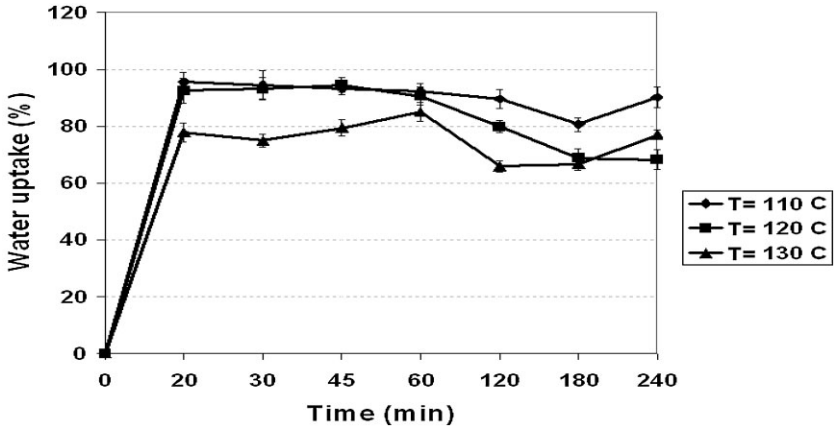

Figure 1.
Water uptake percentage for samples prepared as described in Table 1 (The results are expressed as the mean ± SD, n = 5).

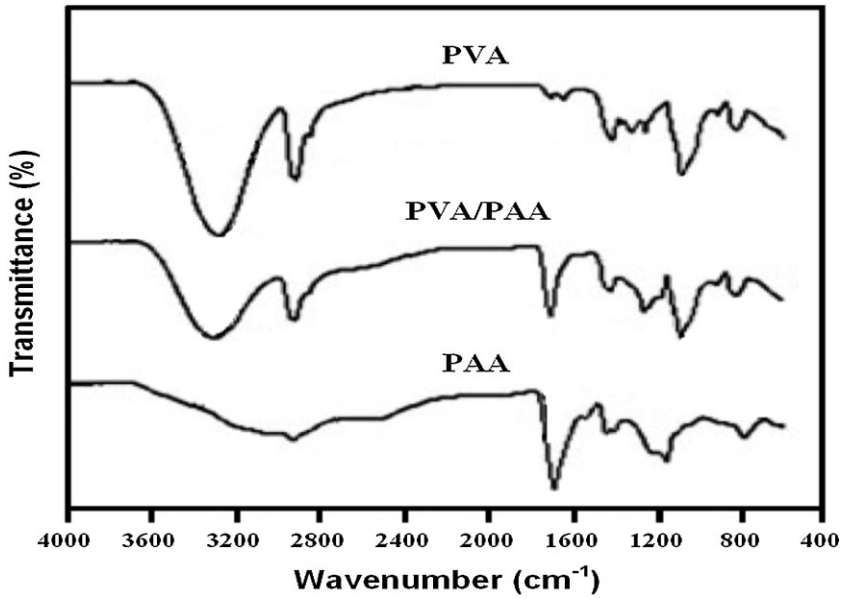

Figure 2.
IR spectra of the PVA, PAA and PVA/PAA hydrogels treated for 60 min at 130 °C.

high wave-number in the structure of PVA/PAA hydrogel. At PVA/PAA hydrogel, it shifted the highest wave-number 1718 cm^{-1}. This shows new strong hydrogen bond interactions between PVA and PAA has replaced hydrogen bond interactions in pure PAA. These differences indicate that new hydrogen bonds of PVA/PAA hydrogel were formed.

From the Tg measurement, only a single glass transition temperature (Tg) for the PVA/PAA hydrogel was detected (Figure 3) and this is attributed to the compatible network structure in hydrogel

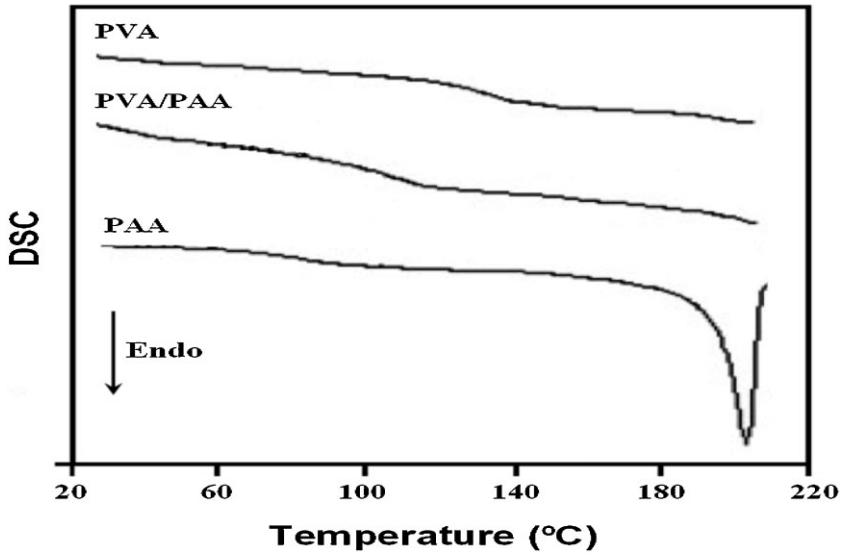

Figure 3.
DSC curves of PVA, PAA and PVA/PAA hydrogels treated for 60 min at 130 °C.

components. The single Tg observed for PVA/PAA hydrogel sample also indicates that both hydrogel network components have excellent miscibility to each other in the hydrogel they have formed.

Were swollen in water and NaOH concentration can be mainly effective on pH sensitivity of PVA/PAA blends. As can be observed in Figure 4, sample 1 that has been swelled in water before boiling in 1M NaOH solution shows more sensitivity to pH value change. The comparison of the swelling elongation ratios (%) between the sample 2 and sample 3 indicates that increasing NaOH concentration from 1M to 2M causes an increase in pH sensitivity of the PVA/PAA blend.

Because of the presence of carboxylic acid, the swelling behavior of PAA hydrogel was highly dependent on the pH of surrounding medium.[28] The pK_a of PAA is 4.28.[29] Below this value, COOH groups of PAA associate and above this value the same groups dissociate. In the dissociation state of COOH groups, there will be more interactions between these groups and water molecules which lead to higher swelling of hydrogel.

Percentage of longitudinal change of the PVA/PAA blend (sample 2) which was calculated according to expression 1 is shown in Figure 5. When the pH value rises above the pK_a of PAA, the ionized carboxylic groups will absorb cations inside the gel network to replace H^+ ions. This will raises free ions concentration inside the network. Therefore swelling pressure of free ions will rise and results in gel swelling. On the other hand gel tends to expand in order to reduce repulsion between carboxylic groups. With increasing pH values and ionization degree, polymer network becomes more hydrophilic. Longitudinal increase reaches its maximum value around pH = 11.

When the pH value exceeds 11, effect of ionic strength plays a dominant role and as shown in Figure 5 , the swelling elongation ratio of sample 2 decreases monotonously with further increasing of pH. The swelling elongation ratio of gel has a jump at about pH 8, much higher than the pKa of polycarboxylic groups. The authors think this is mainly due to the effect of network elasticity of the hydrogel limiting the swelling of gel. Electrostatic repulsion of carboxylic groups will surpass the ionic strength in the pH range decreasing from 11 to 5 values. After that, around pKa of PAA, gel loses its swelling behavior because of

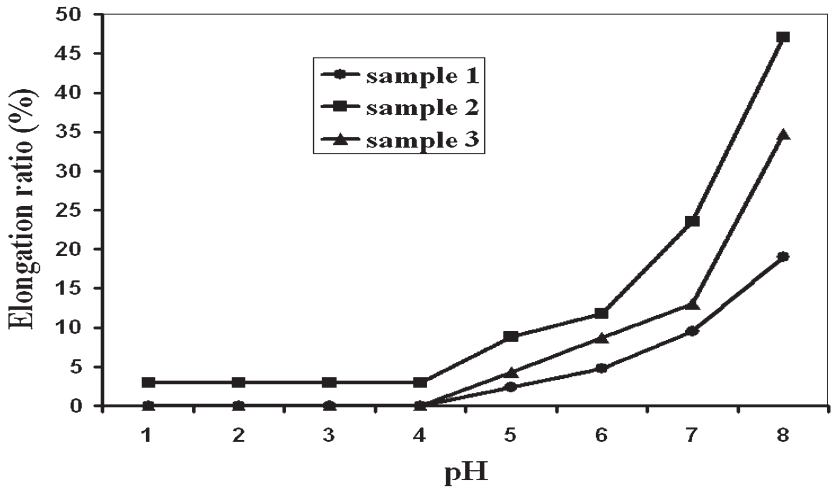

Figure 4.
Swelling elongation ratio (measured by (1)) at different pH values (from 1 to 8) for 3 different samples (Table 2). The swelling state and value of NaOH concentration has effect on the pH sensitivity of samples.

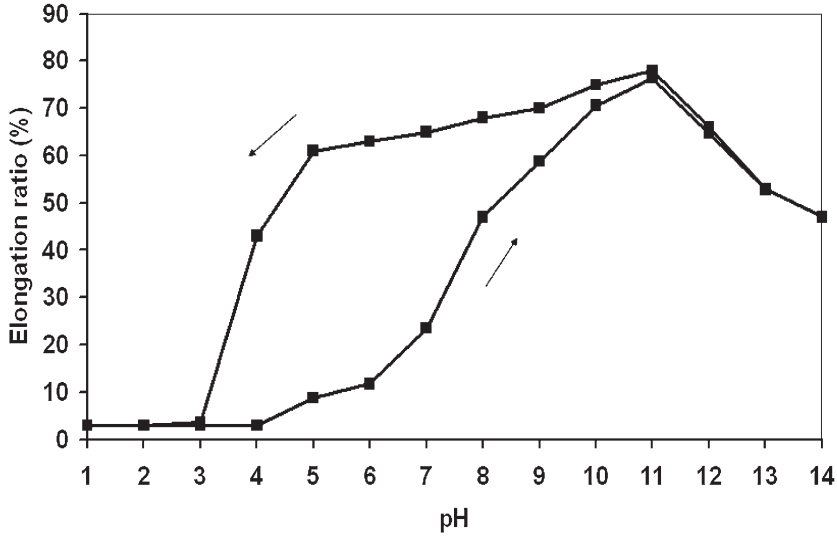

Figure 5.
Equilibrium swelling elongation ratio of sample 1 as a function of pH. The direction of arrows represents the trace from acidic to basic medium and the opposite trace.

network elasticity limitation and lack of electrostatic repulsion between carboxylic groups. During the pH decreasing process when the pH is lower than the pKa of PAA, electrostatic repulsion between the polycarboxylate groups within the network disappear and so the network collapses.

Change in pH values and ionic strength have little effect on PVA network because of its neutral acidic and basic nature. This may be also the reason why the hysteresis loop of the PVA/PAA hydrogel does not disappear, but it disappears in the case of PAN hydrogel sample via controlling ionic strength. Only when the swelling force of PAA network surpasses the contracting force of PVA network gel samples swell abruptly. It is important to mention that different acidic solutions by similar pH values have different effects on gel behavior. For example immersing PVA-PAA blends in acetic acid solution causes gel

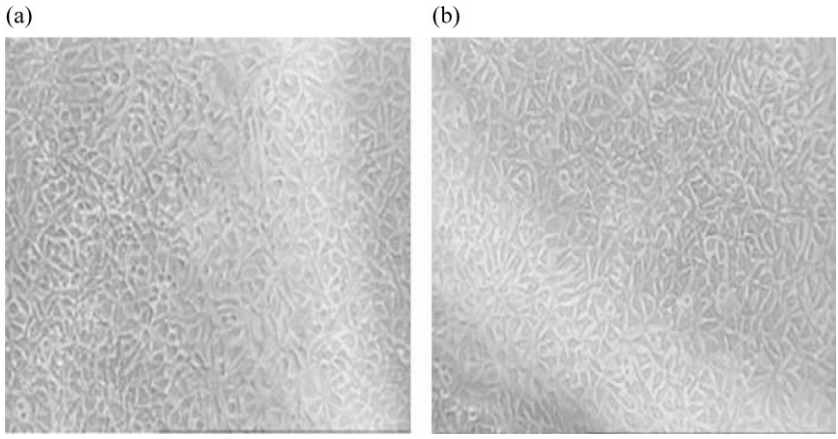

Figure 6.
L929 cell culture and their morphology in (a) PVA/PAA hydrogel sample and (b) control sample after 1 week.

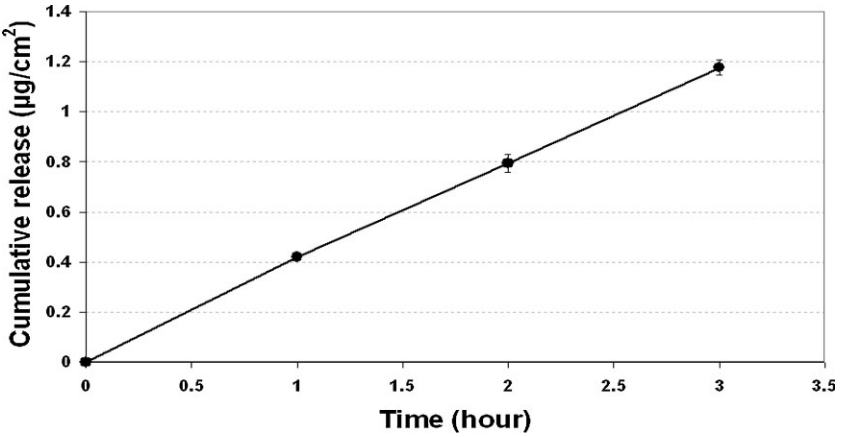

Figure 7.
Teofilin release in acidic solution pH = 2 (The results are expressed as the mean ± SD, n = 4).

expansion while gel remains constant in hydrochloric acid solution by the same pH value.

Cytotoxicity evaluation of hydrogels is shown in Figure 6. Cellular morphology of cultured cells on PVA/PAA hydrogels is like their morphology in control sample. All samples exhibit cell attachment, spindle like cells formation and pseudo pods development.

Lack of toxic leaching compounds, swelling unique properties and results of cytotoxicity test of gels as well as their pH-sensitivity behavior approve their ability to release incorporated drug. Because of physically crosslinking, this hydrogel contains no toxic materials and can be useful to drug release systems, so gel is placed in acidic solution by pH = 2 and release of Teofilin is measured for 3h in acidic solution as the needed time to digest nutrients in human's stomachs.

Release of Teofilin in acidic solution has approximately constant rate as shown in Figure 7 and this approves the gel stability in acidic solutions. After that, gel has been immersed in basic solution by pH= 7.4, while release of Teofilin is also measured.

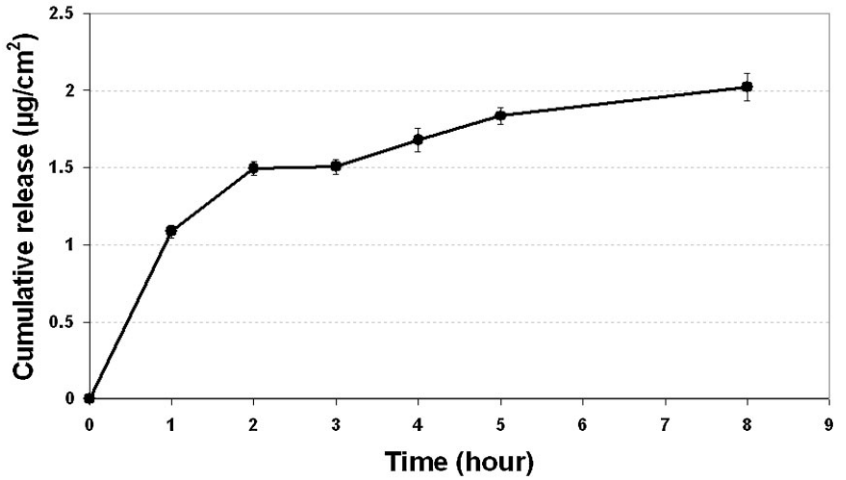

Figure 8.
Teofilin release in basic solution pH = 7.4 (The results are expressed as the mean ± SD, n = 4).

As shown in Figure 8, slope of Teofilin release curve increases while increasing time and drug releases in a relatively regular rate in basic solution.

Conclusion

In this work, nontoxic hydrogels have been prepared through thermal crosslinking condition. The compatibility of PVA/PAA was confirmed by suing FTIR and DSC. Percentage of water uptake is strongly dependent on duration time and temperature of heat treatment. The maximum water uptake percentage accrues for the PVA/PAA blend that was heated for 20min at 120 °C. Synthesized hydrogels show considerable sensitivity to alternative pH values. The sensitivity to pH values was increased by boiling samples in NaOH solution (1M and 2M). The sample that was swelled before boiling in 1M NaOH solution when compared with that of unswelled one shows more elongation ratios (%) in response to different pH values. We found increasing NaOH solution concentration from 1M to 2M is an effective way to increase pH-sensitivity of samples. The PVA/PAA blend hysteresis on elongation/contraction behavior of PVA/PAA hydrogel samples has been analyzed in this research and because of their constancy and resistance in acidic solution; they can be promising candidates for transfer drugs such as insulin and peptides orally to intestine or blood, where they can be released. Release of Teofilin as a model drug through hydrogel samples by alternating pH values was investigated. The results of UV-spectroscopy evaluation confirmed the potential of these hydrogel films to be useful to drug release systems.

[1] D. Kuckling, A. Richter, K. F. Arndt, *Macromol. Mater. Eng.* **2003**, *288*, 144.
[2] R. W. Ğulch, A. Weible, T. Wallmersperger, in: "*Proceedings of the SPIE on Smart Structures and Materials*", Y. Bar-Cohen, Ed., Electroactive Polymer Actuators and Devices, San Diego **2002**, p.367.
[3] H. Li, J. Int, *Solids Struct.* **2008**, *46*, 6.
[4] K. F. Arndt, A. Richter, S. Ludwig, J. Zimmermann, J. Kressler, D. Kuckling, H. J. Adler, *Acta. Polym.* **1999**, *50*, 383.
[5] D. J. Beebe, J. S. Moore, J. M. Bauer, Q. Yu, R. H. Liu, C. Devadoss, B. H. Jo, *Nature.* **2000**, *404*, 588.
[6] R. Bashir, J. Z. Hilt, O. Elibol, A. Gupta, N. A. Peppas, *Appl. Phys. Lett.* **2002**, *81*, 3091.
[7] M. Guenther, G. Suchaneck, J. Sorber, G. Gerlach, K. F. Arndt, A. Richter, Fine. *Mech. Opt. (Olomouc).* **2003**, *48*, 320.
[8] A. J. Marshall, J. Blyth, C. A. B. Davidson, C. R. Lowe, *Anal. Chem.* **2003**, *75*, 4423.
[9] A. Richter, A. Bund, M. Keller, K. F. Arndt, *Sens. Actuat. B.* **2004**, *99*, 579.
[10] G. Gerlach, M. Guenther, G. Suchaneck, J. Sorber, K. F. Arndt, A. Richter, *Macromol. Symp.* **2004**, *210*, 403.
[11] K. F. Arndt, D. Kuckling, A. Richter, Polym. *Adv. Technol.* **2000**, *11*, 496.
[12] K. F. Arndt, T. Schmidt, H. Menge, *Macromol. Symp.* **2001**, *164*, 313.
[13] X. Liu, X. Zhang, J. Cong, J. Xu, K. Chen, *Sens. Actuat. B* **2003**, *96*, 468.
[14] K. T. Kurauchi, T. Shiga, Y. Hirose, A. Okada, In: "*Polymer Gels: Fundamentals and Biomedical Applications*", D. De Rossi K, Y.Ž. Kajiwara, A. Osada, E. Yamauchi, Eds., Plenum, New York **1991**, p. 237.
[15] Y. Bar-Cohen, Y S. Leary, M. Shahinpoor, J. O. Harrison, J. Smith, "*Proceedings of the SPIE Smart Structures and Materials Symposium*", Electroactive Polymer Actuators and Devices, **1999**, p.51.
[16] C. He, S. W. Kim, DS. Lee, J *Control. Release.* **2008**, *3*, 127.
[17] A. S. Hoffman, "*Polymer Gels Fundamentals and Biomedical Applications*", Plenum, New York **1991**, p. 289.
[18] Y. Lu, D. Wang, T. Li, X. Zhao, Y. Cao, H. Yang, Y. Y. Duan, *Biomaterials.* **2009**, *25*, 30.
[19] H. Vazquez-Torres, J. V. Cauich-Rodriguez, C. A. Cruz-Ramos, *J. Appl. Polym. Sci.* **1993**, *50*, 777.
[20] M. G. Katz, T. Wydeven, *J. Appl. Polym. Sci.* **1981**, *26*, 2935.
[21] D. Brunelli, T. Z. Atravrs, *J. Appl. Polym. Sci.* **1995**, *55*, 889.
[22] J. Lehoussel, D. Watteis, E. Marechal, *Eur. Polym. J.* **1997**, *15*, 915.
[23] H. S. Shin, S. Y. Kim, Y. M. Lee, K. H. Lee, S. J. Kim, C. Rogers, *J. Appl. Polym. Sci.* **1998**, *69*, 479.
[24] J. Byun, Y. M. Lee, C. Cho, *J. Appl. Polym. Sci.* **1996**, *61*, 697.
[25] H. S. Shin, Y. S. Kim, Y. M. Lee, *J. Appl. Polym. Sci.* **1997**, *65*, 685.
[26] M. Metayer, O. M. Chamekh, *React. Func. Polym.* **1996**, *33*, 321.
[27] S. P. Marra, K. T. Ramesh, A. S. Douglas, *Mater. Sci. Eng. C.* **2001**, *14*, 25.
[28] Y. Y. Liu, J. Lu, Y.-H. Shao, *Macromol. Biosci.* **2006**, *6*, 452.
[29] J. Fei, Z. Zhang, L. Zhong, L. Gu, *J. Appl. Polym. Sci.* **2002**, *85*, 2423.

