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Preparation and Characterization of pH- and Temperature-responsive by Different Composition Chitosan-P(MAA-co-NIPAM) Hydrogel for Drug Delivery System

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ABSTRACT

In this paper, (chitosan-poly(methacrylic acid-co-N-isopropylacrylamide)) [Chitosan-P(MAA-co-NIPAM)], a multi-responsive hydrogel was successfully fabricated by free-radical polymerization in aqueous media. Chitosan was crosslinked with P(MAA-co-NIPAM) network as a suggestion to improve the efficiency of chitosan as a drug carrier. Due to the hydrophilic and hydrophobic characteristics of MAA and NIPAM monomers presented in the network, the swelling behaviour at different pH and temperature was investigated in this paper. The composition, morphological stability, swelling behavior at various pH and temperature environment of the Chitosan-P(MAA-co-NIPAM) hydrogel were studied using Fourier transform infra-red (FT-IR), Field Emission Scanning Electron Microscopy (FESEM) and swelling test by weight ratio of the hydrogel. This study found that additional feed of MAA and NIPAM monomers during polymerization increased the amount of PMAA and PNIPAM crosslinked in the hydrogel. As a result, the value of LCST increased from 32℃ to more than 37℃. The swelling ratio of the hydrogel was found to be maximum at pH 7 which is suitable to be used in body system especially in human blood of pH 7.4. Thus, the role of Chitosan-P(MAA-co-NIPAM) as a carrier of a drug in drug delivery system has been approved.

Keywords: *Hydrogel; Chitosan; pH-Responsive; Temperature-Responsive; Drug Delivery.*

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Introduction

Hydrogel has become a popular carrier in drug delivery system in more recent years with special capabilities with respect to pH- and temperature-responsive. The hydrogel is normally synthesized from either natural polymer with biocompatibility and biodegradable properties or from a synthetic polymer that contributes to different responsive behavior of hydrogel. The advantages of hydrogel in drug delivery are established through simple preparation, and by having continuous drug release behavior, less toxicity and the most interesting part is stimuli-responsive behavior [1]. Chitosan as one of the interesting polymers used in drug delivery from long previous years still requires improvement due to weakness. One of the weaknesses is poor solubility above pH 6.5 which encourages researchers to modify the properties of chitosan with other moieties in order to enhance water solubility.

Numerous investigations have been employed to develop pH-responsive hydrogel due to variations of pH in human digestive tract. The pH-responsive polymers contain acid or basic functional group which responds to the fluctuations in the external environmental pH [2]. The hydrogel tends to swell or deswell in response to the changes in pH due to the pH-dependent ionizable groups on the polymer side chain. This ability which able to change the properties of the polymer has tempted many researchers to use this material in biomedical application. Based on this factor, methacrylic acid (MAA) was introduced as a co-monomer with chitosan, and this monomer has been widely used in the preparation of pH-sensitive hydrogels designed as drug delivery devices due to its biocompatibility and its convenient to copolymerize. The swelling behaviour of the hydrogels is drastically changed at appropriate pH values even in a very small amount due to the ionization of -COOH group. The swelling of hydrogels containing pH-responsive moieties is controlled by the internal osmotic pressure that arises due to the motions of the ions and counter-ions. It is expected that counter ion interactions balance the internal electrostatic repulsion.

As well as pH-responsive, temperature-responsive is also considered as one of the ways to control the drug release within the human body. The drug would be the most desirable if it could match with the physiological need with the right dosage at the right time via the right route [3, 4]. The thermosensitive polymers can respond to temperature changes of the external environment, which results in a large change on its interesting features such as conformation, solubility, and hydrophobic/hydrophilic balance. In this research, (N-isopropylacrylamide)NIPAM was introduced into the network to lower the temperature aproaching the human body temperature.These features are generally and quantitively described by the lower critical solution temperature (LCST). The aqueous solution of thermosensitive polymer exhibits LCST phenomenon which below the LCST the polymer solution has

one phase, while above the LCST the solution is phase-separated as a result of collapse and aggregation of polymer chains and expelled water [5]. Crosslink between chitosan with PNIPAM shows that de-swelling/shrinkage occurs in the temperature range of 36–39 °C [6]. This approves that the LCST of PNIPAM can be adjusted from 31– 32.8 ℃ to around the physiological temperature of 37℃. However, LCST is also affected by the ratio of hydrophilic and hydrophobic monomers in the thermosensitive polymers [7]. Therefore, the combination of this NIPAM, chitosan and MAA feed composition is worthwhile to be investigated for their pH and temperature responsive behavior on drug delivery system. Physical and chemical properties of the hydrogels were investigated by FTIR, SEM, and DSC, while the swelling behavior was examined by weight ratio of the swollen hydrogel at various pH and temperature environment.

Experimental Procedure

Materials

N, N-methylenebisacrylamide (MBA), N-isopropylacrylamine (NIPAM) and chitosan (Mw≈ 160,000 g/mol, the degree of deacetylation ≈ 91 %) were purchased from Aldrich (St. Louis, MO, USA). Methacrylic acid (MAA) was further purified by distillation under reduced pressure, whereas all other chemicals were used as received and without further purification. Deionized distilled water (ddH2O) used for all reactions and solution preparations.

Preparation of Chitosan-P(MAA-co-NIPAM) Hydrogel

All of the hydrogels with different compositions (as shown in **Table 1**) were synthesized by the free-radical copolymerization of chitosan, MAA, and MBA, using APS as an initiator. Briefly, approximately 300 mg of chitosan were dissolved with different amounts of MAA and NIPAM in 100 mL doubly distilled and deionized water, under constant magnetic stirring for approximately 18–20 h, in a three-neck round-bottom flask equipped with an N_2 gas inlet and condenser. After 20 h of stirring under N_2 purging at 30 °C, MBA (which was used as the crosslinker) was added to the reaction flask. The temperature of the reaction mixture was slowly raised to 70 °C under N_2 purging along with continuous stirring and gradual heating in a silicon-filled oil bath. After 30 min of constant heating at 70 °C, 5 mL of APS (0.05 M) were added to the reaction mixture in order to start the polymerization reaction.

Table 1. The composition of Chitosan-PMAA-PNIPAM Hydrogel.

Sample	Chitosan (g)	MAA (g)	NIPAM (g)
M1	0.3	1	1
M2	0.3	2	1
M3	0.3	3	1
M4	0.3	4	1
N1	0.3	1	3

Several minutes after the addition of the initiator, the color of the reaction mixture turned milky. The polymerization reaction was allowed to proceed for 5 h at constant stirring under N_2 purging at 70°C. The obtained copolymer hydrogels were then purified by centrifugation (Sorvall RC-6 Plus super speed centrifuge, Thermo Electron Co., Waltham, MA, USA) and decantation. Next, each resultant hydrogel was further purified by dialysis for 1 week by using Spectra/Por molecular porous membrane tubing from Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA, cut-off 12,000–14,000, the same tubing was used against a frequently changed distilled water at room temperature (~ 25℃).

Characterization of Chitosan-P(MAA-co-NIPAM) Hydrogel.

Fourier Transform Infra-red (FTIR)

Fourier transform infra-red (FTIR) (PerkinElmer) (Waltham, MA, USA) was used to analyse the chemical structure of multi-responsive hydrogel and identify different functional groups present in the hydrogel. All FTIR spectra of the dried sample were obtained in the range 500-4000 cm^{-1}.

Microscopic Study

The morphological examination of the chitosan-P(MAA-co-NIPAM) hydrogels was carried out using field emission scanning electron microscopy (FESEM) (LEO SUPRA 35VP, Carl Zeiss, Germany). The freeze-dried hydrogels were stubbed lightly on double-sided carbon tapes and were then sputter-coated with gold to cover the hydrogels with a thin layer of conducting material.

Swelling Hydrogel

The swelling properties, which usually use the degree of swelling to define hydrogels, depend on many factors such as network density, solvent nature, polymer-solvent interaction parameter. Each sample was testing at different pH (1.68, 4.08, 7.2 and 10) and at different temperature (25℃, 37℃, and 40℃). Samples were stirred for 24 hours and the swelled hydrogel was weight and swelling ratio was calculated by using Eq. 1.

$$Swelling\ ratio(\%) = \frac{W_2 - W_1}{W_1} x\ 100\% \quad (1)$$

Which, W_2 is the weight polymer after swelling and W_1 is the weight polymer before swelling (dry).

Results and Discussion

Fourier Transform Infra-red (FTIR)

Different peaks at various positions for different functional groups are presented in Figure 1. For sample M1, M2, M3, and M4, the broad bands at approximately 3300–3200 cm^{-1} were assigned to the stretching vibrations of hydroxyl groups. At the peak of 3350-3380 cm^{-1}, N–H stretching vibrations corresponded to the chitosan and NIPAM. The absorption peaks at 2900-2980 cm^{-1} were associated with the C–H stretching of methylene and methyl groups of glycol chitosan.

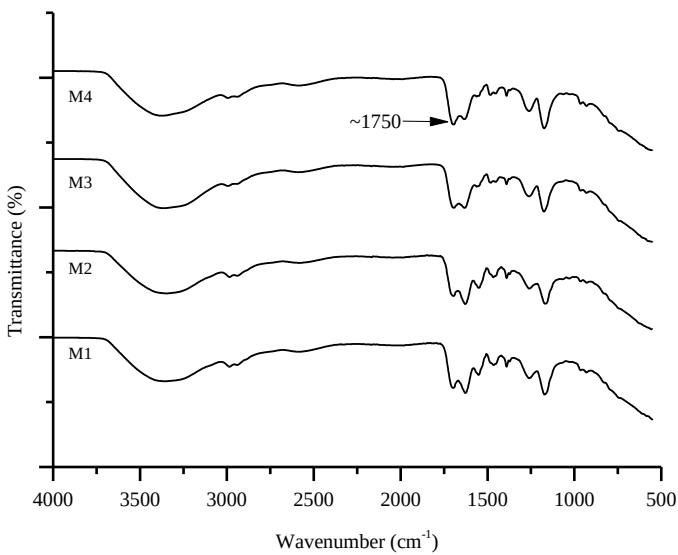

Figure 1: FTIR spectra to Chitosan-P(MAA-co-NIPAM) hydrogel with different composition of MAA.

The characteristic absorption peaks of the chitosan at 1390cm^{-1} were assigned to the vibrations of C–H. Peaks at approximately 938, 1174 and

1556 cm^{-1}, which corresponded to the saccharide structure of chitosan, were also present. Furthermore, the peaks at 1170-1180 cm^{-1} can be assigned to C–N stretching in the amino group of chitosan. The peaks at 163, 1390 and 1385cm^{-1}, could represent the characteristic peaks of amide I, amide II and the isopropyl group, respectively. The increasing amount of the MAA in the hydrogel has increased the intensity of the C=O at the characteristic peak of 1750 cm^{-1}.

The absorption peaks of M1 and N1 are shown in Figure 2. The broad bands at approximately 3300–3200 cm^{-1} were assigned to the stretching vibrations of hydroxyl groups. At the peak of 3384 cm^{-1}, the N–H stretching vibrations were detected which could be due to the chitosan and NIPAM. The absorption peaks at 2900-2980 cm^{-1} were associated with the C–H stretching of methylene and methyl groups of glycol chitosan. The characteristic absorption peaks of the chitosan at 1690–1600 cm^{-1} can be assigned to carbonyl stretching and the stretching vibrations of the amino group (amide bands) of the amino acetyl group of chitosan. Meanwhile, the vibration of C–H was found at 1392 cm^{-1}. Peaks at approximately 938, 1174 and 1556 cm^{-1}, which corresponded to the saccharide structure of chitosan, were also present. Furthermore, the peak at 1174 cm^{-1} can be assigned to C–N stretching in the amino group of chitosan.

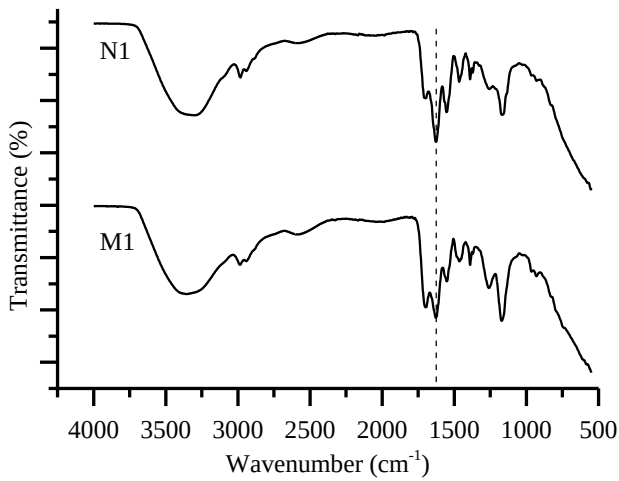

Figure 2: FTIR of Chitosan-P(MAA-co-NIPAM) hydrogel with different composition of PNIPAM.

The spectrum which exhibited significant peaks at 1692, 1395 and 1388 cm^{-1}, could be assigned to the characteristic peaks of amide I, amide II and isopropyl group, respectively. Different composition gave different

intensity of transmittance. For instance, the asymmetric stretching of -CH_3 from the isopropyl groups of NIPAM could be observed at peak of 2978 cm^{-1}, and the amide groups of NIPAM were presented at the peak of 1634 cm^{-1} (line in the graph). . Both peaks showed that for sample N1, the intensity was higher than that of M1. This can prove that by increasing the NIPAM may also increase the number of -CH_3 and amide group of the hydrogel.

Morphology Properties

Morphology analysis was performed using FESEM to investigate the phase separation between Cs and MAA in Chitosan-P(MAA-co-NIPAM) hydrogel. The samples have been justified by the different loading of MAA in Chitosan-P(MAA-co-NIPAM) hydrogel and SEM micrographs of Chitosan-P(MAA-co-NIPAM) hydrogel as shown in Figure 3.

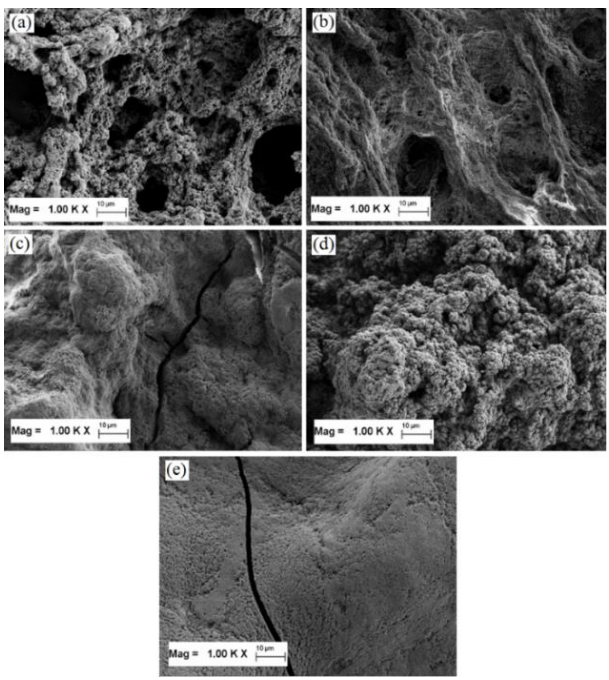

Figure 3: SEM photographs of surface of Chitosan-P(MAA-co-NIPAM) hydrogel at different concentration of MAA (a) M1, (b) M2, (c) M3, (d) M4 and NIPAM (e) N1.

The hydrogels were agglomerated and were not well dispersed. This is due to the MAA moieties which are insoluble at the pH of 3 and also influenced by the ionic interaction between Cs and MAA. As the amount of MAA increased, the particles size of the hydrogel became smaller in

sequence of M1 > M2 > M3. However, the particle sizes for sample M2 were greater, which could be due to the saturation of MAA that prevented the interaction of MAA moieties with Cs.

By increasing the MBA loading (Figure 3e) while fixing the amount of Cs and MAA composition of MAA and Cs, the Chitosan-P(MAA-co-NIPAM) hydrogel also resulted in an agglomeration and not well dispersed. This might be due to the expanding of Cs chain as a result of an intermolecular repulsion between the positively charged amino groups with amide groups of PNIPAM.

Swelling Hydrogel

The swelling behaviour of Chitosan-P(MAA-co-NIPAM) hydrogel in Figure 4 showed that the swelling was slightly higher in the acidic environment. This behaviour was obviously contributed from the amino group of chitosan (NH_3^+) which deprotonated in the acidic environment particularly at pH 1.68. Since the pKa of chitosan was approximately 6.5, most of the amino groups were protonated as the pH increased, while some of the PMAA with pKa ≈ 5.5 were protonated at low pH and the numbers of counter ions were very less. At pH 4, swelling had decreased as compared to that of pH 1.68. Some of the amino groups of chitosan started to be deprotonated into NH_2 and there were also interaction occurred between NH_3^+ with –COO- which reduced the repulsion within the same group. As the pH increased, more $–COO^-$ groups in the MAA chains were gradually protonated, however the amino group of chitosan was less deprotonated. Increasing the amount of MAA had increased the swelling ratio of the hydrogel at pH 4.08. This is due to the increasing amount of conjugated MAA that were started to be deprotonated.

The maximum swelling was attained for all samples of hydrogels at approximately pH 7.2. This is due to the large osmotic swelling force from the ionization of the carboxylic group and a large degree of hydrogels swelling [8]. However, sample M1 which has a low amount of PMAA showed a different trend of swelling. The hydrogel was less swelled as the pH of the environment increased and the hydrogel deswelled at pH 10. This behaviour was attributed to the less amount of PMAA in the hydrogel compared to other samples. Above pH 7, most of the amino groups have been protonated, and swelling of PMAA has obviously started as the pH increased. However, the swelling decreased when the pH approaching 10. Shielding effect from a high ionic strength of buffer solution reduced the ionized carboxylic group, and decreased the electrostatic repulsion and swelling of the hydrogel [9]

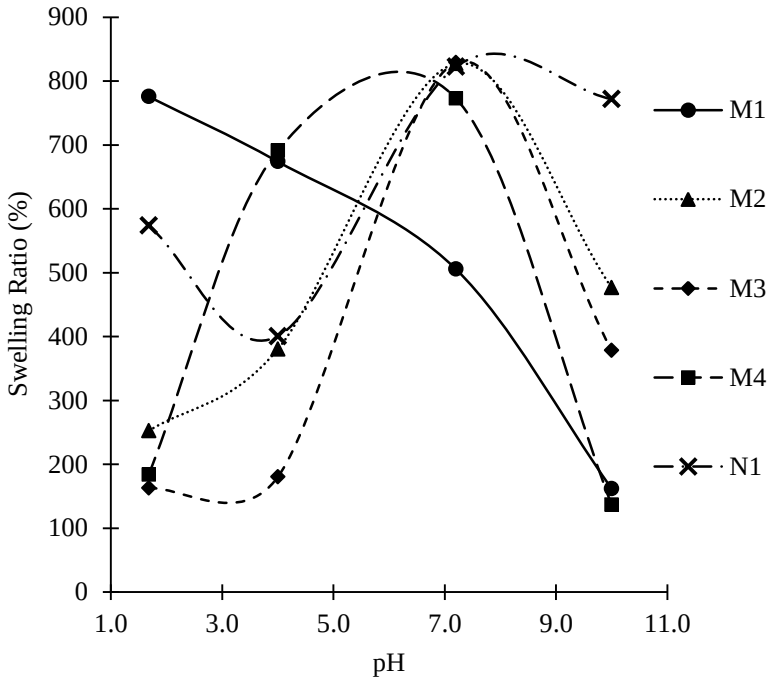

Figure 4**:** Effect of swelling ratio of Chitosan-P(MAA-co-NIPAM) hydrogel for different concentration of MAA at various pH values and temperature 37℃.

The swelling ratio of different temperature of the Chitosan-P(MAA-co-NIPAM) hydrogel is shown in Figure 5. By increasing the temperature, most of the hydrogel capabilities on swelling were achieved above 700% before the hydrogel started to collapse as shown in Figure 5. Different trend was found for hydrogel which has less amount of PMAA (M1), the swelling decreased as the temperature increased. This is due to the less amount of hydrophilic behaviour on the hydrogel which caused the LCST value almost similar to PNIPAM which was almost 32℃. The behaviour showed that the amount of PMAA has a tendency to change the LCST value and has the potential to control the suitable temperature for human body application. From the graph, the hydrogel started to collapse at temperature more than 37℃ and this shows that copolymerization of MAA in the hydrogel system has increased the value of LCST from almost 32℃ to more than 37℃ [10]. Hydrogel which has more NIPAM (N1) showed less swelling ratio at 25℃.

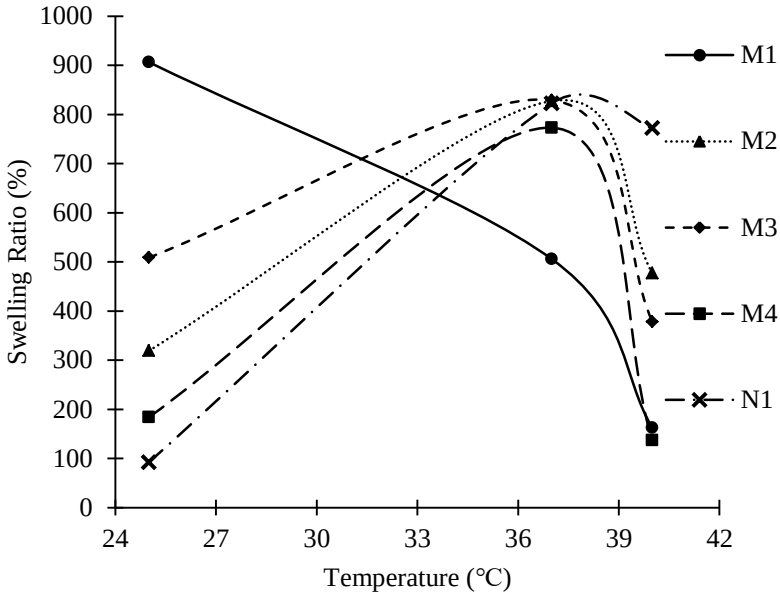

Figure 5: Effect of swelling ratio of Chitosan-P(MAA-co-NIPAM) hydrogel at the different concentration of MAA at various temperature value and pH 7.

Conclusion

The (chitosan-poly(methacrylic acid)-poly(N-isopropylacrylamide) [Chitosan-P(MAA-co-NIPAM)] multi-responsive hydrogel has been successfully fabricated by free-radical polymerization in aqueous media. The influence of different loading of MAA and NIPAM toward morphological stability and swelling properties of the Chitosan-P(MAA-co-NIPAM) hydrogel series was studied. The resulting Chitosan-P(MAA-co-NIPAM) hydrogel series were characterized by Fourier transform infra-red (FT-IR). Results also showed that the intensity of carbonyl group have increased demonstrating the reinforcing of MAA in Chitosan-P(MAA-co-NIPAM) hydrogel series. The SEM micrograph displayed the colloidal Chitosan-P(MAA-co-NIPAM) hydrogel series were agglomerated and not well dispersed. Increasing the amount of MAA during synthesis increased the value of LCST from the previous LCST PNIPAM which was almost 32℃ to more than 37℃. The swelling ratio of the hydrogel was found to be maximum at pH 7, which is suitable to be used in body system especially in human blood of pH 7.4.

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References

[1] K. Wang, Q. Fu, X. Chen, Y. Gao, K. Dong, "Preparation and characterization of pH-sensitive hydrogel for drug delivery system," *RSC Advances* 2 (20), 7772-7780 (2012).

[2] S. Lü, M. Liu, B. Ni, C. Gao, "A novel pH-and thermo-sensitive PVP/CMC semi-IPN hydrogel: Swelling, phase behavior, and drug release study," *Journal of Polymer Science Part B: Polymer Physics* 48 (15), 1749-1756 (2010).

[3] P. Shao, B. Wang, Y. Wang, J. Li, Y. Zhang, "The Application of Thermosensitive Nanocarriers in Controlled Drug Delivery," *Journal of Nanomaterials* 2011 (2011).

[4] Y. Qiu, K. Park, "Environment-sensitive hydrogels for drug delivery," *Advanced drug delivery reviews* 53 (3), 321-339 (2001).

[5] I. Bischofberger, V. Trappe, "New aspects in the phase behaviour of poly-N-isopropyl acrylamide: systematic temperature dependent shrinking of PNiPAM assemblies well beyond the LCST," *Scientific reports* 5 (2015).

[6] A. Khan, M.B.H. Othman, B.P. Chang, H.M. Akil, "Preparation, physicochemical and stability studies of chitosan-PNIPAM based responsive microgels under various pH and temperature conditions," *Iranian Polymer Journal* 24 (4), 317-328 (2015).

[7] R. Salehi, N. Arsalani, S. Davaran, A.A. Entezami, "Synthesis and characterization of thermosensitive and pH-sensitive poly (N-isopropylacrylamide-acrylamide-vinylpyrrolidone) for use in controlled release of naltrexone," *Journal of biomedical materials research. Part A* 89 (4), 919-28 (2009).

[8] J. Zhang, N.A. Peppas, "Synthesis and characterization of pH-and temperature-sensitive poly (methacrylic acid)/poly (N-isopropylacrylamide) interpenetrating polymeric networks," *Macromolecules* 33 (1), 102-107 (2000).

[9] J. Klier, A.B. Scranton, N. Peppas, "Self-associating networks of poly (methacrylic acid-g-ethylene glycol)", *Macromolecules* 23 (23), 4944-4949 (1990).

[10] X. Yin, A.S. Hoffman, P.S. Stayton, "Poly (N-isopropylacrylamide-co-propylacrylic acid) copolymers that respond sharply to temperature and pH," *Biomacromolecules* 7 (5), 1381-1385(2006).