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Synthesis and Characterization of Acrylic Based Copolymeric Hydrogel Nanoparticles: An Approach to Drug Delivery

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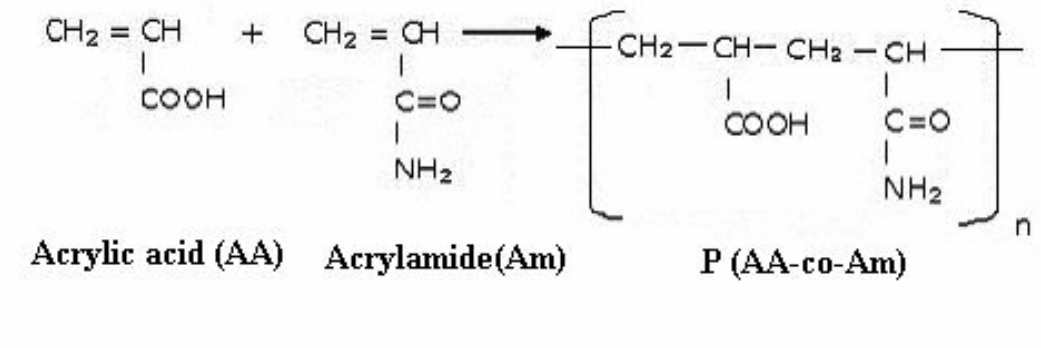
Abstract: Poly(acrylamide-co-acrylic acid) copolymeric hydrogel nanoparticles(HN) were prepared by precipitation polymerization technique using potassium per sulphate(PS) as reaction initiator and *N, N* - methylene-bis-acrylamide (MBA) as crosslinking agent.The prepared HN were characterized by FTIR and copolymerization was confirmed. From the swelling studies of the HN , it was found that the prepared copolymer showed comparatively higher swelling in rat caecal medium which is having higher pH. Among the formulations, formulation A1 showed comparatively higher swelling in all the biological media. Degradation studies of HN in simulated physiological isotonic solution indicated slow rate of degradation for extended period of time which is useful for controlled delivery of drug. HN produced were of submicron size and of low polydispersity, which indicated a relatively narrow particle size distribution that is suitable for targeting the entrapped drug to specific site.

Key Words: Poly acrylamide; Poly acrylic acid; Hydrogel; Bio degradability; Swelling properties

Introduction:

Now -a-days hydrogels have become popular carriers for drug delivery applications due to their biocompatibility and resemblance to biological tissues [1–6]. From a structural point of view, hydrogels are three-dimensional hydrophilic polymer networks that swell in water or biological fluids without dissolving as a result of chemical or physical crosslinks[7]. Hydrogels can be used to target the release of a drug or protein to a specific area of the body [8–12] and simultaneously control the release kinetics due to their three-dimensional structure [13–15]. Hydrogel-based devices belong to the group of the swelling-controlled drug delivery systems [16]. When the polymer network comes in contact with aqueous solutions, the thermodynamic compatibility of the polymer chains and water causes the polymer to swell. As water penetrates inside the glassy network, the glass transition temperature of the polymer decreases and the hydrogel becomes rubbery. The drug trapped inside the hydrogel dissolves with the imbibed water and starts diffusing out of the network. There are three driving forces that contribute to this phenomenon: a penetrant concentration gradient, a polymer stress gradient and osmotic forces. In the case of nonswelling controlled delivery systems, the relaxation rate of the polymer is very slow in comparison to the water transport inside the hydrogel. Then, the transport mechanism in this type of systems follows Fickian diffusion. When the macromolecular chain relaxation is the dominating driving force, Case II transport is observed. However, in many swelling-controlled delivery systems, anomalous transport mechanism has been observed, characterized by an intermediate Fickian diffusion and Case II transport. Hydrogels may also exhibit ionic characteristics. In ionic hydrogels, positive or negative charges are created due to

the pendant ionizable groups in the polymer chains.Depending on the pH and ionic strength of the surrounding environment, swelling or collapse of the hydrogel may occur [17–19]. Certain pH-responsive hydrogels can also exhibit complexation properties. Complexation hydrogels have been previously studied as very promising controlled release devices for drugs and proteins [20–30]. These hydrogels are characterized by the association of chemical groups belonging to different polymer chains. Hydrogen bonding, among others, is one of the interactions responsible for these chemical associations between macromolecular chains [31–33].The present study aims at developing hydrogel nanoparticles of synthesized poly(acrylic acid-co-acrylamide) and finding its suitability for drug delivery studies.



Scheme1. Synthesis of Poly(acrylic acid-co-acrylamide) copolymer

Material and Methods:

Acrylamide (Am) and Glutaraldehyde were purchased from S.D. fine chem. Ltd., Mumbai, India, acrylic acid (AA) from Himedia laboratories Ltd., Mumbai, potassium persulfate (PS) and sodium dodecyl sulfate from Loba chemie, Mumbai. *N, N* - methylene-bis-acrylamide (MBA) 3 % crystal extra pure AR was purchased from Sisco research laboratories Pvt. Ltd., Mumbai. All other reagents used were of analytical grade.

Synthesis of P(AA-co-Am) hydrogel nanoparticles

P(AA-co-Am) hydrogel nanoparticles were prepared by precipitation polymerization[34]. AA and Am were charged into the reaction vessel at different monomer feed (wt.%) ratios followed by addition of sodium dodecyl sulfate (0.02 % w/v) and MBA (0.01mol dm^{-3}) in distilled water (50 ml) at room temperature, were purged with nitrogen and stirred for 30 min, and then heated to 70 ^{0}C. PS (0.16g) in 20 ml water was added to initiate polymerization. The reaction was maintained at 60^{0}C under nitrogen for 6 h. After cooling to room temperature, the resultant nanoparticles were dialyzed for 1 week to remove surfactant and unreacted molecules. The dialysis water was changed three times every day. The cut off molecular weight of the dialysis membrane was 13,000.The dialyzed particle dispersion was condensed by evaporation of water and dried to get hydrogel nanoparticles.

Preparation of rat caecal medium (RCM)

Male rats, weighting 200–250 g maintained on a normal diet, were lightly anesthetized under ether and then sacrificed by decapitation. Central Animal House facility, Jayadev College of

Pharmaceutical Sciences, India approved the study. The caecum was exteriorized, ligated at two ends (2.0 cm distances), cut loose, and immediately removed from the rat body. The formed caecal bag was then opened, its content weighted, pooled, and suspended in two volumes of cold bicarbonate - buffering saline (BBS, pH 7.0: $NaHCO_3$, 110 mM; $Na_2HPO_4{\cdot}12H_2O$, 20 mM; NaCl, 8.0 mM; KCl, 6.0 mM; $CaCl_2{\cdot}2H_2O$, 0.5 mM; $MgCl_2{\cdot}6H_2O$, 0.4 mM) to give a final caecal dilution of 33% (w/v). The suspension was filtered through 400-mesh grit twice to remove debris. Supernatants were then centrifuged at 15,000×*g* for 30 min in order to obtain a clear supernatant containing extracellular enzymes[35].

Swelling behavior of P(AA-co-Am) hydrogel nanoparticles

The equilibrium swelling of the P(AA-co-Am) HN were determined by swelling the dried HN in Simulated Gastric Fluid (SGF), Simulated Intestinal Fluid (SIF) and Rat Caecal Medium (RCM) until equilibrium was attained. The swollen weights of the HN were determined by blotting every hour until equilibrium was attained.

The swelling behavior was computed by calculating the percentage swelling (%S).

$\%S = [(M_t - M_o) / M_o) \times 100$

Where, M_t and M_o are the masses of the swollen and the dry samples respectively.

Fourier Transform Infrared (FTIR)

FTIR studies of P(AA-co-Am) HN were carried out at room temperature by FTIR spectrophotometer (FTIR, Paragon-500) using KBr pellet. All the spectra were recorded in the range of 500-4000 cm^{-1}.

Degradation Study

The degradation of P(AA-co-Am) HN was studied on weight loss basis with about 1.5 g weight, over a period of 60 days [36]. They were conditioned to minimum weight at 37 ± 1 ^{0}C in an oven containing desiccant prior to being immersed into 100 ml of a simulated physiological isotonic solution (0.154 M NaCl aqueous solution at pH 7.4). The specimens were removed at regular intervals of 3, 7, 14, 30 and 60 days, being taken out of the solution, blotted on filter paper to remove surface solution and dried in an oven at 50 ^{0}C to constant weight in order to determine eventual weight loss, taking an average of two readings.

Transmission Electron Microscopy (TEM)

The HN were examined by transmission electron microscopy (CM12 Philips, Eindhoven, Netherlands). Samples were stained with 2% phospho tungistic acid for 10 minutes, immobilized on copper grids and dried overnight for viewing.

Size distribution of hydrogel nanoparticles

The size distribution of HN before and after loading 5-FU was determined using photon correlation spectroscopy (PCS) (PCS System, Malvern Instruments Ltd.). The analysis was performed at a scattering angle of 90 ^{0} and at a temperature of 25 ^{0}C using samples appropriately diluted with filtered water. For each sample, the mean diameter ± standard deviation of six determinations was calculated. Values reported (Table 1) are the mean diameter ± standard deviation of two replicate samples.

Results and discussion

Swelling behavior studies

Hydrogel is the combination of the chains and as the prepared polymer is made up of

P(AA-co-Am), the acidic group bound to their polymer chains, from where the H^+ comes off and combines with OH^- to form H_2O. The charge is compensated by cations that enter the gel together with another OH^-, thus charge neutrality is maintained. The increased cation concentration gives rise to an osmotic pressure that causes the gel to swell/de swell. An equilibrium ionic gel occurs when the elastic restoring force of the network balances the osmotic forces. The Hydrogel formed by the homogenous copolymer of AA and Am with MBA as crosslinker where the acidic group bound to the polymer chains are carboxyl groups which made the gels pH sensitive. The swelling behavior of the copolymer in physiological solutions were studied and it was found that the prepared copolymer showed comparatively higher swelling in rat caecal medium which is having higher pH as shown in Figure 1.Among the formulations, formulation A1 showed comparatively higher swelling in all the biological media.

FTIR of P(AA-co-Am) hydrogel nanoparticles

The FTIR spectra of Polyacrylamide had peaks at 3365.73, 1654.55, 1451.21, 1325.44 and 634.31 cm^{-1} (Figure 2). The spectrum of the P(AA-co-Am) HN showed peaks at 3401.41, 2756.22, 1702.51, 1247.41 and 634.50 cm^{-1}. The band for Polyacrylic acid occurred at 1715.11 cm^{-1}. The band at 1702.51 cm^{-1} in P(AA-co-Am) HN was due to the carbonyl component of the carboxylic acid group of polyacrylic acid. The carbonyl absorption bands of the remaining amide groups overlapped with the carboxylic absorption bands. The broad absorption bands from 3401.41cm^{-1} to 2956.22 cm^{-1} in P(AA-co-Am) HN were assigned to the –OH from the carboxylic group.

Degradation study

Now-a-days investigators have focused on controlling degradation behavior of hydrogels, as well as on enhancing their biological interactions with body components, in order to design and tailor appropriate vehicles for drug delivery. Controlling degradation behavior has been one of critical issues in general biomaterials research, and has been widely investigated to date. In general, especially for drug delivery applications, biomaterials need to be cleared from the body once they complete their roles in the body, and degradable materials could be ideal for this purpose. The degradation studies of the prepared HN were studied on the basis of % wt. loss in simulated physiological isotonic solution (0.154M NaCl aqueous solution at pH 7.4) at regular intervals of 3, 7, 14, 30 and 60 days. From the degradation study of P(AA-co-Am) HN, over a period of 60 days (Figure 3), it was found that with increase in the immersion time, the weight loss increased to become stabilized after about 30 days and extended up to 60 days. The degradation period of the prepared HN reflected its strength, which might be due to effective cross-linking of molecules. The slow and continual degradation behavior of cross-linked HN might be attributed to the attachment points that only allow complete disintegration of the cross-linking molecules once all attachment points are lost.

Size distribution of hydrogel nanoparticles

The size distribution of HN was determined using photon correlation spectroscopy (PCS) as shown in Table 1. The sample was illuminated by a laser beam and the particles undergoing Brownian motion were detected. Light scattering by these particles was received by a fibre-optic cable placed at a particular angle and the fluctuations in scattering intensity were analyzed .The polydispersity (PI) is an important parameter that gives an idea about the reliability of the data obtained with PCS analysis. PI is a dimensionless number extrapolated from values of 0.010 for monodispersed polystyrene standard latex particles upto values around 0.5-0.7. Values greater than 0.7 are characteristic of samples with a very broad size distribution. The particle size distribution data shows that HN produced were of submicron size and of low polydispersity (Table 1), which indicated a relatively narrow particle size distribution that is suitable for targeting the entrapped drug to specific site.

Conclusion:

From the above study it can be concluded that in future studies, drug can be entrapped and released from the submicron hydrogel nanoparticles (A1) due to its good swelling, bio degradability and size distribution properties.

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Table 1. Particle size distribution study of hydrogel nanoparticle by Photon correlation spectroscopy.

Batch	Monomer feed(wt.%) AA :Am	Concentration of MBA (mol dm^{-3})	Temperature (^{0}C)	Exposure time (hour)	Empty HN size ±S.D (nm) (Polydispersity)
A1	25:75	0.01	60	6	18.31±0.65(0.11±0.02)
B1	50:50	0.01	60	6	24.6±1.31(0.12±0.07)
C1	75:25	0.01	60	6	38.4±1.16(0.09±0.02)
D1	90:10	0.01	60	6	54.1±1.9(0.07±0.03)

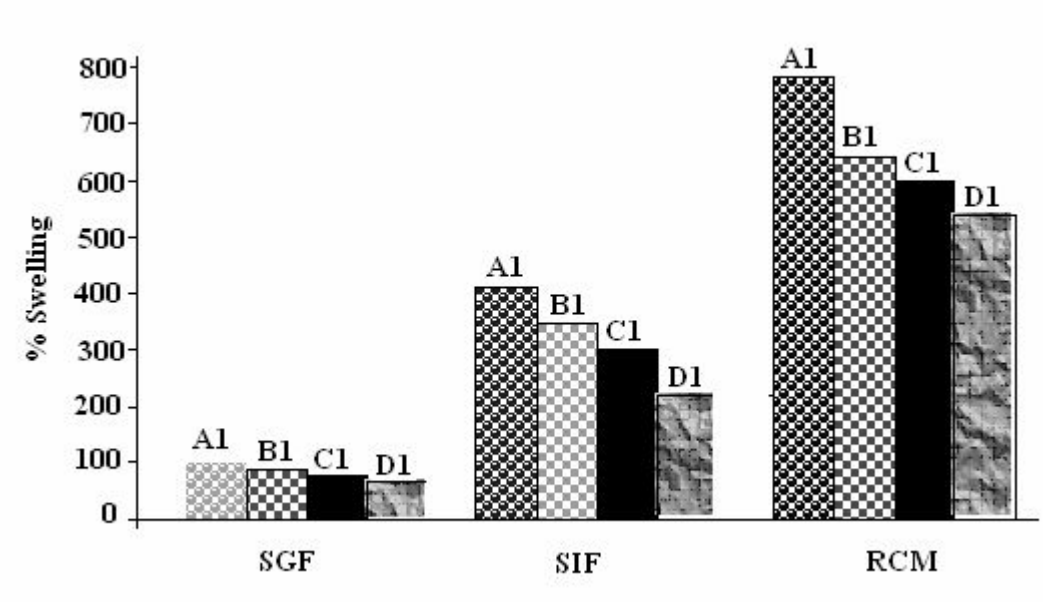


Figure 1. Swelling studies of Poly(acrylic acid-co-acrylamide) hydrogel nanoparticles in biological medium.

Figure2. FT-IR spectra of (a) Poly(acrylic acid), (b) Poly acrylamide, (c) Poly(acrylic acid-co-acrylamide) hydrogel nanoparticles

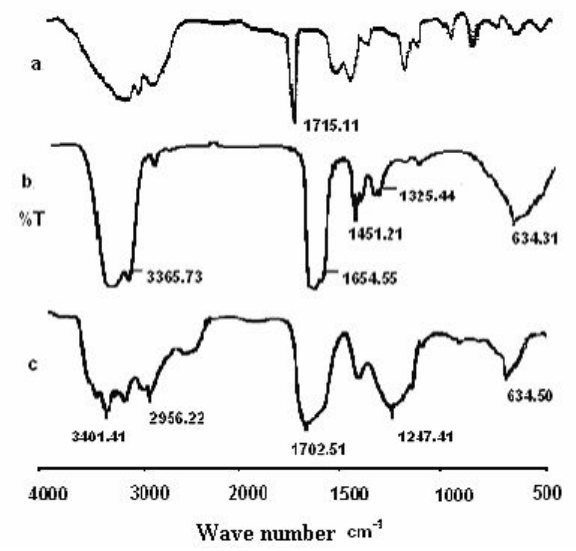


Figure 3. Degradation study of Poly(acrylic acid-co-acrylamide) hydrogel nanoparticles in physiological isotonic solution

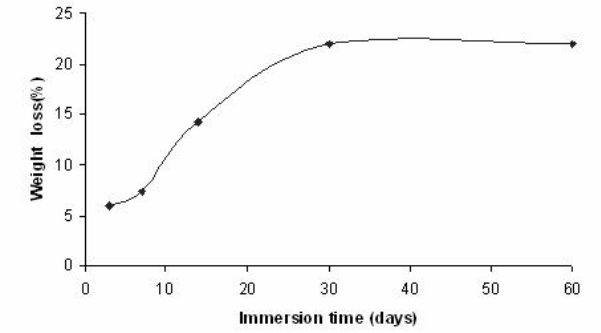
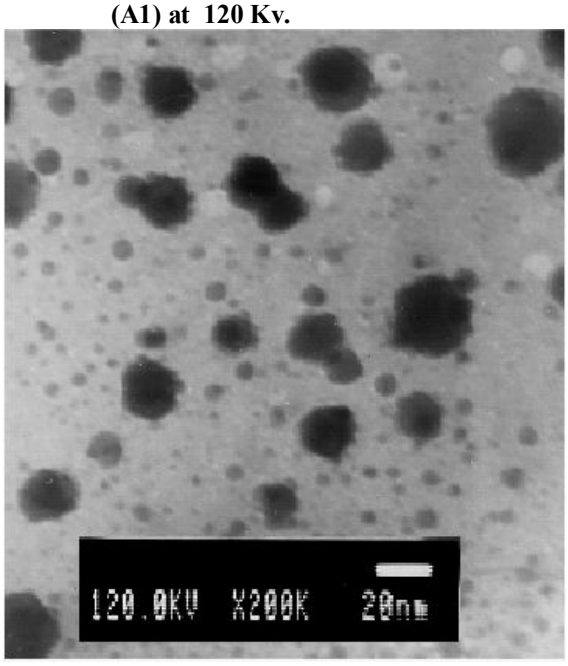


Figure 4. TEM image of Poly(acrylic acid-co-acrylamide) hydrogel nanoparticle (A1) at 120 Kv.



References:

1. Langer R. and Peppas N.A., Advances in biomaterials, drug delivery, and bionanotechnology. AIChE, 2003,49, 2990–3006.
2. Peppas N.A., Huang Y., Torres-Lugo M., Ward J.H. and Zhang J., Physicochemical foundations and structural design of hydrogels in medicine and biology. Ann.Rev. Biomed. Eng., 2000,2 ,9–29.
3. Lowman A.M., Dziubla T.D., Bures P. and Peppas N.A., Structural and dynamic response of neutral and intelligent networks in biomedical environments. In: Peppas NA, Sefton MV, editors. Molecular and cellular foundations of biomaterials. New York, Academic Press,2004, pp. 75–130.
4. Peppas N.A., Wood K.M. and Blanchette J.O., Hydrogels for oral delivery of therapeutic proteins. Expert Opin. Biol. Ther., 2004,4,881–887.
5. Ichikawa H. and Peppas N.A. Synthesis and characterization of pH responsive nanosized hydrogels of poly(methacrylic acid-g-ethylene glycol) for oral peptide delivery. In: Barratt G, Duche^ ne D, Fattal F, Legendre JY, editors. New trends in polymers for oral and parenteral administration: from design to receptors. Paris, Editions de Sante´ ,2001, pp. 261–264.
6. Torres-Lugo M. and Peppas NA., Preparation and characterization of P(MAA-g-EG) nanospheres for protein delivery applications. J. Nanopart. Res., 2000,4,73–81.
7. Peppas N.A. and Khare A.R., Preparation, structure and diffusional behavior of hydrogels in controlled release. Adv. Drug Deliv. Rev., 1993,11,1–35.
8. Peppas N.A., Bures P., Leobandung W. and Ichikawa H., Hydrogels in pharmaceutical formulations. Eur. J. Pharm. Biopharm., 2000,50,27–46.
9. Peppas N.A., Devices based on intelligent biopolymers for oral protein delivery. Int. J. Pharmaceut., 2004,277,11–7.
10. Serra L. and Peppas N.A., Novel poly(ethylene glycol) containing polymers as mucoadhesive drug delivery systems. AAPS Pharm. Sci., 2003, M1302.
11. Peppas N.A., Molecular design and cellular response of novel intelligent mucoadhesive carriers for oral delivery of proteins. In: Saltzman WM, Chilkoti A, Luo D, Uhrich K, editors. Biomaterials for drug delivery: architecture and application of biomaterials and biomolecular materials. Pittsburgh, PA: MRS Proceedings, 2004,
pp. 381–92.
12. Serra L., Dome´ nech J. and Peppas N.A., Disen˜ o y sı´ ntesis de hidrogeles acrı´ licos modificados para la liberacio´ n controlada de fa´ rmacos. Proc. Spanish Soc. Ind. Galenic Pharm., 2005,7,271–73.
13. Scott R.A. and Peppas N.A., Highly crosslinked, PEG-containing copolymers for sustained solute delivery. Biomaterials, 1999,20,1371–80.
14. Brazel C.S. and Peppas N.A., Mechanisms of solute and drug transport in relaxing, swellable, hydrophilic glassy polymers. Polymer, 1999,40, 3383–98.
15. Lowman A.M. and Peppas N.A., Solute transport analysis in pHresponsive, complexing hydrogels of poly(methacrylic acid-g-ethylene glycol). J. Biomater. Sci. Polym. Ed., 1999,10, 999–1009.
16. Colombo P., Santi P., Bettini R., Brazel C.S. and Peppas N.A., Drug release from swelling-controlled systems. In: Wise DL, Brannon-Peppas L, Klibanov AM, Langer RS, Mikos AG, Peppas NA, Trantolo DJ, Wnek GR, Yaszemski MJ, editors. Handbook of pharmaceutical controlled release technology. New York, Dekker, 2000, pp. 183–209.
17. Ostroha J., Pong M., Lowman A., and Dan N., Controlling the collapse/ swelling transition in charged hydrogels. Biomaterials, 2004,25, 4345–53.
18. Kim B., La Flamme K. and Peppas N.A., Dynamic swelling behavior of pH-sensitive anionic hydrogels uses for protein delivery. J. Appl. Polym. Sci., 2003,89,1606–13.
19. Berger J., Mayer J.M., Felt O., Peppas N.A., and Gurny R., Structure and interaction in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. Eur. J. Pharmaceut. Biopharmaceut., 2004,57,19–34.
20. Lowman A.M., Morishita M., Kajita M., Nagai T., and Peppas N.A., Oral delivery of insulin using pH-responsive complexation gels. J. Pharmaceut. Sci., 1999,88,933–37.
21. Foss A.C., Goto T., Morishita M., and Peppas N.A., Development of acrylicbased copolymer for oral insulin delivery. Eur. J. Pharmaceut. Biopharmaceut., 2004,57,163–9.
22. Torres-Lugo M,, Garcia M,, Record R., and Peppas N.A., pH-Sensitive hydrogels as gastrointestinal tract absorption enhancers: transport mechanisms of salmon calcitonin and other model molecules using the caco-2 cell model. Biotechnol. Prog.,2002,18, 612–16.
23. Torres-Lugo M., and Peppas N.A., Molecular design and in vitro studies of novel pH-sensitive hydrogels for the oral delivery of calcitonin. Macromolecules,1999,32,6646–51.
24. Donini C., Robinson D.N., Colombo P., Giordano F., and Peppas N.A., Preparation of P(MAA-g-EG) nanospheres for pharmaceutical applications. Int. J. Pharmaceut., 2002,245,83–91.
25. Kim B., and Peppas NA., PEG-containing hydrogel microparticles for oral protein delivery applications. Biomed. Microdevices., 2003, 5,333–41.
26. Kim B., and Peppas N.A., In vitro release behavior and stability of insulin in complexation hydrogels as oral drug delivery carriers. Int. J. Pharmaceut., 2003,266,29–37.
27. Nakamura K., Murray R.J., Joseph J.I., Peppas N.A., Morishita M., and Lowman A.M., Oral insulin delivery using P(MAA-g-EG) hydrogels: effects of network morphology on insulin delivery characteristics. J. Controlled Release, 2004,95,589–99.
28. Morishita M., Goto T., Peppas N.A., Joseph J.I., Torjman M.C., and Munsick C., et al. Mucosal insulin delivery systems based on complexation polymer

hydrogels: effect of particle size on insulin enteral absorption. J Controlled Release, 2004,97,115–24.
29. Kavimandan N.J., and Peppas N.A., Complexation hydrogels as oral delivery vehicles for insulin–transferrin conjugates. In: Peppas NA, Anseth K, Dillow AK, Schmidt CE, editors. Advances in biomaterials, bionanotechnology, biomimetic systems and tissue engineering. New York: AIChE, 2004, pp. 179–81.
30. Blanchette J.O., and Peppas N.A., Oral chemotherapeutic delivery: design and cellular response. Ann. Biomed. Eng., 2005,33,142–9.
31. Lowman A.M., and Peppas N.A., Molecular analysis of interpolymer complexation in graft copolymer networks. Polymer, 2000,41,73–80.
32. Kim B., and Peppas N.A., Analysis of molecular interactions in poly (methacrylic acid-g-ethylene glycol) hydrogels. Polymer, 2003, 44,3701–7.
33. Lowman A.M., Complexing polymers in drug delivery. In: Wise DL, Brannon-Peppas L, Klibanov AM, Langer RS, Mikos AG, Peppas NA, Trantolo DJ, Wnek GR, Yaszemski MJ, editors. Handbook of pharmaceutical controlled release technology. New York, Dekker, 2000, pp. 89–98.
34. Pelton R. H., and Chibante P., Preparation of aqueous latices with *N*-isopropylacrylamide. Colloids Surf., 1986**,** 20,247-56.
35. Zhang H., and Neau S.H., In vitro degradation of chitosan by bacterial enzymes from rat cecal and colonic contents. Biomaterials, 2002, 23, 2762-74.
36. Elvira C., Mano J. F., Roman J. S., and Reis R. L., Starch-based biodegradable hydrogels with potential biomedical applications as drug delivery systems. Biomaterials, 2002, 23, 1955-66.

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