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Controlled release of 5-aminosalicylicacid from chitosan based pH and temperature sensitive hydrogels

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ABSTRACT

A series of temperature and pH responsive hydrogels based on chitosan and poly(N-isopropyl acrylamide) (PNIPA) was prepared by redox polymerization. Effect of the composition on swelling behavior of the hydrogels and the release of 5-aminosaylcilic acid (5-ASA) at different temperatures and pHs have been investigated. Ammonium persulphate and TEMED were used as a redox pair at room temperature. As a cross linker, methacrylated chitosan was synthesized through the reaction of chitosan with glycidyl methacrylate (GMA). Introduction of the cross-linker provided the hydrogels with pH and temperature sensitivities. The phase transition temperatures of the hydrogels were determined by derivative differential scanning calorimeter (DDSC). Their phase transition temperatures were increased by chitosan content. Swelling behaviors and the release of 5-ASA varied significantly with pH, temperature and the gel composition. The release of 5-ASA from the hydrogels was followed by UV–Vis and fluorescence spectroscopy.

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1. Introduction

Stimuli-responsive hydrogels are well known “smart materials” in terms of responding to environmental signals such as pH, temperature, ionic strength, charge density by changing their shape and volume. Chitosan, which is a high molecular weight polysaccharide, is a well known pH responsive natural polymer, derived by fully or partly N-deacetylation of natural chitin. Its free reactive amino and hydroxymethyl groups are convenient functional groups for further modifications. Amino groups can easily be protonated in aqueous medium to form positively charged chitosan soluble in aqueous acids and insoluble in alkaline and neutral solutions, as well as in common organic solvents. This property therefore makes chitosan pH sensitive. Additionally, protonation of free amino groups makes chitosan bind to some drugs and bio-macromolecules, such as proteins, DNAs, enzymes and antibodies, which makes chitosan suitable for transportation applications of bio-molecules and some drugs [1–3]. Moreover, due to its biodegradable, biocompatible and non-cytotoxic properties, and antimicrobial, antifungal and anticancer activities, chitosan and its copolymers and blends have been attracting increasing attention for various biomedical applications, such as wound healing [4–8], immunology, drug and gene delivery [9–15] and antibacterial food packaging [16]. On the other hand, relatively poor mechanical properties of chitosan cause some limitations in its applications as film materials. Thus, improvement in its physical properties through blending and copolymerization has been the subject of various research groups [17,18].

Thermo-sensitive hydrogels undergo volume phase transition with temperature, among which cross-linked poly(N-isopropyl acrylamide) (PNIPA) is a well known one, having a lower critical solution temperature (LCST). It has a volume transition at around 32 °C from the swollen to collapse state, which makes it suitable for controlled drug delivery in biomedical applications as the transition temperature is close to human body temperature [19–21]. pH and thermo sensitive hydrogels are hydrophilic network structures, carrying both thermo and pH sensitive segments. By considering the varying local pH values and the body temperature, preparation of thermo and pH sensitive hydrogels, leading to specific drug delivery processes, is particularly important. Thus, pH sensitivity of chitosan and the volume transition temperature of PNIPA have been the interest of various groups to develop materials for biomedical applications, such as drug carriers. Syntheses and

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characterizations of hydrogels based on PNIPA grafted chitosan, using gamma-irradiation, were previously studied by Cai et al. [22]. They reported the effect of NIPA concentration and irradiation dose on grafting efficiency and swelling behavior of the hydrogels. Han et al. prepared chitosan-PNIPA hydrogels, using photo polymerization technique, who studied acid orange (AO8) and 5-flurouracil (5FU) release [23]. Therapeutic effect of 5-ASA loaded chitosan capsules for ulcerative colit was investigated by Tozaki et al. [24]. They observed that chitosan capsules are useful 5-ASA carriers for colon-specific drug delivery. Separately, a pH sensitive methacrylated 5-ASA as a pro-drug was prepared and its swelling characteristics and release properties were studied [25]. On the other hand, to the best of our knowledge, there is no report available in the literature on controlled release of 5-ASA by thermal and pH sensitive chitosan-PNIPA hydrogels.

Although 5-ASA is an important anti-inflammatory drug used to treat inflammatory bowel diseases such as ulcerative colit and Crohn's disease, it causes gastric irritation when administrated through oral system. However, controlled release of 5-ASA by pH and temperature sensitive hydrogel in intestine system, rather than stomach, reduces such irritation. A report demonstrated the release of 5-ASA from chitosan capsules in a controlled way in bowel system for the treatment of Crohn's disease [24].

As independent and mutual influences of both pH and temperature on release kinetics of a drug, complete release of 5-ASA in small and/or large intestine is possible by tuning the chitosan/NIPA ratio. This study focused on the syntheses of pH and temperature sensitive chitosan-PNIPA based hydrogels, employing redox polymerization technique and determination of their potential use in controlled release of 5-ASA in intestinal system. Methacrylated chitosan was synthesized and applied as a cross-linker in redox polymerization of NIPA. Phase transition, swelling and collapsing behaviors of the hydrogels and their controlled release of 5-ASA as a function of temperature, pH and composition were examined.

2. Materials and methods

2.1. Materials

Chitosan was prepared by deacetylation of chitin from shrimp shell. N-isopropyl acrylamide (NIPA) (minimum 98% purity), glycidyl methacrylate (GMA, minimum 97% purity), N,N,N′,N′-tetramethylethylenediamine (TEMED, minimum 99% purity) and potassium peroxydisulphate (KPS, minimum 99% purity) were supplied from Aldrich, Fluka, Analyticals Carlo-Erba and J.T. Baker, respectively, and they were used as received. Acetic acid with 99–100% purity was supplied from Carlo Erba and used without further purification. Hydroquinone was supplied from Aldrich (minimum 99% purity) and used as inhibitor for undesirable polymerization of GMA in the course of methacrylated chitosan synthesis. Acetonitrile (AN) and tetrahydrofurane (THF) were supplied from Aldrich (99% purity) and used for isolation and purification of methacrylated chitosan. 5-Aminosalicilic acid (5-ASA) was 95% purity, obtained from Alfa Aeser and used without purification.

2.2. Instrumentation

Thermal gravimetric analyses (TGA) were recorded on Seiko-EXSTAR-TG/DTA7300 model thermal analyzer. Measurements (5–7 mg of the samples) were performed at heating rate of 10 °C/min under dynamic argon atmosphere at a flow rate of 20 ml/min. The phase transition temperatures were determined using Perkin Elmer Jade type differential scanning calorimeter (DSC) under argon atmosphere at 10 °C/min heating rate. Derivative of the DSC curves (DDSC) were plotted using Pyris TA software. Temperature calibration of DSC was conducted according to the indium melting point and melting enthalpy. The molar mass and molar mass distribution of chitosan were determined by gel permeation chromatography (GPC) system, equipped with Perkin Elmer-series 200 GPC high pressure pump, injector, serial connected four Water columns (Ultrahydrogel 120 + Ultrahydrogel 250 + Ultrahydrogel 500 + Ultrahydrogel 1000), Wyatt Optilab differential refractive index detector (RI) at 654 nm and Dawn Heleos multiangle light-scattering (LS) detector. The mobile phase was 0.1 M solution of sodium nitrate ($NaNO_3$) in 2% acetic acid water mixture, which had a flow rate of 1.0 ml/min. Measurements were carried out at 25 °C. Polymer concentrations were in the range of 0.5–2.0 mg/ml and all the samples were filtered through 0.2 μm filter prior to use. Drug release was followed in phosphate buffer, using Perkin Elmer Lambda-35 UV–Vis spectrophotometer and Scinco FS-2 fluorescence spectrophotometer, having Fluoro Master Plus Wave Scan software. The degree of deacetylation (DD) and methacrylation of glucosamine groups of chitosan were determined by ^1H NMR (Varian 600 MHz NMR) at 50 °C, applying a solvent system of 2% CF_3COOH in D_2O. Proton chemical shifts were recorded in ppm downfield from tetramethylsilane (TMS).

2.3. Preparation and characterization of chitosan

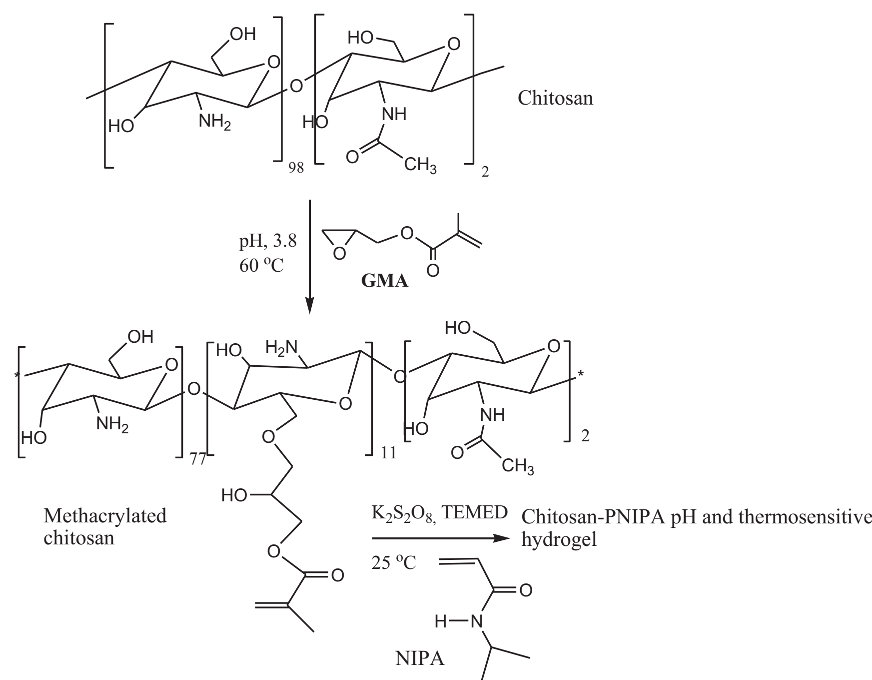
Chitosan was prepared from chitin of shrimp shell according to the literature procedure [26–28]. Briefly, chitin was isolated and purified by removing the minerals, proteins and pigments. Deacetylation of chitin was carried out in a 50% aqueous solution of NaOH at 80 °C for 8 h. The degree of deacetylation (DD) of chitosan was determined as 98% by ^1H NMR spectra. The specific refractive index increment (dn/dc) was found to be 0.128 ml/g in 0.1 M solution of sodium nitrate ($NaNO_3$) in a 2% acetic acid water mixture. The molar-mass and molar-mass distribution were determined by on-line GPC-LS technique. The polydispersity (Mw/Mn), absolute Mw and Mn values were found to be 1.6, 117.000 g/mol and 73.000 g/mol, respectively.

2.4. Synthesis of methacrylated chitosan [29]

5 g of chitosan (35 mmol glycosamine groups) and 250 ml of 3% (v/v) acetic acid/water solution were introduced into 500 ml three-necked round bottom flask (RB), equipped with a magnetic stirrer, gas inlet and a rubber septa. The solution was stirred under Ar atmosphere until a clear and yellowish solution was observed and 0.01 mg hydroquinone was added to prevent undesirable polymerization of GMA. pH was adjusted to 3.8 with KOH solution (0.1 M) and 5 ml GMA was slowly added with a vigorous stirring in an ice bath. After the reaction was allowed to stir at 60 °C for 4 h, the RB was placed into an ice bath to terminate the reaction. The product was precipitated in acetonitrile, washed twice with THF and kept under reduced pressure at room temperature. The conversion of chitosan was determined to be 12% by ^1H NMR spectroscopy.

2.5. Synthesis of chitosan-PNIPA hydrogels [30]

A solution of 0.2 g methacrylated chitosan (1.1 mmol of functional group based on 12% conversion) in 10 ml of 5% acetic acid was placed in a 20 ml reaction tube, to which 1.5 g of NIPA (13.2 mmol) and 0.2 ml of APS (0.05 M in water) were added. After the viscous solution was purged with argon for 10 min to remove oxygen, 0.1 ml of TEMED (0.05 M in water) was added and the solution was further purged with argon for 2 min. It was then transferred to a plastic straw of 5 mm diameter and 10 cm long, which was sealed and kept at room temperature until the cross-linking reaction was complete. The hydrogel was purified by consecutive swelling (pH 4 at 30 °C)



Scheme 1. Syntheses of chitosan-PNIPA hydrogels.

and collapsing (pH 8 at 40 °C) cycle, and dried under vacuum at 50 °C.

The presence of chitosan and PNIPA components in the hydrogels were proved by TGA, considering the distinctive thermal decomposition behaviors of pure chitosan and PNIPA. The syntheses of the chitosan-PNIPA hydrogels were outlined in Scheme 1.

2.6. Determination of swelling ratio

Swelling measurements were performed in acetate (pH 4) and phosphate (pH 8) buffers at 30 °C and 40 °C. For each measurement, about 0.5 g of dry gel was added into buffer solution. After reaching to swelling equilibrium, the gel was removed from the buffer and quickly blotted with dry filter paper and weighed again. The swelling ratio (Q) of the hydrogels were calculated gravimetrically by the following equation.

$$Q = \frac{W_2}{W_1}$$

where W_1 and W_2 are weights of the dry and swollen hydrogels, respectively.

2.7. 5-ASA loading and release studies

21 mg of 5-ASA was dissolved in 30 ml of acetate buffer solutions (pH 4) at 30 °C and about 0.5 g of dry hydrogels were added into the solutions. After reaching to a swelling equilibrium, the hydrogels were removed quickly, blotted with a filter paper and weighed. The equilibrium swelling and weight percentage of absorbed 5-ASA were determined gravimetrically as indicated below,

$$\text{5-ASA (wt.\%)} = \frac{((W_2 - W_1) \times (0.7/1000))}{W_1} \times 100$$

where W_2 and W_1 are weights of the swollen and dry gels, respectively. Quantity of 5-ASA was determined as weight-percent based on dry weight of the hydrogel. 5-ASA loaded hydrogels were added into the pH 8 phosphate buffer at 37 °C and the release was followed by UV–Vis and fluorescence spectrophotometers, as 5-ASA is a UV and fluorescence active material [31]. Spectroscopic measurements were performed with 1 ml samples at different time intervals. The composition of the hydrogels and their 5-ASA contents are collected in Table 1.

3. Results and discussion

3.1. NMR characterization of chitosan and methacrylated chitosan

The methacrylated chitosan was prepared with a minor modification of the literature method [29]. ^{1}H NMR spectrum of the chitosan is depicted in Fig. 1. Its deacetylation degree was calculated to be 98% from the integrations of the peaks at 2.05 ppm (—CH_3 protons of residual GlcNAc groups) and 4.9 ppm (anomeric proton), which was clearly observed when the spectrum was recorded at 50 °C. The singlet at 3.2 ppm is due to H-2 of GlcN and the multiplet between 3.7 and 4.0 ppm are due to the H-3–H-6 of GlcN and GlcNAc groups. The ^{1}H NMR spectrum of the methacrylated chitosan at 50 °C is shown in Fig. 2, in which the new peaks at 5.75 and 6.09 ppm are due to the =CH_2 protons of methacrylate groups and the peak at 1.9 ppm was assigned to be the —CH_3 group of GMA. These peaks confirmed that the methacrylation reaction was successful. Degree of methacrylation was calculated to be 12% from the integrations of the peaks at 1.9 and 5.75 ppm.

3.2. Thermal characterization of hydrogels

Hydrogels were prepared using redox polymerization technique. A series of hydrogels, having four different methacrylated chitosan/NIPA ratios were synthesized, applying APS-TEMED redox initiation system [30]. In order to explain the presence of both components, i.e. chitosan and PNIPA, in hydrogels, initially, their TG curves were recorded, since this technique can provide useful information, regarding the composition of the copolymers, polymer blends and composites, if the components have distinguishable

Table 1
Hydrogel compositions and their 5-ASA contents.

Hydrogel code	Chitosan content (wt.%)	Dry gel (mg)	Swollen gel (mg)	5-ASA absorbed into gel (mg)	wt.% of dry gel
HG-1	15	19.6	1078	0.74	3.78
HG-2	20	20.9	944	0.64	3.10
HG-3	25	22.7	987	0.68	2.99
HG-4	40	22.6	855	0.58	2.57

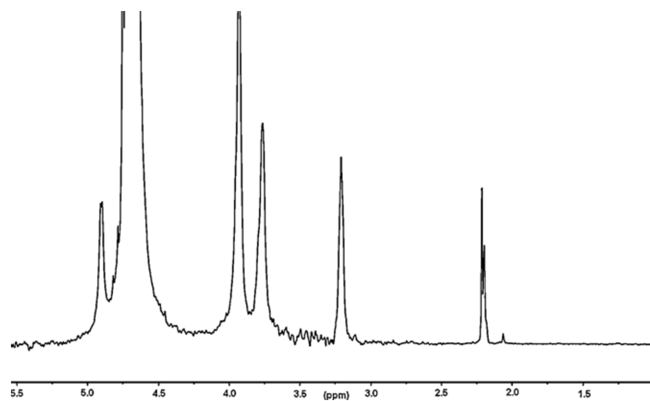


Fig. 1. ^{1}H NMR spectrum of deacetylated chitosan at 50 °C.

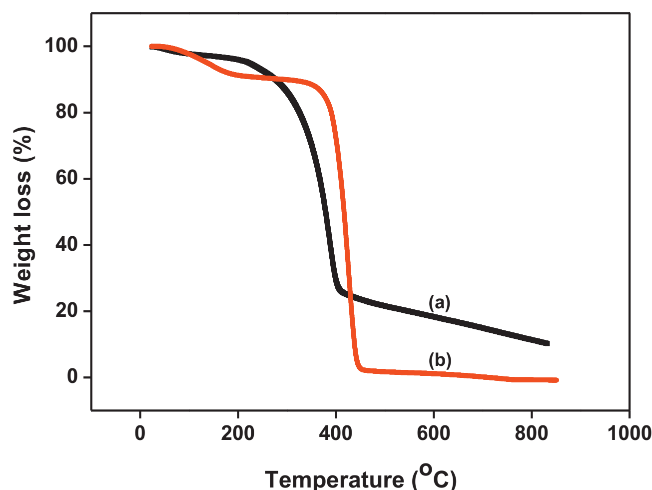


Fig. 3. TG curves of pure chitosan (a) and pure PNIPA (b).

thermal decomposition characteristics. As shown in Fig. 3, pure chitosan and PNIPA have quite different thermal behaviors. In TG curve of the chitosan (Fig. 3a), while the first step, corresponding to 5% weight-loss within 80–150 °C, is due to the elimination of free and bounded waters present in chitosan, the second weight-loss is due to the decomposition of cyclic moiety, which starts at 220 °C and ends at 400 °C. As for the thermal decomposition of PNIPA (Fig. 3b), the weight-loss starts and ends at much higher temperatures compare with that of chitosan, i.e. between 360 and 435 °C. On the other hand, three weight-loss steps were observed for HG-4 (Fig. 4). As the first step between 80 and 200 °C is due to the elimination of bonded and free waters in hydrophilic structure of the hydrogel, the second weight-loss between 220 and 320 °C is very similar to the second step weight-loss of pure chitosan. The third and the main weight-loss, observed between 320 and 435 °C, is due to the thermal decomposition of the remaining portion of the chitosan and PNIPA. Each weight-loss can easily be observed from derivative TG (DTG) curve of the HG-4, in which a strong derivative peak at 407 °C (between 320 °C and 435 °C) quantitatively covers the main decomposition step of both chitosan and PNIPA components. This is a clear indication for the presence of both components in the hydrogel networks.

3.3. Swelling behaviors of the hydrogels

Hydrogel characteristics such as swelling ratio and swelling kinetics are particularly important in controlled drug delivery and for biomedical applications. In dual sensitive hydrogels, these parameters can be tuned according to the environmental signals (pH and temperature) and location of the delivery by controlling the content of the cross-linker and the stimuli sensitive components. Swelling degree and swelling rate of the chitosan-PNIPA hydrogels were examined at above and below of the corresponding critical phase transition values of chitosan (pH 4 and pH 8) and PNIPA (T = 30 °C and T = 40 °C). The swelling characteristics at pH 4 in acetate buffer solution are shown in Fig. 5.

Remarkable differences were noticed in the rate and swelling degree, depending on temperature and composition. The swelling degree at 30 °C, which is below the critical phase transition

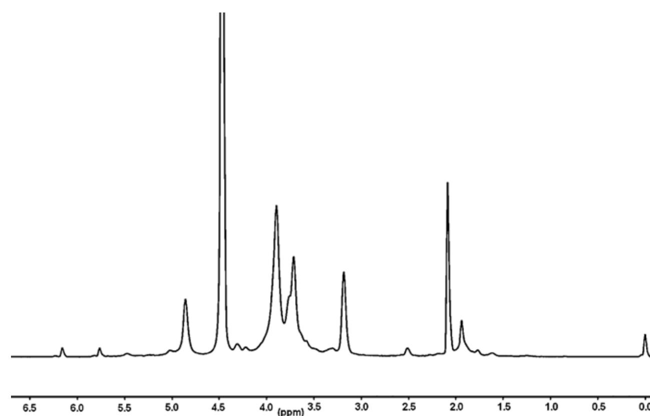


Fig. 2. ^{1}H NMR spectrum of methacrylated chitosan.

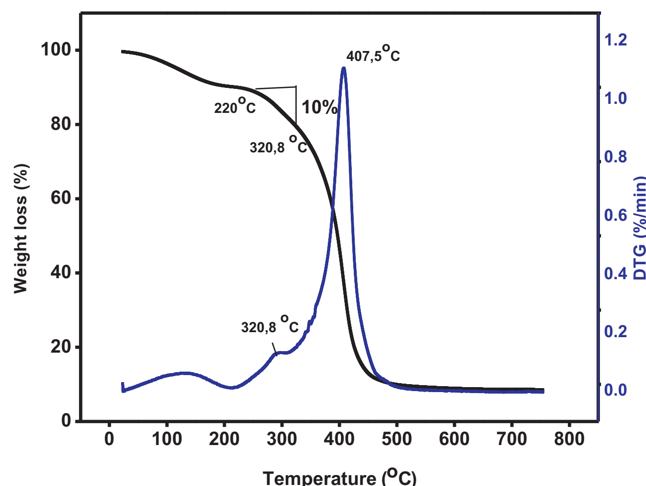


Fig. 4. TG and DTG curves of HG-4.

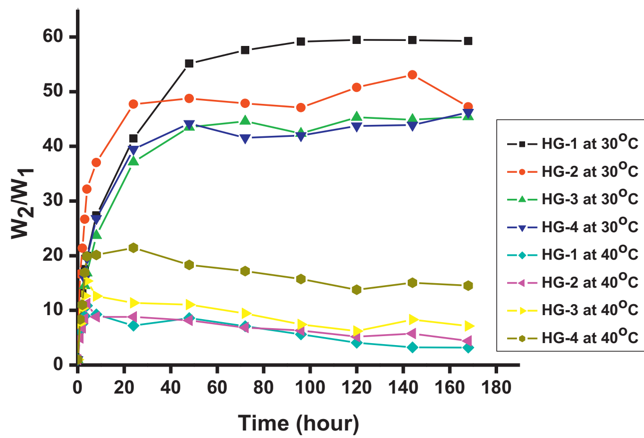


Fig. 5. Temperature and composition dependence of swelling behavior of hydrogels at pH 4.

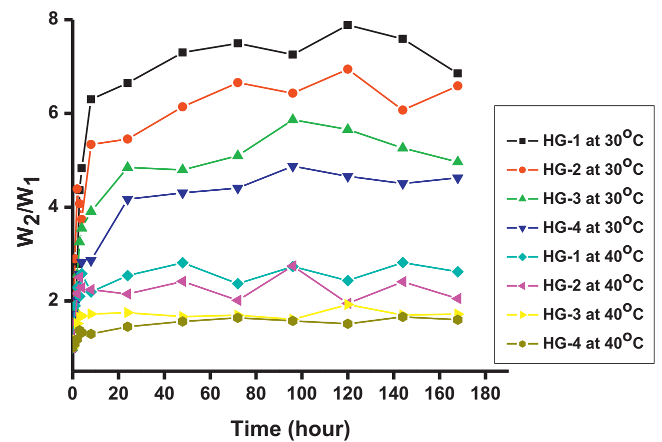


Fig. 6. Temperature and composition dependence of swelling behavior of hydrogels at pH 8.

temperature of PNIPA, was observed to be higher than that of at 40 °C. This is due to good solubility of chitosan and PNIPA at this pH and temperature. The swelling degree also depends on the amount of chitosan in hydrogels. Incorporation of partly methacrylated chitosan, which acts as a cross-linker, led to the increase in cross-link density, which resulted in decrease at swelling ratio. The results indicated that the swelling degree of the hydrogels could be controlled by the amount of chitosan incorporated into the hydrogels and are consistent with the previous findings of Cai et al. [22], where the swelling ratio was increased with the increasing content of the NIPA. On the other hand, at 40 °C, an opposite behavior was observed. Swelling ratio of the hydrogels decreased with the increasing content of chitosan, which is due to the volume change of the PNIPA at this temperature.

Similarly, swelling behaviors of the hydrogels were examined in phosphate buffer (pH 8) at 30 and 40 °C. As depicted in Fig. 6, equilibrium degree of swelling at 30 °C is notably higher than the swelling degree at 40 °C, and the degree of swelling decreases with increasing chitosan content, which is due to the lower solubility of chitosan at pH 8. It means that only PNIPA contributes to the swelling at 30 °C and pH 8. If the swelling behaviors of the hydrogels at pH 4 and pH 8 (Figs. 5 and 6, respectively) are compared, it is worth noting that, below their phase transition values, the highest equilibrium degree of swelling was observed with HG-1 (Q = 60). This is the result of the lowest methacrylated chitosan content, lowest cross-link density and contribution of both components (chitosan and PNIPA) to the swelling. Furthermore, the lowest degree of swelling was observed with HG-4 at 40 °C and pH 8 (Q is in the range of 1.5–2.5), which indicates that as the chitosan content increases in the hydrogels, cross-link density increases, resulting in a lower swelling degree. It could be due to partly protonation of the amino groups of chitosan, observed between pH 2.2 and 8.0 [23].

3.4. Phase transition of the hydrogels

DSC is an important technique for characterization of heat absorption or release behaviors of materials. The peak area and the temperature obtained by DSC are characteristic and quantitative values for phase transitions. PNIPA is a temperature sensitive component of the hydrogels, the hydrophilic N-isopropyl amine groups of which can make reversible hydrogen bonding with water. Over the phase transition temperature, i.e. body temperature, N-isopropyl groups become more energetic and lead to endothermic cleavage of hydrogen bonding, resulting in simultaneous phase separation and systemic delivery of 5-ASA in intestinal system. Thus, it could be important to determine sensitively the changes

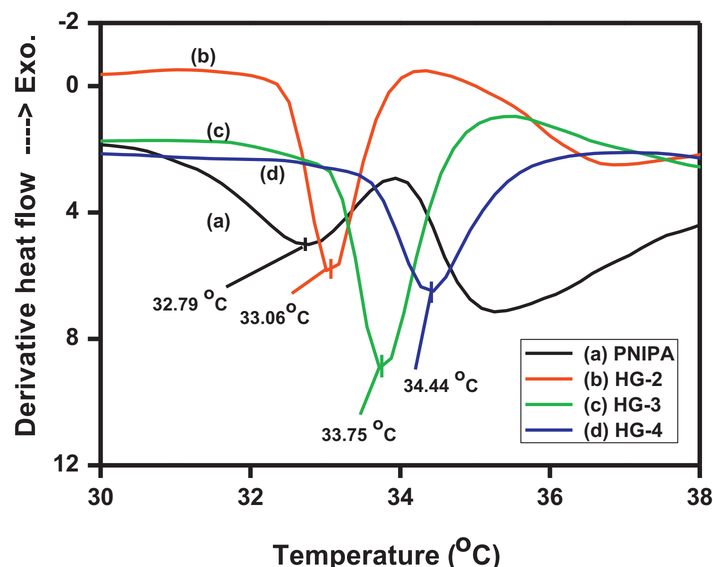


Fig. 7. DDSC curves of PNIPA and 5-ASA loaded hydrogels (HG-2, HG-3 and HG-4) in acetate buffer (pH 4.0).

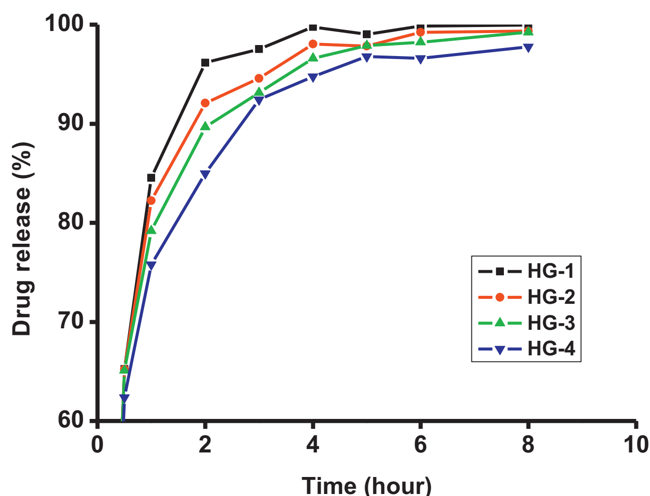


Fig. 8. Release profile of 5-ASA obtained by UV–Vis spectroscopy.

at phase transition temperatures of 5-ASA loaded hydrogels. Fig. 7 shows DSC curve of pure PNIPA and 5-ASA loaded PNIPA-chitosan hydrogels at pH 4. While the phase transition temperature of pure PNIPA was determined to be 32.73 °C, it increased from 33.07 °C to 34.44 °C with the increase of chitosan content in the hydrogels. This was attributed to the increasing interactions of the side chains of PNIPA with more protonated amino groups of chitosan in acidic media. These results are of critical for therapeutic effect of 5-ASA in intestinal system.

3.5. 5-ASA release from hydrogels

Release characteristics of 5-ASA from the hydrogels were determined, using UV–Vis and fluorescence spectroscopy techniques by mimicking intestinal conditions (pH 8 and 37 °C). For each measurement, 1 ml of sample was analyzed for the release of 5-ASA. Samples were introduced back to the solution after each measurement to avoid any error, resulting from a volume change. In Fig. 8, the release profiles of 5-ASA from the hydrogels are shown as a function of time. Within 2 h, 5-ASA was released from HG-1 in nearly 90%. Considering the transit time in small intestine, which is roughly 3 h, 5-ASA is expected to be released largely in small intestine. The amount of chitosan, influencing the swelling degree and swelling rate of hydrogels, also affects the rate of 5-ASA release.

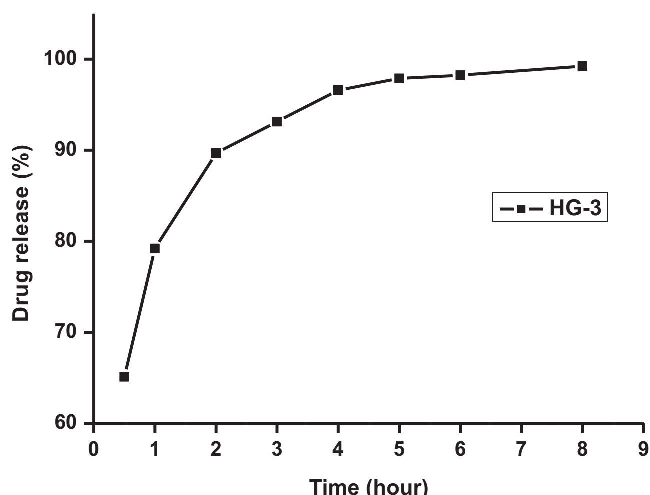


Fig. 9. Release profile of 5-ASA obtained by fluorescence spectroscopy.

As the chitosan content in hydrogels increased, the rate of 5-ASA release decreased, and while 5-ASA was largely released within 4 h from HG-1, its release from HG-4 was completed within 6 h, which is due to the high sensitivity of PNIPA to temperature.

The release from HG-3 was followed by fluorescence spectroscopy. Initially, a calibration curve was prepared and the amount of released 5-ASA was determined. 5-ASA had a strong fluorescence emission peak at 500 nm on excitation at 335 nm and increasing release of 5-ASA at pH 8 caused a remarkable increase in fluorescence peak intensity. The release profile of 5-ASA, obtained from fluorescence measurements is plotted in Fig. 9. Compatible results were observed with fluorescence and UV measurements.

4. Conclusion

In conclusion, controlled release of 5-ASA from chitosan/PNIPA hydrogels has been studied. Swelling and release experiments indicated that the rate of 5-ASA release, which is quite important for the treatment of ulcerative colit and Crohn's diseases, can be tuned by controlling the chitosan/PNIPA ratio in hydrogels. It has been demonstrated that the increase of chitosan content in hydrogels reduces the rate of 5-ASA release. It was released in high extent within 4 h from the hydrogel, containing 15% chitosan (HG-1). Considering the gastrointestinal time, these results are evidence of the completed release of 5-ASA, before reaching the hydrogels to large intestine. It was observed that chitosan has an effect to increase the phase transition temperature of hydrogels, varying from 32.79 °C to 33.44 °C. The anomeric proton of chitosan shifted to further downfield and become distinctive when ^{1}H spectrum was recorded at 50 °C. This result provided a quantitative characterization of chitosan modification by NMR spectroscopy. As 5-ASA is a fluorescence active molecule, its release was also followed by fluorescence spectroscopy, which gave more sensitive results.

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