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Preparation and swelling behavior of carrageenan-graft-polymethacrylamide superabsorbent hydrogel as a releasing drug system

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Abstract

The polysaccharide, kappa-carrageenan (κC) was chemically modified to achieve a novel superabsorbent hydrogel via graft copolymerization of acrylamide (AAm) onto the substrate followed by alkaline hydrolysis. Ammonium persulfate (APS) and N,N'-methylene bisacrylamide (MBA) were used as a free-radical initiator and a crosslinker, respectively. The saponification reaction was carried out using sodium hydroxide aqueous solution. Either κC-g-PAAm or hydrolyzed κC-g-PAcA was characterized by FTIR spectroscopy. The effect of media variables such as alkaline hydrolysis conditions (i.e., NaOH concentration, hydrolysis time and temperature) were systematically optimized to achieve a hydrogel with swelling capacity as high as possible. Absorbency of superabsorbing hydrogels was examined in buffer solutions with pH ranged 1-13. Also, the pH reversibility and on-off switching behavior, at pH 2.0 and 9.0, makes the synthesized hydrogels as a good candidate for controlled delivery of bioactive agents. Finally, the κC-g-PAcA hydrogel exhibited a pH-responsiveness character so that a swelling-deswelling pulsatile behavior was recorded at pH 1.2 and 7.4. In addition, the delivery behavior of Acetaminophen from the hydrogel bead was studied.

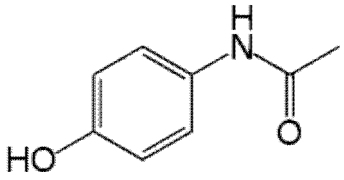
Keywords: Carrageenan; hydrogel; superabsorbent; pH-sensitive; Acetaminophen.

Introduction

Hydrogels, chemically or physically crosslinked three-dimensional networks composed of hydrophilic polymers, can absorb and retain large amounts of aqueous fluids, and the absorbed water is hardly removable even under some pressure. Due to excellent properties compared with traditional water absorbing materials, hydrogels have drawn much attention in a wide variety of fields such as drug delivery system, wound dressings, gel actuators, artificial organs, medical pharmaceuticals and contact lenses (Buchholz & Graham 1997; Chu, 2007; Cheng, 2008).

Carrageenans as the natural polymer are relatively new polysaccharides to synthesize of natural-based superabsorbent polymers. These biopolymers are linear sulfate polysaccharides that are obtained from certain species of red seaweeds (Kakinoki, 2007). The presence of hydrophilic sulfate groups with high ionization tendency and less sensitivity to salt solution was our main idea for synthesis of carrageenan-based superabsorbent hydrogels. Swelling of such hydrogels in the stomach is minimal and thus the drug release is also minimal. Due to increase in pH, the swelling degree increases as the hydrogels pass down the intestinal tract. A variety of synthetic or natural polymers with acidic or basic pendant groups have been employed to fabricate pH-sensitive hydrogels for getting the desired controlled release of drugs (Sadeghi, 2011). The use of natural polymers in the design of pH sensitive hydrogels has received much attention due to their excellent biocompatibility and so,the aim of this work was not only to characterize new type of superabsorbents, but also to evaluate the usefulness and feasibility of these polysaccharide-based superabsorbents for orally administered drug delivery system (DDS).

Fig.1. Chemical structure of drug Acetaminophen.



Experimental

Materials

The polysaccharide, kappa-carrageenan (κC, from Condinson Co., Denmark); N,N'-methylene bisacrylamide (MBA, from Fluka), ammonium persulfate (APS, from Fluka), acrylamide (AAm, from Merck), were of analytical grade and were used as received. The drug, Acetaminophen, was obtained from Jaberebne Hayan Pharmaceutical Co. (Tehran, Iran). The chemical structure of Acetaminophen is shown in Fig. 1.

Graft copolymerization

The graft copolymerization reactions were carried out using APS as an initiator and MBA as a crosslinker in an aqueous solution. A general procedure was conducted as follows: κC (0.50 g) was dissolved in 50 mL degassed distillated water in a three-neck reactor equipped with mechanical stirrer (Heidolph RZR 2021, three blade propeller type, 600 rpm). The reactor was placed in a water bath preset at 80 °C. After complete dissolution of the polysaccharide to form a homogeneous solution, a definite amount of APS solution (0.10-0.5 g in 5 mL H_2O) was added into the mixture and was allowed to stir for 15 min. Then certain amounts of AAm (0.50-3.50 g in 5 mL H_2O) and MBA (0.05-0.50 g in 5 mL H_2O) were simultaneously added to the reaction mixture. After 60 min, the reaction product was allowed to cool to ambient temperature. The

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obtained hydrogel was then poured into methanol (500 ml). After complete dewatering for 24 h, the hardened gel particles product were filtered, washed with fresh methanol (2 × 50 mL) and dried at 50 °C. After grinding, the powdered superabsorbent hydrogel was stored away from moisture, heat and light.

Alkaline hydrolysis

The graft copolymer (0.50 g) was saponified using 20.0 mL of NaOH (0.5-3.5 M) in a two-neck flask fitted with a mechanical stirrer and a reflux condenser. The hydrolysis temperatures of 60-100 ºC; and hydrolysis times of 30-180 min were studied. The mixture was allowed to cool to room temperature and neutralized to pH 8.0 by addition of 10 %wt aqueous acetic acid solution. Then the product was poured into methanol (200 mL) to dewater for 10 h. The hardened particles were filtered off, dried in oven (50 ^{o}C, 5 h), and kept in a dry and cool place.

pH-sensitivity

pH-sensitivity of the hydrogel was investigated in terms of swelling and deswelling of the final product at two buffered solutions (pHs 2.0 and 9.0). Swelling capacity of the hydrogels at each pH was measured according to Eq. 1 at consecutive time intervals (30 min).

$$SwellingRatio(g/g) = \frac{Weight\ of\ swollen\ gel - Weight\ of\ dried\ gel}{Weight\ of\ dried\ gel} \quad (1)$$

Standard absorbance curve

The standard calibration curve of the absorbance as a function of drug concentration was studied at 256 nm on the UV spectrophotometer.

In vitro drug release

The samples (0.1±0.0001 g) were immersed into 50 mL of the release medium (simulated gastric and intestinal fluids, SGF and SIF) with different pH values (pH 1.2 or 7.4) at 37^{o}C with agitation. At given time intervals, 1 mL of the release medium was removed; using a syringe attached with a 0.45 μm Millipore filter and after suitable dilution, the concentration of released drug was measured by UV spectrophotometer at 210 nm.

Infrared Spectroscopy

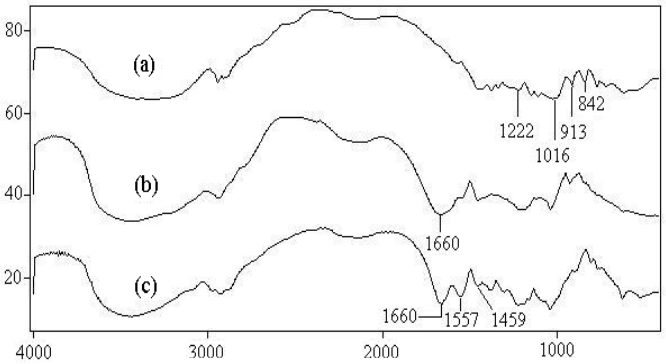
The samples were powdered and mixed with KBr to make pellets. Spectra were taken using an ABB Bomem MB-100 FTIR spectrophotometer.

Results and discussion

Synthesis and spectral characterization

Scheme 1 shows a simple mechanism for crosslinking graft copolymerization of AAm onto κC backbones and alkaline hydrolysis of the resulted graft copolymer. At the first step, the thermally dissociating initiator, i.e. APS, is decomposed under heating (65 ^{o}C) to produce sulfate anion-radical. Then the anion-radical abstracts hydrogen from the hydroxyl group of the κC substrate to form corresponding alkoxy radicals. So, these macroradicals initiate AAm grafting onto κC backbones led to a graft copolymer so called κC-g-PAAm. In addition, crosslinking reaction was occurred in the presence of a crosslinker, i.e., MBA. The κC-g-PAAm was then hydrolyzed with NaOH solution to produce a super swelling hydrogel, H-κC-g-PAAm-co-AcA. During the partially saponification, NH_3 gas was evolved and some of the 'non-ionic' carboxamide groups are converted to 'ionic' carboxylate salt.

Fig. 2. FTIR spectra of κC (a), κC-g-PAAm (b), and H-κC-g-PAAm-co-AcA (c).



Infrared spectroscopy was carried out to confirm the chemical structure of the hydrogel. Fig. 2 shows the FTIR spectra of κC, κC-g-PAAm, and H-κCg-PAAm-co-AcA. The bands observed at 842, 913, 1016, 1222, and 3200-3400 cm^{-1} can be attributed to D-galactose-4-sulfate, 3,6-anhydro-D-galactose, glycosidic linkage, ester sulfate stretching, and stretching of -OH groups of non-modified κC, respectively (Fig. 2a). The graft copolymer, κC-g-PAAm, comprise a κC backbone with side chains that carry carboxamide functional groups that are evidenced by a new peak at 1660 cm^{-1} (Fig. 2b). This peak attributed to C=O stretching in carboxamide functional groups of PAAm. The stretching band of -NH overlapped with the -OH stretching band of the κC portion of the copolymer. After alkaline hydrolysis, the new absorption modes at 1459 and 1557 cm^{-1} can be attributed to symmetric and asymmetric stretching modes of carboxylate groups, respectively (Fig. 2c) (Siepmann & Peppas, 2001). As shown in Fig. 2c and Scheme 1, after partially alkaline

Fig.3. Influence of NaOH concentration on swelling capacity

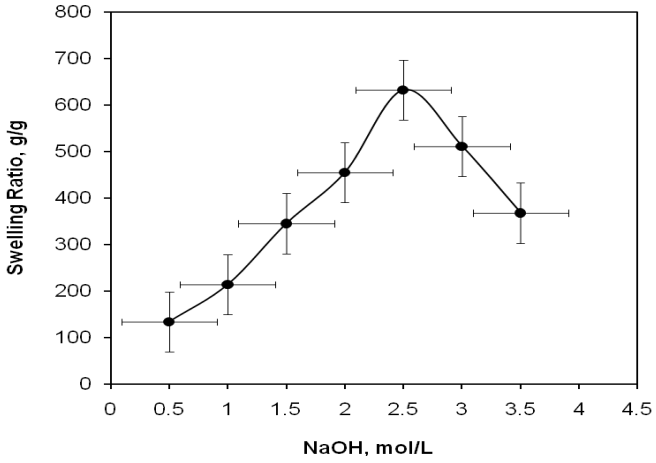


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Fig. 4. Effect of hydrolysis temperature on swelling capacity of H--κC-g-PAAm-co-AcA superabsorbent hydrogel.

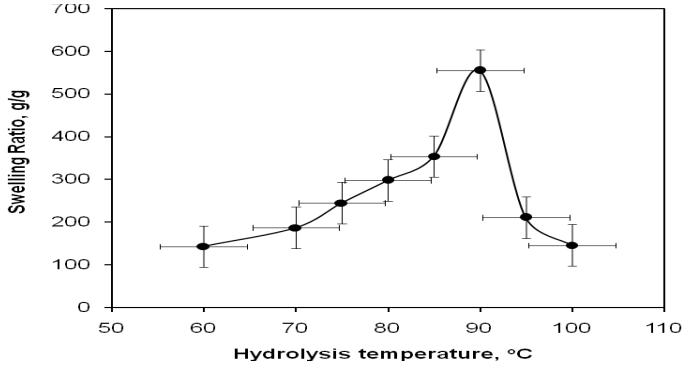


Fig.5. Effect of hydrolysis time on swelling capacity of H--κC-g-PAAm-co-AcA superabsorbent hydrogel.

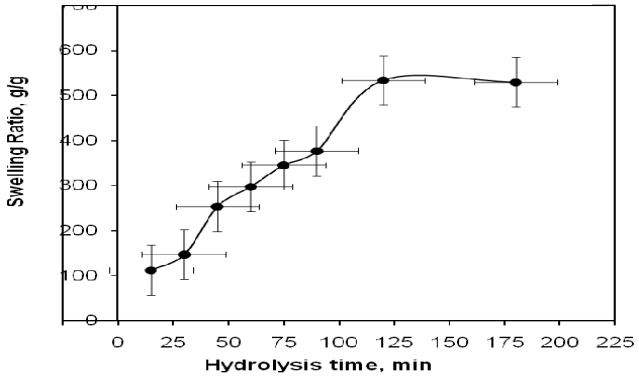
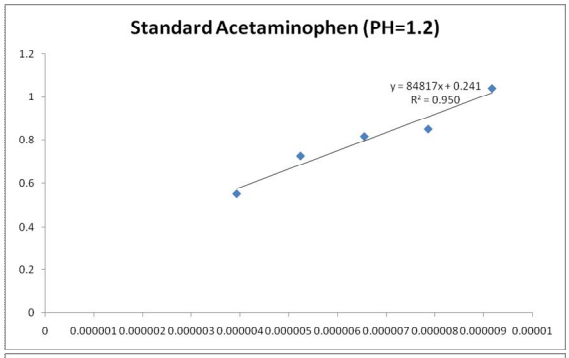


Fig. 6. The standard calibration curve of the absorbance as a function of Acetaminophen concentration at 256 nm on the UV spectrophotometer at pH 1.2 (a) and pH 7.4 (b).



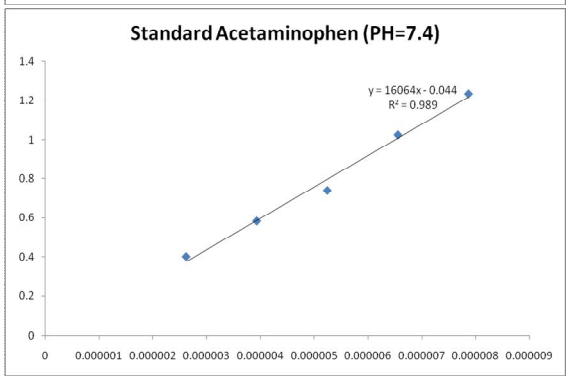
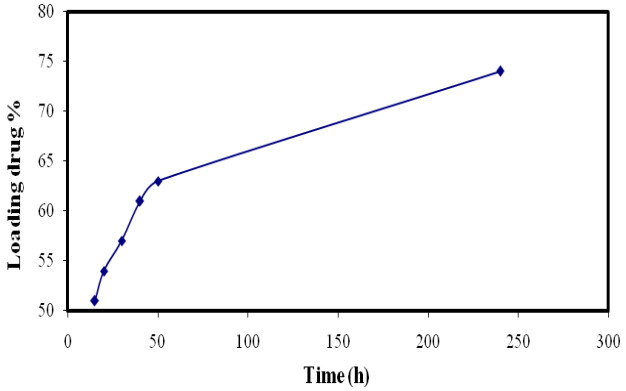


Fig. 7. The dependency of the drug loading amount to the loading time in pH=1.2 and 7.4.



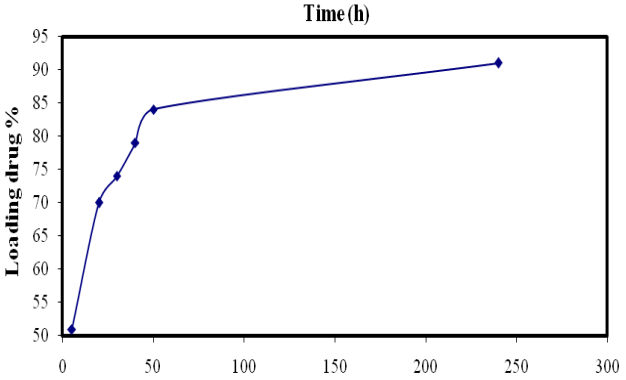


Fig. 8. Effect of pH of buffered solution on swelling of H--κC-g-PAAm-co-AcA superabsorbent hydrogel.

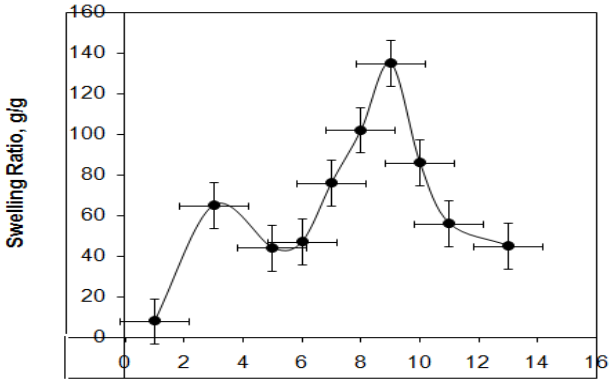
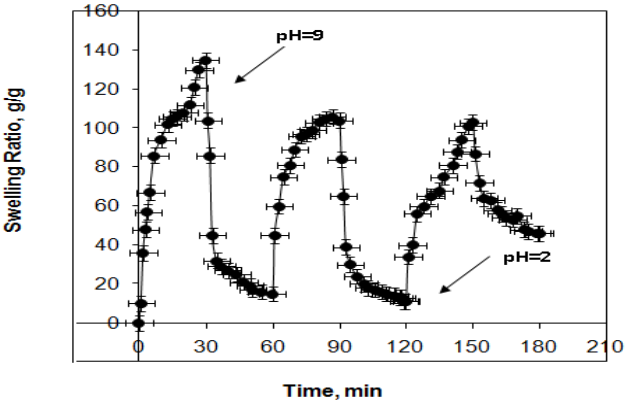


Fig. 9. On-off switching behavior as reversible pulsatile swelling (pH 9.0) and deswelling (pH 2.0) of the H-κC-g-PAAm-co-AcA hydrogel.



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hydrolysis of the κC-g-PAmM, some of the amide groups are converted to carboxylate anions.

Optimization of the alkaline hydrolysis conditions

In this work, optimization of the alkaline variables (i.e. concentration of NaOH and hydrolysis time and temperature) as well as the swelling behavior of the resulted pH-responsive superabsorbent hydrogels were investigated.

Effect of NaOH concentration

The alkaline hydrolysis of the graft copolymer, κC-g-PAmM, was carried out using different concentration of NaOH (0.5-3.5 N). As shown in Fig.3, swelling capacity is increased with increasing the NaOH concentration from 0.5 to 1.5 N and then it is considerably decreased with a further increase in the concentration of hydrolyzing NaOH. Initial increase in swelling values is due to enhancement of the repulsive action of the increasing carboxylate anions. Swelling decrease after the maximum can be attributed to residual alkaline, after completion of alkaline hydrolysis. The excess cations shield the carboxylate anions, as well as the sulfate anions of the polysaccharide backbone, and prevent effective anion-anion repulsion (screening/shielding effect). Additionally, alkaline degradation of the polysaccharide backbone can be another reason of the swelling decrease in highly concentrated alkaline hydrolytic media. The proposed mechanism for this alkaline degradation is reported in the previous work (Kim, 2006). Similar alkaline degradation behaviors were already reported in the case of other polysaccharides (Sadeghi & Yarahmadi, 2011).

Effect of hydrolysis temperature: The relationship between the hydrolysis temperature of κC-g-PAAm and swelling capacity of H-κC-g-PAAm-co-AcA was studied (Fig. 4). By increasing the hydrolysis temperature up to 85 ^{o}C the kinetics of alkaline hydrolysis increased which, in turn, result in carboxylate anions increment and consequently absorbency enhancement. Thereafter, decreasing the swelling capacity may be attributed to alkaline degradation of the κC part of the hydrogel.

Effect of hydrolysis time: In this series of experiments, swelling capacity of the final hydrogel, H-κC-g-PAAm-co-AcA, was studied as a function of the hydrolysis time of κC-g-PAAm (Fig. 4). It is observed that the absorbency is increased substantially with increasing the hydrolysis time up to 90 min and then it is gradually decreased. The initial increasing in swelling value can be attributed to increase carboxylate-to-carboxamide ratio values. Intensive electrostatic repulsion of the anions leads to higher swelling of the hydrogel. The swelling-loss after the optimum time (90 min) may be attributed to degradation of the κC part of the hydrogel under relatively alkaline conditions (1.5 M NaOH, 85 ^{o}C).

Standard calibration curve: The calibration curve of the absorbance as a function of the Acetaminophen concentration at 256 nm, shown in Fig. 6, has a linear relationship with a correlation coefficient (r) of 0.950 and 0.989 at pHs 1.2 and 7.4, respectively.

Drug loading: The amounts of the loaded drug in superabsorbent hydrogels was also significantly affected by the impregnation times (Fig. 7). It is obvious that with increasing the loading time, the amount of drug loaded is initially increased and then begins to level off(in both pH=1.2 and 7.4). The initial increment in the amounts of the loaded drug with increasing the loading time can be ascribed to the increased drug diffusion into the swollen matrix. The most efficient time of loading efficiency in pH=7.4 was 240 h, where a major amount of drug was encapsulated.

Effect of pH on equilibrium swelling: Since the H-κC-g-PAAm-co-AcA hydrogel comprise anionic sulfate and carboxylate groups, they exhibit sharp swelling changes at a wide range of pHs. Therefore, the equilibrium swelling of H-κC-g-PAAm-co-AcA hydrogel was measured at various buffer solutions with pH ranged from 1 to 13 (Fig. 8). Under acidic pHs, most of the carboxylate anions are protonated, so the main anion-anion repulsive forces are eliminated and consequently swelling values are decreased. At higher pHs (5-9), some of carboxylate groups are ionized and the electrostatic repulsion between COO^- groups causes an enhancement of the swelling capacity. Again, a charge screening effect of the counter ions (cations) limit the swelling at higher basic pHs (pHs>9).

Investigation of pH-responsiveness behavior of the hydrogel **:** In this series of experiments, swelling reversibility for the synthesized hydrogels was measured in the solutions with two different pHs 1.2 and 7.4 (Fig. 9). Since the swelling capacity of all “anionic” hydrogels is appreciably decreased by the addition of counter ions (cations) to the swelling medium, no buffer solutions were used. Therefore, stock NaOH (pH 10.0) and HCl (pH 1.0) solutions were diluted with distilled water to reach desired basic (7.4) and acidic (1.2) pH, respectively.

The figure 9 shows a stepwise reproducible swelling change of the hydrogel at 25^{o}C with alternating pH between 1.2 and 7.4. At pH 7.4, the hydrogel swells up to 135 g/g due to anion-anion repulsive electrostatic forces, while, at pH 1.2, it shrinks within a few minutes due to protonation of carboxylate groups. This sharp swelling-deswelling behavior of the hydrogels makes them suitable candidates for controlled drug delivery systems.

In vitro Acetaminophen Release in the Simulated Human Gastrointestinal System: To determine the potential application of starch-based superabsorbent containing a

Table 1. The percent of released Acetaminophen from the polymeric carriers as a function of pH

pH	1.2	2	3	4	5	6	7	7.4	9	10	11	12
Concentration (10^{-4} mol/L)	0.15	2.25	3.44	3.41	3.44	3.94	4.93	5.12	5.09	4.87	3.33	2.52
Percent released	7%	13%	48%	47%	48%	57%	65%	69%	68%	64%	42%	22%

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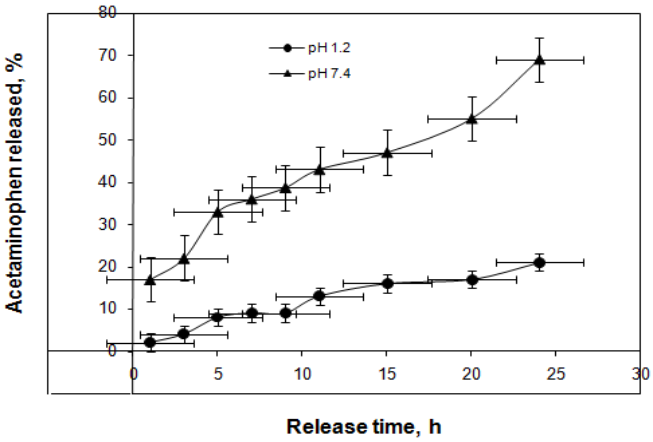
pharmaceutically active compound, we have investigated the drug release behavior Acetaminophen form this system under physiological conditions. The percent of released drug from the polymeric carriers as a function of pH is shown in Table 1. The concentration of Acetaminophen released at selected pH intervals was determined by UV spectrophotometer. The Acetaminophen-loaded hydrogels with high degrees of drug loading (>91%) were prepared by the swelling-diffusion method. The amount of Acetaminophen released in a specified time from the carrageenan-based hydrogel decreased as the pH of the dissolution medium was lowered, indicating better release in a medium with a pH much higher than that of the stomach.

At low pH values, electrostatic repulsion between the carboxylic acid groups of backbone is low, thus decreases gel swelling and minimizes release of Acetaminophen via diffusion. However, in alkaline media the presence of OH^- increases the electrostatic repulsion between carboxylate groups, thus increases the gels swelling degree and so the release of Acetaminophen was increased (Hamidi, 2008; Koo, 2009).

Fig.10. Release of Acetaminophen from hydrogel carrier as a function of time and pH at 37ºC.



The release rate experiments were also performed in SFG (pH 1.2) and SIF (pH 7.4) solutions at 37 ^oC (Fig.10). As can be seen from Table 1, when pH of the medium is 1.2, the cumulative release ratio of Acetaminophen from the test hydrogels is below 21% at the end of the experiment (24 h), whereas almost 47% of the loaded drug is released within 15 h in pH 7.4 medium. Again, these results indicate that the higher swelling ratios of the hydrogel create larger surface areas to diffuse the drug. In basic solutions (pH 7.4), the electrostatic repulsion between COO^- anions of grafted poly (sodium methacrylate) on the hydrogel accelerates the release of Acetaminophen from the hydrogel.

Conclusion

In this work, we prepared a novel superabsorbent hydrogel, H-κC-g-PAAm-co-AcA, by graft copolymerization of AAm onto κC backbones followed by alkaline hydrolysis of the κC-g-PAAm graft copolymer. So, swelling capacity of the hydrogels was recognized to affect by the alkaline hydrolysis variables. Also, the superabsorbent hydrogels exhibited high swelling capacity at basic pH as well as reversible pH-responsiveness property. Therefore, this natural-based superabsorbent intelligently responding to pH may be considered as an excellent candidate to design novel drug delivery systems. The release value of Acetaminophen from hydrogels at pH 7.4 was higher than that at pH 1.2 due to the electrostatic repulsion between carboxylate groups. Overall, the hydrogels presented in this study may serve as a platform for a wide range of pharmaceutical uses to improve the bioavailability of non-steroidal anti inflammatory drugs.

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