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Preparation of pH-sensitive carrageenan-*g*-poly (acrylic acid)/bentonite composite hydrogel for 5-fluorouracil controlled release in colon

Hossein Hosseinzadeh

Department of Chemistry, University of Payame Noor, West Azerbaijan, Miandoab, Iran.

ABSTRACT

A series of pH-sensitive composite hydrogels, carrageenan-*g*-poly (acrylic acid)/bentonite was prepared for colonic delivery of 5-fluorouracil (5-FU) as the model drug. The structure and surface morphology of the composite hydrogels were characterized by FTIR and SEM, respectively. The swelling and pH-sensitive properties of the hydrogels were investigated, and the drug loading and controlled release properties of the hydrogels were also evaluated. Results indicate that the swelling of the developed hydrogels was influenced by pH of the test medium and bentonite content. The results also showed that the composite hydrogels had good pH-sensitivity. The cumulative release ratios of 5-FU from the hydrogels were 35% in pH 1.2 solution within 24 h and 88% in pH 7.4 solution within 2 h, respectively. The release rate of the drug from the composite hydrogels is remarkably slowed down, which indicated that incorporating bentonite into the composite can improve the burst release effect of the drug. Overall, the drug delivery results demonstrated that this hydrogel could successfully deliver a drug to the colon without losing the drug in the stomach, and could be a good candidate as an orally administrated drug delivery system.

Keywords: Carrageenan, Hydrogel, Acrylic Acid, 5-Fluorouracil, Controlled Release.

**Corresponding author*

INTRODUCTION

Drug delivery systems (DDS) recently received great interests because they realized the effective and targeted delivery of drug and minimized the side effects resulting from the traditional drug-dosage form in the pharmaceutical field. Hydrogels are cross-linked hydrophilic polymers absorbing large quantities of water or aqueous solutions without dissolving. Because of softness, smartness and the capacity to store water, hydrogels are a unique carrier matrix for DDS [1].

Utilization of natural polymers for the design of DDS has long been the subject of great interest during the past decades due to their excellent properties such as biocompatibility, biodegradability and long-term safety of drugs [2–4]. However, such polymers are known to have some shortcomings when used as drug carriers: (1) a high burst effect due to the accumulation of hydrophilic drugs near the surface of the carriers; (2) causing undesirable drug degradation because an acidic environment is formed inside the carriers during hydration and degradation of the polymers [5–8]. Additionally, the higher production cost and low gel strength of these superabsorbents restrict their application widely. To improve these limitations, inorganic compounds with low cost can be used. The introduction of inorganic fillers to a polymer matrix increases its strength and stiffness properties. Among inorganic compounds, special attention has been paid to clay minerals in the field of nanocomposites because of their small particle size and intercalation properties. Mineral powders are hydrated layered aluminosilicate with reactive -OH groups on the surface. The interaction of mineral powders, reactive site of natural polymers and monomers results in a superabsorbent composite.

Recently, many polymer/clay composite hydrogels are found proper to be used as drug delivery carriers because they frequently exhibit remarkably improved properties compared with the pure polymer hydrogels owing to the synergistic effects of polymer and clay [9-11]. Also, the introduction of clay can decrease the swelling ratio of polymer, reduce the burst effect, increase the stability of drug and provide a slower and more continuous release mode of drugs [12, 13].

The properties of the swelling medium (e.g. pH and ionic strength) affect the swelling characteristics. SAPs responding to external stimuli such as heat, pH, electric field, and chemical environments are often referred to as "intelligent" or "smart" polymers. Among these, pH-sensitive hydrogels have been extensively investigated for potential use in site-specific delivery of drugs to specific regions of the gastrointestinal tract and have been prepared for delivery of low molecular weight drugs. Therefore, these hydrogels have important applications in the field of medicine, pharmacy, and biotechnology [14, 15].

Carrageenan is a collective term for linear sulfated polysaccharides that are obtained commercially by alkaline extraction of certain species of red seaweeds [16]. Schematic diagram of the idealized structure of the repeat units for the most well-known and most important type of carrageenan family, kappa-carrageenan (κ C), is shown in Fig. 1.

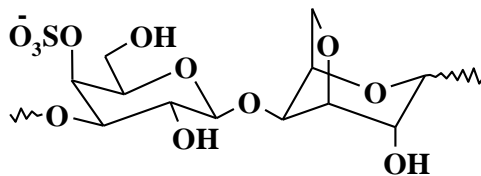


Figure 1. Repeating disaccharide units of kappa-carrageenan (κ C).

In the present study, swelling properties and pH-sensitivity of the composite hydrogels were investigated. Their drug loading and controlled delivery behaviors were also systematically evaluated using 5-fluorouracil (Fig. 2), as a model drug. 5-Fluorouracil is one of the most widely used anti-cancer chemotherapeutic drugs. It is used for colon and rectal cancer, breast cancer, gastrointestinal cancers, head and neck cancer and ovarian cancer.

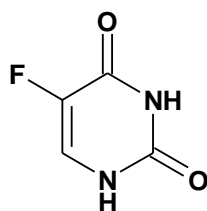


Figure 2. Chemical structure of 5-FU.

EXPERIMENTAL

Materials

The polysaccharide, kappa-carrageenan (κ C, MW=100,000, from Condinson Co., Denmark), N,N'-methylene bisacrylamide (MBA, from Merck), ammonium persulfate (APS, from Fluka) and bentonite (from Aldrich, particle size $5\mu\text{m}$) were used as received. Acrylic acid (AA from Merck) as ionic monomer was used after vacuum distillation for removing inhibitor. 5-Fluorouracil was obtained from Jaberebne Hayan Pharmaceutical Co. (Tehran, Iran). The simulated gastric fluid (SGF, pH 1.2) composed of 21.25 mL HCl, 11.18 g KCl and 1000 mL distilled water and the simulated intestinal fluid (SIF, phosphate buffer solutions, PBS, pH 7.4) composed of 3.6 g KH_2PO_4 , 4.8 g Na_2HPO_4 and 1000 mL distilled water were prepared as described in US Pharmacopoeia 30.

Preparation of composite hydrogels

A pre-weighed amount of κ C (0.30-0.70 g) was added to 40 mL degassed distilled water in a 1-L reactor equipped with a mechanical stirrer (RZR 2021, a three-blade propeller type, Heidolph, Schwabach, Germany) and stirred for 10 min. The reactor was placed in a thermostated water bath to control the reaction temperature at 80 °C. After complete dissolution of κ C, various amounts of bentonite powder (0.70-0.30 g) were added to the κ C solution and allowed to stir for 10 min. Then, APS initiator (0.05-0.50 g, dissolved in 5 mL water)

was added to the reaction mixture and the mixture was stirred for 10 min. MBA (0.05-0.25 g, dissolved in 5 mL water) and AA (1.0-5.0 g, completely neutralized with NaOH) were poured into the reactor. All of the reactions were carried out at 80 °C under an argon gas atmosphere. At the end of the propagation reaction, the gelly product was poured in ethanol (300 ml) and allowed to dewater for 24 h. Then, the product was filtered and washed with 100 mL ethanol. The filtered product was dried in an oven at 50 °C for 10 h. After grinding, the powdered superabsorbent composite was stored away from moisture, heat and light.

pH-Sensitivity

Composite hydrogel (0.25 g) were immersed in 250 mL solution with various pH values (pH 1.2 and pH 7.4) at 37 °C to reach swelling equilibrium. Swollen samples were then separated from unabsorbed water by filtering through a 100-mesh screen under gravity for 30 min without blotting the samples. The equilibrium swelling (ES) capacity in buffer solution was calculated according to the following equation:

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

Determination of encapsulation efficiency and drug loading

Composite hydrogel (0.10 g) was immersed in 10 mL of the phosphate buffer solution (pH 7.4) in a 50 mL beaker for completely swelling. The swollen hydrogels were crushed in an agate mortar with a pestle and transferred into a conical flask, and then about 20 mL of the fresh phosphate buffer solution was added to the conical flask and the homogeneous mixture was sonicated for 20 min. The 5-FU solution was separated from the mixture after being centrifuged for 20 min at 5000 rpm. The amount of 5-FU was determined using UV spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan). The drug loading (%) and encapsulation efficiency (%) were calculated using the following equations, respectively:

$$\text{Drug Loading (\%)} = \frac{\text{Weight of drug in hydrogel}}{\text{Weight of hydrogel}} \times 100 \quad (2)$$

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Weight of drug in hydrogel}}{\text{Theoretical drug loading}} \times 100 \quad (3)$$

In vitro drug release experiment

The release rate experiments were performed in SFG (pH 1.2), and SIF (pH 7.4) solutions at 37 °C under unstirred conditions. The hydrogel (0.1 g) containing a known amount of drug (5-FU) was added to the release medium (50 mL). At a given time interval, 1 mL filtered samples were withdrawn and assayed for the amount of released drug as a function of time. The amount of released drug was determined by UV spectroscopy at characteristic λ_{max} using a calibration curve constructed from a series of drug solutions with known concentrations. The

results were expressed as cumulative release ratio (amount of released drug/all amount of loaded drug).

Statistical analysis

All the tests including determination of drug content, swelling and *in vitro* drug cumulative release were carried out in triplicate and the averages were reported. Statistical data analysis was performed using the Student's *t*-test with $p < 0.05$ as the minimal level of significance.

Instrumental analysis

Fourier transform infrared (FTIR) spectra of samples were taken in KBr pellets, using an ABB Bomem MB-100 FTIR spectrophotometer (Quebec, Canada), at room temperature. The surface morphology of the gel was examined using scanning electron microscopy (SEM). After Soxhlet extraction with methanol for 24 h and drying in an oven, superabsorbent powder was coated with a thin layer of gold and imaged in a SEM instrument (Leo, 1455 VP).

RESULTS AND DISCUSSION

Synthesis and spectral characterization

The hydrogel composite was prepared by graft copolymerization of acrylic acid onto κ C in the presence of a crosslinking agent and powdery bentonite. Ammonium persulfate was used as an initiator. The persulfate is decomposed under heating and produced sulfate anion-radicals that abstract hydrogen from $-OH$ groups of κ C backbones. So, this persulfate-saccharide redox system results in active centers capable to radically initiate polymerization of acrylic acid led to a graft copolymer. Since a crosslinking agent, e.g. MBA, is presented in the system, the copolymer comprises a crosslinked structure.

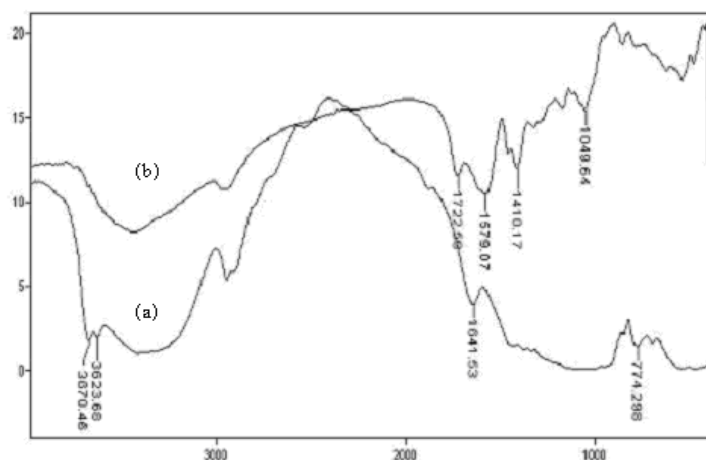
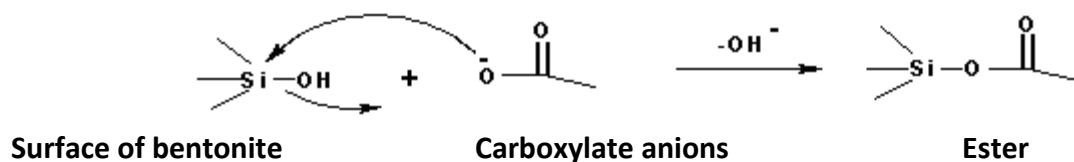


Figure 3. FTIR spectra of the physical mixture of κ C and bentonite (a) and κ C-g-PAANA/bentonite composite hydrogel.

FTIR spectroscopy was used for identification of the hydrogel. Figure 3a represents the spectrum of the physical mixture of κ C and bentonite. In the layer silicate structure of bentonite, the hydroxyl groups show absorption bands at $3630\text{-}3680\text{ cm}^{-1}$. The broad band at $3200\text{-}3400\text{ cm}^{-1}$ is due to stretching of -OH groups of the polysaccharide. In the spectrum of the composite (Figure 3b), two new absorption peaks at 1579 and 1722 cm^{-1} are appeared. The characteristic band at 1572 cm^{-1} is due to C=O asymmetric stretching in carboxylate anion that is reconfirmed by another peak at 1410 cm^{-1} which is related to the symmetric stretching mode of the carboxylate groups. The absorption band at 1722 cm^{-1} can be corresponding to the ester groups that can be formed during the graft polymerization reaction. The carboxylate groups of the grafted poly(acrylic acid) can be react with the -OH groups on the bentonite surface results in the ester formation. The reaction can be shown as follows:



One of the most important properties that must be considered is hydrogel microstructure morphologies. Figure 4 shows the scanning electron microscope images of the hydrogel. This picture verifies that the synthesized polymer in this work have a porous structure. It is supposed that these pores are the regions of water permeation and interaction sites of external stimuli with the hydrophilic groups of the graft copolymers.

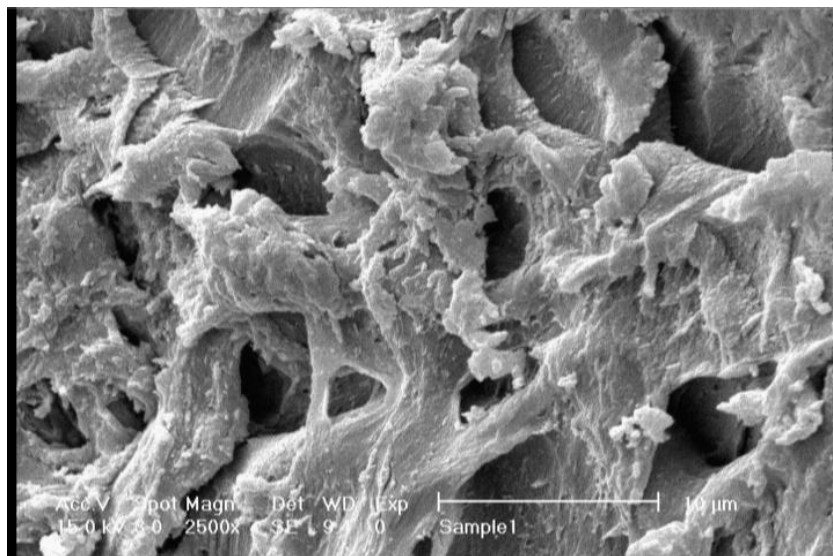


Figure 4. SEM photograph of the hydrogel. Surfaces were taken at a magnification of 2500, and the scale bar is 10 μm .

pH-Responsiveness behavior of composite hydrogel

It is known that the pH environment of the gastrointestinal tract varies from acidic in the stomach to slightly alkaline in the intestine. Thus, as an oral drug carrier, pH-sensitivity of polymer/clay hydrogels is a highly significant parameter controlling oral drug release behavior. Thus, pH-sensitivity of the composite hydrogels is studied as shown in Fig. 5. In order to simulate the possible effect of pH on drug release rate, a swelling study was conducted in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) at physiological temperature of 37 °C. At pH 7.4, the hydrogel swells due to anion-anion repulsive electrostatic forces, while at pH 1.2, it shrinks within a few minutes due to protonation of the carboxylate anions. This swelling-deswelling behavior of the hydrogels makes them as suitable candidate for designing drug delivery systems.

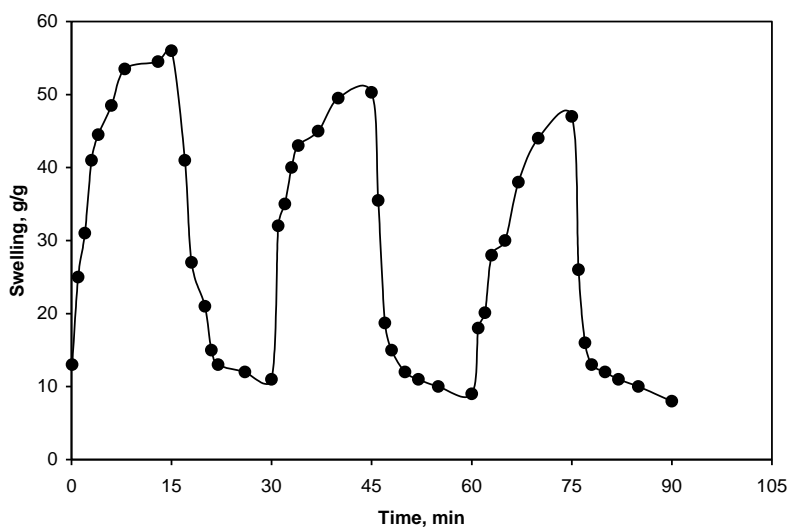


Figure 5. On-off switching behavior as reversible pulsatile swelling (pH 7.4) and deswelling (pH 1.2) of the κ C-g-poly(acrylic acid) composite hydrogel.

Measurement of the extinction coefficient of drug

The 5-FU drug was dissolved in water at five different concentrations. The UV absorption spectra of these solutions were measured in a 1.00 cm UV cell and plotted versus concentrations. The plot gave straight line that obey to the Beer–Lambert law ($A = \epsilon cl$, where A is the absorbance of the solution, ϵ is the extinction coefficient, l is the cell length and c is the drug concentration). According to these results, the extinction coefficient at $\lambda_{max} = 266$ is $1.25 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

Drug encapsulation efficiency and drug loading

In this series of experiments, the encapsulation efficiency and the drug loading of the composite hydrogels with different kaolin content were shown in Fig. 6. As can be seen, the

amount of drug loaded in the composite hydrogel beads decrease with increasing the content of kaolin. The decrease tendency may be attributed to the crosslinking nature of kaolin. Indeed, kaolin acts as crosslinking points in the network, which caused the increase of the crosslinking density of hydrogel with the increase of clay content. The greater the crosslinking density, the worse the elasticity of the polymer chains [17], which could restrict the penetration of 5-FU into composite hydrogel, and then leads to the decrease of the encapsulation efficiency and the loading amount for drug. Additionally, the excess kaolin acts as filler and prevents 5-FU entering the polymeric network with further increasing kaolin content, which is also responsible for the decrease of encapsulation efficiency and drug loading.

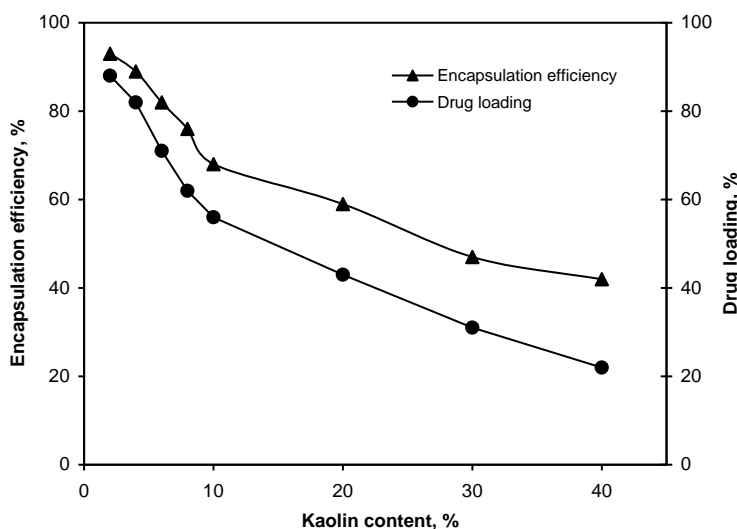


Figure 6. Effect of kaolin content on the encapsulation efficiency and drug loading of the composite hydrogel.

***In vitro* release behavior of hydrogels**

In order to determine the potential application of κC-based superabsorbent containing a pharmaceutically active compound, we have investigated the drug release behavior from this system under physiological conditions. The percent of released drug from the polymeric carriers as a function of time and pH of solution is shown in Figure 7. The concentration of 5-FU released at selected time intervals was determined by UV spectrophotometer. As can be seen from Fig. 5, when pH of the medium is 1.2, the cumulative release ratio of 5-FU from the test hydrogels is below 35% at the end of the experiment (24 h), whereas almost 90% of the loaded drug is released within 15 h in pH 7.4 medium. This difference of their swelling behavior is responsible for the difference of the drug release ratio with changing pH of the medium. Indeed, the drug in the hydrogel could be released as a result of the hydrogel volume change and interaction between the polymer network and drug. The fractional release is directly proportional to the swelling ratio of the hydrogels. This result indicates 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 acrylate) on the hydrogel accelerates the release of 5-FU from the hydrogel.

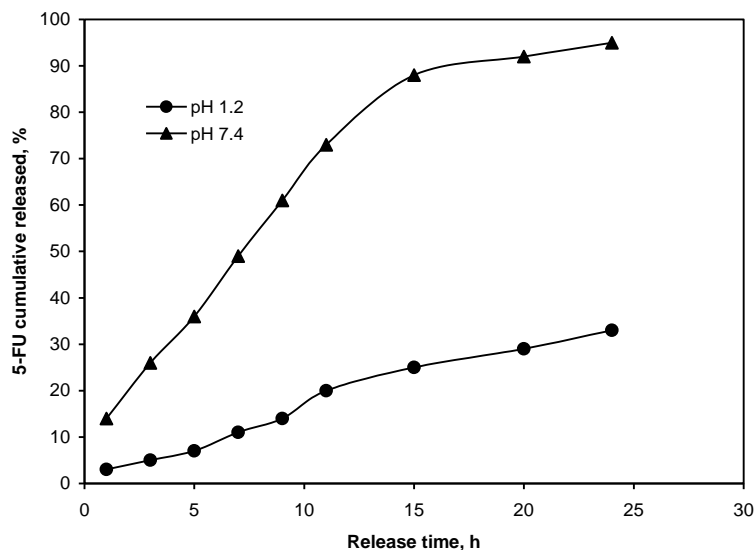


Figure 7. Release of 5-FU from hydrogel carrier as a function of time and pH at 37°C.

The amount of the released 5-FU from composite hydrogels was also significantly affected by the drug-loaded content (Figure 8). It was generally noticed that there is an immediate release of drug at the time of immersing the sample in the release medium. This might be due to the surface drug on the composite. This explanation is supported by the fact that this immediate release depends largely on the weight percent of the drug-loaded in the hydrogel. The higher the weight percent of the drug, the higher the percent of the drug immediately released. This immediate drug release is desirable from the practical point of view at the beginning of the applications which must be followed by a period of a controlled release of the drug.

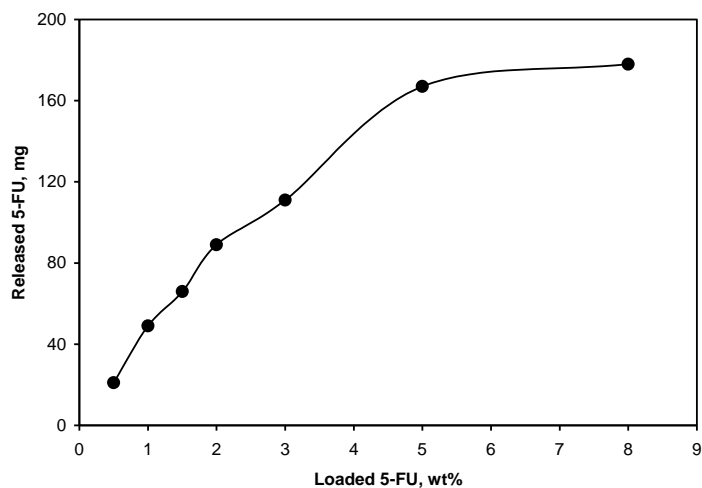


Figure 8. The dependency of the released drug amount to the 5-FU loaded content.

Figure 9 shows the kaolin-dependent cumulative release of the drug from the hydrogels in pH 7.4 buffer solution. As can be seen from Fig. 6, the release time of 5-FU from composite hydrogel prolongs with the increase of kaolin content. This may be attributed to the role of kaolin playing in composite hydrogels. As has been reported by Sorby and Wang, kaolin could be used as vehicles for the slow-release of promazine and diclofenac sodium [18-20]. Thus, the 5-FU adsorbed on kaolin need to migrate out of the composite hydrogels through a longer path, and then the improved release behavior was observed.

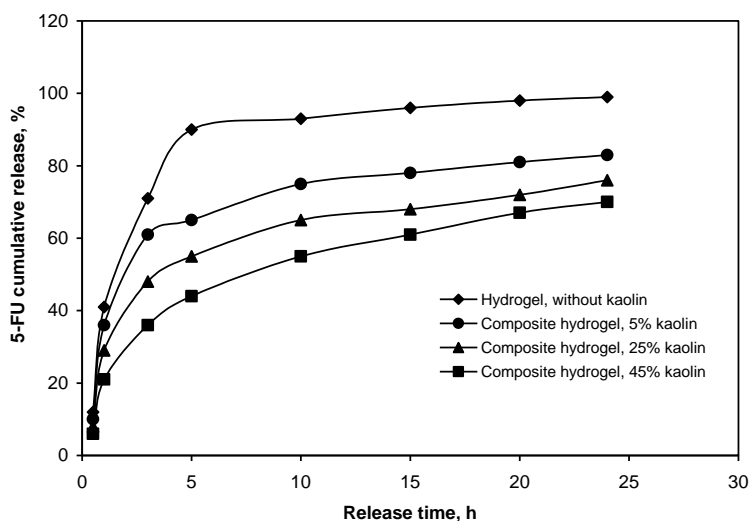


Figure 9. *In vitro* cumulative release of 5-FU from the composite hydrogel with different kaolin content at pH 7.4 and 37°C.

CONCLUSION

In the present work, we have developed a new drug delivery system based on kappa-carrageenan for controlled 5-FU release. The kaolin was used as an inorganic crosslinking agent to reduce the burst effect and improve the efficiency of the drug. The controlled 5-FU release experiments of composite hydrogels demonstrated that the major factors that influence the drug-release profile are the amount of 5-FU loaded into the copolymer, pH of swelling medium, and kaolin content. It was observed that the release of 5-FU was much higher in SIF compared to SGF, indicating that the release system is controllable and can be as a release system for intestine specific drug delivery. The drug release rate of the hydrogels was also influenced by the kaolin content. The cumulative drug release of 5-FU from the composite hydrogels was decreased with increasing kaolin content. In addition, the drug encapsulation and drug loading efficiency was decreased with increasing clay content. Overall, the results demonstrated that this hydrogel could successfully deliver the drug to the colon without losing the drug in the stomach, and could be a good candidate as an orally administrated drug delivery system for drugs such as 5-FU.

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