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Preparation of chitosan/chondroitin sulfate complex microcapsules and application in controlled release of 5-fluorouracil

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ABSTRACT

Chitosan/chondroitin sulfate complex microcapsules were prepared to encapsulate the anti-cancer drug 5-fluorouracil (5-Fu) by emulsion-chemical crosslinking method. The microcapsules were characterized by Fourier transform infrared spectroscopy (IR), scanning electron microscopy (SEM) in conjunction with energy dispersive X-ray spectrometry (EDS), and laser diffraction particle size analysis. The in vitro drug release behavior of 5-Fu from the microcapsules was studied by UV–visible spectrophotometry. Results showed that the microcapsules were in spherical form with diameter mostly in the range of 20–60 μm. Result of EDS showed that 5-Fu was in the internal core of the microcapsules. The IR spectrum indicated that electrostatic interactions exit between chitosan and chondroitin sulfate, with the sulfate group and free carboxyl group reacted with the amino groups of chitosan. The release kinetic experiments showed that the release performance of the microcapsules was influenced by the amount of crosslinking reagent, drug loading and the pH of the release medium.

1. Introduction

Over the past few decades, the rise of modern pharmaceutical technology and the growth of the biotechnology industry have greatly accelerated the process of drug discovery and development. The most common method of administration of drugs is in the form of pills or injections, which meet the requirements of efficacy for several drugs. However, these methods are inadequate for many new drugs, such as hormones and vaccines, which are usually high molecular weight proteins and are very sensitive to environmental conditions. To overcome these difficulties, new technologies, like the microencapsulation, have been developed (Herrero, Del Valle, & Galan, 2006; Schwinger, Klemenz, Busse, & Kressler, 2004). The microencapsulation of chemicals, drugs, enzymes or cells has been used not only in biochemical engineering, but also in biomedical fields (Gong, Luo, Yang, & Wu, 2006; Qi et al., 2006), as well as in the development and production of improved food and drug delivery systems, normally used to control the released active ingredients or enhance core material activities (Chen, Chen, & Lin, 2006; Sinha et al., 2004; Tanaka, Kogoma, & Ogawa, 2006).

Chitosan is obtained by alkaline deacetylation of chitin from crab and shrimp shells, which is the second abundant polysaccharide next to cellulose (Ruel-Gariepy, Leclair, Hildgen, Gupta, & Leroux, 2002; Sinha et al., 2004). As a natural polyaminosaccharide, chitosan has advantages such as nontoxicity, biocompatibility and biodegradation, and is thus widely used as a pharmaceutical excipient in oral drug formulations in order to improve the dissolution of poorly soluble drugs or for the sustained release of drugs by a process of slow erosion from a hydrated compressed matrix (Higuera-Ciapara, Felix-Valenzuela, Goycoolea, & Arguelles-Monal, 2004; Qi, Xu, Jiang, Hu, & Zou, 2004). In recent years, chitosan microcapsules were developed for site specific drug delivery such as intestine selective drug and anti-cancer agents (Tozaki et al., 2002; Zhao et al., 2007). Chitosan has one primary amino and two free hydroxyl groups for each C_6 sugar unit. It may carry a positive charge which in turn interacts with many negatively charged substances. Chondroitin sulfate (CS) is a polysaccharide found in bone, cartilage, and connective tissue, composing of acetylgalactosamine and glucuronic acid residues alternately linked to each other by the β1–4 and β1–3 bonds, respectively. In addition, chondroitin sulfate is the major glycosaminoglycan of the arterial wall, and it has been shown to have anti-atherogenic effect in animal models in the literature (Chen et al., 2007). Chondroitin sulfate is an acidic mucopolysaccharide, which is able to form ionic complexes with positively charged substance (Lee et al., 2007). Therefore chitosan can react with chondroitin sulfate, with electrostatic interactions.

Antimetabolite 5-fluorouracil (5-Fu) is one of the major anticancer agents used clinically for the treatment of stomach, colorectal, head and neck cancers (Wang et al., 2003). Traditionally, it is administered in feeds, in drinking water, or by injection given several times a day. However, it shortens in vivo half-lives of drugs in the body and has toxic side effects (Fu, Shyu, Su, & Yu, 2002). One way to increase the therapeutic efficacy of the drugs is to encapsu-

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late them in sustained delivery systems by using biopolymers. For this reason, preparation of microcapsules smaller than 50 μm using biodegradable polymer for long-term release of the anti-tumor drug should be investigated. In this paper, we have used 5-Fu as a reference drug and the chitosan/chondroitin sulfate as the coating polymer to make microcapsules. The properties of microcapsules have been studied.

2. Materials and methods

2.1. Materials

Chitosan (CHS) (Deacetylation degree 90%) was purchased from Haidebei Bioengineering Co. (Jinan, China). Chondroitin sulfate (CS) was obtained from Guanglong Biochemical Co. (Qufu, China). 5-Fluorouracil (5-Fu) was provided from Longsheng Co. (Jinan, China). White oil (36#, food grade) was purchased from Jinan Yiduo Economic & Trade Co. (Jinan, China). Span-80 (Chemical Pure) was purchased from Yaoshun Impart & Export Co. (China). Magnesium stearate (Chemical Pure) and glutaraldehyde (25% water solution) was obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). All other chemicals and reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of microcapsules

The microcapsules were prepared by emulsion-chemical cross-link method.

First, the solutions of chitosan and chondroitin sulfate were prepared separately. Solution A: 200 mg chitosan was dissolved in 20 mL 1.0% (v/v) acetic acid, and 200 mg 5-Fu was dissolved in it for drug-loading microcapsules. Solution B: 200 mg chondroitin sulfate was dissolved in 5 mL distilled water. Then microcapsules were prepared by the following steps: Solution A was added to 100 mL of white oil which contains 5% (v/v) Span-80 and 5 g/L magnesium stearate, and the system was stirred for 30 min at 60 °C. Then solution B was added into the white oil solution dropwise during one hour, and kept stirring for 30 min. Finally 1 mL 25% glutaraldehyde water solution was added to the white oil solution slowly, and the system was kept stirring for 1 h. Afterwards, the microcapsules were obtained by centrifugal separation, washed with petroleum ether and absolute ethyl alcohol, filtered and dried under vacuum overnight until constant weight.

2.2.2. Fourier transform infrared spectroscopy measurement

Infrared (IR) spectra were recorded with a NEXUS-470 spectrophotometer (NICOLET Co., USA). The samples were prepared by processing compressed KBr disks.

2.2.3. Analysis of morphology, internal element and size distribution

The external morphology of microcapsules was analyzed by scanning electron microscopy (SEM). The powder of microcapsules was fixed on support with carbon-glue, and coated with gold using a gold sputter module in a high-vacuum evaporator for SEM images. Samples were then observed with a scanning electron microscope (S-2500 scanning microscope, Hitachi, Japan) at 20 kV. The internal morphology was studied by embedding the microcapsules in modified acrylate adhesive (Trade name: 302, Fushun, China). After drying, the blocks were cut with a razor blade and the fragments obtained were deposited on SEM stubs. At the same time, the blocks were observed with the energy X-ray dispersive spectrometer (EDS) to study the internal element of the microcapsules. All microcapsules were analyzed for their size distribution using a laser diffraction particle size analyzer (LS 13320 Particle Size Analyzer, Beckman Coulter Co., USA).

2.2.4. Drug loading and in vitro release

The amount of 5-Fu encapsulated within microcapsules was determined by spectrophotometry (Shao & Leong, 1995).

5-Fu solution of different concentration was prepared in the release medium at pHs of 1.0, 7.0 and 7.4, respectively. The absorbance of 5-Fu solutions was analyzed at 266 nm by UV spectrophotometry (UV-260, Shimadzu, Japan). Three calibration curves, each for every pH, were obtained for the analysis of the concentration of 5-Fu.

Fifty milligrams dry microcapsules was ground and added to 100 mL water placed in a water bath at 37 °C. The mixture was treated with ultrasonic for several times to release the drug completely, followed by filtration. The absorbance of the filtrate was measured and the amount of 5-Fu was obtained through the standard curve.

For the in vitro release experiments, 50 mg of 5-Fu-loaded microcapsules was placed in the dialysis bag and suspended in 200 mL release medium. The dissolution medium was kept under stirring at 100 rpm at 37 °C. Aliquots of the dissolution medium (5 mL) were withdrawn at predetermined time intervals and replaced by fresh buffer. Samples were analyzed at 266 nm by spectrophotometry.

3. Results and discussion

3.1. Structure of microcapsules confirmed by FTIR

Structures were confirmed by FTIR as shown in Fig. 1. Compared the spectrum of CHS/CS blank microsphere (c) with that of CHS (a) and CS (b), the peak at 1648 cm^{-1} ($CONH_2$) in the spectrum of CHS shift to 1642 cm^{-1}, and the peak of amino group disappeared, indicating that the amino group reacted with CS. The peak at 1253 cm^{-1} (S═O) in the spectrum of CS turn weaker and shift to 1250 cm^{-1}, the coupling peak of the C–O stretch vibrations and O–H variable-angle vibration turn weaker and divide into two peaks (the peak at 1413 cm^{-1} divide into 1411 and 1402 cm^{-1}, the peak at 1380 cm^{-1} divide into 1383 and 1378 cm^{-1}), indicating that the sulfate group and some of the free carboxyl group of CS reacted with CHS. Polyelectrolyte complex was obtained after the interaction between the amino group of CHS and the sulfate group and the free carboxyl group of chondroitin sulfate.

In IR spectrum of 5-Fu (d), the characteristic absorption peaks are the following: 1673 cm^{-1} (overlapped stretching vibration absorption of C═O and C═C), 1431 cm^{-1} (inplane bending vibration of C–H in –CF═CH–), 1247 cm^{-1} (absorption of C–N stretching

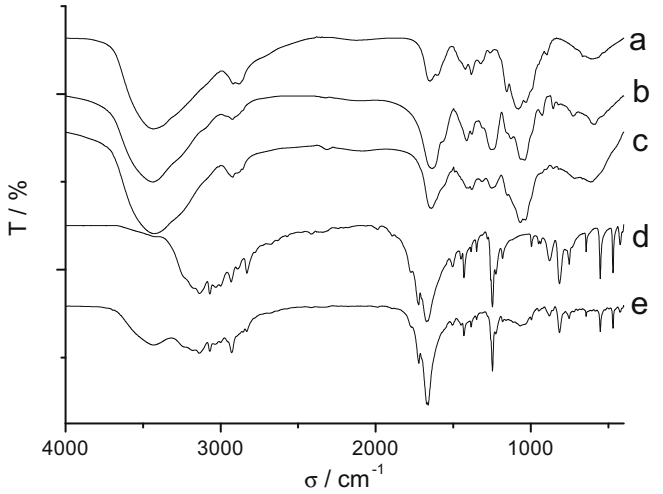

Fig. 1. FT-IR spectra of (a) CHS, (b) CS, (c) CHS/CS blank microcapsules, (d) 5-Fu and (e) 5-Fu loaded microcapsules.

vibration), 879 cm^{-1} (out-of-plane bending vibration of C–H in –CF=CH–), 815 cm^{-1} and 752 cm^{-1} (out-of-plane bending vibration absorption of C–H in –CF=CH–).

Compared with CHS/CS blank microcapsule (c) and 5-Fu (d), the IR spectrum of 5-Fu loaded microcapsule (e) is just the combination of both of (c) and (d), with the peaks' position of 5-Fu in the drug loaded microcapsules have little or no change, indicating that 5-Fu was encapsulated in the microcapsule without interaction with CHS/CS.

3.2. Morphology, internal composition and size distribution

3.2.1. External morphology

SEM images of the microcapsules in Fig. 2 show that the microcapsules are in the spherical form mostly. Most of the microcapsules have a relatively smooth surface (Fig. 2A–C), but some of the microcapsules have a rough surface (Fig. 2D). Water is easy to diffuse into the microcapsules, which is advantageous for the swelling of the microcapsules. The rough surface is also advantageous for the diffusion of the drug that loaded in the microcapsules.

3.2.2. Internal morphology

The microcapsules were embedded in a modified acrylate adhesive, then the dried blocks were cut with a razor blade and the fragments obtained were studied by SEM. Fig. 3 shows that the internal morphology of blank microcapsule (A) is compact, while the drug loaded microcapsule (B) is porous, since the internal structure of the microcapsules may be influenced by the drug loading. Without drug loading, the close combination of CHS with CS makes the internal structure of the microcapsule very compact (Fig. 3A), which can be made loose and porous as more core materials (5-Fu) was encapsulated in the microcapsule (Fig. 3B).

3.2.3. Internal component

Fig. 4 is the internal EDS spectra of microcapsules corresponding to blank microcapsules (A) and drug loaded microcapsules (B). There was no F element in the internal of the blank microcapsules (Fig. 4A), indicating that there was no 5-Fu in the inside of the blank microcapsules. The internal EDS spectrum of drug loaded microcapsules (Fig. 4B) shows the presence of the F element inside microcapsules, indicating the conclusion of 5-Fu was encapsulated in the internal of the microcapsules.

3.2.4. Size distribution of microcapsules

The microcapsules were analyzed for their size distribution by a laser particle size analyzer as shown in Fig. 5. The size distribution of the microcapsules is normal distribution. The microcapsules are mostly in the size range of 20–60 μm in diameter, and the mean particle size is 40 μm.

3.3. In vitro release of 5-Fu in the microcapsule

The concentration C (mg L^{-1}) of 5-Fu in different pH solutions was obtained through the standard calibration curve shown below, where A is the absorbance at 266 nm by spectrophotometry and R is linear correlation coefficient of the standard curve.

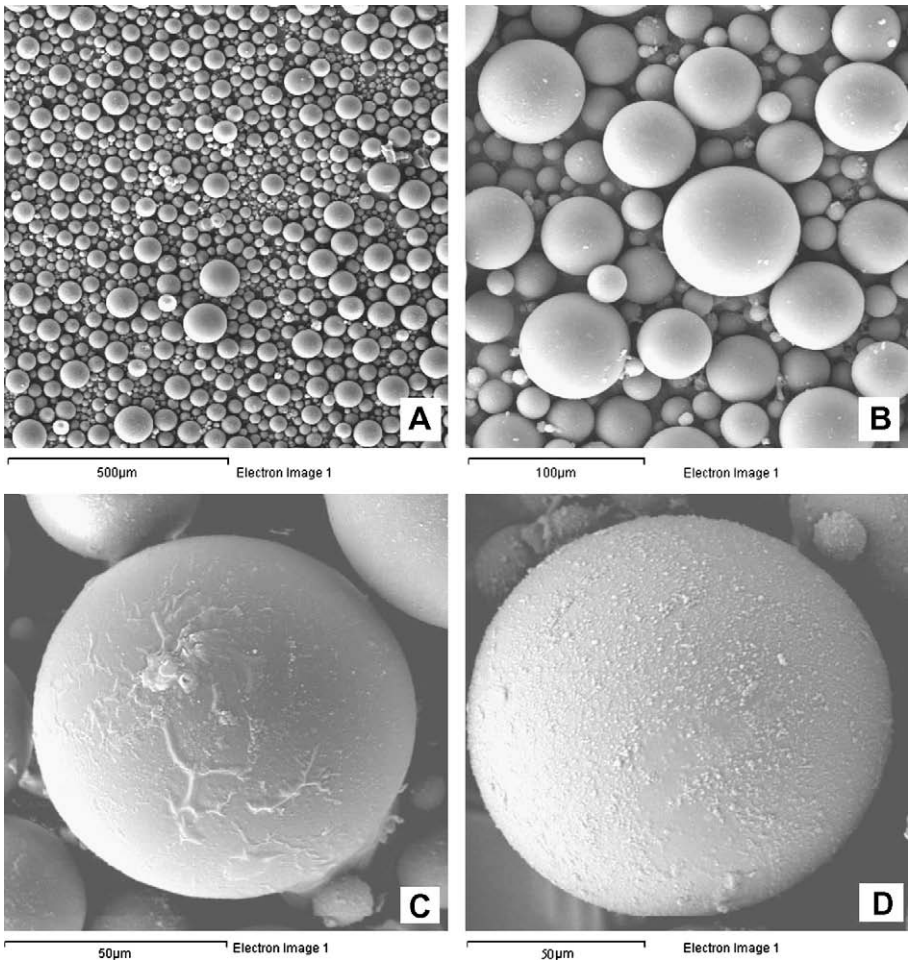

Fig. 2. SEM images in different dimension to show the external surface morphology of the microcapsules. (A) scale bar = 500 μm, (B) scale bar = 100 μm, (C) scale bar = 50 μm, (D) scale bar = 50 μm.

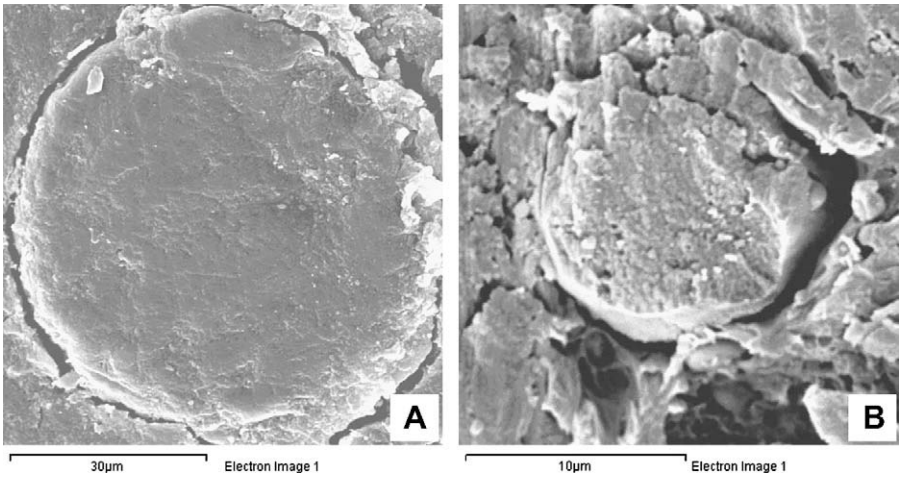

Fig. 3. SEM images of internal morphology of blank microcapsule (A) and drug loaded microcapsule (B).

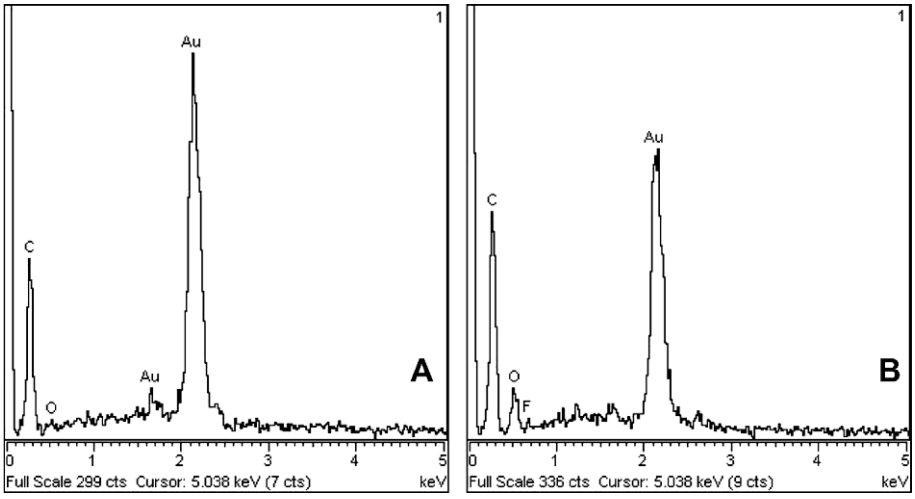

Fig. 4. Internal EDS spectra of blank microcapsules (A) and drug loaded microcapsules (B). Taking at the internal center of the microcapsule compared with the outside of the microcapsule.

$$C = -0.2304 + 18.04A \quad R = 0.99994 \ (\mathrm{pH} = 1.0)$$
$$C = -0.2270 + 19.35A \quad R = 0.99994 \ (\mathrm{pH} = 7.0)$$
$$C = -0.0426 + 20.46A \quad R = 0.99996 \ (\mathrm{pH} = 7.4).$$

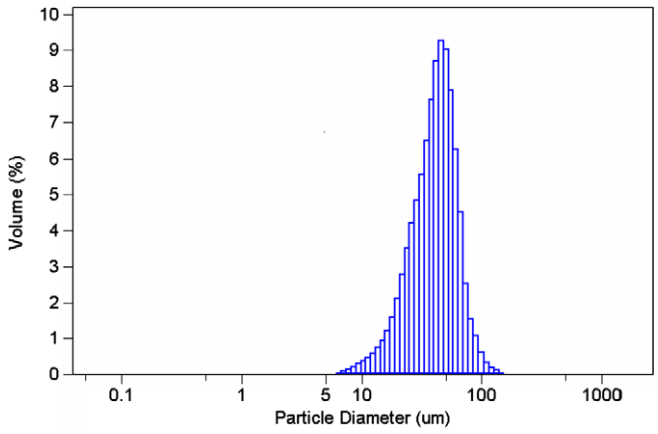

Fig. 5. Size distribution of the microcapsules taking by laser particle size analyzer.

3.3.1. Influence of the amount of crosslinking reagent on the release performance

Fig. 6 shows the release profiles of 5-Fu from microcapsules which were prepared with different molar ratio between the –CHO of glutaraldehyde and the $-NH_2$ of chitosan. An initial burst effect was followed by a slowly release. This might be related to 5-Fu adsorbed on the surface of the microcapsules. As the ratio of n (–CHO): n ($-NH_2$) increases, the release of 5-Fu becomes lower, due to increased amount of crosslinking, which also limits the swelling capacity of the wall materials of microcapsules. The results show that the release rate of 5-Fu in the microcapsules can be controlled by the amount of crosslinking agent.

3.3.2. Influence of drug loading on the release performance

Fig. 7 is the release profiles of 5-Fu from microcapsules prepared with different mass ratio between 5-Fu and wall materials. At the same amount and concentration of the wall materials, lower amount of 5-Fu slow down the release rate. As the amount of 5-Fu decreases, the relative amount of the wall materials increases, and the thickness of microcapsule wall increases, which is disadvantageous to the release of 5-Fu.

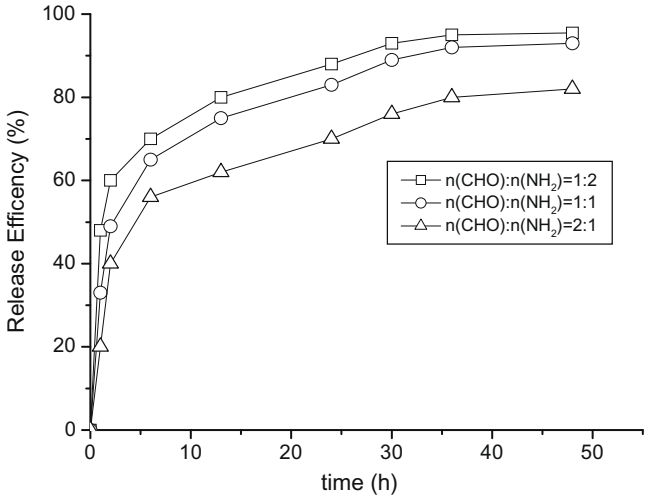

Fig. 6. Release profiles of 5-Fu from microcapsules prepared with different molar ratio between the –CHO of glutaraldehyde and the $–NH_2$ of chitosan in pH 7.0 solution.

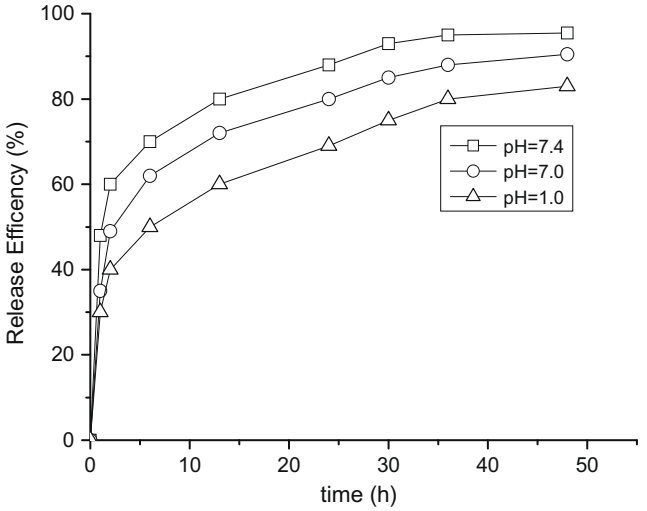

Fig. 8. Release profiles of 5-Fu from microcapsules at different medium pH of 1.0 (internal environment of stomach), 7.0 (neutrality) and 7.4 (internal environment of intestine) phosphate buffers.

3.3.3. Influence of release medium pH on the release performance

Fig. 8 shows the release profiles of 5-Fu from microcapsules at different medium pH of 1.0 (internal environment of stomach), 7.0 (neutrality) and 7.4 (internal environment of intestine) phosphate buffers. In the alkaline medium, 5-Fu was released in shorter periods than in the acidic mediums, and the released amount was higher. 5-Fu is an acidic drug, it becomes ionized and negative charged in the alkaline medium, so its solubility is thus higher and releases faster.

4. Conclusions

5-Fluorouracil could be successfully microencapsulated by emulsion-chemical crosslink method, with chitosan and chondroitin sulfate as the wall materials. IR spectrum indicated that there were electrostatic interactions between chitosan and chondroitin sulfate. The microcapsules were in spherical form with a size range of 20–60 μm; 5-Fu was inside the microcapsules; the internal structure of the blank microcapsules is compact, while the internal structure of the microcapsules loaded with the drug was porous.

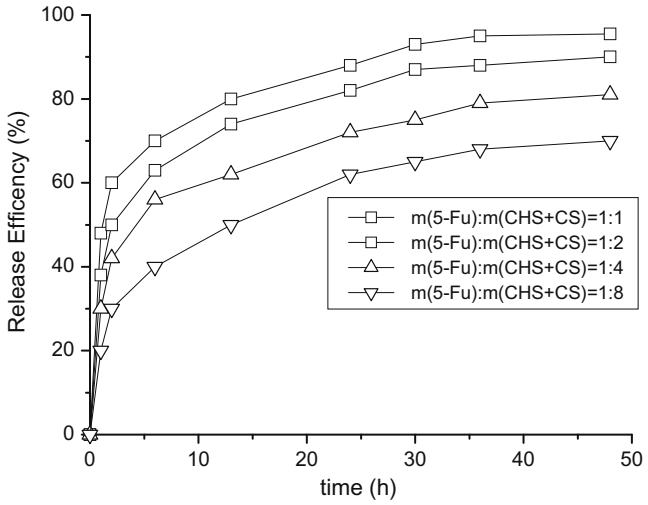

Fig. 7. Release profiles of 5-Fu from microcapsules prepared with different mass ratio between the 5-Fu and wall materials of CHS and CS in pH 7.0 solution.

The release performance of the microcapsules can be controlled by the degree of crosslinking, drug loading and pH of the release medium.

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