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Sustained release of antineoplastic drugs from chitosan-reinforced alginate microparticle drug delivery systems

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Abstract

Alginate based microparticle drug delivery systems were prepared for the sustained release of antineoplastic drugs. Two drugs, 5-fluorouracil (5-FU) and tegafur, were encapsulated into the microparticles. The drug loaded microparticles were fabricated using a very convenient method under very mild conditions, i.e., directly shredding the drug loaded beads into microparticles in a commercial food processor. The mean sizes of the obtained microparticles were between 100 and 200 μm. To effectively sustain the drug release, alginate microparticles were reinforced by chitosan during gelation. The drug release from the chitosan-reinforced alginate microparticles was obviously slower than that from the unreinforced microparticles. The effect of the reinforcement conditions on the drug release property of the microparticles was studied, and the optimized concentration of chitosan solution for reinforcement was identified. The effects of drug feeding concentration and pH value of the release medium on the drug release were investigated.

Keywords: Microparticles; Drug delivery; Alginate; Chitosan; 5-Fluorouracil; Tegafur

1. Introduction

Natural polymers, such as polysaccharides, are widely used in pharmaceutical applications due to their good biocompatibility and biodegradability. Alginate is one of the most extensively studied polysaccharides and has shown great potential as a drug carrier. The simple, mild, aqueous-based gel formation of sodium alginate in the presence of divalent cations is extremely suitable for encapsulating various drugs with different properties (George and Abraham, 2006).

Compared with protein drugs, the controlled release of anticancer drugs with low molecular weights through encapsulation into polysaccharides still suffers from certain limitations. Due to the high water content of the polysaccharides based release matrices, the release of low molecular weight drugs usually cannot be effectively controlled and the release rate is very fast with an obvious burst release (George and Abraham, 2006; Arıca et al., 2002).

So one purpose of this research is to realize the sustained release of anticancer drugs with low molecular weights from the alginate based drug delivery systems. Through reinforcing by chitosan during gelation of alginate and optimizing the reinforcement conditions, the release of anticancer drugs, 5-FU and tegafur, could be effectively sustained.

Microparticle drug delivery systems have received a great deal of attention due to their injectable property which could avoid the inconvenient surgical insertion of large implants (Pica et al., 2006), and are very suitable for localized release of anticancer agents to achieve sequential and simultaneous drug delivery and to reduce the side effects associated with the systemic delivery of anticancer agents. Direct injection into the tumor site could localize the treatment to cancer cells (Pica et al., 2006). Besides, the microparticles which could protect the

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encapsulated drugs against the harsh environment of the gastrointestinal tract are of special interest for oral drug delivery because their small size and their large specific surface area favor their absorption compared to larger carriers (Silva et al., 2006).

Compared with the well established production procedure of alginate beads based on external gelation, i.e., the mixture of alginate and drug is extruded drop-wise through a needle into a gelling bath containing divalent cations to form gelated alginate beads, as far as we know, the fabrication of drug loaded alginate microparticles still suffers from certain limitations. For example, traditional milling and grinding techniques are not very amenable to expensive drugs which can often be obtained only in small amounts because milling and grinding both require large amounts of materials. Besides, milling and grinding may also result in denaturation of protein drugs due to the increased pressure at the pushing inlet and grinding inlet (Nykamp et al., 2002; Schlocker et al., 2006). The widely used emulsification/gelation technique need to disperse the polymer, drug and divalent cations in an oil phase such as paraffin oil or other organic solvents in the presence of an emulsifier and the microspheres obtained must be washed thoroughly to remove the residual oil or organic solvents (Ribeiro et al., 2005; Silva et al., 2006). Besides, the emulsification requires many steps, and rigid control of the fabrication conditions. Through extrusion under an electrostatic field, microspheres can be produced (Gåserød et al., 1998). Obviously, the use of the electrostatic field with a high voltage is the limitation of this method. Layer-by-layer (LBL) self-assembly technique can produce microparticles with a narrow size distribution. However, the fabrication procedure is complicated and the template cores are needed for the formation of microparticles (Ye et al., 2005; Tao et al., 2007).

Therefore, another goal of this work is to develop a new method for fabricating microparticle drug delivery systems with polysaccharides as matrices. In this study, we used a very convenient method, i.e., directly shredding the drug loaded alginate beads in a commercial food processor to obtain microparticles. As distinguished from other conventional methods, the method used in this report is very rapid, convenient, solvent free, and easy to scale-up with a high particle yield.

2. Materials and methods

2.1. Materials

Sodium alginate (viscosity ~0.02 Pa·s in 1% aqueous solution at 20 °C) was supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Chitosan (M_w 8000–20000, degree of deacetylation ≧90%) was purchased from Sigma. Calcium chloride ($CaCl_2$) was provided by Kermel Chemical Reagent Co. Ltd. (Tianjing, China). 5-Fluorouracil (5-FU) was from Sigma. Tegafur was purified from the tegafur tablets (Jinan Pharmaceutical Factory, China). The drug pellets were ground and dissolved in ethanol. After the removal of starch and other impurities by filtration, tegafur was obtained by recrystallization in ethanol. All other reagents were of analytical grade and used as received.

2.2. Preparation of chitosan-reinforced and unreinforced drug loaded alginate microparticles

Sodium alginate (1.6 g for samples **1**, **2**, **3**, **4** and **6** with drug feeding concentration of 20 wt.% or 1.8 g for samples **5** and **7** with drug feeding concentration of 10 wt.%) was dissolved in distilled water (98 mL). Then a particular amount of drug (0.4 g for samples **1**, **2**, **3**, **4** and **6** with drug feeding concentration of 20 wt.% or 0.2 g for samples **5** and **7** with drug feeding concentration of 10 wt.%) was added to the solution under stirring until a uniform suspension was obtained. The suspension was introduced into a 10 mL syringe and then extruded through a needle with an internal diameter of 0.45 mm into a 150 mL of solution containing 6% (w/v) $CaCl_2$, a particular amount of chitosan (0.2%, 0.4% or 0.8%, w/v), and 1% (v/v) acetic acid at room temperature. The distance between the edge of the needle and the surface of the solution was 5 cm. The formed drug loaded beads were allowed to harden for 15 min in the solution to form beads with a diameter of 2.5 mm, and then were shredded into microparticles, with the presence of original gelation solution containing $CaCl_2$, chitosan and acetic acid, in a commercial kitchen-type food processor (Model JYL-350, 350 W, Jiu-Yang Corp., China) with the blade rotating at 22,000 rpm for 3 min (3 × 1 min). The obtained microparticles were centrifuged, rinsed with distilled water three times, and then freeze-dried in a LABCONCO freeze dry system.

For comparison, the unreinforced drug loaded alginate microparticles were formed in a 150 mL of solution containing 6% (w/v) $CaCl_2$ in the absence of chitosan and acetic acid. Other conditions were the same as that for chitosan-reinforced microparticles.

2.3. Characterizations of drug loaded beads and microparticles

The images of the drug loaded beads and the microparticle suspension before freeze-drying were taken by a digital camera (Canon S60). The images of drug loaded microparticle suspensions before freeze-drying were also taken by an inverted microscope (Olympus IX70 equipped with a digital camera). The surfaces of freeze-dried unreinforced and chitosan-reinforced drug loaded microparticles were visualized by a Hitachi X650 scanning electron microscope (SEM). The samples were coated with gold before observation.

The size and size distribution of the drug loaded microparticles before freeze-drying were measured by a Coulter LS230 particle size analyzer (Beckman).

The drug loading content and encapsulation efficiency were determined as follows.

Drug loading content = (drug recovered in microparticles/microparticles recovered) × 100%.

Encapsulation efficiency = (drug recovered in microparticles/drug fed) × 100%.

Table 1
Formulation variables and properties of unreinforced and chitosan-reinforced alginate microparticle drug delivery systems

Sample	Drug loaded	Drug feeding concentration (wt.%)	Chitosan concentration (%, w/v)	Drug loading content (wt.%)	Encapsulation efficiency (%)
1	5-FU	20	0	1.6	7.8
2	5-FU	20	0.2	1.8	9.2
3	5-FU	20	0.4	2.2	11.2
4	5-FU	20	0.8	2.0	10.0
5	5-FU	10	0.4	0.7	6.9
6	Tegafur	20	0.4	0.9	4.5
7	Tegafur	10	0.4	0.4	4.2

2.4. In vitro drug release study

The freeze-dried drug loaded microparticles (100 mg) were immersed in 3 mL of Tris buffer (pH 7.4) except for studying the effect of pH on the drug release where a Tris buffer solution with pH 5.0 was used for comparison. The test tube was incubated in a shaking water bath at 37 °C. At specific times, after centrifuging at 5500 rpm for 5 min, the supernatant was completely removed and replaced by 3 mL of fresh buffer solution. The drug concentration in the supernatant was determined by an UV–vis spectrophotometer (PerkinElmer Lambda Bio 40) at 267 nm for 5-FU or 270 nm for tegafur. The release data were given as mean ± standard deviation (S.D.) based on three independent measurements.

3. Results and discussion

3.1. Preparation and characterizations of drug loaded microparticles

The formulation variables, drug loading content, and encapsulation efficiency of the alginate based microparticle drug delivery systems are summarized in Table 1. As listed in Table 1, the encapsulation efficiency values for 5-FU loaded microparticles are higher than that of the tegafur loaded microparticles. This is mainly due to the lower water solubility of 5-FU as compared with tegafur. Compared with the drugs with high molecular weights, the encapsulation efficiency of the drugs with low molecular weights such as 5-FU and tegafur in the current study is much lower (Arıca et al., 2002; Ribeiro et al., 2005) because of the higher drug loss during preparation due to the faster diffusion of the drugs with lower molecular weights.

In this study, the alginate based microparticle drug delivery systems were prepared using a very convenient method, i.e., directly shredding the drug loaded chitosan-reinforced alginate beads into microparticles in a commercial food processor. Using this method, all beads could be shredded into microparticles, and all data presented (images, particle size distributions, and drug release properties of the microparticles) were obtained after shredding directly without filtering or sieving. The images of the beads and microparticles before freeze-drying taken by a digital camera are shown in Fig. 1. As depicted in Fig. 1a, the mean diameter of the beads is about 2.5 mm. After shredding, the images of 5-FU loaded and tegafur loaded chitosan-reinforced alginate microparticles before freeze-drying observed by an inverted microscope are shown in Fig. 2. (After freeze-drying, the particle size will become smaller.) The microscopy observation is in good agreement with the data obtained by the particle size analyzer. As depicted in Fig. 2, the mean diameters of the microparticles are determined to be 118.2 and 146.3 μm for 5-FU loaded and tegafur loaded microparticles, respectively. The microparticles with such sizes could be delivered through injection administration for tumor localized treatment and oral administration.

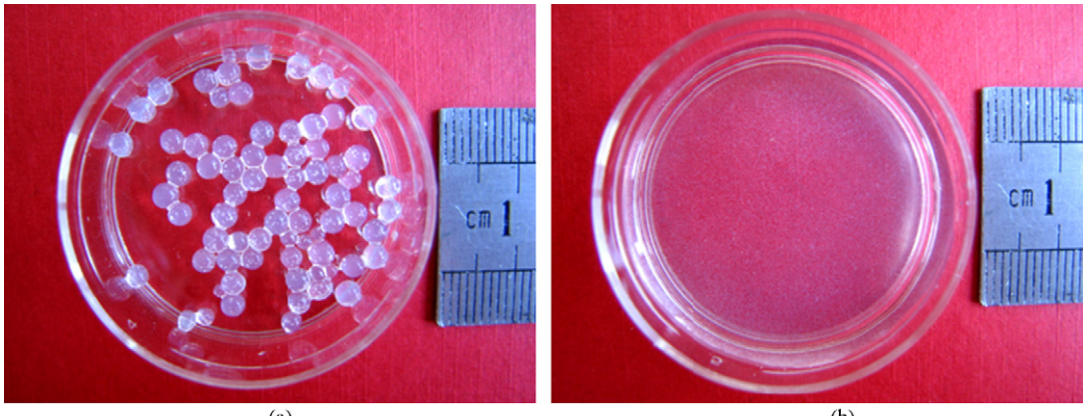

Fig. 1. Images of chitosan-reinforced alginate beads and microparticles taken by a digital camera. (a) Beads for preparation of sample **3** before shredding, and (b) sample **3**: 5-FU loaded microparticles.

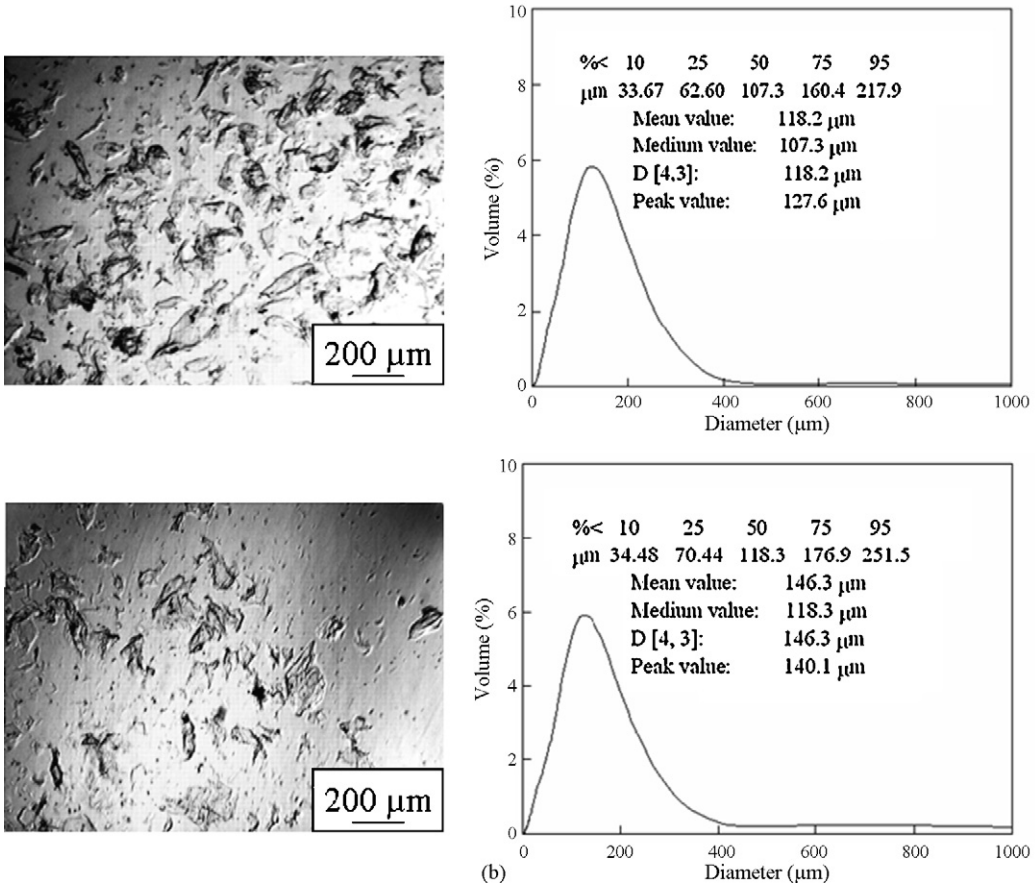

Fig. 2. Images observed by an inverted microscope and particle size distribution determined by a particle size analyzer of chitosan-reinforced alginate microparticles. (a) Sample **3**: 5-FU loaded microparticles, and (b) sample **6**: tegafur loaded microparticles.

The SEM images of unreinforced and chitosan-reinforced 5-FU loaded alginate microparticles are presented in Fig. 3. Compared with the crannied unreinforced surface (Fig. 3a), the chitosan-reinforced surface shows a denser morphology free of cracks can be observed (Fig. 3b) due to the formation of chitosan–alginate complex. In the preparation of microparticles, the chitosan contained solution existed during the shredding process and the microparticles were immersed in the chitosan contained solution, so the microparticles were also reinforced by chitosan. The SEM observation is in accordance with the

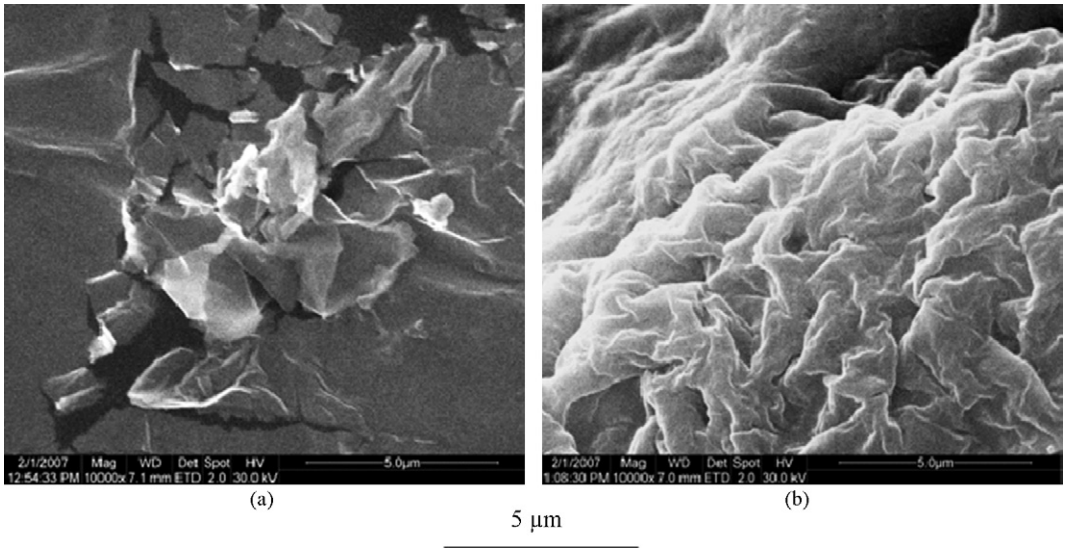

Fig. 3. SEM images of the surfaces of 5-FU loaded alginate microparticles. (a) Sample **1**: unreinforced microparticles, and (b) sample **3**: chitosan-reinforced microparticles.

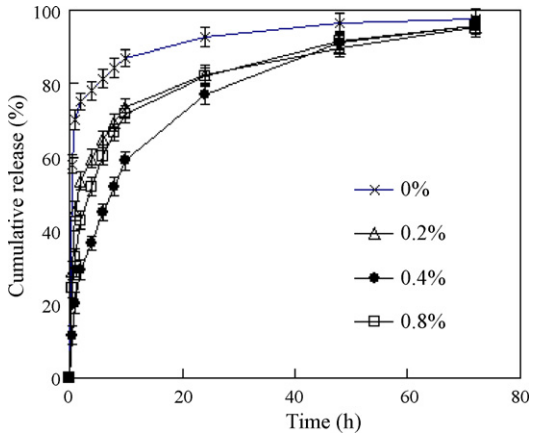

Fig. 4. 5-FU release profiles from unreinforced microparticles (sample **1**) and chitosan-reinforced alginate microparticles (samples **2**, **3**, **4**) with different chitosan concentrations for the reinforcement.

fact that no obvious burst release could be observed for the chitosan-reinforced microparticles (Mi et al., 2002).

3.2. *In vitro* release study

To investigate the effect of chitosan reinforcement on the drug release, the *in vitro* releases of both unreinforced and chitosan-reinforced alginate microparticles were carried out in Tris buffer at pH 7.4. The results, as shown in Fig. 4, reveal that the 5-FU releases from all chitosan-reinforced microparticles are much slower, as opposed to the relatively faster release from unreinforced microparticles, in the initial release stage. This is due to the interaction between carboxylate groups of alginate and ammonium groups of chitosan, leading to the formation of chitosan–alginate complex. As a result, the drug diffusion inside the matrix is retarded and the release of 5-Fu is suppressed (Mi et al., 2002; Wittaya-areekul et al., 2006). Comparing the release profiles from chitosan-reinforced microparticles with different chitosan concentrations of 0.2%, 0.4% and 0.8% (w/v) for the reinforcement, we can find that the release from the microparticles reinforced by 0.4% (w/v) chitosan solution is slowest, indicating the sigmoidal dependence of reinforcement efficiency on chitosan concentration. If the chitosan concentration is too low, the reinforcement is not sufficient. With an increase in the chitosan concentration, more chitosan–alginate complexes could be formed. However, if the chitosan concentration is too high, the reinforcement efficiency would be reduced since the high viscosity would retard the diffusion of chitosan chains and is unfavorable for the complexion between the chitosan chains and the alginate chains. The drug release is a comprehensive result of many factors. In this study, the reinforcement efficiency is the dominant effect on the release rate. Our experiments also show the release rates of different samples are in accordance with the reinforcement efficiency. The microparticles with chitosan concentrations of 0.4% (w/v) for the reinforcement have the best reinforcement efficiency, and correspondingly have the slowest drug release rate. Our results are in agreement with previous studies on the chitosan reinforcement by other researchers (Wittaya-areekul et al., 2006).

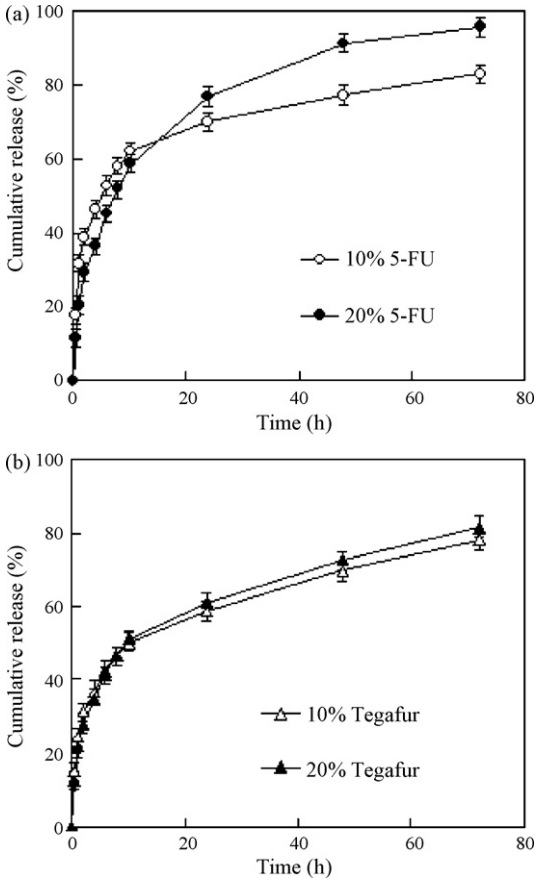

Fig. 5. Release profiles of chitosan-reinforced alginate microparticles with different drug feeding concentrations. (a) Samples **3**, **5**: 5-FU loaded microparticles, and (b) samples **6**, **7**: tegafur loaded microparticles.

As we know, the release rate and entrapment efficiency of different drugs, encapsulated in polysaccharides based drug delivery systems, differ significantly (Bouhadir et al., 2001). Since generally polysaccharides have strong affinity with water and the polysaccharide matrices are highly hydrolyzed, the drugs with low molecular weights could be diffused out quickly and easily, leading to the fast release and the low encapsulation efficiency. In the current study, we used two drugs, 5-FU and tegafur, both with low molecular weights to evaluate the efficiency of our systems for sustaining the drug release. Under the optimized reinforcement conditions, the drug release from the microparticles (sample 3) could be effectively sustained and the time for 50% release, $T_{50\%}$, is about 8 h, which is much longer than the reported values for the release of low molecular weight drug such as 5-FU and indomethacin in previous literatures on polysaccharides based drug delivery systems (Denkbaş et al., 2000; Shantha and Harding, 2000; Arıca et al., 2002; Chang et al., 2007)

Fig. 5 demonstrates the release profiles from chitosan-reinforced microparticles with different drug feeding concentrations of 10 and 20 wt.%. For the samples with different drug loadings, an increase in the drug loading is expected to result in elevated amount of drug released because diffusion driving

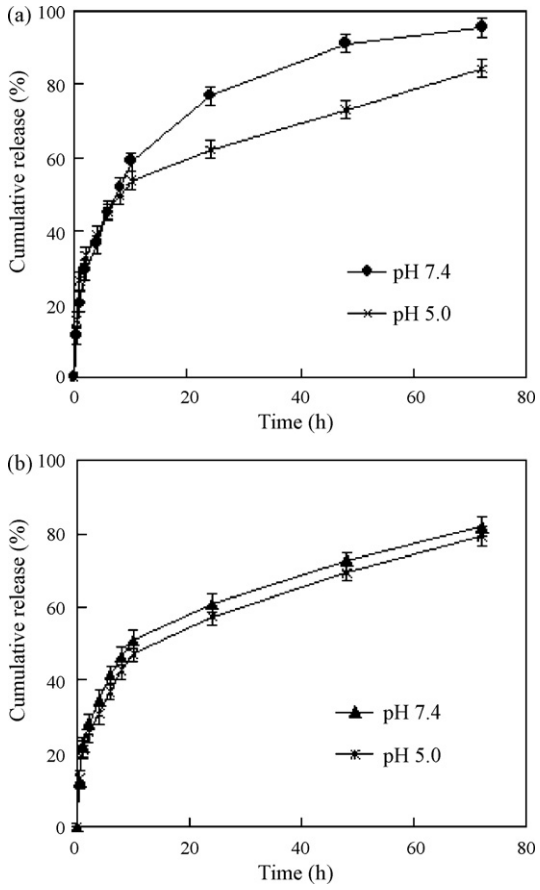

Fig. 6. Release profiles of chitosan-reinforced alginate microparticles at different pH values. (a) Sample **3**: 5-FU loaded microparticles, and (b) sample **6**: tegafur loaded microparticles.

force of concentration gradient is enhanced with greater concentration of encapsulated drug. However, the difference in the percentages of the accumulative release is not so obvious as compared with the difference in the amounts of the drug release. As shown in Fig. 5, the microparticles with a higher drug loading have a slightly higher percentage of accumulative release. Our results are in agreement with previous studies of other researchers (Denkbaş et al., 2000).

The drug releases at different pH values are compared in Fig. 6. Alginate with carboxylate groups shrinks at low pH and dissolves at high pH. As a result, a faster drug release from the alginate matrix occurs at a higher pH. However, in the current study, the alginate microparticles are reinforced by chitosan. So the faster drug release is retarded by the reinforcement. As we know, the chitosan with ammonium groups dissolves at low pH and is insoluble at high pH. Upon mixing, the carboxylate groups of alginate and the ammonium groups of chitosan ionically interact to form the polyelectrolyte complex. Complexation of alginate with chitosan reduces the porosity of the matrix and decreases the diffusion of encapsulated drugs. Moreover, the possible dissolution of alginate at high pH could be prevented by the chitosan (Ribeiro et al., 2005). As an overall result, the drug releases at pH 7.4 are slightly faster than the releases at pH 5.0 for both 5-FU and tegafur as illustrated in Fig. 6.

4. Conclusions

Microparticle drug delivery systems based on chitosan-reinforced alginate were prepared by directly shredding the drug loaded beads into microparticles in a commercial food processor. Two anticancer drugs, 5-FU and tegafur, were loaded in the microparticle drug delivery systems. The *in vitro* release shows that chitosan reinforcement could effectively sustain the release of the drugs with low molecular weights. And the efficiency of reinforcement is affected by the concentration of chitosan solution. The drug releases at pH 5.0 and 7.4 do not exhibit obvious difference, implying the complexation of alginate with chitosan could efficiently decrease the diffusion of the encapsulated drugs and thus retard the drug release.

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