Chitosan/Alginate Nanoparticles Stabilized by Poloxamer for the Controlled Release of 5-Fluorouracil

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ABSTRACT: In this study, stable 5-fluorouracil (5-FU)-loaded chitosan (CS)/alginate (Alg) nanoparticles (NPs) were prepared with poloxamer as a surfactant. The effects of the Alg concentration, CS/Alg weight ratio, and poloxamer concentration on the properties of the 5-FU-loaded CS/Alg NPs were studied. The results of dynamic light scattering and transmission electron microscopy indicated that stable 5-FU-loaded CS/Alg NPs of around 200 nm with low-size polydispersities were achieved. Furthermore, the *in vitro* release of the 5-FU-loaded CS/Alg NPs was investigated in phosphate buffer solution at pH 7.4. The results show that the encapsulation efficiency of 5-FU depended on the drug feeding amount (DFA), poloxamer concentration, Alg concentration, and CS concentration. However, the *in vitro* release rate of the 5-FU-loaded CS/Alg NPs was only related to the DFA, Alg concentration, and CS concentration and was independent of the poloxamer concentration. The time of 5-FU release from the CS/Alg NPs could be controlled to be sustained for more than 12 h. According to this study, CS/Alg NPs stabilized by poloxamer could serve as a suitable candidate for the controlled release of 5-FU. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 2354–2359, 2010

Key words: chitosan; biocompatibility; drug delivery systems

INTRODUCTION

Polymeric microparticles/nanoparticles (NPs) have shown interesting promise for drug, protein, and peptide delivery in controlled release applications. Particularly, the idea of using microparticles/NPs made from natural biodegradable polymers to deliver drugs, proteins, and peptides in controlled release applications has attracted great interest.[1–5] Widely used natural polymers for the controlled release of drugs include alginate (Alg), chitosan (CS), cellulose derivatives, and guar gum.[6,7] Among those, Alg, a naturally occurring an anionic polysaccharide consisting of guluronic acid and manuronic acid, is widely used in pharmaceutical applications. The pK_a value of Alg acid ranges from 3.4 to 4.4, depending on the type of Alg and the salts present in the mixture.[8,9] CS is a cationic polysaccharide consisting of β-[1-4]-linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose; it has a macro-pK_a value in the range 6.3–6.5.[10,11] The use of polyelectrolyte complexing between the oppositely charged macromolecules of sodium alginate and CS to prepare microparticles/NPs has been widely studied.[12–16] Many researchers have prepared microparticles/NPs made of Alg and CS for the delivery of drugs, proteins, and peptides.[13–22]

5-Fluorouracil (5-FU) is one of the most widely used antineoplastics drugs in the treatment of breast cancer,[23,24] gastric cancer,[25] and pancreatic cancer,[26] but it metabolizes so fast that the half-life is only 5–10 min. To obtain an effective clinical blood drug concentration, people often choose to augment drug mass or to administer the drug to patients continually or repeatedly, which enhances the toxic side effect of 5-FU.[27] To solve this defect, various microparticles/NPs encapsulating 5-FU have been prepared to control 5-FU to release slowly over certain ranges of time.[28–31]

In the case of intravenous injection, microparticles/NPs are required to circulate stably and to release the loaded drug in controlled release mode *in vivo*. In this study, poloxamer was introduced as a surfactant to prepare stable 5-FU-loaded CS/Alg NPs. The factors in the relationships of the size of 5-FU-loaded CS/Alg NPs, such as Alg concentration, CS/Alg ratio, and poloxamer concentration, were studied. Furthermore, the morphology, drug

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TABLE I
DLA and EE Values of Samples 1–12 with Different Component

Samples	Drug feeding amount (mg)	[Poloxamer] (μg/mL)	[Alg] (mg/mL)	[CS] (mg/mL)	[$CaCl_2$] (mg/mL)	DLA (wt %)	EE (%)
1	160	8	5.4	4	8.1	45.7 ± 1.68	47.7 ± 1.82
2	180	8	5.4	4	8.1	42.1 ± 1.52	37.9 ± 1.26
3	200	8	5.4	4	8.1	48.3 ± 1.20	42.2 ± 1.93
4	200	4	5.4	4	8.1	48.7 ± 1.23	42.8 ± 1.62
5	200	6	5.4	4	8.1	53.3 ± 1.79	51.4 ± 1.20
6	200	8	5.4	4	8.1	48.2 ± 1.50	41.9 ± 1.05
7	200	8	4.6	4	8.1	57.2 ± 1.72	56.1 ± 1.89
8	200	8	5.4	4	8.1	55.0 ± 1.35	55.1 ± 1.28
9	200	8	6.2	4	8.1	54.4 ± 1.48	57.2 ± 1.95
10	200	8	4.6	3	8.1	69.1 ± 1.78	77.3 ± 2.01
11	200	8	4.6	4	8.1	58.6 ± 1.56	59.6 ± 1.96
12	200	8	4.6	5	8.1	58.2 ± 1.35	69.9 ± 2.20

loading, and *in vitro* release properties of 5-FU-loaded CS/Alg NPs were evaluated.

EXPERIMENTAL

CS (weight-average molecular weight = 50,000, degree of deacetylation = 95%) was purchased from Golden-Shell Biochemical Co., Ltd. (Zhejiang, China). Sodium alginate was supplied by Bordi Chemical (Tianjin, China). 5-FU was obtained from Bangcheng Chemical, Ltd. (China), and Poloxamer 188 was purchased form BASF (Germany). Other chemicals (Tianjin, China) were all analytical grade.

Preparation of the 5-FU-loaded NPs

Samples 1–12 of the prepared 5-FU-loaded NPs are shown in Table I. Here, the process of preparation is given with sample 1 as an example. 5-FU (160 mg) was dispersed in 50 mL of sodium alginate solution (5.4 mg/mL) under magnetic stirring, and this mixture was added dropwise to 25 mL of an ethyl acetate solution containing poloxamer (0.2 mg). After that, under gentle stirring, 60 mL of $CaCl_2$ solution (8.1 mg/mL) was added dropwise to the formed emulsion to prepare Alg pregel particles. Then, the obtained latex was added dropwise to 80 mL of CS solution (4 mg/mL). After the mixture was sonicated for 30 s at 200 W and stirred for 4 h at 40°C to evaporate the ethyl acetate, the 5-FU-loaded NPs dispersion was obtained. The obtained NPs were centrifuged, rinsed with distilled water three times, and then lyophilized overnight.

Characterization of the 5-FU-loaded NPs

Dynamic light scattering was used to measure the diameters of the 5-FU-loaded NPs with a BI 90Plus instrument of Brookhaven Corp (Texas, USA). with a laser source (678 nm) at an angle of 90°. The temperature was controlled at 25 ± 0.5°C. The data of the NPs are given as mean plus or minus standard deviation on the basis of three independent measurements.

Transmission electron microscopy (TEM) was used to observe the morphology of the 5-FU-loaded NPs. We prepared the samples by dipping a Formvar-coated copper grid into a 5 wt % solid content solution. After 3 min, the excess solution was removed by filter paper, and the sample was washed with water and dried in air. Measurement was carried out on a JEM-100CX II instrument (JEOL Ltd., Tokyo, Japan) operating at an accelerating voltage of 100 kV.

In vitro release of the 5-FU-loaded NPs

A certain volume of 5-FU-loaded NP dispersion was placed in a dialysis bag, which was immersed in 90 mL of phosphate buffer solution (PBS) as a receptor fluid. *In vitro* release was carried out in an incubator shaker (SHZ-88, Jintan Medical Treatment Instruments Manufactory, Jiangsu, China) at 70 rpm and 37.0 ± 0.1°C. At appropriate time intervals, a 20-mL aliquot of PBS outside the dialysis bag was removed to measure the drug amount released from the 5-FU-loaded NPs, and then, a 20-mL aliquot of fresh PBS was supplemented. The standard curve of 5-FU was calibrated by an UV–visible spectrophotometer (WFZ-26A UV–visible spectrophotometer, Tianjin Science Instrument Plant, China) at 265 nm. Drug release was determined with UV-absorption spectrum analysis. The data are given as mean plus or minus standard deviation on the basis of three independent measurements. The drug concentration was calculated according to a standard curve, and accumulative release was obtained by the following formula:

$$E = \frac{V_E \sum_{1}^{n-1} C_i + V_0 C_n}{m_0} \times 100\%$$

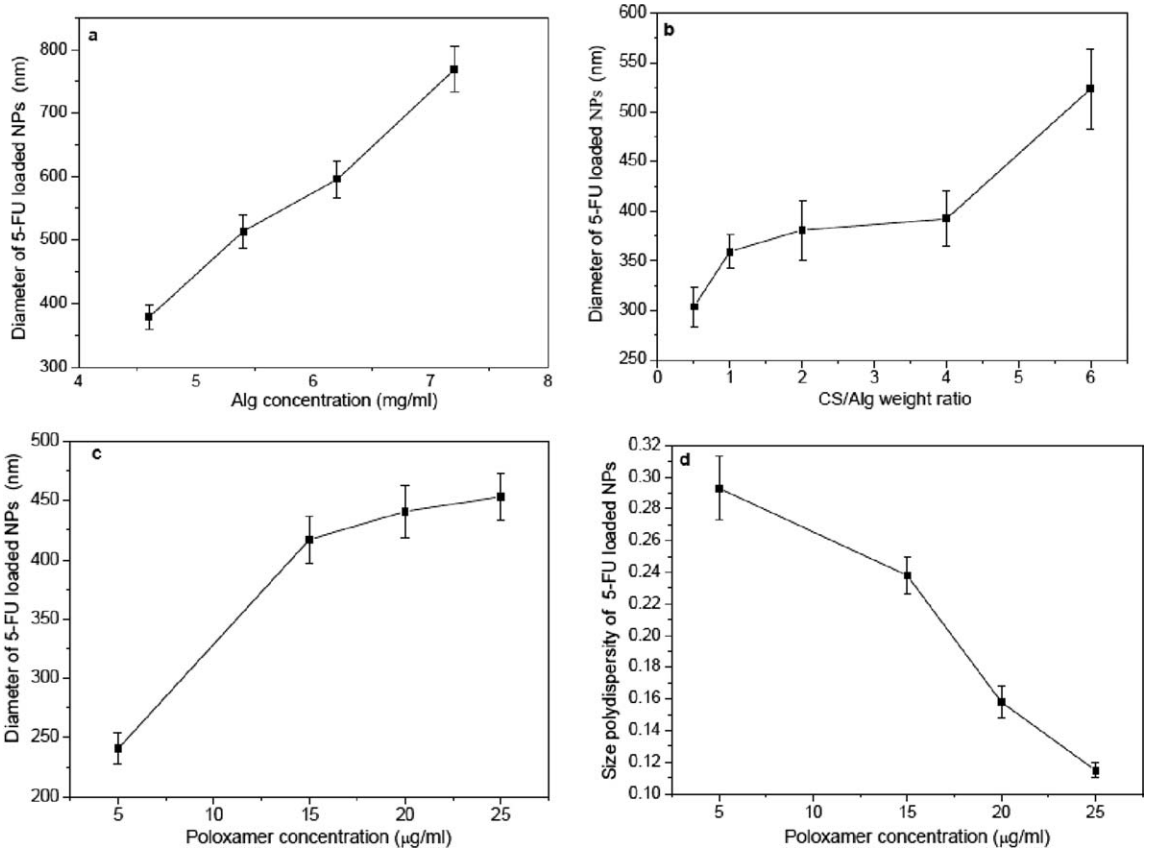

Figure 1 Diameter change of 5-FU-loaded NPs as a function of (a) Alg concentration, (b) CS/Alg weight ratio, and (c) poloxamer concentration and (d) size polydispersity change of 5-FU-loaded NPs as a function of poloxamer concentration.

where E is the accumulative release (%) of 5-FU, V_E is the sampling volume (20 mL), V_0 is the initial volume of receptor fluid (90 mL), C_i and C_n are the drug concentrations (μg/mL) of the receptor fluid, i and n are the sampling times, and m_0 is the mass of 5-FU in the drug-loaded NPs (μg).

The *in vitro* release behavior of the 5-FU-loaded NPs was divided into two stages. The first stage was the release of dissociated drug, and the release rate in this stage was fast. The second stage was the release of the drug encapsulated in the 5-FU-loaded NPs, and the release rate in this stage was very slow. So the encapsulation efficiency (EE) and drug loading amount (DLA) was obtained from the release curve in the second stage.

RESULTS AND DISCUSSION

Investigation of factors affecting the size of the 5-FU-loaded NPs

The stability of the NPs *in vivo* was related to their size. In this research, the size of the 5-FU-loaded NPs depended on the Alg, CS, $CaCl_2$, and poloxamer amounts. For simplicity, the effect of Alg, CS, and poloxamer amounts on the size of the 5-FU-loaded NPs was investigated with a set amount of $CaCl_2$.

The influence of the Alg concentration on the size of the 5-FU-loaded NPs is shown in Figure 1(a). The size of the 5-FU-loaded NPs increased rapidly with increasing Alg concentration because of the thickness of the reinforced 5-FU-loaded NPs. Therefore, Alg concentrations were chosen in the range from 4.6 to 7.2 mg/mL to prepare samples to obtain 5-FU-loaded NPs with both small sizes and appropriate compact cores.

The influence of the CS/Alg weight ratio on the size of the 5-FU-loaded NPs is presented in Figure 1(b). The size of the 5-FU-loaded NPs increased slightly when the CS/Alg weight ratio was less than 4 : 1. However, it increased rapidly when the CS/Alg weight ratio was more than 4 : 1. The compact membrane of CS probably began to form on the surface of Alg when the CS/Alg weight ratio reached 4 : 1. When the CS/Alg weight ratio continually increased, a mass of CS absorbed on the surface of the 5-FU-loaded NPs, which resulted in a rapid increase in the size of the 5-FU-loaded NPs. By combining the effect of the CS/Alg weight ratio on the size of the NPs and the reinforcement for the CS/Alg NPs in favor of drug controlled release, we

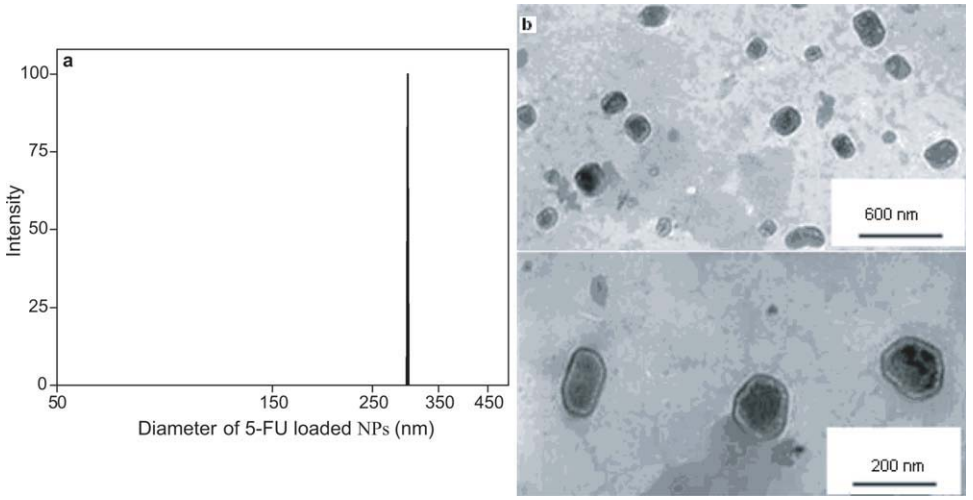

Figure 2 (a) Size distribution of sample 11 with an Alg concentration of 4.6 mg/mL, a poloxamer concentration of 8 μg/mL, a CS concentration of 4 mg/mL, and 5-FU of 200 mg and (b) TEM micrograph of sample 11.

chose a CS/Alg weight ratio equal to 4 : 1 to prepare samples 1–9.

Figure 1(c) displays influence of the poloxamer concentration on the size of the 5-FU-loaded NPs. The size of the 5-FU-loaded NPs increased from 240 to 453 nm as the poloxamer concentration increased from 5 to 25 μg/mL. Generally, an increment of surfactant concentration results in a decrease in the droplets size. However, a continuous increment of surfactant concentration led to the viscosity enhancement of the solution and a liquidity decrease of the droplets when the surfactant concentration reached a certain extent. Low liquidity did not guarantee that the droplets were broken into small droplets, and eventually, they were prone to form large ones. On one hand, to obtain small 5-FU-loaded NPs, the concentration of surfactant should be reduced. On the other hand, the poloxamer concentration as a function of the polydispersity of the 5-FU-loaded NPs, shown in Figure 1(d), indicated that the size distribution of the 5-FU-loaded NPs became wider with decreasing surfactant concentration. Therefore, poloxamer concentrations from 4 to 8 μg/mL were chosen to prepare the 5-FU-loaded NPs.

Figure 2(a) shows the size distribution of sample 11 with an Alg concentration of 4.6 mg/mL, a poloxamer concentration of 8 μg/mL, a CS concentration of 4 mg/mL, and 200 mg of 5-FU. Clearly, the size of the 5-FU-loaded NPs had a narrow distribution with a mean size around 299 nm. Figure 2(b) shows TEM micrograph of sample 11. Obviously, the 5-FU-loaded NPs had a capsule configuration. According to our experimental procedure, we deduced that the drug and Alg were encapsulated by the membrane consisting of CS. From the TEM picture of sample 11, we also observed that the size of the 5-FU-loaded NPs had a relatively narrow distribution, which was similar to the size distribution of those shown in Figure 2(a), and the size of the 5-FU-loaded NPs was approximately 200 nm. The sizes of sample 11 shown in Figure 2(b) were smaller than those analyzed by dynamic light scattering because the particles shrank during the drying process before TEM analysis.

In vitro release

In addition to the Alg, CS, $CaCl_2$, and poloxamer amounts, the drug amount also affected the *in vitro* release rate of the drug-loaded NPs. Similar to the investigation of the size change tendency mentioned previously, the effects of the drug, Alg, CS, and poloxamer amounts on the *in vitro* release rate of the 5-FU-loaded NPs were investigated with a constant $CaCl_2$ amount.

The EEs of samples 1–3, as shown, in Table I, were 47.7, 37.9, and 42.2% when the drug feeding amount (DFAs) were 160, 180, and 200 mg, respectively. Generally, the EE of the drug-loaded NPs decreased with the increment of DFA. The reason was that more DFA resulted in a larger opportunity of drugs to diffuse across the membrane, which led to a decreasing EE. Therefore, sample 1 had a comparatively higher EE compared to samples 2 and 3. However, the EE of sample 3 was higher than that of sample 2 because of a higher crystal degree of 200 mg of 5-FU compared to 180 mg of 5-FU. Figure 3 shows influence of DFA on the *in vitro* release of the 5-FU-loaded NPs. On the basis of the considerations mentioned previously, the *in vitro* release rate from sample 2 should have been faster than that from samples 1 and 3, which was consistent with the

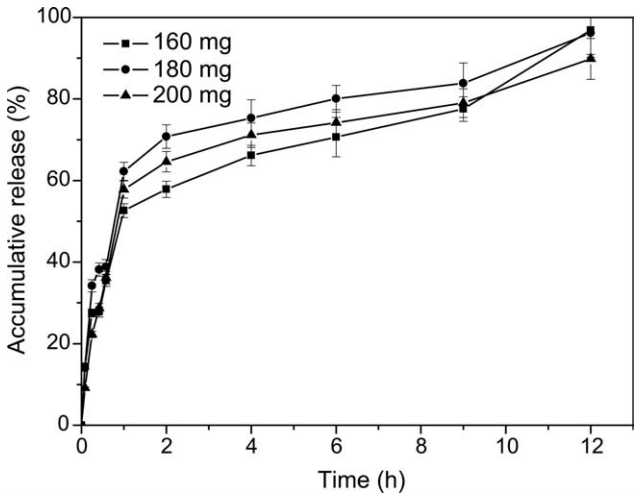

Figure 3 Influence of the DFA on the *in vitro* release of the 5-FU-loaded NPs.

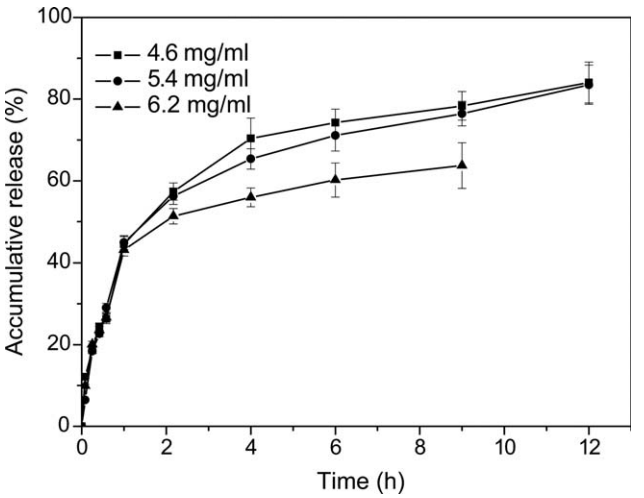

Figure 5 Influence of the Alg concentration on the *in vitro* release of the 5-FU-loaded NPs.

experimental results shown in Figure 3. The *in vitro* release rate from sample 3 was slightly faster compared to that of sample 1 when the release time was less than 12 h.

The EE values of samples 4–6, as shown in Table I, were 42.8, 51.4, and 41.9%, respectively, for the 5-FU-loaded NPs prepared with poloxamer amounts of 4, 6, and 8 μg/mL. It is known that the interfacial tension of the droplets decreased with increasing surfactant concentration, which was beneficial to the improvement of the stability of the dispersed droplets. However, the medium concentration (6 μg/mL) helped to improve the EE of the 5-FU-loaded NPs. Figure 4 shows influence of the poloxamer concentration on the *in vitro* release of 5-FU-loaded NPs. The poloxamer concentration had little effect on the *in vitro* release behavior of the 5-FU-loaded NPs, and the *in vitro* release rates of samples 4–6 were similar.

The EE values of samples 7–9, as shown in Table I, were 55.1, 56.1, and 57.2% when the Alg concentrations were 4.2, 5.4, and 6.2 mg/mL, respectively. The results suggest that EE did not strongly depend on the Alg concentration in the range from 4.2 to 6.2 mg/mL. Figure 5 shows the influence of the Alg concentration on the *in vitro* release of the 5-FU-loaded NPs. The *in vitro* release rate of sample 9 was slower than those of samples 7 and 8. Obviously, the increment of the Alg concentration with the deep encapsulation of 5-FU was helpful for the controlled release of the 5-FU-loaded NPs. The main reason was that the increment of the Alg concentration improved the nucleophilicity of CS, which resulted in the increment of the crosslinking degree of the inner Alg core.

The EE values of samples 10–12, as shown in Table I, were 77.3, 59.6, and 69.9% when the CS concentrations were 3, 4, and 5 mg/mL, respectively. Figure 6 shows the influence of the CS concentration

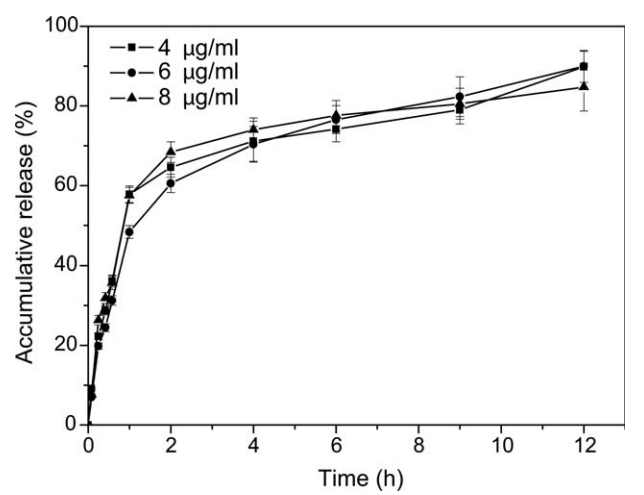

Figure 4 Influence of the poloxamer concentration on the *in vitro* release of the 5-FU-loaded NPs.

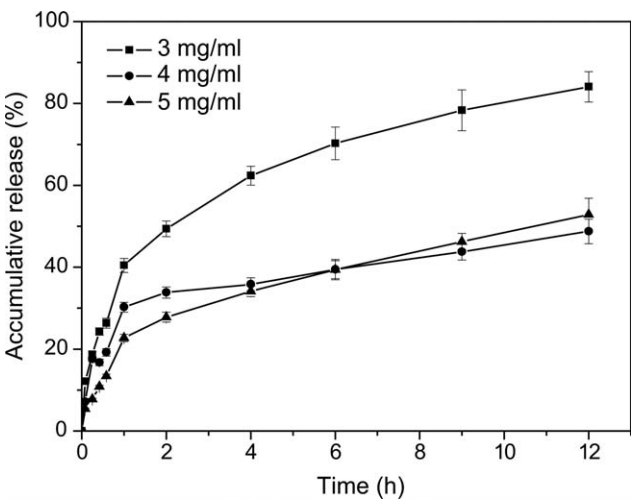

Figure 6 Influence of the CS concentration on the *in vitro* release of the 5-FU-loaded NPs.

on the *in vitro* release of the 5-FU-loaded NPs. The *in vitro* release rate of sample 10 was faster than those of samples 11 and 12. The accumulative release of sample 11 was less than 50% at 12 h. The accumulative release of CS microspheres entrapping 5-FU and microspheres designed for the colonic delivery of 5-FU even reached almost 90% in phosphate buffer systems at pH 7.4 for 1 h.[28,29] Yu et al.[30] reported that, under optimized reinforcement conditions, the drug release from CS/Alg microparticles could be effectively sustained, and the time for 50% release was about 8 h. Alg beads containing 5-FU were prepared by the gelation of Alg with calcium cations, and the accumulative release was more than 80% at 8 h.[31] The effect of the CS/Alg NPs in this study for the controlled release of 5-FU was comparable to that reported by Yu et al.[30] The CS/Alg complex membrane with a high CS concentration became thick because of the forced diffusion induced from the concentration gradient. The formation of a thick CS/Alg complex skin layer led to a decrease in the drug-release rate.

CONCLUSIONS

The size of our 5-FU-loaded CS/Alg NPs strongly depended on the Alg concentration, CS/Alg weight ratio, and poloxamer concentration. The size distribution of the 5-FU-loaded CS/Alg NPs could be modulated by poloxamer concentration. Stable 5-FU-loaded CS/Alg NPs (sample 11) of around 200 nm with a narrow size polydispersity were prepared with an Alg concentration of 4.6 mg/mL, a poloxamer concentration of 8 μg/mL, a CS concentration of 4 mg/mL, a $CaCl_2$ concentration of 8.1 mg/mL, and 200 mg of 5-FU. The results of the *in vitro* release of sample 11 in PBS at pH 7.4 showed that the accumulative release of 5-FU-loaded NPs was less than 50% at 12 h. CS/Alg NPs stabilized by poloxamer are promising for application in the controlled release of 5-FU.

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