Controlled Release of Berberine Hydrochloride from Alginate Microspheres Embedded Within Carboxymethyl Chitosan Hydrogels

Ai Mei Zhu,[1] Jian Hua Chen,[2] Qing Lin Liu,[1] Yu Liang Jiang[1]

[1]*Department of Chemical and Biochemical Engineering, National Engineering Laboratory for Green Chemical Productions of Alcohols, Ethers and Esters, The College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China*
[2]*Department of Chemistry and Environmental Science, Zhangzhou Normal University, Zhangzhou 363000, China*

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ABSTRACT: Berberine hydrochloride is a natural medicine with wide clinical application. In this article, berberine hydrochloride was entrapped into alginate microspheres via an emulsification/gelation method. The size distribution of the microspheres was determined by a laser particle sizer. Drug distribution within the microspheres was determined by confocal laser scanning microscopy. Those drug-loaded microspheres were further entrapped into carboxymethyl chitosan (CMC) hydrogel to form a new drug-delivery system (DDS). The surface morphology of the DDS was observed using metallographic microscopy and scanning electron microscopy (SEM). The compression strength of the DDSs with alginate microspheres was found significantly higher than that of the pure hydrogel. The drug-release performances of the DDS in phosphate buffer solution (PBS, pH 7.4), saline solution (pH 6.3), and hydrochloric acid solution (HAS, pH 1.2) were also studied. Decay of the DDS in PBS within 72–80 h results in a faster release; however, the steady release in saline solution could last for all the testing period without cleavage of the DDS. In HAS, because of the shrinkage of the DDS, release is fast in the first period and remains steady later. The DDS exhibits prospective in controlled steady release of drugs. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 120: 2374–2380, 2011

Key words: alginate microsphere; carboxymethyl chitosan hydrogel; berberine hydrochloride; controlled release

INTRODUCTION

Controlled delivery systems provide an alternative approach to regulate the bioavailability of therapeutic agents. In controlled drug-delivery systems (DDSs), an active therapeutic is incorporated into a polymeric network structure in such a way that the drug is released from the material in a predefined manner.[1,2] Researchers have strived to engineer the physical and chemical properties of DDSs to regulate their permeability, environmental response, surface functionality, biodegradability, and biorecognition sites to produce "intelligent" DDSs.[3–5] Tremendous efforts have currently focused on naturally occurring polymers such as sodium alginate and chitosan, which are gaining importance in the pharmaceutical field due to their unique properties such as good biocompatibility,[6,7] nontoxicity,[2,8] biodegradability,[3,4] and antimicrobial property.[9]

With well-documented properties and other positive traits (e.g., hydrophilicity, functional amino groups, and a net cationic charge), chitosan is a suitable polymer for the intelligent delivery of macromolecular compounds, such as peptides, proteins, antigens, oligonucleotides, and genes. Chitosan is either physically associated or chemically crosslinked to form a polymeric hydrogel, which does not require any toxic covalent linker molecules and is always safe for clinical applications. However, its widespread application is limited due to the weak mechanical strength and uncontrolled dissolution,[10] and the microsphere/hydrogel combination system is becoming a useful effective means.[11–13]

Calcium alginate microspheres have been used to deliver bioactive compounds, such as riboflavin,[14] interleukin-2,[15] and antigens.[16] However, the Na^+ ions present in the external solution undergo ion exchange with the Ca^{2+} ions that are binding with $-COO^-$ groups in the alginate microspheres.[17] As a

Correspondence to: Q. L. Liu (qlliu@xmu.edu.cn).
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result, the electrostatic repulsion among negatively charged $-COO^-$ groups increases, which ultimately enhances the gel swelling and induces a very serious burst effect. Many methods including the combination of a polymer and alginate were used to reduce the burst effect. Investigation showed that releases of the therapeutic could be lowered to 10–25% with increasing polymer crosslinking.[18–20]

With fluorescence, berberine hydrochloride has a variety of pharmacological effects, such as anticancer action,[21] antibiotic property,[22,23] and anti-inflammatory effect.[24] However, the absorption effect of oral berberine hydrochloride is poor, and the content of berberine hydrochloride[25] in the body by injection reduces too fast to take effect for 24 h. So, researchers prepared all kinds of DDS to solve this problem. Tsai et al.[26] incorporated berberine into chitosan hydrogel to prepare ointments and then investigated the physicochemical properties of the ointments and the release profile of berberine. The objective of this work is thus to develop a new intelligent DDS for controlled release of berberine hydrochloride. The drug-loaded alginate microspheres were entrapped into amphoteric carboxymethyl chitosan (CMC) hydrogel to overcome weak mechanical strength of chitosan and burst of alginate beads. The mechanical strength was tested. Swelling characteristics of these complex systems under different pHs were investigated. Additionally, profiles of drug from testing complex system in simulated media were studied.

MATERIALS AND METHODS

Materials

Berberine hydrochloride (98%) was purchased from Xian Feida Bio-tech Co., China. Alginate of high viscosity (1.05–1.15 Pa s), Tween-80, and ethylene diamine tetra-acetic acid (EDTA) were purchased from Xilong Chemical Plant, Guangdong, China. CMC with DS (degree of substitution) value of 80% was purchased from Zhejiang Aoxing Biotechnology Co. The other solvents and reagents including light liquid paraffin, of reagent grade, were purchased from Sinopharm Chemical Reagent Co.

Preparation of microspheres and hydrogels

Preparation of microspheres

Microspheres were prepared using a modified emulsion/gelation method.[27,28] Alginate (0.4 g) and berberine hydrochloride (0.1 g) were dissolved in deionized water to form a water phase. Twenty milliliters of the water phase were added into 40.0 mL of the oil phase (light liquid paraffin) with 2.0 mL of surfactant (span 80: tween 80 = 9 : 1, v/v) in a flask under mechanical agitation. Emulsion I was thus formed 5 min later. To avoid aggregation of microspheres, 10.0 mL of 25% $CaCl_2$ solution and 2.0 mL of ethanol were added into 20.0 mL of the oil phase with 1.0 mL of the surfactant (span 80: tween 80 = 9 : 1, v/v) to make emulsion II under vigorous agitation. The emulsion II was then added into the emulsion I to start the reaction for 10 min. The microspheres were then centrifuged and washed with ethyl acetate, ethanol, and deionized water successively to remove the oil completely.

Preparation of pure hydrogels

Five grams of CMC were dissolved in 100 mL of deionized water under agitation to form a 5% (w/v) CMC solution. Four milliliters of the resulting solution were then put into a weighing bottle with diameter of 24.0 mm. About 0.2 mL of 2.5% glutaraldehyde solution was added into the solution to initiate the crosslinking reaction. Pure hydrogel was formed in a minute. The sample was washed with distilled water and then preserved.

When the CMC was replaced by the berberine hydrochloride and CMC, the drug-loaded hydrogels were thus prepared.

Incorporation of microspheres into hydrogels

The steps for preparing the pure hydrogels were repeated to prepare the samples of DDS-I and DDS-II containing 10.0 and 20.0 mg of alginate microsphere, respectively, for property and release measurements. The weighed microsphere was dispersed into a few drops of water and then mixed with CMC solution under agitation. The mixture of CMC solution and microsphere was used instead of CMC solution in preparing the DDS.

Microsphere size determination

Microsphere size was determined by a laser particle analyzer (LS-POP III; OMEC, China). The microspheres were dispersed in distilled water and ultrasonically treated for 2 min before each measurement. The size distribution is calculated by the following equation:

$$\text{Span} = \frac{D_{90} - D_{10}}{D_{50}} \quad (1)$$

where D_{90} means 90% of the microspheres being smaller than this size, D_{50} means 50% of the microspheres being smaller than this size, and D_{10} means 10% of the microspheres being smaller than this size. Span factor reflects the size distribution.

Mechanical property

DDS should have certain mechanical strength, which is evaluated by the mechanical testing. The wet pure hydrogel and the DDS were freshly prepared to characterize their mechanical property using an electronic testing machine (WDS-5, Hongshan Equipment Plant, China). Sample was placed on the top surface of a cylindrical-shaped plate and compressed by another cylindrical-shaped plate at a constant speed of 1.0 mm min^{-1} until fragmentation occurred. Compression strength was calculated from compressive force at the time of fragmentation divided by the top surface area of the samples.

Morphology studies

Confocal laser scanning microscopy

Berberine hydrochloride is a natural drug with fluorescence. Confocal laser scanning microscopy (MRC1024, Bio-Rad, UK) was used to investigate the distribution of the berberine hydrochloride within the microspheres. The berberine hydrochloride-loaded microspheres were suspended in distilled water. One drop of the suspension was placed directly onto glass slides for examination, and the image was recorded on a computer linked with laser scanning confocal microscope. The berberine hydrochloride was detected using an argon laser with an excitation wave length of 488 nm.

Optical microscopy of DDSs

To determine the distribution of the microspheres in the CMC hydrogel, the freshly made DDS-I and DDS-II were placed onto glass slides and observed with a metallographic microscope (Nikon MA100, Japan). The surface morphology of the hydrogels was observed, and the image was recorded on a computer.

Scanning electron microscopy of DDSs

The freshly made DDS was frozen at −20°C for 12 h and then freeze-dried. Before observation, the resulting samples were sputtered with gold for 25 s. The surface morphology of the DDS was investigated using field emission scanning electron microscopy (FE-SEM LEO1530, Germany).

Swelling studies

The freeze-dried DDS was incubated in buffer within a pH range 1.0–10.0 in a sealed container at room temperature until the equilibrium was reached. The DDS was taken out and weighed after blotting the excess water on the surface with filter paper. Each experiment was done in triplicate. The swelling ratio (SR) is calculated using the following equation:

$$\mathrm{SR} = \frac{W_s - W_d}{W_d} \tag{2}$$

where W_s is the weight of the swollen DDS and W_d is the weight of the dried DDS. Buffer solution with a pH range 2.0–10.0 was prepared according to the literature.[29] Solution of pH 1.0 was prepared using hydrochloric acid. The ionic strength was adjusted to 0.15*M* by the addition of NaCl.

In vitro release of berberine hydrochloride

To study the drug-release performance, 4 mL of freshly made DDS was put into a 20-mL centrifuge tube. Five milliliters of release medium (solution with different pHs) were then added into the tube, which was subsequently left in a shaking water bath with a rotation rate of 50 r min^{-1} at 37°C ± 0.1°C. The tube was centrifuged at regular intervals, and the supernatant medium was taken out and analyzed using UV spectrophotometer (TU-1900, Purkinje, China) at 345 nm for the released berberine hydrochloride, and the release medium was replaced with the same amount of fresh solution. According to the amount of microsphere added and the supernatant drug content measured, the percentage of cumulative amount of the released berberine hydrochloride was calculated and plotted against time. Data in the figures show the mean ± standard deviation for each experiment that was repeated for at least three times.

Release ratio was calculated by the amount of accumulated release/the total amount of berberine in alginate microspheres. A little amount of NaOH was added into the 0.5% (w/v) EDTA solution to make the EDTA dissolved completely. Twenty milligrams of microspheres were put into the EDTA solution to accelerate the decomposition of the microspheres. And the solution was analyzed by UV spectrophotometer, and the concentration of berberine released from 20 mg of microspheres was further determined. Thus, the entrapment efficiency of berberine hydrochloride can be obtained.

RESULTS AND DISCUSSION

Preparation and size distribution of the microspheres

Dispersed alginate microspheres were prepared via a modified emulsification/external gelation method. Conventional method for preparing alginate microspheres usually included dropping calcium chloride solution directly into alginate emulsion, which

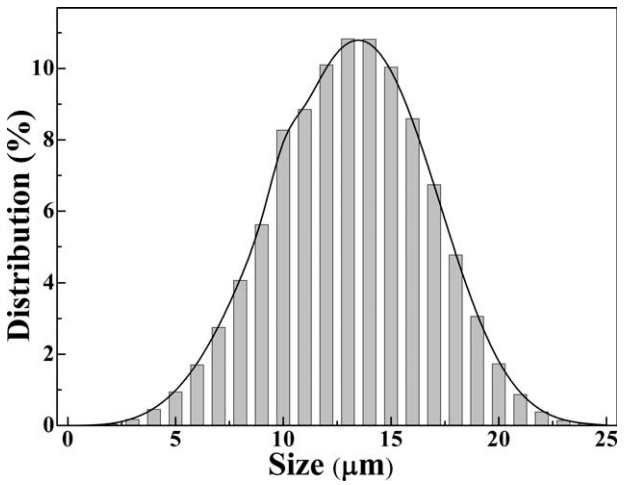

Figure 1 Size distribution of the microspheres prepared by a modified emulsion/gelation method.

would easily cause clumping of prepared microspheres as noted by literature[30] and our experiments. Therefore, the calcium chloride solution was replaced by emulsion in this work, and clumping was thus avoided.

Within a size range 1.0–25.0 μm, the microspheres percentage distribution is shown in Figure 1. The percentage distribution at size n ($1.0 \leq n \leq 25.0$) was obtained with the cumulative percentage at n minus the cumulative percentage at $n - 1$. The value of D_{10}, D_{50}, and D_{90} measured using LS-POP(III) was 7.61, 12.45, and 17.45 μm, respectively. Span factor was thus estimated to be 0.79.

The size of microsphere directly affects the drug release of microsphere and bioavailability, the amount of drug loaded, and the *in vivo* distribution and targeting. If the size of microsphere increased, the amount of drug loaded increased accordingly, whereas the release of drug from microspheres slowed down.

Morphology studies

The berberine hydrochloride shows bright fluorescence in the CLSM image (Fig. 2). The microspheres were scanned layer-by-layer from the top to the bottom. As can be seen from the picture (the middle layer), most of the berberine hydrochloride was well encapsulated into the microspheres, and the drug distribution was uniform.

The surface morphology of the DDS was characterized using both metallographic microscopy and SEM. Figure 3 shows that the two results agree with each other. Metallographic microscopy image is formed by reflected light, but some microspheres inside the hydrogel can also be seen as spherical doming in the image. This is because the hydrogel does not totally reflect light as metal does. Part of the light went into the hydrogel and then was reflected. For metallographic microscopy, samples should be prepared with smooth surface at the same height level as far as possible to avoid light scattering, which would affect the quality of image. More alginate microspheres can be seen in the DDS-II as expected. It seems to be that the microspheres can stay spherical after the process of incorporation into the hydrogels. Those microspheres embedded in the hydrogel network can be distinguished by combining the SEM with the metallographic photos.

Swelling performance

Swelling performance of the pure hydrogel, DDS-I, and DDS-II was tested in solution with different pHs. The experimental results suggest that samples are all "intelligent" DDSs and show similar swelling performance (Fig. 4). At pH below 8.0, a slight decrease in SR of DDSs was found when the amount of incorporated microspheres increased, which was accordant with the literature.[31] For the pure hydrogel, there existed isoelectric point (IEP) of CMC with a pH range 2.0–4.0. At IEP, the amount of NH_3^+ and $-COO^-$ in the amphoteric hydrogel is equal, intraionic attraction between opposite charges results in rare residual ionic group.[32] Hence, the mobile ions inside the hydrogel are in minimum number, and osmotic pressure makes the hydrogel shrink to a maximum degree. SR decreased with increasing microsphere content in the DDSs. This may be because alginate contains $-COO^-$ and the increase of $-COO^-$ content could enhance the electrostatic force between $-COO^-$ and NH_3^+. This hinders the stretch of polymer chain and lowers the SR.[33] A decrease in SR happened in a pH range 8.0–10.0. This is probably

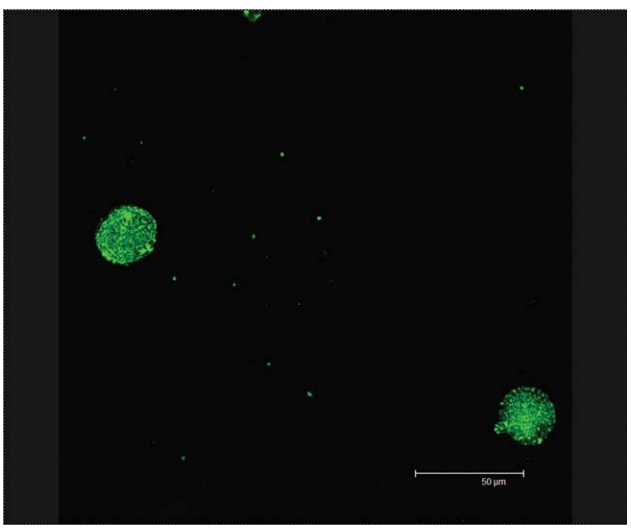

Figure 2 CLSM image of the berberine hydrochloride-loaded alginate microspheres immersed in water. [Color figure can be viewed in the online issue, which is available at www.wileyonlinelibrary.com.]

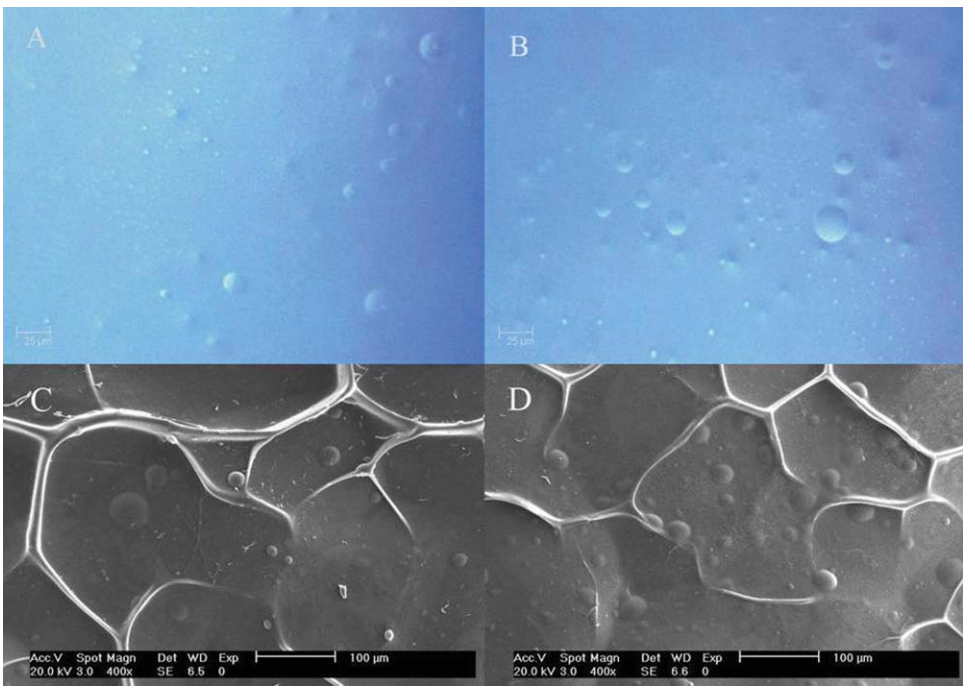

Figure 3 Surface morphology of the DDSs. Metallographic microscopy of the freshly made DDS-I (A) and DDS-II (B); SEM image of the freeze-dried DDS-I (C) and DDS-II (D). [Color figure can be viewed in the online issue, which is available at www.wileyonlinelibrary.com.]

that new crosslinks are formed through hydrogen bonding and totally deprotonated amino groups, leading to a decrease in polymer solubility.[32]

Mechanical property

The initial compression strength of the pure hydrogel, DDS-I, and DDS-II was estimated to be about 17.1, 25.6, and 23.3 kPa, respectively. A great increase in compressive property of the hydrogel was noted with the incorporation of microspheres. A little decrease in compressive property was observed with further increasing the amount of microsphere, as noted in the literature[33] in which compression modulus decreased from 12.5 to 11.6 kPa with increasing microsphere loading from 2 to 20%. The author gave an explanation that exclusion volume of microspheres would result in an increase in free volume of the crosslinked network. Increasing microsphere amount would cause some clumping of microspheres, which could be found in the literature[33] and some of our microscopy photos (not shown). And at those places with aggregated microspheres, hydrogel network would be not so well crosslinked and could not stand high force. This may be one reason for the decrease of compressive property.

In vitro release of berberine hydrochloride

The experimental results showed the entrapment efficiency of berberine hydrochloride being 81.3%. The content of berberine hydrochloride in microspheres was 10.6 wt %. Release of the berberine hydrochloride from the freshly made DDS was investigated at pHs 7.4, 6.3, and 1.2 for 80 h. Figure 5 shows the release performance of the berberine hydrochloride from DDS immersed in a phosphate buffer solution (PBS; pH 7.4) at 37°C ± 0.5°C. The results showed that the release was the fastest from the pure alginate microspheres, slower from both the DDS-I and DDS-II than the pure hydrogel, and the slowest from the DDS-I. Less than 20% of the loaded berberine hydrochloride was released within the first 24 h, whereas about 40% of the loaded

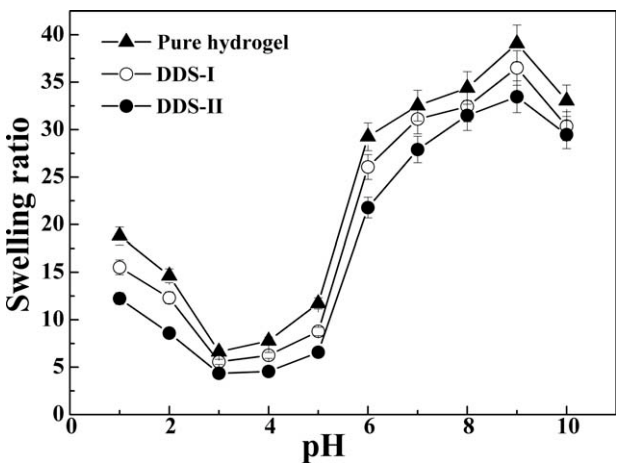

Figure 4 Swelling performance of the pure CMC hydrogel and the DDSs immersed in buffer with a pH range 1.0–10.0.

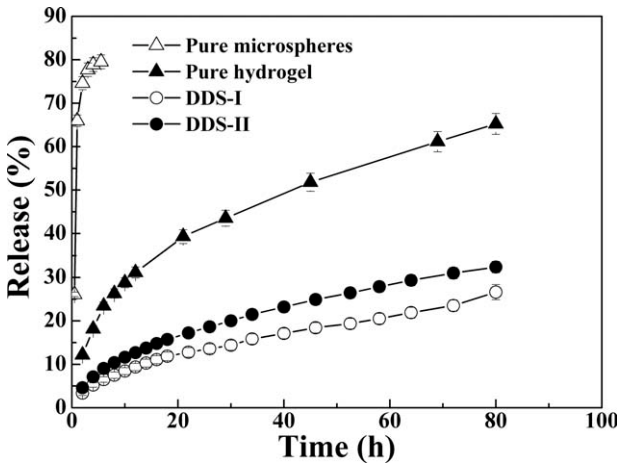

Figure 5 Release of the berberine hydrochloride from the DDSs immersed in a pH 7.4 PBS.

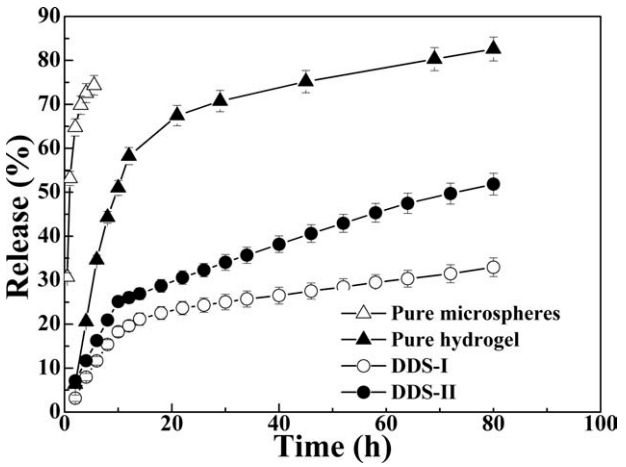

Figure 7 Release of the berberine hydrochloride from the DDSs immersed in a pH 1.2 hydrochloric acid solution.

berberine hydrochloride was released from the pure hydrogel. However, 79.55% of the loaded berberine hydrochloride was released from the pure alginate microspheres within 5.5 h. So, burst release was unremarkable for both the DDS-I and DDS-II. After a period of 20 h, the berberine hydrochloride was steadily released at a nearly constant rate, indicating the release mechanism being close to zero-order. Within 80 h, 65.24, 26.6, and 32.4% of the loaded berberine hydrochloride released from pure hydrogel, the DDS-I, and DDS-II, respectively. The DDSs composed of alginate microspheres and CMC reduced the burst effect of alginate microspheres.[34] However, the DDS-I began to decay, and berberine hydrochloride release became faster within a time range 72–80 h. Because of the ion exchange between calcium and sodium, erosion of alginate microspheres happened.[35] It is generally recognized that osmotic pressure and polymer elasticity are the opposite force to balance a system such as microsphere and hydrogel in solution.[36] In PBS, dissociative alginate is negatively charged. Thus, mobile ions inside DDSs caused high-ion osmotic pressure and accelerated the fission of DDSs,[37] which could be explained by Donnan equilibrium.[38]

Then, release study was performed by immersing the DDS into a 0.9% (w/w) saline solution (pH 6.3). The curves were similar to the results for PBS (pH 7.4) except that steady release could last for all the testing period without cleavage of the DDS. Within 80 h, 69.67, 32.2, and 46.2% of the loaded berberine hydrochloride released from the pure hydrogel, DDS-I, and DDS-II, respectively (Fig. 6).

To further understand the effect of pH on the release performance, we studied the berberine hydrochloride release by immersing the DDS into a hydrochloric acid solution (pH 1.2). As shown in Figure 7, 74.34% of the loaded berberine hydrochloride was released from the pure alginate microspheres within 5.5 h, while the release was biphasic for all the other samples. Release was faster in the first stage, because more than 35% of the loaded berberine hydrochloride released in the first 10 h; afterward, the release slowed down. Within the first stage, the DDS shrunk and the size of the DDS was about two-third of that of the freshly made DDS. This is mainly because —COO— ions convert into —COOH,[39] and the berberine hydrochloride solution was expelled out of the DDS. About 82.6, 33.0, and 51.9% of the loaded berberine hydrochloride released within 80 h for the pure hydrogel, DDS-I, and DDS-II, respectively.

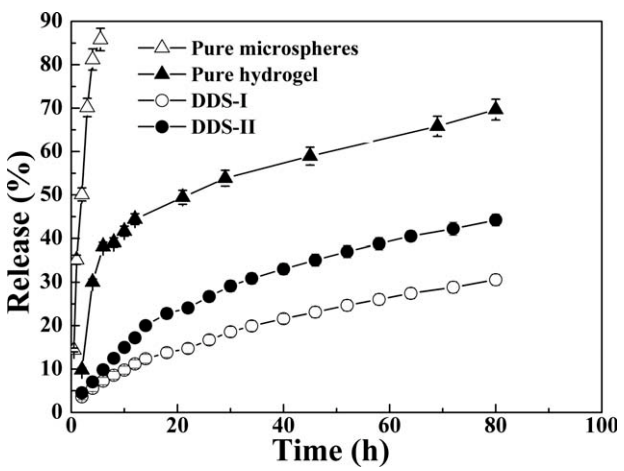

Figure 6 Release of the berberine hydrochloride from the DDSs immersed in a pH 6.3 saline solution.

CONCLUSIONS

With combination of alginate microspheres and CMC hydrogel, DDSs (DDS-I and DDS-II) are prepared for delivery of a natural drug (berberine hydrochloride).

The as-prepared DDSs (DDS-I and DDS-II) with alginate microspheres have better mechanical property than the pure CMC hydrogel. And the content of alginate microspheres affected the DDSs mechanical property. The DDSs with alginate microspheres and the pure hydrogel are all "intelligent" DDSs and follow a similar swelling discipline. The SR has a minimum at around pH 3.0 and a maximum within a pH range 8–10. DDSs composed of alginate microspheres and CMC decrease the burst effect of the alginate microspheres, which is supposed to be prospective in controlled release of drugs.

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