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# Preparation, Characterization, and Release Behavior of Ceftiofur-Loaded Gelatin-Based Microspheres

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**ABSTRACT:** Drug-loaded microspheres prepared from biomacromolecules have received considerable interest. In this article, we report a facile method for preparing ceftiofur-loaded gelatin-based microspheres for controlled release. We investigated the effects of factors, including the rotational speed, concentration of surfactant, concentration of gelatin, and ratio of water to oil (W/O), on the morphologies of gelatin microspheres and obtained the optimized conditions; for a typical average diameter of about 15 $\mu$m, these were 1000 rpm, a concentration of span 80 of 2.0%, a gelatin concentration of 20%, and a W/O of 1:20. Gelatin microspheres loaded with ceftiofur, ceftiofur-Na, and ceftiofur-HCl were prepared and characterized by scanning electron microscopy and laser light scattering. *In vitro* release studies were carefully performed for microspheres prepared with different crosslinker contents, loaded with different drugs, and blended with chitosan. The loaded ceftiofur showed an obviously longer release time compared with pure ceftiofur powder. A higher content of crosslinker led to a longer release time, but when the content reached 5%, the microspheres had a significantly cracked surface. The results also indicate that the blending of a small amount of chitosan could greatly prolong the release time. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 2369–2376, 2013

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## INTRODUCTION

Conventional oral drug administration does not usually provide rate-controlled release or target specificity. Over the past a few decades, much research has also been focused on degradable polymer microspheres for drug delivery.[1] Functional microspheres show many features, such as a large specific surface area, high diffusibility and mobility, stable dispersions, high uniformity, and variety in surface chemistry and texture, and hence have been widely used in various fields.[2,3] The administration of medication via such microspheres is advantageous because microspheres can be ingested or injected; they can be tailored for desired release profiles and, in some cases, can even provide organ-targeted release. Until now, various microspheres prepared from polymers or biomacromolecules have been used for drug release; these polymers and biomacromolecules include gelatin, chitin, chitosan, amylase, poly(lactic acid), and poly(glycolic acid).[1]

Gelatin is a denatured, biodegradable protein obtained by the acid and alkaline processing of collagen. The biosafety of gelatin has been proven through its long clinical usage as a plasma expander in surgical biomaterials and as an ingredient in drugs. It is extensively used for industrial, pharmaceutical, and medical purposes, including in microspheres for drug release.[4] To modulate the properties of gelatin microspheres, especially their release kinetics, modified microspheres have been synthesized by methods such as blending,[5–9] grafting,[10,11] chemical reactions such as amination,[12,13] and mineralization.[14–16] For example, Manjeshwar et al.[6] prepared semi-interpenetrating polymer network hydrogel blend microspheres from gelatin and hydroxyethyl cellulose by a water-in-oil (W/O) emulsion technique and used them to investigate the controlled release of theophylline, an anti-asthamatic drug. Tang et al.[8] designed and prepared poly(L-lactide-*co*-glycolide)/gelatin composite microspheres in porous scaffolds to prolong the release of bioactive factors. Curcio et al.[10] reported a novel class of microspheric hydrogels synthesized by the free-radical grafting of *N*-isopropylacrylamide and commercial gelatin. They observed that depending on the temperature of the surrounding environment, diclofenac sodium salt release took place by abrupt volume

**Table I.** Orthogonal Experiments for the Preparation of the Gelatin Microspheres

| Run | Rotational speed (rpm) | [Span 80] (% v/v) | [Gelatin] (% w/v) | W/O | Average diameter ($\mu$m) | SV |
|---|---|---|---|---|---|---|
| 1 | 1000 | 1.0 | 10 | 1:10 | 13.57 | 1.43 |
| 2 | 1000 | 1.5 | 15 | 1:15 | 18.41 | 4.44 |
| 3 | 1000 | 2.0 | 20 | 1:20 | 15.84 | 1.40 |
| 4 | 1250 | 1.0 | 15 | 1:20 | 16.52 | 1.86 |
| 5 | 1250 | 1.5 | 20 | 1:10 | 13.95 | 1.76 |
| 6 | 1250 | 2.0 | 10 | 1:15 | 24.48 | 2.80 |
| 7 | 1500 | 1.0 | 20 | 1:15 | 8.62 | 1.42 |
| 8 | 1500 | 1.5 | 10 | 1:20 | 17.78 | 1.99 |
| 9 | 1500 | 2.0 | 15 | 1:10 | 15.18 | 2.24 |

changes in the hydrogels and by the diffusion of the therapeutic drug through the polymeric network. Wang et al.[12] modified gelatin microspheres as a nasal drug-delivery system for peptide drugs. They proposed that aminated gelatin, a positively charged gelatin derivative, could significantly increase the nasal absorption of some kinds of drugs. Microspheres with organic–inorganic structures have also received considerable interest in recent years. Leeuwenburgh et al.[14] investigated the functional properties of gelatin–apatite composite microspheres for potential use as injectable bone substitutes. Habraken et al.[15] also found that gelatin microsphere/calcium phosphate cement composites could be applied for the sustained release of growth factors. Among these methods, blending is mostly used to prepare microspheres with desirable performances because of its low cost and simplicity.

Gelatin microspheres have been used for the controlled release of various kinds of drugs, including proteins such as lysozyme,[17] insulin,[12,13] anti-asthamatic drugs such as theophylline,[6] antihypertensive drugs such as Nifedipine,[11] a large number of growth factors,[16,18–21] anti-inflammatory drugs such as diclofenac sodium salt,[10] pesticides such as endosulfan,[22] and some other chemicals such as allyl isothiocyanate.[23] Ceftiofur is a broad-spectrum, third-generation cephalosporin that is approved for intramuscular use in food animals; it is often used in the form of ceftiofur sodium (ceftiofur-Na) and ceftiofur hydrochloride (ceftiofur-HCl). The release of ceftiofur has only been scarcely reported.[24–26] The objective of this study was to develop a model system based on gelatin microspheres for the release of ceftiofur by optimization of the parameters for preparing gelatin microspheres and to compare the release time with or without the addition of chitosan, which has a large number of crosslinkable amino groups. We expected to increase the release time by blending a small amount of chitosan, which would be useful in the design of drug-release materials.

## EXPERIMENTAL

### Materials

Gelatin (sample number 20090304) and carboxymethyl chitosan (chitosan, 75–85% deacetylated) was purchased from Sigma and was used as received. Ceftiofur, including ceftiofur sodium (ceftiofur-Na) and ceftiofur hydrochloride (ceftiofur-HCl), was received from Zhejiang Hisun Pharmaceutical Co., Ltd. (China). Span 80, paraffin oil, amino acetic acid, glutaraldehyde (GA), isopropyl alcohol, and the other chemicals were analytical grade and were commercially obtained from Sinopharm Chemical Reagent Co. (China).

### Preparation of the Gelatin Microspheres

The preparation of the gelatin microspheres was carried out according to a modified procedure reported by Tabata and Ikada.[27] Typically, a mixture of 50 mL of paraffin oil and 1 mL of surfactant Span 80 (sorbitan monooleate) was placed in a 100-mL sampling bottle, and then, 3 mL of an aqueous solution containing 0.6 g of gelatin, which was previously fully swollen by heating to 50°C overnight, was slowly added to the mixture with vigorous stirring; this was followed by emulsification by stirring for 15 min. The resulting emulsion was kept in an ice–water bath for another 30 min with vigorous stirring. Then a certain amount of GA (2, 3, 4, and 5% v/v) was added for crosslinking for 1 h followed by the addition of amino acetic acid to terminate any exposed aldehyde group introduced by GA. The resulting microspheres were filtered and successively washed by isopropyl alcohol two times and finally washed by acetone. After drying, gelatin microspheres were obtained. To further investigate the effects of factors such as rotational speed, concentration of span 80, concentration of gelatin, and W/O on the morphologies of microspheres, we designed orthogonal experiments with four factors and three levels, as shown in Table I.

### Preparation of the Ceftiofur-Loaded Gelatin Microspheres

The procedure for the preparation of ceftiofur-loaded gelatin microspheres was similar to the aforementioned method for the preparation of gelatin microspheres. A certain amount of ceftiofur (ceftiofur-Na or ceftiofur-HCl) and carboxymethyl chitosan, which has a large number of amino groups, were dissolved in a gelatin aqueous solution in which gelatin was previously fully swollen. For ceftiofur-Na and ceftiofur-HCl, to prevent extraction by isopropyl alcohol, the filtered microspheres were only washed by acetone and then dried by freeze dehydration.

### Morphological Observation of the Microspheres

The microspheres were observed by optical and scanning electron microscopy (SEM). For optical observation, the

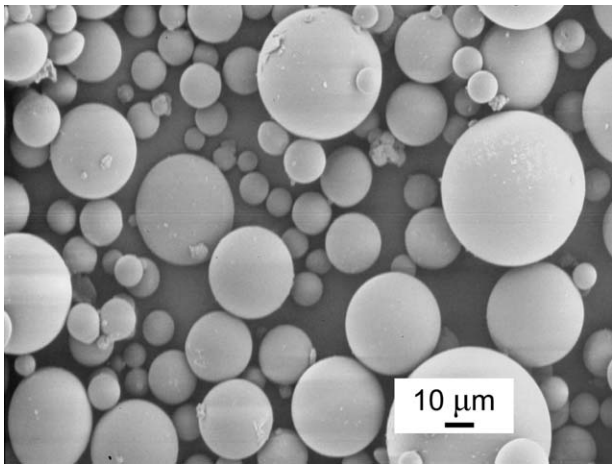


**Figure 1.** Morphology of the gelatin microspheres of run 3 observed by SEM.

microspheres were dispersed in saline and dropped on a clean glass slide. For SEM, the microspheres were sprinkled on double-sided adhesive tape attached to an aluminum stub and fixed onto a graphite surface. Excessive samples were then removed, and the stub was sputter-coated with gold. The coated samples were viewed under SEM at 25 kV to reveal the surface quality and porosity of the microspheres.

### Average Diameter and Particle Size Distribution of the Microspheres

The average diameter and size distribution of the microspheres were measured by a MasterSizer 2000 particle size analyzer on the basis of laser light scattering. Before measurement, the weighed microspheres were suspended in distilled water and vortexed. The resulting homogeneous suspension was used to determine the average diameter and particle size distribution. The average diameter was reported as the volumetric mean diameter, and the particle size distribution was evaluated by span value (SV), as defined as the following expression:

$$\mathrm{SV} = \frac{D_{90\%} - D_{10\%}}{D_{50\%}}$$

where $D_{N\%}$ ($N = 10$, 50, and 90) is that the volume percentage of microspheres with diameters up to $D_{N\%}$ equals to $N\%$. The smaller the SV is, the narrower the particle size distribution is (Table I).

### ζ Potential Measurement

ζ potential measurements were performed with a Zetasizer Nano ZC instrument (Malvern Instruments). Measurements were performed at a concentration of 100 g/mL, and the average values of at least 10 measurements were adopted as the ζ potential at pH 7.4.

### Loading Ratio of Ceftiofur

The microspheres were dispersed in 5 mL of 0.1*M* phosphate buffer (pH 6.0) under ultrasonic action for 30 min, and the resulting solution was kept on a horizontal shaker at 50 rpm for 2 h at room temperature. Afterward, the supernatant obtained by centrifugation of the solution was analyzed by high-performance liquid chromatography (HPLC). In brief, HPLC was performed with the chromatographic system consisting of an HPLC pump (model G1311A solvent delivery module, Agilent Co.), a variable-wavelength UV absorbance detector at 254 nm (model G1314A variable-wavelength detector, Agilent Co.) and an analytical column [TC-C18 (2), Agilent Co. 15 cm × 4.6 mm i.d.]. The mobile phase consisted of a mixture of 0.02*M* disodium hydrogen phosphate dihydrate buffer (pH 6.0, adjusted with ortho-phosphoric acid) and acetonitrile at a 78:22 ratio. The mobile phase was pumped into the column at a flow rate of 1.0 mL/min. The chromatographic system was kept at room temperature (23 ± 1°C). The injection volume was 20 μL. Afterward, the mobile phase was filtered through a 0.45-μm membrane filter and degassed under ultrasonic action for 30 min. Then, quantitative analysis was performed with external standardization and measurement of the peak area. All of the samples were determined in triplicate. The theoretical loading ratio ($R_{th}$), experimental loading ratio ($R_{exp}$), and entrapment ratio of the drugs ($R_{en}$) were determined with the following formulas:

$$R_{th} = \frac{m_0}{m_0 + M_1} \times 100\%$$

$$R_{exp} = \frac{m_1}{m_1 + M_1} \times 100\%$$

$$R_{en} = \frac{R_{exp}}{R_{th}} \times 100\%$$

where $m_0$ and $m_1$ are weights of the drug in the feed and the loaded drug, respectively, and $M_0$ and $M_1$ are the weights of the polymer in the feed and the loaded polymer, respectively.

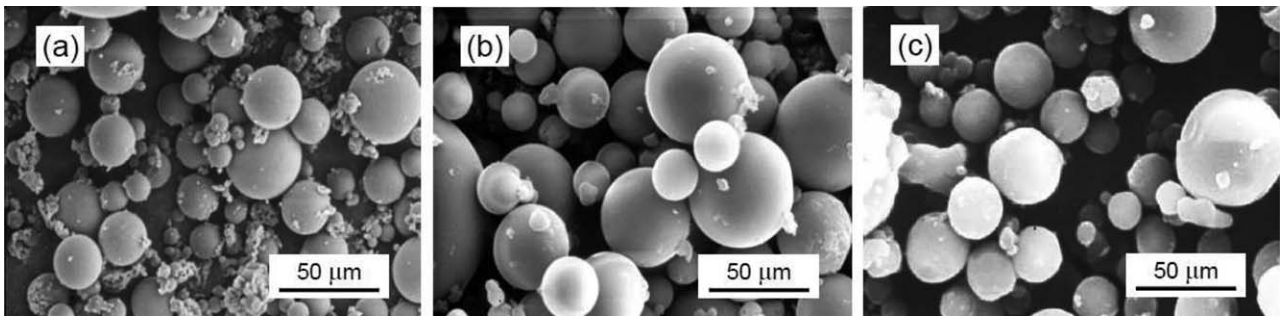


**Figure 2.** SEM images of the gelatin microspheres with a loading of (a) 15.79% ceftiofur-Na, (b) 13.85% ceftiofur-HCl, and (c) 13.68% ceftiofur.

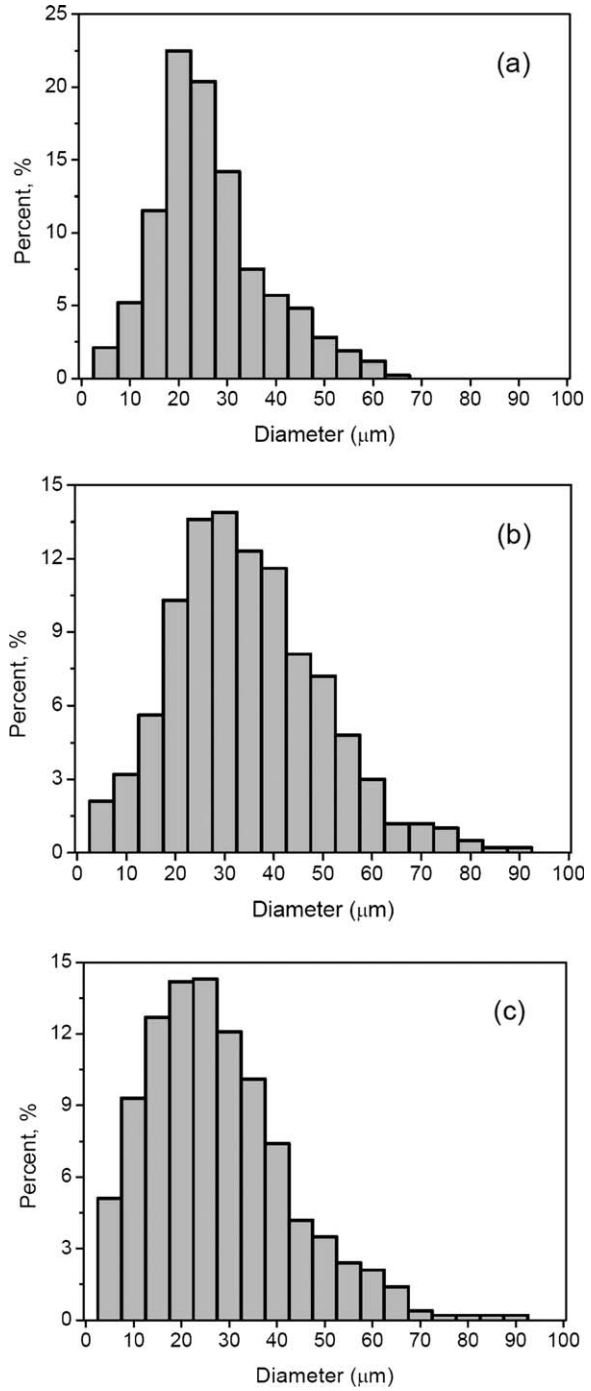


**Figure 3.** Size distributions of the gelatin microspheres with loading ratios of (a) 15.79% ceftiofur-Na, (b) 13.85% ceftiofur-HCl, and (c) 13.68% ceftiofur.

### *In Vitro* Drug-Release Studies

Ceftiofur released from the microspheres was investigated *in vitro* by a dialysis method. The drug-loaded microspheres (30 μg) were sealed into dialysis bags (Millipore dialysis tube, molecular weight cutoff = 8–14 kDa) and dialyzed against 50 mL of phosphate buffered saline (pH 7.4) at 37 ± 0.2°C in an water-bath shaker at 50 rpm for 48 h. The system was protected from light. The released medium was collected at 0.5, 1, 2, 4, 5, 8, 12, 16, 20, 30, and 40 h, and the whole medium was replaced with fresh phosphate buffered saline. The released amount of ceftiofur was determined by a UV spectrophotometer (UV-384 plus, Molecular Devices Corp.) at 254 nm. As for the control, all of the experimental conditions and procedures were the same as those of the ceftiofur-loaded microspheres, except that ceftiofur was released from 30 mg of ceftiofur powder. All of the experiments were run in triplicate.

## RESULTS AND DISCUSSION

A variety of methods have been developed for preparing microspheres; these include some new methods based on templating films with holes.[28–35] Some commonly used are the solvent evaporation technique (or the double-emulsion technique) and the spray-drying technique.[1] In this study, we prepared gelatin microspheres by an emulsion method. It is well known that many factors can affect the morphologies and size distribution of the resulting microspheres. These factors mainly include the rotational speed, concentration of surfactant, concentration of gelatin, and W/O. Therefore, we designed orthogonal experiments with four factors and three levels to optimize the synthesis of the gelatin microspheres. The experimental conditions and results are shown in Table I. The average diameter of the microspheres ranged from 8.62 to 24.48 μm. Furthermore, SV, which indicates the particle size distribution, also changed a lot with the synthesis conditions. Figure 1 shows the morphology of the microspheres, which were round, smooth, and without large pores. With the size distribution, average diameter, and yield taken into account, in run 3, which proceeded at a rotational speed of 1000 rpm, a concentration of span 80 of 2.0%, a gelatin concentration of 20%, and W/O of 1:20 were preferred.

Under the optimized conditions, ceftiofur-loaded gelatin microspheres were prepared. Figure 2 shows SEM micrographs of the gelatin microspheres with loadings of 15.79% ceftiofur-Na, 13.85% ceftiofur-HCl, and 13.68% ceftiofur. The use of the crosslinker had a great influence on the microspheres. Hiwale et al.[17] characterized the microstructure and the performance of gelatin microspheres crosslinked by two different crosslinkers, namely, D-glucose and GA, which produced two different crosslinked structures. They found that the surface of the GA-crosslinked microspheres was smoother than that of the crosslinked glucose. In this study, GA was used for our model system (it should be noted that it is better to avoid with some chemicals, e.g., GA, in the controlled release system for clinical application, although it can be easily removed by rinsing). Similarly, microspheres with a smooth surface were obtained. In addition, we found that the particles were round in shape. It should be noted that there were some very small particles for the ceftiofur-Na sample [Figure 2(a)]; this may have been due to the existence of ceftiofur crystalline. The results of the size distribution (Figure 3) indicated that the average diameter and size distribution were slightly different for these three kinds of microspheres and were induced by the properties of ceftiofur, such as solubility. The ζ potential was also measured. For gelatin microspheres with loadings of 15.79% ceftiofur-Na, 13.85% ceftiofur-HCl, and 13.68% ceftiofur, the ζ potential was −21.23, −22.16, and

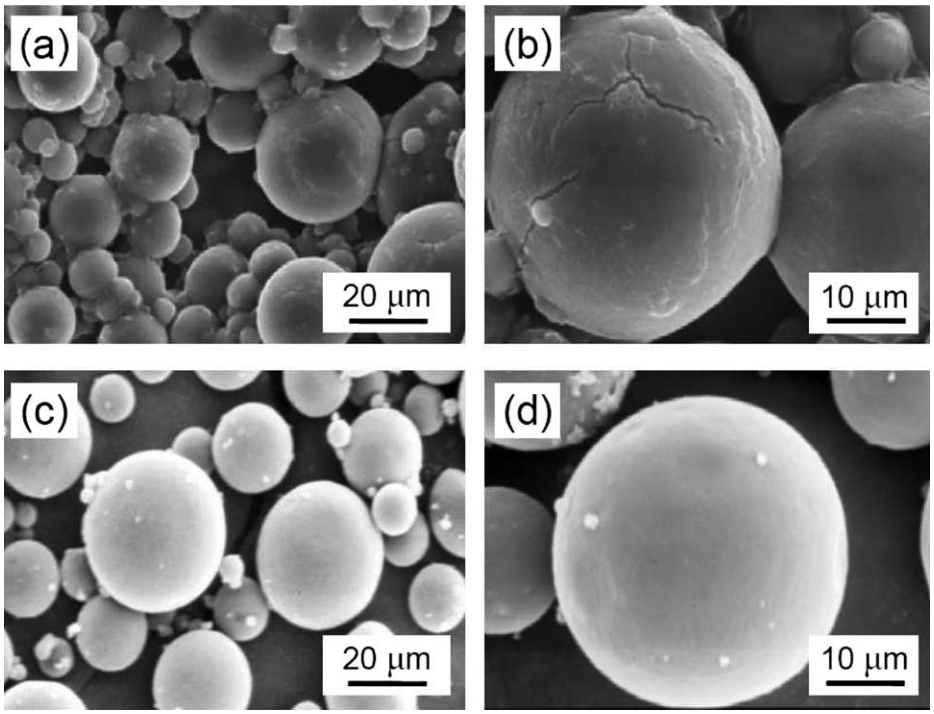


**Figure 4.** Effects of (a,b) 5% and (c,d) 4% GA on the morphology of the gelatin microspheres loaded with a loading ratio of 13.68% ceftiofur.

−20.09 mV, respectively. The ζ potential changed little as ceftiofur was mainly encapsulated in the microspheres.

We further investigated the effects of the concentration of the crosslinker GA on the morphology of the gelatin microspheres (Figure 4). A higher crosslinker concentration resulted in a denser particle structure and could hence prolong the release time. We examined a series of GA concentrations of 2, 3, 4, and 5%. The results reveal that the surface texture changed when it reached 5%. The cracks may have been caused by intense shrinking under too high a crosslinker concentration.

In fact, the addition of ceftiofur and the kind of ceftiofur (ceftiofur, ceftiofur-Na, or ceftiofur-HCl) affected the resulting drug-loaded gelatin microspheres. The detailed results are summarized in Tables II, III, and IV, in which microspheres loaded with different contents of ceftiofur-Na, ceftiofur-HCl, and ceftiofur are shown, respectively. It is clear that the average diameter increased first and then declined with the amount of ceftiofur in the feed, whether ceftiofur-Na, ceftiofur-HCl, or ceftiofur. The $R_{en}$ values also exhibited similar trends. In addition, we could obtain acceptable microspheres that had a good morphology and could be well dispersed with an $R_{th}$ value of up to 50% for the ceftiofur-Na system. However, it was limited to an $R_{th}$ of up to 33.33% for the ceftiofur-HCl and ceftiofur systems.

Then, we carefully studied the *in vitro* release of loaded ceftiofur from the microspheres. The most desirable release profile would show a constant release rate with time. However, in many cases, the release profiles are more complicated and often contain two main expulsion processes: the first with an initial burst of expelled medication from the sphere surface and the second, a usually more constant stage, with release rates dependent on diffusion and degradation.[1] Figure 5 shows the ceftiofur release profiles of the gelatin microspheres crosslinked with different contents of GA. In the case of the 2% microspheres, a sudden burst release of about 55% of drug was observed within the first 2 h, and a sudden burst release of about 62% was observed for the 3 and 4% microspheres. In the second stage, all three samples showed similar profiles, and the release times of the 3 and

**Table II.** Results of Gelatin Microspheres Loaded with Different Contents of Ceftiofur-Na

| Sample | Average diameter (μm) | $R_{th}$ (%) | Loading ratio (%) | $R_{en}$ (%) |
|---|---|---|---|---|
| 1 | 16.67 ± 3.21 | 11.11 | 8.00 ± 0.78 | 72.01 ± 5.22 |
| 2 | 20.25 ± 4.12 | 14.29 | 10.71 ± 0.65 | 74.95 ± 4.55 |
| 3 | 22.56 ± 4.09 | 20.00 | 15.79 ± 0.97 | 78.95 ± 4.85 |
| 4 | 27.10 ± 4.46 | 33.33 | 29.13 ± 1.22 | 87.40 ± 3.66 |
| 5 | 25.57 ± 4.33 | 40.00 | 32.48 ± 0.98 | 81.20 ± 2.45 |
| 6 | 23.94 ± 4.30 | 50.00 | 37.40 ± 1.46 | 74.80 ± 2.92 |

**Table III.** Results of Gelatin Microspheres Loaded with Different Contents of Ceftiofur-HCl.

| Sample | Average diameter (μm) | $R_{th}$ (%) | Loading ratio (%) | $R_{en}$ (%) |
|---|---|---|---|---|
| 1 | 18.23 ± 2.99 | 11.11 | 7.59 ± 0.33 | 68.32 ± 4.35 |
| 2 | 24.22 ± 4.21 | 14.29 | 9.18 ± 0.47 | 64.24 ± 5.12 |
| 3 | 29.71 ± 4.59 | 20.00 | 13.85 ± 0.71 | 69.25 ± 5.13 |
| 4 | 33.14 ± 4.63 | 33.33 | 21.37 ± 0.62 | 64.12 ± 2.90 |

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**Table IV.** Results of Gelatin Microspheres Loaded with Different Contents of Ceftiofur

| Sample | Average diameter ($\mu$m) | $R_{th}$ (%) | Loading ratio (%) | $R_{en}$ (%) |
|---|---|---|---|---|
| 1 | 17.78 ± 3.33 | 11.11 | 5.97 ± 0.48 | 53.74 ± 4.32 |
| 2 | 23.13 ± 4.25 | 14.29 | 8.01 ± 0.49 | 56.05 ± 3.43 |
| 3 | 25.44 ± 4.30 | 20.00 | 13.68 ± 0.71 | 68.40 ± 3.55 |
| 4 | 25.71 ± 4.67 | 33.33 | 18.76 ± 0.77 | 56.28 ± 2.31 |

4% microspheres were longer than that of the 2% system. They released about 90% at a time of 20 h. It is known that a drug encapsulated in a slowly degrading matrix provides the opportunity for slower release effects, but polymer degradation is not the only mechanism for the release of a drug. The drug release is also diffusion-controlled as the drug can travel through the pores formed during sphere hardening; this will be affected by the concentration of the crosslinker. We also tried to fit the release curves with some diffusion models but found they did not fit well; this may have been due to complicated diffusion behavior and other factors such as polymer swelling.[36,37]

The kinds of ceftiofur also influenced the release profiles of the corresponding microspheres. Ceftiofur-Na exhibited a fastest release rate when compared with ceftiofur and ceftiofur-HCl (Figure 6). This result was reasonable because it has been suggested that the factors affecting the drug-release rate revolve around the structure of the matrix where the drug is contained and the chemical properties associated with both the polymer and the drug. In some cases, drugs containing nucleophilic groups can cause increased chain scission of the polymer matrix; this also increases the rate of drug expulsion.[38]

Figure 7 presents the ceftiofur-Na release profiles of the gelatin microspheres with different loadings. Compared to the ceftiofur-Na powder, which released nearly 85% at the time of 2 h, the microspheres possessed significantly slower release profiles. The concentrations of ceftiofur-Na released from the

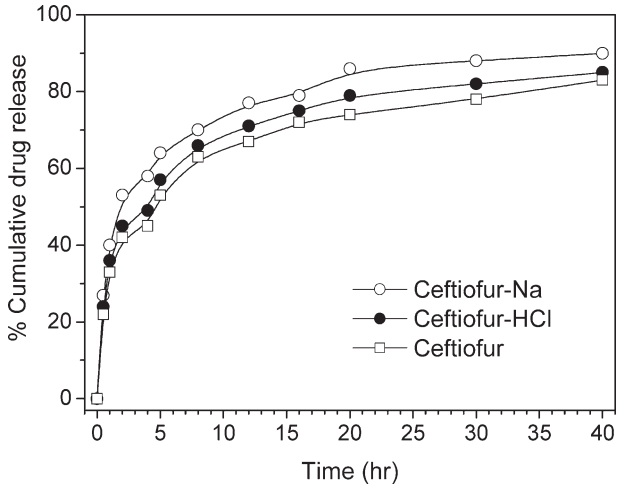


**Figure 6.** Release profiles of ceftiofur, ceftiofur-Na, and ceftiofur-HCl from the gelatin microspheres. The content of GA was 4%. The drug loadings were 15.79, 13.85, and 13.68% for ceftiofur-Na, ceftiofur-HCl, and ceftiofur, respectively.

microspheres loaded with 29.13% ceftiofur-Na were about 36, 48, and 77% at times of 0.5, 1, and 8 h, respectively. With decreasing drug loading, the release rate decreased accordingly.

To further modulate the release time of ceftiofur-Na from the gelatin microspheres, chitosan, with a large number of amino groups, was blended with gelatin to form microspheres. The physical blending of two polymers affects the release profiles of the polymer spheres. For example, Bidone et al.[5] prepared composite particles of poly(3-hydroxybutyrate), which is a biodegradable and biocompatible polymer with gelatin, for prolonged ibuprofen release from the microspheres. Our results also confirmed this point. As shown in Figure 8, the amounts of ceftiofur-Na released from the microspheres without chitosan were 27, 53, 70, and 90% at times of 0.5, 2, 8, and 40 h, respectively. However, if 3% chitosan was introduced into the gelatin microspheres, the release amounts of ceftiofur-Na significantly decreased to 19, 34, 51, and 77%, respectively. These results

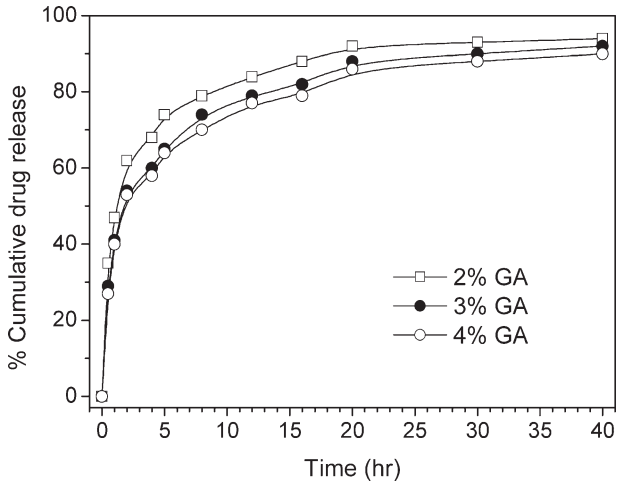


**Figure 5.** Ceftiofur-Na release profiles from gelatin microspheres crosslinked with different contents of GA. The loading of ceftiofur was 15.79%.

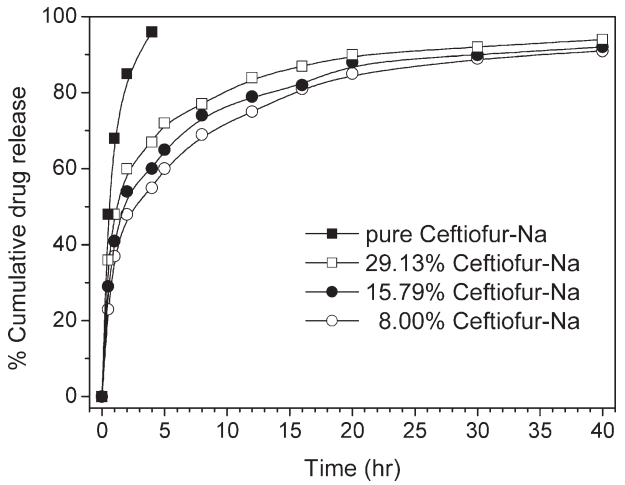


**Figure 7.** Ceftiofur-Na release profiles from the gelatin microspheres with different loadings. The content of GA was 3%.

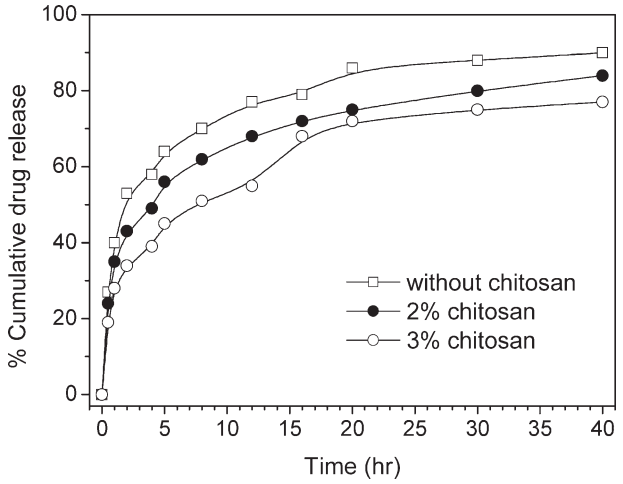


**Figure 8.** Ceftiofur-Na release profiles from the gelatin microspheres with or without chitosan. The content of GA was 4%, and the drug loading was 15.79%.

suggest that the gelatin microspheres blended with a small amount of chitosan are promising for the controlled release of ceftiofur. It should be noted that the release time was still not long enough for actual use in drug controlled release, although it was greatly extended by the introduction of chitosan. The release time may be improved by further optimization of some factors, such as the size of microspheres, which could affect the diffusion of drugs, or with other additives.

## CONCLUSIONS

Gelatin microspheres were prepared by the optimization of factors of rotational speed, surfactant concentration, gelatin concentration, and W/O. Ceftiofur, ceftiofur-Na, and ceftiofur-HCl were successfully introduced into the microspheres. We found that the properties of drugs showed slight influences on the morphology, average diameter, and particle size distribution of the gelatin microspheres. The loaded ceftiofur showed an obviously longer release time compared with the pure ceftiofur powder. A higher content of crosslinker led to a longer release time, but when the content reached 5%, the microspheres had a significantly cracked surface. The results also indicate that the blending of a small amount of chitosan greatly increased the release time; this is promising for the controlled release of ceftiofur.

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