Preparation of uniform sized chitosan microspheres by membrane emulsification technique and application as a carrier of protein drug

Lian-Yan Wang, Guang-Hui Ma*, Zhi-Guo Su

State Key Lab of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100080, PR China

Received 27 October 2004; accepted 18 April 2005
Available online 26 May 2005

Abstract
The control of size and size distribution of microspheres is necessary for obtaining repeatable controlled release behavior. The chitosan microspheres were prepared by a membrane emulsification technique in this study. Chitosan was dissolved in 1 wt.% aqueous acetic acid containing 0.9 wt.% sodium chloride, which was used as a water phase. A mixture of liquid paraffin and petroleum ether 7:5 (v/v) containing PO-500 emulsifier was used as an oil phase. The water phase was permeated through the uniform pores of a porous glass membrane into the oil phase by the pressure of nitrogen gas to form W/O emulsion. Then GST (Glutaraldehyde Saturated Toluene) as crosslinking agent was slowly dropped into the W/O emulsion to solidify the chitosan droplets. The preparation condition for obtaining uniform-sized microspheres was optimized. The microspheres with different size were prepared by using the membranes with different pore size, and there was a linear relationship between the diameter of microspheres and pore size of the membranes when the microspheres were in the range of micron size. The smallest chitosan microspheres obtained was 0.4 μm in diameter. This is the first report for preparing the uniform-sized chitosan microspheres by membrane emulsification technique. Uniform chitosan microspheres were further used as a carrier of protein drug. Bovine serum albumin (BSA) as a model drug was loaded in the microspheres and released in vitro. The effects of pH value, diameter and crosslinking degree of microspheres, and BSA concentration on loading efficiency and release behavior were discussed.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Chitosan; Microspheres; Uniform size; Membrane emulsification; Controlled release

1. Introduction
Chitosan is a functional linear polymer and can be derived by partial deacetylation of chitin. It is the most abundant natural polysaccharide on the earth after cellulose and can be obtained from exoskeleton of marine crustaceans such as crabs, lobsters, shrimps and krill. Chitosan is a copolymer consisting of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units linked with beta-(1→4) bonds [1,2]. Because of its excellent properties such as non-toxicity, biocompatibility, mucus-adhesion and biodegra-
tion, chitosan has been developed for a variety of biomedical applications including wound dressings and drug delivery systems [3,4]. In the last decade it has been proven that chitosan was a useful excipient, and it can be formed to microspheres with many kinds of drugs and vaccines. Chitosan-based drug delivery systems of microspheres for various vaccines have been developed and studied recently, such as influenza, pertussis and diphtheria antigens [5].

As a controlled release system for protein, peptide and vaccine, chitosan shows many advantages as follows: (1) chitosan is soluble in a weak acidic solution, and the use of organic solvent can be avoided, which is favorable for maintaining bioactivity of protein and peptide drug; (2) the amino group of chitosan are protonated in an acidic solution and the resultant soluble polysaccharide is positively charged, which can bind strongly to negatively charged surface such as cell surface and mucosa. Therefore, chitosan formulation can greatly improve the residence time of drug on tissues and cells and release the drug sustainedly there, as a result, the bio-availability of drug can be improved, the administration frequency of drug can be reduced; (3) as a vaccine delivery system, chitosan can stimulate immunity system and works as an adjuvant [6]. L. Illum et al. [5] found that chitosan-based influenza antigen produced much higher antibody level than other polymer-based antigen system.

There are a few methods for preparation of chitosan microspheres, such as emulsification/solvent evaporation [7,8], spray drying [9,10], ionotropic gelation [11–13] and coacervation technique [14,15]. In these methods, the emulsion is prepared by mechanical stirring method or spraying method firstly, then, the droplets are solidified with a proper method. The size of droplets or microspheres is difficult to control and the size distribution is very broad. This disadvantage will bring some limitations in applications: (1) the reproducibility of microspheres is poor among batches, which will result in poor repeatability of the release behavior and efficacy of drug among doses; it is also difficult to investigate the relationship between doses and treatment effects; (2) because the accumulated locations of the microspheres containing drug depend on the size of the particles, the bio-availability of drug will be low and the side-effects of the drug will be increased, especially for anticancer agents if the size distribution of microspheres is broad. Therefore, it is necessary to prepare uniform-sized microspheres and control the size of microspheres for their application in drug delivery system.

In order to obtain uniform-sized chitosan microspheres and control the size easily, a novel membrane emulsification technique was developed in this study. A porous glass membrane mainly consisting of Al₂O₃–SiO₂ was used. The pore size distribution of this membrane is very narrow, but it is highly hydrophilic, which is only suitable for preparing uniform-sized emulsion of O/W type. However, when W/O emulsion was prepared by it, the size distribution is very broad, because the necessary condition for obtaining uniform-sized droplets is that the interfacial tension between dispersed phase and the membrane should be high enough. Therefore, the membrane was modified to hydrophobicity in this study, then the chitosan aqueous solution was permeated through the pores of the membrane into the oil phase to form uniform-sized W/O emulsion under adequate pressure, finally the droplets of emulsion were crosslinked by glutaraldehyde. The effects of chitosan concentration, type of oil phase and emulsifier, volume ratio of water to oil phase on the uniformity of emulsion were investigated. Usually, the drug can be encapsulated in microspheres just by dispersing the drug in the chitosan aqueous phase when preparing W/O emulsion. However, in the case of protein drug which has amino group, the drug will be crosslinked inside the microspheres when the chitosan microspheres were solidified by crosslinking method. In this study, therefore, the BSA (model drug) was loaded in microspheres by adsorption method after the uniform chitosan microspheres were obtained, and the drug release behavior was studied in detail.

2. Experimental

2.1. Materials

Chitosan was purchased from Putian Zhongsheng Weiye Co., Ltd. (Fujian, China), and the degree of deacetylation is 89% and Mv (viscosity-average molecular weight) is 780000. Glutaradehyde and BSA (Bovine Serum Albumin) was obtained from Sigma-Aldrich Inc (Germany). PO-500 (Hexaglycerin penta)
ester) was purchased from Sakamoto Yakuhin Kogyo Co., Ltd. (Japan). KP-18C is provided by Shin-Etsu Chemical Co. (Japan).

2.2. Apparatus

The mechanism of membrane emulsification is shown in Fig. 1. A schematic diagram of the membrane emulsification equipment is shown in Fig. 2. A tubular porous glass membrane with the size of 2 cm (L) × 1 cm (φ) was installed into a module. The dispersed phase (aqueous phase) was stored in water tank (Teflon tank, 10 ml) which was connected to a nitrogen gas inlet. The dispersed phase was pressed by the nitrogen gas through the pores of the membrane wall into the continuous phase to form the droplets, and the continuous phase (oil phase) was stirred gently with a magnet bar in a beaker to prevent the creaming of the droplets. Under an adequate pressure of nitrogen gas, the uniform droplets were formed, and then stabilized by emulsifier (PO-500) dissolved in the oil phase. The pressure was different according to the pore size.

2.3. Modification of SPG membrane

A silane coupling agent with a C₁₈ hydrophobic chain (KP-18C) was used to react with the Si–OH

![Fig. 1. Principle of membrane emulsification (W/O emulsion).](image1)

![Fig. 2. Schematic diagram of small scale equipment for membrane emulsification.](image2)

![Fig. 3. SEM photographs of chitosan microspheres prepared by membrane emulsification technique. (a) before and (b) after modification of porous glass membrane.](image3)
group on the surface of the glass membrane to modify
glass membrane to hydrophobicity. The modification
process and conditions are as follows: the porous
membrane was ultrasonic wetted in distilled water
under sonification with intensity of 80 Hz for 30
min at first, then in 3% (v/v) KP-18C aqueous solu-
tion under vacuum of 0.05–0.06 MPa for 2.0 h, and
finally was heated under 120–140 °C for 4 h.

2.4. Preparation of chitosan microspheres

2.4.1. Membrane emulsification process

Chitosan microspheres were prepared by mem-
brane emulsification technique combined with chem-
ical crosslinking method. Briefly, chitosan was
dissolved in a 1 wt.% aqueous acetic acid containing
0.9 wt.% sodium chloride. A definite chitosan solu-
tion was poured into the Teflon tank as the dispersed
phase (aqueous phase), and then it was pressed
through the pores of the membrane into continuous
phase (oil phase: liquid paraffin and petroleum ether
in volume ratio of 7:5) under nitrogen pressure to
form W/O emulsion. Then GST (Glutaraldehyde Sat-
urated Toluene) was slowly dropped into the emulsion
to crosslink chitosan droplets at a stirring speed of 300
rpm for 1 h. Finally, the chitosan microspheres were
collected and washed two times with petroleum ether
by centrifugation of 3351×g and four times with
distilled water by filtration, and then the microspheres
were lyophilized. The standard experimental condi-
tions were as follows if it was not specified: pore size
of diameter was 4.7 μm and C.V. value of the mem-
brane was always below 10%, concentration of chit-
osan solution was 1.5 wt.%, oil phase was the mixture

Table 1

<table>
<thead>
<tr>
<th>Chitosan concentration (wt.%)</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number-average diameter (μm)</td>
<td>17.40</td>
<td>13.81</td>
<td>12.92</td>
</tr>
<tr>
<td>C.V. value (%)</td>
<td>21.94</td>
<td>13.35</td>
<td>19.70</td>
</tr>
</tbody>
</table>

The number of measurements (N) was 300.

Table 2

<table>
<thead>
<tr>
<th>Oil phase</th>
<th>Liquid paraffin/petroleum ether (7/5, v/v)</th>
<th>Cotton seed oil</th>
<th>Olive oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number-average diameter (μm)</td>
<td>13.81</td>
<td>13.92</td>
<td>13.83</td>
</tr>
<tr>
<td>C.V. value (%)</td>
<td>13.35</td>
<td>24.36</td>
<td>22.85</td>
</tr>
</tbody>
</table>

The number of measurements (N) was 300.
of liquid paraffin and petroleum ether 7:5 (v/v) containing 4 wt.% PO-500 emulsifier, volume ratio of water and oil phase was 1:10, amount of crosslinking agent was set as that the molar ratio between amino groups and aldehyde groups was 1:1, and the crosslinking time was 60 min.

2.4.2. Mechanical stirring method

Chitosan microspheres were also prepared by mechanical stirring emulsification method to compare with the results by membrane emulsification technique. Briefly, the chitosan aqueous phase and oil phase were prepared as the same as described above, then the W/O emulsion was formed by mixing oil phase with aqueous phase in a high speed of 1000 rpm agitating for 30 min. The chemical crosslinking, washing and drying processes and other experimental conditions were the same as those in membrane emulsification process.

2.5. Characterization of chitosan microspheres

2.5.1. Optical microscopic observation

Droplets before and after crosslinking were observed with an optical microscope. Diameter of approximately 300 droplets was counted to calculate average diameter. The number-average diameter (\(\bar{d}\)) and size distribution (C.V., coefficient of variation) were defined as follows:

\[
\bar{d} = \frac{\sum_{i=1}^{n} d_i}{N}
\]

\[
CV = \left( \frac{\sum_{i=1}^{n} (d_i - \bar{d})^2}{N} \right)^{1/2} / \bar{d} \times 100\%
\]

where \(d_i\) is the diameter of each droplet, \(N\) is the total number of droplets measured and \(\bar{d}\) is the number-average diameter.

2.5.2. SEM observation

The shape and surface feature of chitosan microspheres after drying were observed by a JEM-6700F scanning electron microscopy (SEM, JEOL, Japan). Microspheres were suspended in distilled water and the dispersion was dropped on aluminum foil and dried at ambient atmosphere. The sample was placed on a metal stub and coated with platinum under vacuum by an ion sputter (JFC-1600, JEOL, Japan).

2.5.3. Analysis of particle size distribution

The volume-average diameter (\(\mu m\)) and C.V. value of chitosan microspheres after drying were measured by laser diffractometry. Freeze-dried microspheres were re-dispersed in distilled water and sized by laser diffractometry using Ls230 Coulter (Coulter Co., USA). The diameter and C.V. values were calculated by averaging the results of three measurements.

2.6. BSA loading

After 5 ml of BSA aqueous solution [PBS (phosphate buffer solutions) with different pH value] containing different amount of BSA was mixed
with 5 mg of chitosan microspheres, the suspension was kept at 4 °C for 48 h under shaking to load BSA by adsorption. After a predetermined interval, the BSA-loaded chitosan microspheres were separated from the medium and the amount of BSA in the supernatant was determined by Coomassie Brilliant Blue protein assay. The loading efficiency (LE) for BSA was calculated according to the following equation:

$$\text{LE} = \frac{m_0 - m}{W} \text{ (mg BSA/mg microspheres)},$$

where $m_0$ and $m$ are the BSA mass with respect to the BSA medium before and after adsorption by chitosan microspheres. $W$ is the dry mass of chitosan microspheres used in the adsorption experiments. The effects of pH value, crosslinking degree and crosslinking time of chitosan microspheres, and BSA concentration on loading efficiency were studied.

### 2.7. In vitro BSA release study

BSA release from chitosan microspheres was studied in pH 7.4 PBS at 37 °C using a shaking air bath (100 rpm). Accurately weighted chitosan microspheres loaded with BSA were dispersed in 2

---

**Table 3**

<table>
<thead>
<tr>
<th>Pore size of membrane (μm)</th>
<th>0.5</th>
<th>4.7</th>
<th>5.7</th>
<th>10.2</th>
<th>19.6</th>
<th>Regression equations$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical pressure (kgf/cm$^2$)$^b$</td>
<td>2.00</td>
<td>0.134</td>
<td>0.104</td>
<td>0.021</td>
<td>0.006</td>
<td>$y = 2.3848x + 2.9201$</td>
</tr>
<tr>
<td>Diameter of droplets before crosslinking (μm)$^c$</td>
<td>0.78</td>
<td>13.84</td>
<td>17.30</td>
<td>26.54</td>
<td>49.87</td>
<td>$y = 1.1606x + 3.6957$</td>
</tr>
<tr>
<td>Diameter of droplets after crosslinking (μm)$^c$</td>
<td>0.65</td>
<td>8.7</td>
<td>10.06</td>
<td>16.62</td>
<td>26.06</td>
<td>$y = 0.6804x + 0.8764$</td>
</tr>
<tr>
<td>Diameter of microspheres after drying (μm)$^d$</td>
<td>0.39</td>
<td>3.92</td>
<td>5.30</td>
<td>7.26</td>
<td>14.38</td>
<td></td>
</tr>
</tbody>
</table>

The number of measurements ($N$) was 300.

$y$ stands for diameters of droplet before or after crosslinking or microspheres after drying and $x$ stands for pore diameter of membranes.

$^a$ The regression equations were obtained by using the results from the membrane with sizes 4.7, 5.7, 10.2 and 19.6 μm.

$^b$ Critical pressure: the minimum pressure above which the water phase can go through the pores of the membrane into oil phase.

$^c$ Measured by optical photographs.

$^d$ Measured by laser diffraction coulter.

---

Fig. 6. Size distribution of chitosan microspheres prepared by membrane emulsification and mechanical stirring method.
ml of release medium. 0.5 ml of the medium was periodically removed and replaced by the same quantity of PBS. The amount of BSA in the release medium was assayed by Coomassie Brilliant Blue protein assay. The BSA aqueous solution was used as control. The effects of various loading conditions of BSA on release behavior were studied.

Fig. 7. SEM photographs of chitosan microspheres prepared with different amount of crosslinking agent. Amino group: aldehyde group (mol:mol) (a) 1:0.4; (b) 1:0.7; (c) 1:1; (d) 1:1.5; (e) 1:2; (f) 1:3.
3. Results and discussion

3.1. Preparation results of uniform chitosan microspheres

3.1.1. Effect of hydrophobicity of the membrane

Porous glass membrane with uniform pore size used here is hydrophilic and not suitable for the preparation of W/O emulsion. The SEM photographs of chitosan microspheres prepared under the standard preparative condition before and after the modification of membrane by silane coupling agent are shown in Fig. 3, and their particle size distributions are shown in Fig. 4. It was evident that the hydrophilicity and hydrophobicity of the membrane strongly affected the uniformity of microspheres. Microspheres prepared with hydrophilic membrane were polydispersed (Fig. 3a), while those prepared with hydrophobic membrane were uniform (Fig. 3b). Furthermore, in the former case, the smaller particles adhered on the larger particles to form coagulates due to the high specific surface energy of small particles. Therefore, it is necessary to modify hydrophilic membrane to hydrophobicity to obtain uniform W/O emulsion and solidified microspheres.

3.1.2. Effect of chitosan concentration on uniformity of microspheres

Table 1 shows the average diameter and C.V. value of droplets in oil phase before crosslinking, prepared with different chitosan concentrations (1.0, 1.5, 2.0 wt.%) in aqueous phase, and other preparation conditions were as the same as the standard condition. The size of droplets in oil phase decreased with increase of chitosan concentration, and the C.V. value was smallest when the chitosan concentration was 1.5 wt.%. When chitosan concentration was lower (1.0 wt.%), the viscosity of chitosan solution was relatively lower, which resulted in lower critical pressure, therefore a smaller fluctuation of the pressure would affect uniformity of droplets. On the other hand, when the chitosan concentration was higher (2.0 wt.%), the viscosity of chitosan solution became so high that the droplets, formed on the opening of the membrane pore, adhered there for a longer time, resulting in the coalescence between the droplets. Therefore, it is necessary to choose suitable polymer concentration to prepare uniform droplets and microspheres.

3.1.3. Effect of oil phase on uniformity of microspheres

Oil phase is also an important factor affecting the uniformity of droplets and microspheres. A mixture of liquid paraffin and petroleum ether by volume ratio of 7:5, cottonseed oil and olive oil were used as the oil phase, respectively. Other preparation conditions were the same as the standard preparative condition. The number-average diameter and C.V. value of droplets in oil phase before crosslinking, prepared in different oil phase are shown in Table 2. From Table 2, it was known that the size distribution of droplets was broader when the cotton seed oil or olive oil was used. Again, this was because the viscosity of oil phase was higher, which resulted in the coalescence of droplets on the opening of the membrane pore.

From the above results, the optimum condition for obtaining uniform sized chitosan microspheres was as follows: concentration of chitosan solution was 1.5 wt.%, oil phase was the mixture of liquid paraffin and petroleum ether 7:5 (v/v) containing 4 wt.% PO-500 emulsifier, volume ratio of water and oil phase was 1:10 (v/v).

3.1.4. Comparison of membrane emulsification and stirring method

Chitosan microspheres prepared by membrane emulsification technique (membrane pore size: 0.5 μm) and stirring mechanical method, respectively, were compared. Other preparation conditions were

![Swelling degree (%) vs. Molar ratio between amino and aldehyde group](image)

Fig. 8. Effect of the amount of crosslinking agent on swelling degrees of chitosan microspheres.
the same with the standard condition. Figs. 5 and 6 show the SEM photographs and size distribution of chitosan microspheres prepared by membrane emulsification and mechanical stirring method, respectively. Compared with stirring method, microspheres by membrane emulsification were very uniform. Furthermore, uniform-sized microspheres with submicron size were successfully prepared by this novel membrane emulsification technique as shown in Figs. 5a and 6.

Fig. 9. SEM photographs of chitosan microspheres prepared with different crosslinking time (amino group:aldhye group (mol:mol)=1:1). Crosslinking time: (a) 20 min; (b) 40 min; (c) 60 min; (d) 80 min; (e) 100 min.
3.1.5. Control of the diameter of microspheres by pore size of the membrane

The diameter of the chitosan droplets in oil phase before and after crosslinking, and that of microspheres after drying, prepared by different pore size of membranes are summarized in Table 3. The diameter of droplets in oil was reduced after crosslinking by glutaraldehyde, and the size was further reduced after drying. Plotting the average diameter of the droplets in oil before and after crosslinking, and corresponding microspheres after drying as a function of pore size of the membranes, gives a linear relationship, respectively, and the regression equations are also shown in Table 3. By choosing the adequate pore size of membranes, the required size of the droplets or microspheres can be obtained, the membrane emulsification is an ideal method to prepare uniform and size-controllable droplets or particles. The nanosphere about 400 nm was obtained by using the membrane with pore size of 0.5 μm (Fig. 5a), however, it deviates from regression equations shown in Table 3, probably due to its nanosize. Therefore, the regression equations were obtained by using the results from the membranes with pore sizes of 4.7, 5.7, 10.2 and 19.6 μm.

3.1.6. Effect of crosslinking degree and time on morphology of microspheres

The microspheres with different crosslinking degree were prepared by adding different amount of glutaraldehyde (according to the molar ratio of amino group and aldehyde group). The SEM photographs and swelling degree of chitosan microspheres with different amount of crosslinking agent are shown in Figs. 7 and 8, respectively. The effect of crosslinking time on the morphology and swelling degree of microspheres was also investigated, the SEM photographs and swelling degree of chitosan microspheres are shown in Figs. 9 and 10, respectively. The surface of the microspheres with lower amount of crosslinking agent and shorter crosslinking time was wrinkled or not spherical, and the swelling degree was higher. The surface of the microspheres was smooth and spherical when the ratio of amino group and aldehyde group was more than 1:1 and crosslinking time was longer than 1 h. The swelling degree decreased with increase of the amount of crosslinking agent and prolongation of the crosslinking time.

Fig. 10. Effect of crosslinking time on swelling degrees of chitosan microspheres.

Therefore, the microspheres with required adequate swelling degree can be prepared by different amount of crosslinking agent and crosslinking time.

3.2. BSA loading into chitosan microspheres

The BSA loading into chitosan microspheres prepared under the standard preparative condition with membrane pore size of 4.7 μm were used to load BSA in medium with different pH value and various BSA concentrations. The equilibrium BSA loading efficiencies in the medium of various pH values are shown in Fig. 11. The BSA loading efficiency exceeded 0.3 mg BSA/mg microspheres when pH values were between 7 and 8. There showed a maximum BSA loading efficiency at pH 8.09, which reached 0.40 mg BSA/mg microspheres. Therefore, pH 8.09 was accepted in the following experiments. Fig. 12 shows the BSA loading efficiency of chitosan microspheres as a function of the amount of crosslinking agent. When the amino:aldehyde molar ratio decreased from 1:0.4 to 1:0.7, the BSA loading efficiency increased slightly. When crosslinking degree increased further, the BSA loading efficiency decreased gradually. With increase of the crosslinking degree, the rigidity of the microspheres was enhanced and its swelling degree decreased, as a result, the capacity of microspheres adsorbing BSA decreased. Fig. 13 shows the effect of different crosslinking time of chitosan microspheres on the BSA loading efficiency. With the increase of crosslinking time, the crosslinking degree of chitosan microspheres increased, the swelling degree of chitosan microspheres became lower. By the same reason, the BSA loading efficiency became lower. Fig. 14 shows the BSA loading efficiency as...
a function of BSA concentration in solution. When BSA concentration was below 4 mg/ml, the loading efficiency enhanced with increase of BSA concentration. However, little change was observed when BSA concentration was higher than 4 mg/ml, which indicated that there existed a saturated loading efficiency.

3.3. In vitro release profiles of BSA

The chitosan microspheres, prepared at the standard preparative condition with the membrane pore size of 4.7 μm and loaded BSA in pH 8.09, were used to investigate the release profiles of BSA in different pH values, the results are shown in Fig. 15. For each pH value, the initial release, so-called burst effect, was very high (59% and 52% for pH 2.0 and pH 7.4 respectively). After 120 h, the loaded BSA was almost released completely. This release behavior indicated that a large fraction of adsorbed BSA localized near the surface of chitosan microspheres and only less than 50% of adsorbed BSA entered the interior of the microspheres by diffusion. The BSA adsorbed near surface was released rapidly into release medium, which resulted in serious burst effect. The BSA which entered the interior of microspheres was released also by diffusion due to concentration gradient, which was much slower than that of the BSA near the surface. The release profiles of the microspheres prepared with different amount of crosslinking agent and crosslinking time are shown in Figs. 16 and 17, respectively. There showed higher initial release for the sample of higher crosslinking degree, compared with that of lower crosslinking degree. With elevation of crosslinking degree, the chitosan microspheres became more compact, therefore, it was more difficult for BSA to enter the interior of the microspheres, and a larger fraction of adsorbed BSA localized near the surface of the chitosan microspheres. By the same reason, higher initial release was observed as crosslinking time was prolonged, as shown in Fig. 17. No matter which crosslinking degree, a large fraction of adsorbed BSA localized near the surface of microspheres, resulted in serious burst effect and rapid release, so the difference of release rate among different crosslinking degree was not so apparent. Similar results were found by Francesco Castelli et al. [16], who prepared α, β-polyasparahydrazide (PAHy) microparticles crosslinked by glutaraldehyde (GLU).
and loaded diflunisal (DFN) in microparticles. The drug release profiles showed that the sample with higher crosslinking degree seemed to release DFN faster than that with lower crosslinking degree in the first 2 h. But after 2 h, this trend was not apparent. Fig. 18 shows the release behavior of the BSA loaded microspheres where BSA was loaded at different BSA concentrations. When the BSA concentration was lower, the amount of BSA loaded in the microspheres was lower, but the BSA could enter the interior of the microspheres and was released gradually. When the BSA concentration was higher, the burst release was also higher. This was because the amount of BSA entering the interior of the microspheres attained to saturation and a large fraction of adsorbed BSA localized near the surface of the microspheres.

Concluding the above results, the uniform-sized chitosan microspheres can be prepared by membrane emulsification technique, and the crosslinking degree can be adjusted by adding different amount of GST and varying crosslinking time, which not only affected the loading efficiency of BSA but also its release behavior. When decreasing the amount of GST and crosslinking time, the loading of efficiency of BSA increased and the release rate became slower. In addition, the release behavior of drug-loaded microspheres also can be adjusted by changing the preparation conditions of microspheres and loading conditions of drug. A lower burst effect and a relatively ideal release behavior was realized when BSA was loaded into microspheres at a lower BSA concentration. However, the BSA adsorbed was released relatively fast, and all of them were released within 1 week. In order to let the protein drug release more slowly, the drug should be loaded directly during the preparation of chitosan microspheres. Apparently, it is impossible if crosslinking agent is used because the protein drugs will be crosslinked inside of the microspheres, it is necessary...
to develop a physically solidification method, which is under study in our laboratory.

4. Conclusions

The uniform chitosan microspheres were successfully prepared by membrane emulsification technique. The key points for preparation of uniform chitosan microspheres are the modification of membrane into hydrophobicity, adequate chitosan concentration and viscosity of oil phase. Uniform chitosan microspheres whose C.V. value was around 10% can be prepared by optimizing preparative conditions. The optimum condition for preparation of uniform chitosan microspheres is that the chitosan concentration was 1.5 wt.%, the oil phase was a mixture of liquid paraffin and petroleum ether with a volume ratio of 7:5, the volume ratio of water and oil phase was 1:10, the oil emulsifier in oil was PO-500 and the amount was 4 wt.%. The uniform-sized chitosan nanospheres also can be prepared when the pore size of membrane was less than 1.0 \( \mu m \). The chitosan microspheres prepared by the membrane emulsification were used to load BSA by adsorption method. The BSA loading efficiency was highest when pH value was 8.09, and it decreased with an increase of the crosslinking degree. When BSA concentration was below 4 mg/ml, the loading efficiency increased with enhancing of BSA concentration. A maximum loading efficiency (0.4 mg BSA/mg microspheres) was obtained when the ratio between amino group and aldehyde group was 1:0.7, the crosslinking time was 40 min and the BSA concentration was 4 mg/ml. The release rate of BSA can be adjusted by changing crosslinking degree, pH value and BSA concentration in BSA solution.

The preparation of uniform microspheres and control of the diameter are important for drug controlled release. The membrane emulsification technique is a potential method to prepare uniform microspheres as drug carrier.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (20125616 and 20221603) and National High Technology Project (2004AA625040).

References

