Encapsulation of curcumin loaded oil droplets by cryotropic gel formation from O/W emulsion

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Abstract

Cryogel based encapsulation was attempted to entrap oil phase (containing curcumin) with a ternary system of colloidal chitosan, κ-carrageenan, and carboxy methylcellulose sodium salt (NaCMC). The cryotropic gel formation was investigated by varying the cooling rate during freezing and type of polymer suspension. The microstructure of the resulting curcumin cryogels revealed oil droplets entrapped in the cryogel matrix. The encapsulation yield for two types of suspension was found to vary from 83.89 to 99.37%. Controlled release of the curcumin in an aqueous system could be maintained for 4 days, and the released amount of curcumin was found to vary from 41.1-59.9%. The encapsulation yield as well as the released pattern and amount of curcumin were influenced by the cooling protocol used during freezing. The release patterns were found to be sensitive to the ambient aqueous pH and, interestingly, either a burst release or a first order release was achievable simply by changing the freezing condition. These results suggested that freezing could modify the gel formation of the present cryogel, and the resulting structural modification evidently controlled the oil encapsulation manner. The present ternary system (chitosan, κ-carrageenan, and NaCMC) is an interesting matrix for designing controlled release system in a food system.

Keywords: cryogel; curcumin; chitosan; carrageenan; carboxymethylcellulose; freezing

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1. Introduction

In the last decades, nano-micro encapsulation technology attracts wide interests to the food and pharmaceutical industry in various applications such as preservation of efficacy and controlled release of active food ingredients such as vitamin, polyphenol etc. It is anticipated that the increasing focus on food quality will lead to tighter regulations for food manufacturers to ensure the ingredient activity in a food until its consumption. The encapsulation manners could be classified into several types such as reservoir type, core-shell type, matrix type, etc. Several researchers are interested in preparing and applying hydrogel encapsulation to drug delivery system because hydrogels possess three-dimensional networks of hydrophilic polymers that are insoluble and swell in water. As a result, hydrogels have large quantities of space for stabilizing biologically active substances [1]. These gel structures usually possess nano-scale holes formed by cross-linked polymeric chains, and the sizes of these holes are determined by the characteristics of the polymers and the manner of gel formation. Therefore, by controlling the gelation process, a hydrogel of properly selected polymers can control the release behavior. Considering severe security regulations for food and pharmaceutical ingredients, it would be ideal to make various types of encapsulants from a single approved substance. Use of cryogels for encapsulation would be an interesting engineering strategy [2-5]. Cryotropic gelation makes use of sol-gel transition induced by localized concentration increase of the substrate due to dehydration by freezing (ice formation) [6, 7]. Controllable by a simple freezing operation, this cryotropic gelation would be handy and flexible for entrapping dispersed oil microspheres in a gel network. In this study, a technique to encapsulate an oil-soluble food nutrient into a hydrogel system was investigated. A curcumin loaded oil-in-water emulsion was prepared from a chitosan colloidal suspension, and the emulsion was converted into hydrogel by cryotropic gel formation. The obtained frozen samples were freeze-dried for preservation. Curcumin was subsequently extracted by immersion of the dried samples in a phosphate-buffered solution to determine the encapsulation yield in the matrix. The polymeric structure of cryogels was examined to understand the role of freezing. Release behavior of the encapsulated curcumin in an aqueous phase at selected pH conditions was investigated, and the influence of the freezing condition on the release characteristics was reported.

2. Materials and Methods

2.1. Materials

The chitosan granules used in this work were supplied from Tokyo Chemical Industry Co., Ltd., Japan. Measured by the authors using 1H-NMR the degree of deacetylation of this chitosan was about 82% [8]. Curcumin powder was purchased from Thai-China Industry Co., Ltd., Thailand. Commercial-grade carboxy methylcellulose sodium salt and κ-carrageenan powders were supplied from Sigma Co., Ltd., Germany. All the other chemicals used in this work, Tween 80, acetic acid, ethanol, triolein and sodium chloride, were of analytical grade. All chemicals were used as received.

2.2. Preparation of cryogels and freeze-dried specimens

The preparation process of cryogel samples was schematized in Fig. 1. First, a triolein solution containing 0.3 wt% of curcumin (oil phase) was mixed with a 3 wt% chitosan aqueous suspension containing 2 wt% of acetic acid and 5 wt% of Tween80, and this mixture was emulsified with a high-speed homogenizer (Heung Bo Tech. Co. Ltd., Korea) at 12,000 rpm for 5 min. The ratio of the oil phase to the aqueous phase was 10:90 (v/v). Next 5 % (w/v) of sodium chloride was added to the prepared o/w emulsion before being mixed with another polymer suspension consisting of κ-carrageenan, NaCMC and 5% of sodium chloride, and then homogenized at 12,000 rpm for 5 min to obtain a uniform colloidal
suspension. As noted in Fig. 1, two types of the polymer suspensions with different ratios of carrageenan to NaCMC were prepared and investigated.

Sample preparation was carried out by a freezing system with a contact plate heat exchanger. In this setup, the temperature of the sample was controlled by circulated coolant in the heat exchanger. A sample holder with a cylindrical hole (made from PTFE, diameter D=10mm, Height H=10mm) was set on the cooling plate, and the above colloidal suspension solution was placed in the sample holder. The set solution was cooled at a selected cooling rate (-0.5, -1.0, or -2.0 K/min) and frozen at -40°C. The completely frozen samples were subsequently freeze-dried for preservation. Curcumin was then extracted from the prepared dried samples in a solution to determine the encapsulation yield in the matrix.

3. Results and Discussion

Cryogels were prepared as schematized in Fig. 1. This process allows to form core-shell microparticles surrounded by cryogel membranes (as the optical microscopic images depicted in Fig. 1), and these microparticles were further embedded in hydrogel matrix. The purpose of the sodium chloride addition was to restrict gel formation between cationic and anionic polyelectrolytes (chitosan and carrageenan, respectively) caused by electrostatic attraction [9]. Because we aimed to control sol-gel transition that is caused by dehydration due to freezing, the selected amount of NaCl was added to the original polyelectrolytes suspension in order to avoid rapid and strong hydrogel formation at ambient temperature. \(\kappa\)-Carrageenan serves as a counterpart polyelectrolyte of chitosan. The gelling natures of chitosan/\(\kappa\)-carrageenan mixtures were expected to be tuned by replacing some of the \(\kappa\)-carrageenan with a weaker
anionic polyelectrolyte, namely NaCMC [10]. As shown in Fig. 2 (a-A), when sodium chloride was added after the chitosan-containing emulsion and carrageenan-containing suspension had been mixed, fibrous particles were observed because chitosan and carrageenan immediately formed gel by strong attraction between the positive charges of the amino groups of chitosan and the negative charges of the sulfonate groups of κ-carrageenan. As a consequence, chitosan and carrageenan were unavailable for interactions with any subsequently added Na+ and Cl− ions. On the contrary, Fig. 2 (a-B) shows that a nice colloidal suspension was obtained when sodium chloride was added to the emulsion before it was mixed with the κ-carrageenan-containing suspension. Therefore, the latter procedure was adopted throughout the remaining experiments. Some of the frozen specimens were thawed just after the completion of freezing in order to confirm the cryogel formation as shown in Fig. 2 (c and d). The freeze-dried specimens shown in Fig. 2 (b) were confirmed to be spongy.

![Sample images](image1)

Fig. 2. Sample images: (a) ternary mixture of the polymer suspension; (b) freeze-dried samples; (c) colloidal suspensions before and after freeze-thawing; (d) prepared cryogel (freeze-thawed)

![SEM images](image2)

Fig. 3. SEM images of cryogel specimens: (a) 150x magnification; (b) 400x magnification
3.1. Effect of the cooling rate on the yield of encapsulation in cryogels

Encapsulation yield of curcumin in cryogel matrix were listed in Table 1. It was shown that, as the cooling rate increased, the yield of encapsulation rose in tandem. Obviously, the cooling rate had a significant effect on this yield. Fig. 3 shows SEM images of the morphology of cryogel specimens at different magnifications. The specimens were obtained by mixing the o/w emulsion with polymer suspensions of Type A and B, respectively, freezing them at -20°C for 24 h, then thawing and drying them at 25°C. Fig. 3 (a) with a lower magnification shows the general shape of the cryogel network. At a higher magnification, Fig. 3 (b) reveals oil droplets encapsulated in the cryogel matrix.

Table 1. Encapsulation yield of curcumin in cryogels

<table>
<thead>
<tr>
<th>Specimen Code</th>
<th>Oil phase (%)</th>
<th>Mixed polymer Type A and B</th>
<th>Cooling rate (K/min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-1</td>
<td>10</td>
<td>A</td>
<td>-0.5</td>
<td>83.89</td>
</tr>
<tr>
<td>M-1</td>
<td>10</td>
<td>A</td>
<td>-1</td>
<td>94.49</td>
</tr>
<tr>
<td>F-1</td>
<td>10</td>
<td>A</td>
<td>-2</td>
<td>99.93</td>
</tr>
<tr>
<td>S-2</td>
<td>10</td>
<td>B</td>
<td>-0.5</td>
<td>95.15</td>
</tr>
<tr>
<td>M-2</td>
<td>10</td>
<td>B</td>
<td>-1</td>
<td>98.13</td>
</tr>
<tr>
<td>F-2</td>
<td>10</td>
<td>B</td>
<td>-2</td>
<td>99.37</td>
</tr>
</tbody>
</table>

Fig. 4. Release curves of curcumin from dried cryogels in a buffer solution with pH 7.4
3.2. Effect of the cooling rate on the release behavior of curcumin from cryogels

The results of curcumin release tests were plotted in Fig. 4. Release properties of an ingredient from a matrix show indirect but reasonable evidence of the characteristic of polymeric structure formed in cryogels. Note that S-1, M-1 and F-1 were prepared using polymer suspension type A, whereas S-2, M-2 and F-2 used polymer suspension type B. Obviously the released amount of curcumin increased as the cooling rate during freezing increased (Table 2). This means that the resulting microstructure of the gel network was dependent on its gel formation kinetics during sol-gel transition. A comparison between the release curves for specimens F-1 and F-2 made with the same formulation told us additional important information. Whereas F-1 showed a typical burst release curve, F-2 exhibited a moderate first-order release curve. It may be considered that, when the formulation was suitable, the rapid freezing condition would influence the formation of nano-micro structures (i.e. curcumin) of the present cryogels. Therefore, the present cryogel-based technique holds big potential for producing various types of hydrogel structures from a suitable formulation simply by changing the processing parameters.

Table 2. Total amount of released curcumin

<table>
<thead>
<tr>
<th></th>
<th>S-1</th>
<th>S-2</th>
<th>M-1</th>
<th>M-2</th>
<th>F-1</th>
<th>F-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amount released for 4 days [%]</td>
<td>41.1</td>
<td>43.3</td>
<td>48.7</td>
<td>48.3</td>
<td>58.5</td>
<td>59.9</td>
</tr>
</tbody>
</table>

3.3. Effect of the pH on the release behavior

Fig. 5 compares the release behaviors of the same formulation F-2 in aqueous buffers of pH 6.0 and 8.0, respectively. It was found that the total released amount of curcumin increased about 10% when the pH of the buffer solution decreased from 8 to 6. Thus it may be concluded that hydrogel would be a high-potential matrix for realizing a pH-controlled release system.

Fig. 5. Release test comparison in buffers of pH 6.0 and 8.0
4. Conclusions

A ternary system of chitosan, κ-carrageenan, and NaCMC can be used for hydrogel encapsulation of curcumin using cryotropic gel formation. The encapsulation yield and release behaviour of the ingredient was studied in various aqueous systems to obtain the following conclusions.
1. The encapsulation yield was influenced by the cooling protocol during freezing.
2. When the formulations were the same, the cooling protocol during freezing significantly affected the release behaviour of the ingredient.
3. The release patterns were found to be sensitive to the ambient aqueous pH and, interestingly, either a burst release or a first order release was achievable simply by changing the freezing condition. This pH dependency indicated that the present hydrogel matrix had high potential for realizing a pH-controlled release system.

References


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