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Preparation of Gelatin Microbeads With a Narrow Size Distribution Using Microchannel Emulsification

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ABSTRACT The purpose of this study was to prepare monodisperse gelatin microcapsules containing an active agent using microchannel (MC) emulsification, a novel technique for preparing water-in-oil (W/O) and oil-in-water (O/W) emulsions. As the first step in applying MC emulsification to the preparation of monodisperse gelatin microcapsules, simple gelatin microbeads were prepared using this technique. A W/O emulsion with a narrow size distribution containing gelatin in the aqueous phase was created as follows. First, the aqueous disperse phase was fed into the continuous phase through the MCs at 40°C (operating pressure: 3.9 kPa). The emulsion droplets had an average particle diameter of 40.7 μm and a relative standard deviation of 5.1%. The temperature of the collected emulsion was reduced and maintained at 25°C overnight. The gelatin microbeads had a smooth surface after overnight gelation; the average particle diameter was calculated to be 31.6 μm, and the relative standard deviation, 7.3%. The temperature was then lowered to 5°C by rapid air cooling and finally dried. The gelatin beads were dried and could be resuspended well in iso-octane. They had an average particle diameter of 15.6 μm, and a relative standard deviation of 5.9%. Using MC emulsification, we were able to prepare gelatin microbeads with a narrow size distribution. Since this emulsification technique requires only a low-energy input, it may create desirable experimental conditions for microencapsulation of unstable substances such as peptides and proteins. This method is promising for making monodisperse microbeads.

KEYWORDS: gelatin, beads, microcapsule, MC emulsification, narrow size distribution

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INTRODUCTION Gelatin is a fibrous protein that is produced from collagen [1-3]and forms an aqueous gel. Gelatin gels are widely used in various fields of application, such as photography, pharmaceuticals, cosmetics, and the food industry, because of the remarkable mechanical properties of gels and the natural biological origins of gelatin. In addition, gelatin is the most important wall material in the production of pharmaceutical microcapsules that contain an active agent [4,5].

Control of the microcapsule size and the size distribution has several important implications for controlled-release drug delivery [6-8]. For example, there is typically an ideal sphere size that provides the optimal release rate and route of administration. Several methodologies for microcapsule preparation have been described in the literature, including precipitation [9], spraying [10-13], phase separation, and/or emulsion techniques [14-16]. The emulsion approach is commonly used on both bench and industrial scales. Droplet size and size distribution are reproducible but often hard to control. Relative standard deviations equal to 25% to 50% of the mean diameter are not uncommon.

Microchannel (MC) emulsification is a novel technique for preparing water-in-oil (W/O) and oil-in-water (O/W) emulsions [17-19]. The MC plate has uniform microsized channels fabricated on a single-crystal silicon substrate using photolithographic and etching processes. Emulsions with a relative standard deviation of approximately 5% have been successfully prepared by applying this technique.

The purpose of this study was to prepare monodisperse gelatin microcapsules containing an active agent using MC emulsification. Simple gelatin beads with a narrow size distribution must be prepared as the first step in applying MC emulsification to the preparation of monodisperse gelatin microcapsules. MC emulsification was applied in

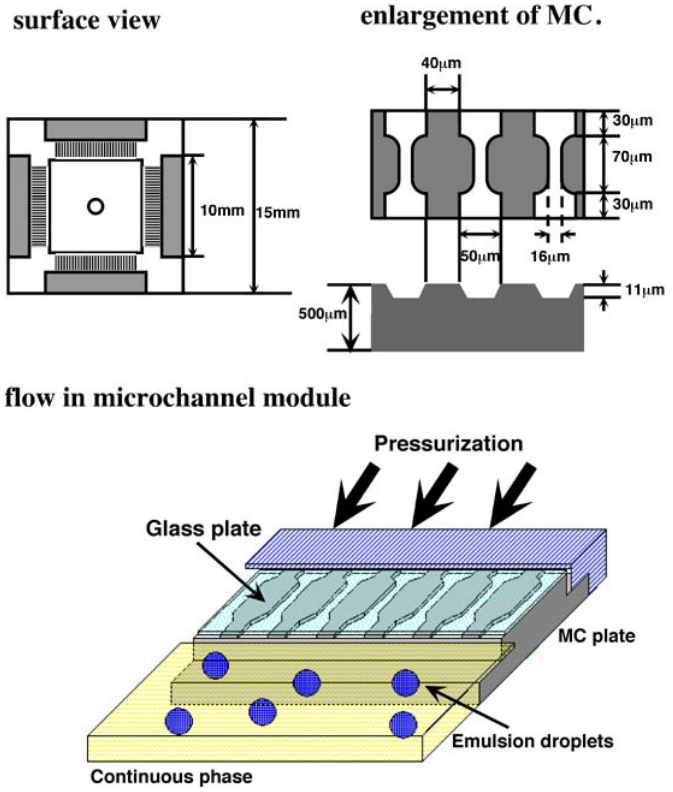

Figure 1. Schematic diagram of a silicon MC plate: (a) surface view of the silicon MC plate, (b) enlargement of the MCs, and (c) flow in the MC module.

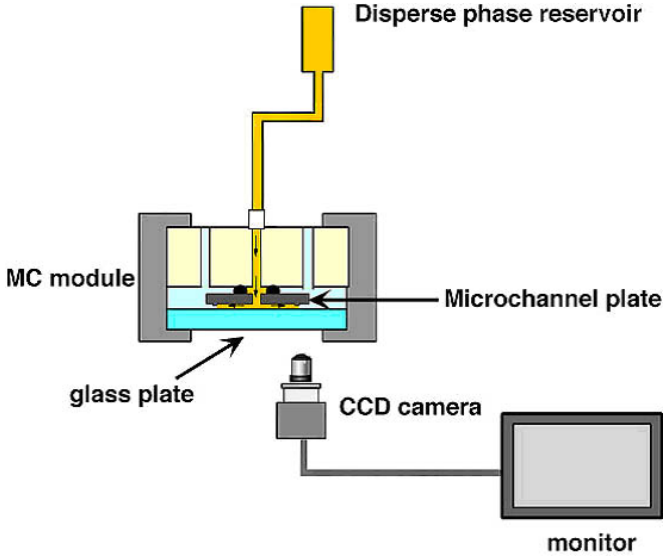

Figure 2. Experimental apparatus of the MC module and microscope video system. CCD indicates charge-coupled devices.

this study to the preparation of a monodisperse W/O emulsion, from which gelatin gel beads were obtained.

MATERIALS AND METHODS

Materials

Gelatin (type B: from calf bone, 300 Bloom) was kindly supplied by Nitta Gelatin Inc (Osaka, Japan). Iso-octane and n-hexane were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Octadecyltrichlorosilane was purchased from Aldrich Chemical Co, Ltd (St. Louis, MO) and used for surface modification of the silicon MC plate and the glass plate. Tetraglycerin condensed ricinoleic acid ester (TGCR) was kindly supplied by Sakamoto Yakuhin Kogyo Co, Ltd (Osaka, Japan). The water used in the experiments was Millipore (Mili-Q SP UF, Tokyo, Japan) purified (resistivity of 18 MW cm).

Methods

MC emulsification

Gelatin of 5% (wt/wt) was suspended in water with the pH adjusted to 7.4 using phosphate and allowed to swell for 30 minutes at room temperature. The sample was then dissolved at 60°C by stirring with a magnetic bar for 30 minutes. The sample temperature was then reduced and maintained at 40°C, to be used as the disperse phase. Iso-octane containing 5% (wt/wt) TGCR was used as the continuous phase.

Figure 1presents a schematic diagram of the MC plate and the MC emulsification process. A glass plate was firmly attached to the MC plate to cover the top of the slits fabricated on the MC plate and to form the MCs between them. Forcing the disperse phase into the continuous phase through the MCs created emulsion droplets. The silicon MC plate and the glass plate were originally hydrophilic because of silanol groups on the surface. As a rule, the disperse phase should not wet the MC plate and the glass plate for MC emulsification [18-20]. They were therefore modified with octadecyltrichlorosilane using a method described in a previous paper [17].

Figure 2depicts the experimental apparatus of the MC module and microscope video system. The emulsification process was monitored in real time through a glass plate by using an inverted microscope (CM-10; Nikon Co, Tokyo, Japan) to observe the MCs and to record the images through the eyepiece with an 8-mm videocamera (CV735; Shimadzu Co, Kyoto, Japan). The MC module was kept at 40°C to prevent gelation of the contained disperse phase. The continuous phase was fed at 30-minute intervals to recover emulsion droplets.

Preparation of gelatin microbeads

The temperature of the collected emulsion was reduced and maintained at 25°C overnight. The temperature was then lowered to 5°C by rapid air cooling and stirring for 60 minutes to completely solidify the droplets of the disperse phase. The gelatin beads were separated by suction filtration, washed quickly with hexane precooled to 5°C, and finally dried.

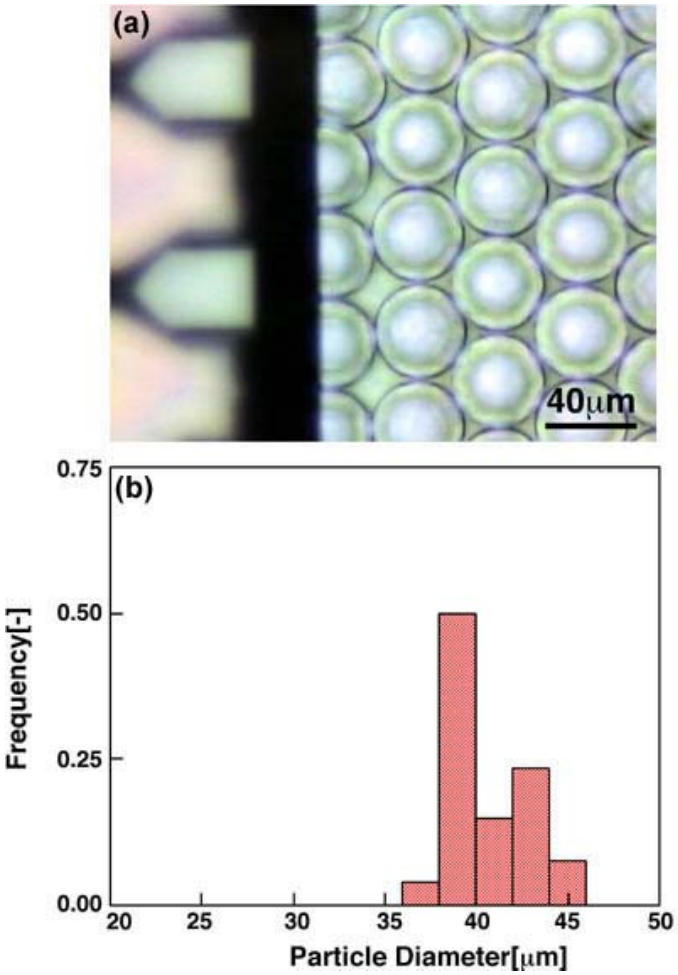

Figure 3. (a) Photomicrograph of the MC emulsification process for making emulsion droplets at 40°C. (b) Droplet diameter distribution of the emulsion. Disperse phase: 5%(wt/wt) gelatin solution; continuous phase: iso-octane with 5%(wt/wt) TGCR.

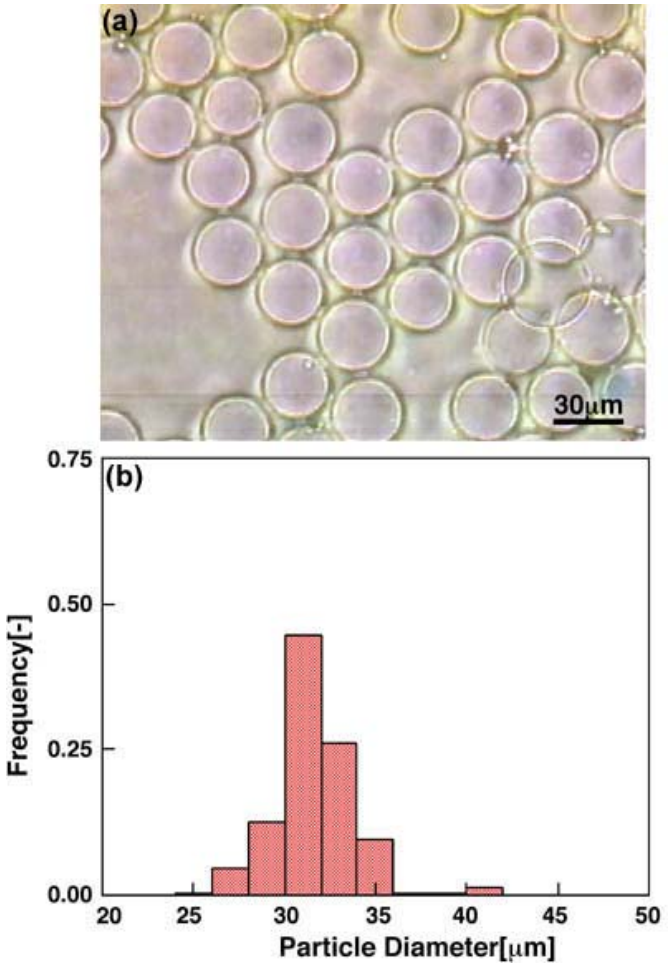

Figure 4. (a) Photomicrograph of gelatin microbeads after overnight gelation. (b) Particle diameter distribution of the microbeads.

Particle diameter analysis

The average particle diameters and relative standard deviations of the gelatin microbeads were determined by averaging the values of more than 200 diameters of gelatin microbeads measured from pictures taken with the microscope video system. The relative standard deviation (RSD) is represented by the following equation:

$$RSD = \frac{\sigma}{D_{av}} \times 100 \quad (1)$$

where D_{av} is the average particle diameter and σ is the standard deviation of the particle diameter. Winroof (Mitani Corporation, Fukui, Japan) software was used to analyze the captured pictures.

RESULTS AND DISCUSSION

Figure 3ashows a photomicrograph of the MC emulsification process for making gelatin solution droplets. Emulsion droplet formation resulting from forcing the disperse phase into the continuous phase through the MCs was observed in real time. The operating pressure of MC emulsification in this study was 3.9 kPa. **Figure 3b**illustrates the droplet diameter distribution of the emulsion made by MC emulsification. A monodisperse emulsion with an average particle diameter of 40.7 μm and a relative standard deviation of 5.1% was prepared.

Figure 4ais a photomicrograph of gelatin microbeads dispersed in iso-octane after overnight gelation. Gelatin microbeads with smooth surfaces were observed. The microbeads had an average particle diameter and relative standard deviation of 31.6 μm and 7.3% (**Figure 4b**). The gelatin microbeads could be well resuspended in iso-octane with ultrasonication after removal of the iso-octane and the drying process (**Figure 5a**). The gelatin microbeads suspended in iso-octane had an average particle diameter of 15.6 μm and a relative standard deviation of 5.9%, as shown in **Figure 5b**.

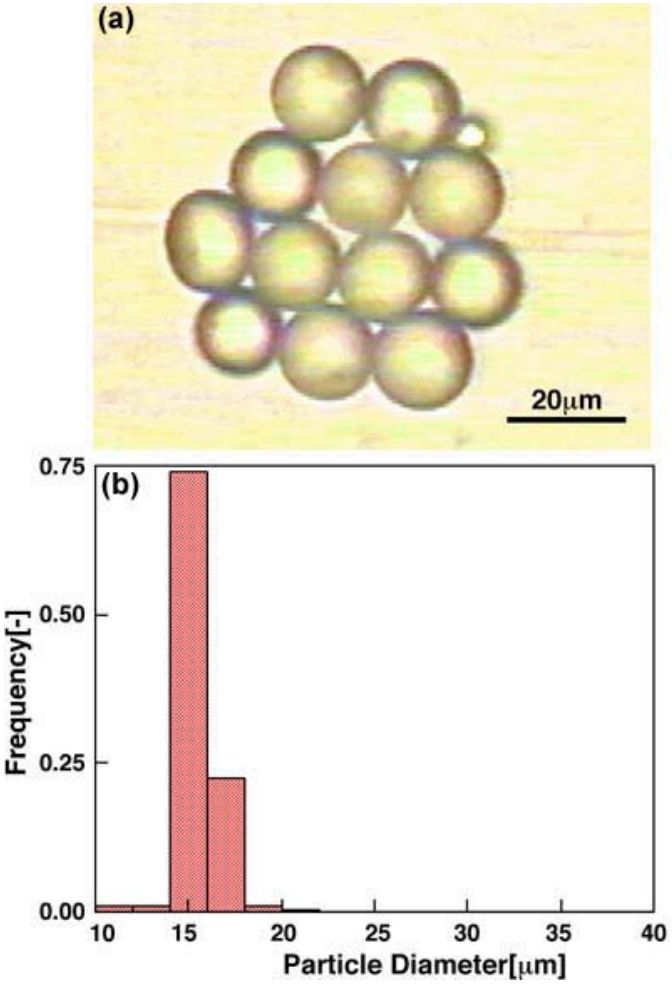

Figure 5. (a) Photomicrograph of the dried gelatin microbeads resuspended in iso-octane. (b) Particle diameter distribution of the microbeads.

Using MC emulsification, it was possible to prepare gelatin microbeads with a narrow size distribution. Monodispersity may play an important role in microcapsule behavior for controlling drug release rate dispersion. Sugiura et al proposed a droplet formation mechanism on an MC array in which a distorted disperse phase was cut off spontaneously into a spherical droplet by interfacial tension [21]. This droplet formation in MC emulsification requires only a low-energy input. It may thus enable microcapsules to be prepared in an environment without high shear stress. Peptides and proteins have attained increased importance as drug substances in recent years [22,23]. They are not very stable in most cases and therefore are less effective when administered orally. Sustained-release formulations must be developed to avoid infusions or to reduce the frequency of injections. Microbeads made of biodegradable polymers such as gelatin offer the potential to create optimal systems for controlled release. MC emulsification can be used for encapsulation of peptides and/or proteins because it requires only a low-energy input to create emulsion droplets.

This method is promising for making monodisperse microbeads. The average particle diameter decreased from 40.7 to 15.6 μm as the gelatin bead preparation proceeded, while the relative standard deviations were between 5.1% and 7.3%. Although the gelatin beads did shrink considerably on drying, the relative standard deviations remained almost constant at 5.1% to 5.9%.

CONCLUSION We succeeded in preparing gelatin microbeads with a narrow size distribution by using MC emulsification, a novel technique for preparing W/O and O/W emulsions. A monodisperse emulsion with an average particle diameter of 40.7 μm and a relative standard deviation of 5.1% was prepared using the technique. Gelatin microbeads dispersed in iso-octane after overnight gelation had smooth surfaces, with an average particle diameter and relative standard deviation calculated to be 31.6 μm and 7.3%. The dried gelatin microbeads could be resuspended well in iso-octane and had an average particle diameter of 15.6 μm and a relative standard deviation of 5.9%. The procedure used in this study is promising for making monodisperse microbeads.

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