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Preparation of calcium chloride-loaded solid lipid particles and heat-triggered calcium ion release

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Abstract−$CaCl_2$-loaded solid lipid particles (SLPs) were prepared by a melt/emulsification/solidification method. $CaCl_2$ microparticles (1-5 μm) could be obtained in a mortar with aid of the dispersant (Tween 80/Span80 (35/65, w/w)) when the ratio of $CaCl_2$ to dispersant was 2 : 0.1 (w/w). SLP was prepared by dispersing 0.42 g of micronized $CaCl_2$ particles in 2 g of molten PBSA, emulsifying the mixture at 85 °C in 40 ml of Tween 20 solution (0.5% w/v), and quenching the emulsion in an ice bath. The diameter of $CaCl_2$-loaded SLP was 10-150 μm. The unenveloped $CaCl_2$ could be removed by dialysis and the specific loading of $CaCl_2$ in SLP was 0.036 mg/mg. An EDS spectrum of $CaCl_2$-loaded SLP, which was dialyzed, showed that the unenveloped $CaCl_2$ was completely removed. Any excipients (dispersant, Tween 20, $CaCl_2$) had little effect on the melting point of SLPs. No appreciable amount of Ca^{2+} was released in 20-50 °C for 22 h. But the release degree at 60 °C was significant (about 2.3%) during the same period. The matrix of the lipid particle was in a liquid state at 60 °C, so $CaCl_2$ particles could move freely and contact the surrounding water, leading to the release. At 70 °C, the release degree at a given time was a few times higher than that obtained at 60 °C.

Keywords: Solid Lipid Particles, Calcium Chloride, Heat-triggered Release, Calcium Ion

INTRODUCTION

Encapsulation is a technology to envelop core materials with a coating or shell [1]. The objective of encapsulation is to reduce the adverse effect, control the release, and enhance the stability of the core materials [2]. Various kinds of capsules have been designed to release the core materials in a stimuli-triggered manner. Temperature change, pH change, light irradiation, magnetic field, electric filed and redox reaction can be used as a trigger for release [3]. Among them, temperature change is one of the most frequently used ones. Liposome (phospholipid bilayer vesicle), which releases its content with the respect to temperature change, has been developed by decorating the liposomal surface with temperature-sensitive polymers [4,5] or by taking advantage of the solid gel-to-liquid crystal transition of the liposomal membrane [6]. Another lipidic particle which undergoes the phase transition (e.g. melting) is solid lipid particles (SLP) [7]. Due to the easy preparation, the high shelf stability, the low toxicity, and the high bio-absorptivity, SLP was proposed to be used as a drug carrier for the transdermal delivery of an anti-inflammatory agent (e.g., Triptolide) [8,9], the intracellular delivery of genes [10,11], and the oral delivery of anti-cancer drugs (e.g., rifampicin, isoniazid and pyrazinamide) [12,13]. In this study, $CaCl_2$-loaded SLP (SLP/$CaCl_2$) were prepared by dispersing the $CaCl_2$ microparticles in molten fatty acid and emulsifying the molten fatty acid containing $CaCl_2$ microparticles in an aqueous solution. $CaCl_2$ microparticles loaded in SLP will hardly release in an aqueous solution once they are completely enveloped by the lipid matrix, which is impermeable to water. If the SLP suspension is heated to a temperature greater than the melting point of fatty acid, the SLP will become an emulsion droplet. Upon the melting of solid lipid matrix, the mobility of $CaCl_2$ microparticles in the lipid particles will markedly increase and the chance of $CaCl_2$ microparticles to be exposed to surrounding aqueous phase will also increase. As a result, $CaCl_2$ will dissolve out of SLP at a temperature greater than the melting point of fatty acid (Fig. 1). The release of $CaCl_2$ out of SLP was investigated by increasing the temperature of SLP suspension in the range of 20 °C-70 °C, and the amount of $CaCl_2$ released was determined by measuring the concentration of Ca^{2+} in the suspension. By taking advantage of the principle of heat-induced meting and release, calcium chloride-loaded SLP was newly developed for heat-triggered calcium ion release. Calcium chloride-loaded SLP was developed so that it can be used as heat-sensitive cross-linking particle for the preparation of bulky alginate hydrogel (it is described in detail in the section of Results and Discussion).

EXPERIMENTAL

1. Materials

Palm-based stearic acid (PBSA, stearic acid 38%/palmitic acid 61.5%) was provided by LG household and Healthcare. Tween 20, Tween 80, Span 80, calcium chloride, and alginate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Water was doubly distilled in a Milli-Q water purification system (Millipore Corp., Billerica, MA, USA) until the resistivity was 18 MΩ/cm. All other reagents were of analytical grade.

2. Preparation of $CaCl_2$ Microparticles

2 g of $CaCl_2$ powder and variable amount of dispersant were put in a mortar so that the weight ratio was 2 : 0.4, 2 : 0.2, 2 : 0.1 and

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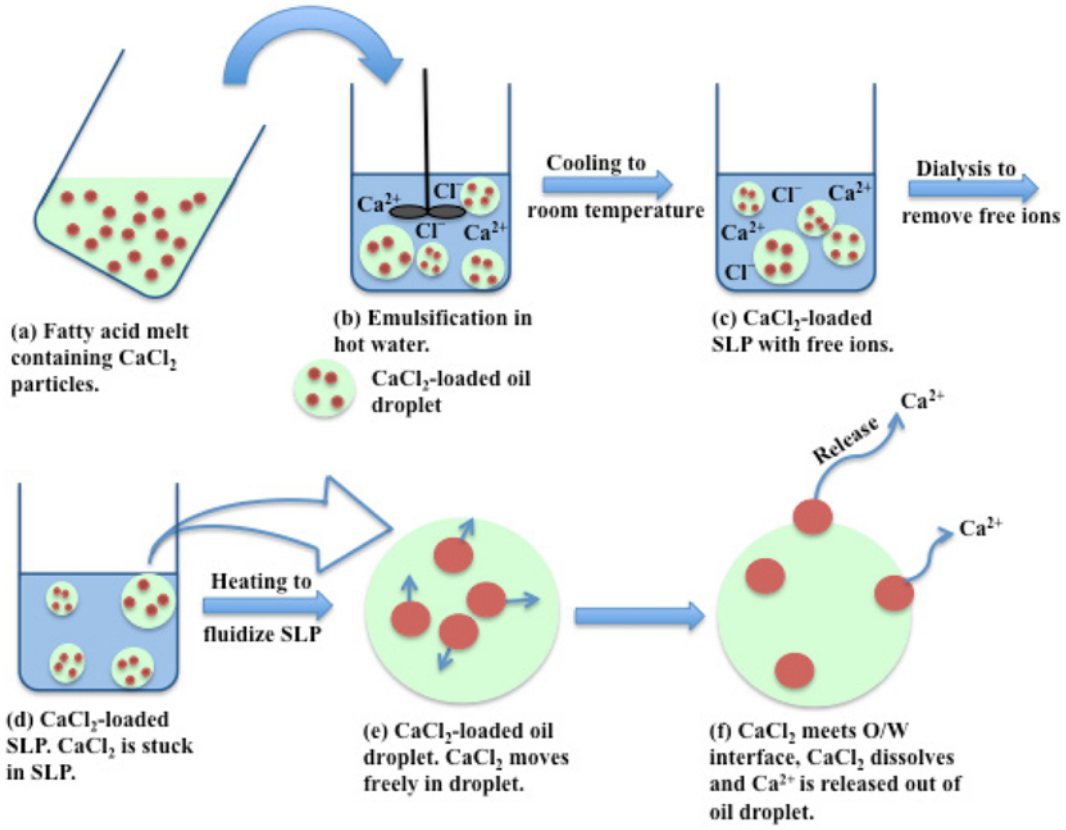

Fig. 1. Schematic diagram for preparation of $CaCl_2$-laoded SLP and heat-triggered calcium ion release out of SLP.

2 : 0. The mixture of Tween 80/Span80 (35/65, w/w) was used as dispersant. $CaCl_2$ powder with or without dispersant were ground in the mortar for 1 hr. The size of $CaCl_2$ microparticles was determined using an image analyzer (Vernier, LQ2-LE). $CaCl_2$ microparticle was prepared using the mixture of which $CaCl_2$/dispersant ratio was 2 : 0.4, 2 : 0.2, 2 : 0.1 and 2 : 0 will be termed as $CaCl_2$ (2 : 0.4), $CaCl_2$ (2 : 0.2), $CaCl_2$ (2 : 0.1) and $CaCl_2$ (2 : 0), respectively.

3. Preparation of Lipid Microparticles

0.2 g of Tween 20 (an emulsifier) was dissolved in 40 ml of distilled water contained in a 100 ml beaker so that the concentration was 0.5%, and the solution was heated to 85 °C. 2 g of PBSA was put in a 20 ml vial and it was melted in a water bath of the same temperature. While the aqueous phase was being stirred using a 3 cm-long magnetic bar at either 200 rpm, 400 rpm or 800 rpm, the molten PBSA was slowly put into the water phase and subsequently the two phase system continued to be stirred for 5 min at 85 °C for emulsification. Immediately after emulsification, the hot emulsion was cooled by immersing the emulsion-containing beaker in 500 ml of ice water (0.5 °C) contained bath and stirring the emulsion at 200 rpm using a 3 cm-long magnetic bar until the temperature of emulsion became around 20 °C.

To investigate the effect of dispersant amount on the size, the shape, and the thermal stability of SLP, variable amount of dispersant was added to 2 g of molten PBSA so that the weight ratio of PBSA to dispersant was 2 : 0.02, 2 : 0.05, and 2 : 0.1, 2 : 1, and 2 : 3. The mixtures of molten SA and dispersant were emulsified at 200 rpm and all other conditions were exactly the same as described previously.

When $CaCl_2$-loaded SLPs were prepared, 0.42 g of $CaCl_2$ (2 : 0.1) ($CaCl_2$ microparticle prepared using a mixture of which $CaCl_2$/dispersant ratio was 2 : 0.1) was added to 2 g of molten PBSA. The mixture of molten PBSA and $CaCl_2$ (2 : 0.1) was emulsified at 200 rpm and all other conditions were exactly the same as described above.

Unenveloped $CaCl_2$ microparticles were removed from SLPs by dialysis. The suspension of SLPs containing $CaCl_2$ (2 : 0.1) was freeze-dried, and 100 mg of the dry SLPs and 10 ml of distilled water were put together into a dialysis bag (MWCO 10,000). It was dialyzed in 800 ml of distilled water contained in a 1 L-beaker for 24 h and dialyzed again for another 24 h after the dialysate was replaced with the same amount of fresh distilled water. The concentration of Ca^{2+} in the dialysate was determined with time lapse using a calcium ion detector (Vernier, LQ2-LE).

4. SEM-EDS

The suspension of SLP containing $CaCl_2$ (2 : 0.1) was freeze-dried before and after unenveloped salt was removed by dialysis. The freeze dried SLPs were laid on metal stubs, coated with gold by sputtering technique. The SEM photos were obtained on a scanning electron microscope (Hitachi S-4800) installed at Central Laboratory in Kangwon National University. The composition of the SLP surface was examined by SEM-EDS analysis on the electron microscope equipped with energy dispersive X-ray spectrometer.

5. Differential Scanning Calorimetry

PBSA, dry SLP, dry SLP containing dispersant, and dry SLP

containing $CaCl_2$ (2 : 0.1), 5 mg of each was weighed into an aluminum pan (Tzero), and the DSC curves were recorded in 20-100 °C on a differential scanning calorimeter (DSC Q2000, TA Instruments, USA) at the heating rate of 2 °C /min.

6. Release of Ca^{2+} from SLP

20 mg of dry SLP containing $CaCl_2$ (2 : 0.1), subjected to dialysis and thus free of free salt, was put into 2 ml of distilled water contained in a glass vial. The temperature of the SLP suspension was kept to 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C for 22 h by immersing the vial in a water bath. The concentration of Ca^{2+} in the SLP suspension was detected using a calcium ion detector (LQ2-LE). The release degree was defined as the percent of the amount of $CaCl_2$ released with respect to the total amount of $CaCl_2$ enveloped in SLP.

RESULTS AND DISCUSSION

1. Preparation of $CaCl_2$ Microparticles

Fig. 2(A) shows the optical microphoto of $CaCl_2$ (2 : 0.4), $CaCl_2$ (2 : 0.2), $CaCl_2$ (2 : 0.1) and $CaCl_2$ (2 : 0). Large lumps rather than particles were found on the photo of $CaCl_2$ (2 : 0.4) (Fig. 2(A)(a)). When $CaCl_2$ to dispersant ratio was 2 : 0.4, $CaCl_2$ powder was completely wet due to the excess amount of dispersant; thus the ground mixture ($CaCl_2$ (2 : 0.4)) was a kind of paste. On the other hand, dispersed particles were found on the photo of $CaCl_2$ (2 : 0.2), $CaCl_2$ (2 : 0.1) and $CaCl_2$ (2 : 0). Fig. 2(B) shows the size distribution of $CaCl_2$ (2 : 0.2), $CaCl_2$ (2 : 0.1) and $CaCl_2$ (2 : 0). The size of $CaCl_2$ (2 : 0.2) was a few to tens of μm and the particles in the range of 3-4 μm were the most frequently found. The particle size greater

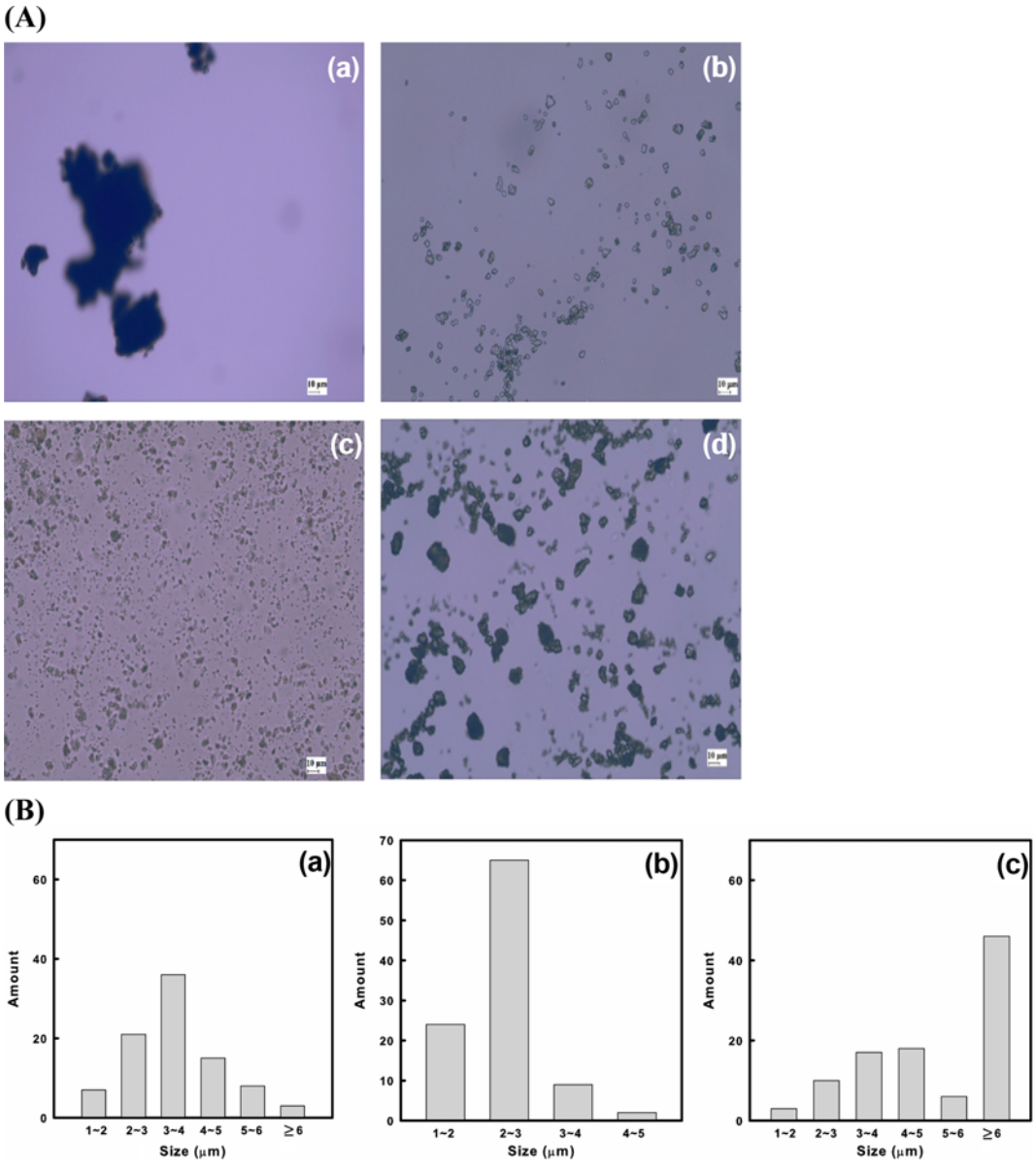

Fig. 2. Optical microphoto (A) of $CaCl_2$ (2 : 0.4) (a), $CaCl_2$ (2 : 0.2) (b), $CaCl_2$ (2 : 0.1) (c) and $CaCl_2$ (2 : 0) (d). Each bar in the photo represents 10 μm. Size distribution (B) of $CaCl_2$ (2 : 0.2) (a), $CaCl_2$ (2 : 0.1) (b) and $CaCl_2$ (2 : 0) (c). Size distribution of $CaCl_2$ (2 : 0.4) could not be determined.

than 10 μm is due to the agglomeration of particles (see Fig. 2(A)(b)). When the $CaCl_2$ to dispersant ratio was 2 : 0.2, the amount of dispersant seemed to be still excessive and it might cause the particle agglomeration. The particle size of $CaCl_2$ (2 : 0.1) was in the range of 1-5 μm and the particles in the range of 2-3 μm were the most frequently found. The particle size greater than 10 μm was seldom found (see Fig. 2(A)(c)). The size of $CaCl_2$ (2 : 0) was a few to tens of μm and particles greater than 10 μm were the most frequently found. When the $CaCl_2$ to dispersant ratio was 2 : 0, $CaCl_2$ could hardly be broken down into smaller particles, possibly because there was no dispersant in the preparation. Since smaller particles could be obtained with the aid of dispersant (Tween 80/Span80 (35/65, w/w)), it can be said that dispersant (Tween 80/Span80 (w/w)) was effective in helping to micronize $CaCl_2$. When the hydrophilic and lipophilic balance of a surfactant falls within 9-11, the surfactant can be used as a dispersant [14]. In fact, the HLB of the mixture of Tween 80/Span80 (35/65, w/w) is estimated to be 11 [15], so the mixture is believed to play a role in dispersing $CaCl_2$ particles. And following the result described above, it can be said that $CaCl_2$ to dispersant ratio of 2 : 0.1 was the best choice among the ratios tested.

2. Preparation of Lipid Microparticles

Fig. 3 shows the optical microphoto and the size distribution of SLPs prepared at the stirring speed of 200 rpm, 400 rpm and 800 rpm. When the stirring speed was 800 rpm, the diameter of particles was in the range of 3-40 μm and the particles in 10-20 μm were the most frequently found. When it was 400 rpm, the diameter was in the range of 10-50 μm and the particles in 10-30 μm were the most frequently found. When 200 rpm, the diameter was in the range of 10-150 μm and the particles in 50-150 μm were the most frequently found. As the stirring speed is higher, the shear force is stronger and the size of oil droplet is smaller. Since one of the objectives of the present work is to envelop $CaCl_2$ in SLP, larger SLPs

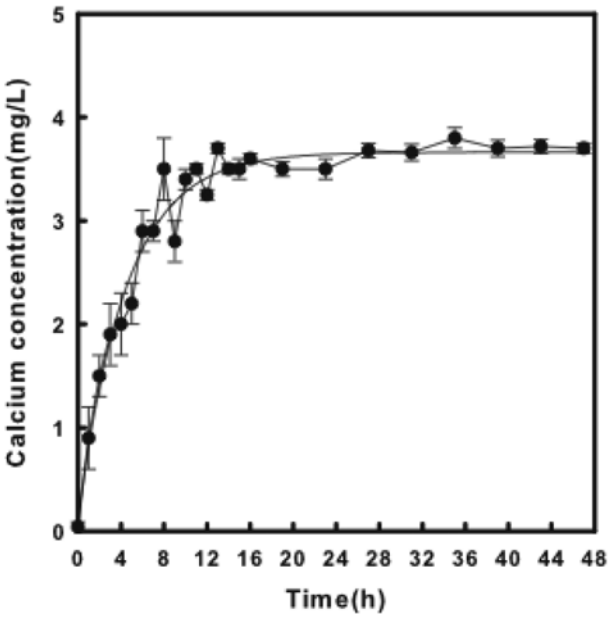

Fig. 4. Concentration of Ca^{2+} in dialysate (water) with time lapse.

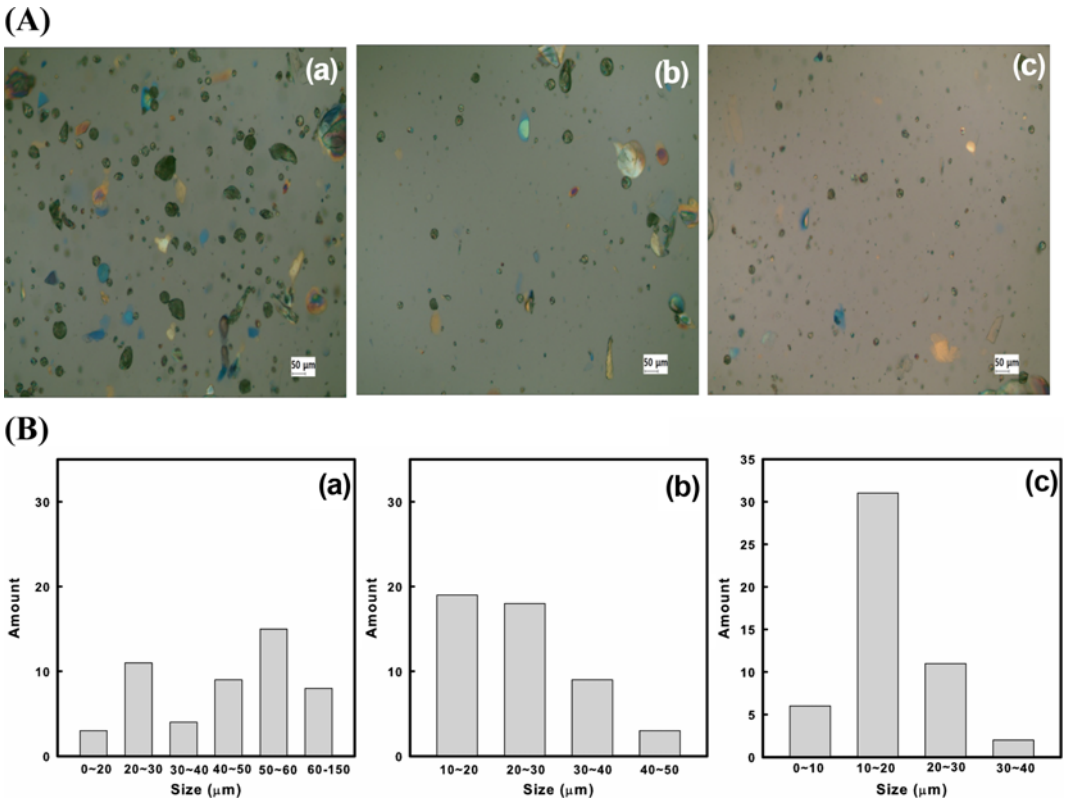

Fig. 3. Optical microphoto (A) of SLPs prepared at the stirring speed of 200 rpm (a), 400 rpm (b) and 800 rpm (c). Each bar in the photo represents 50 μm. Size distribution (B) of SLPs prepared at the stirring speed of 200 rpm (a), 400 rpm (b) and 800 rpm (c).

are likely to be favorable. Thus, the stirring speed of 200 rpm was chosen for the preparation of $CaCl_2$-laoded SLPs.

Fig. 4 shows the concentration of Ca^{2+} in dialysate (water) with time lapse. The concentration increased for the first 8 h and no significant change was observed during the rest of the period. The concentration increase is because unenveloped $CaCl_2$ was dissolved in water and Ca^{2+} released out of the dialysis bag. The almost constant concentration in the later stage is due to the equilibrium between the inside and the outside of the dialysis bag. After the dialysate was replaced with fresh distilled water, the concentration of Ca^{2+} in the dialysate was close to zero and almost constant for 24 h. This means that free Ca^{2+} was removed out of dialysis bag for the first 24 h. Using the concentration of Ca^{2+} detected in the dialysate, the amount of unenveloped $CaCl_2$ and the specific loading were calculated to be 0.034 mg/mg and 0.036 mg/mg, respectively.

3. SEM-EDS

Fig. 5 shows the SEM photos of dry SLPs containing $CaCl_2$ (2 : 0.1) obtained by freeze-drying before and after they were dialyzed. On the SEM photo of the SLP before dialysis, some particles were tens of micrometers in diameter and others were more than 100 μm. On the SEM photo of the SLP after dialysis, particles fell within the size range of particles that were not subjected to dialysis. Since the continuous matrix material, PBSA, was relatively in excess compared with the discontinuous core material, $CaCl_2$, (the weight ratio of PBSA to $CaCl_2$ in SLP containing $CaCl_2$ (2 : 0.1) was 2 : 0.4), SLP would have maintained its integrity even if all $CaCl_2$ particles enveloped in SLP had been leached out during the dialysis. This may account for why the particle size did not change markedly after dialysis. The surface of SLP was rough whether it underwent dialysis or not. SLP was prepared in an aqueous phase; thus $CaCl_2$ particles at the oil/water interface would have been dissolved in water phase, leaving voids on the surface of the particle.

Fig. 6 shows the EDS spectra of dry SLPs containing $CaCl_2$ (2 : 0.1) obtained by freeze-drying before and after they were dialyzed. On the EDS spectrum of the SLP before dialysis, Ca signal was found around 3.7 eV, and the content of Ca on the surface of SLP was evaluated to 3.54% (w/w). Because the suspension of SLP containing $CaCl_2$ (2 : 0.1) was not dialyzed after it was prepared, the suspension would contain not only $CaCl_2$ particles within the SLP matrix but also free Ca^{2+}/Cl^- in its bulk aqueous phase. Upon the freeze-drying of the suspension, the free ions in the bulk phase will be precipitated out and deposited on the surface of the SLP. This can account for why the surface of the SLP was salty. On the EDS spectrum of the SLP after dialysis, no trace of Ca signal was detected. Because the suspension of SLP containing $CaCl_2$ (2 : 0.1) was extensively dialyzed until no free Ca^{2+} was detected, the suspension would contain only $CaCl_2$ particles within the SLP matrix but not free Ca^{2+}/Cl^- in its bulk aqueous phase. Accordingly, no free ions in the bulk phase will be precipitated out and deposited on the surface of the SLP by freeze-drying. This can account for why the surface of the SLP was free of salt.

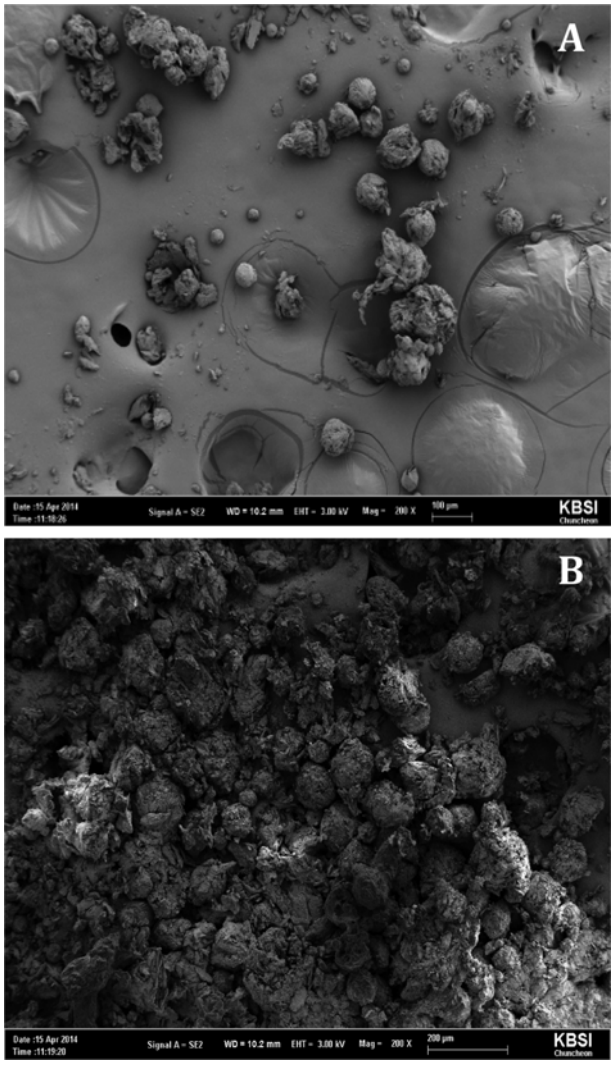

Fig. 5. SEM photos of dry SLPs containing $CaCl_2$ (2 : 0.1) obtained by freeze-drying before (A) and after (B) they were dialyzed.

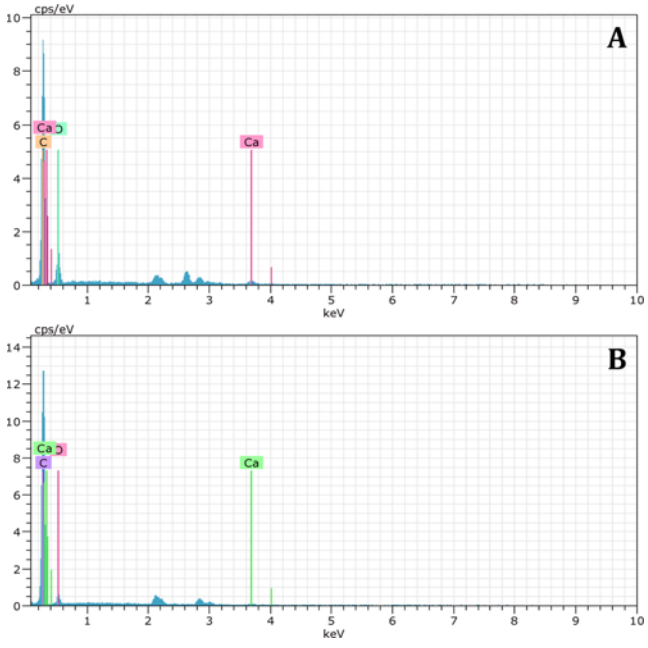

Fig. 6. EDS spectra of dry SLPs containing $CaCl_2$ (2 : 0.1) obtained by freeze-drying before (A) and after (B) they were dialyzed.

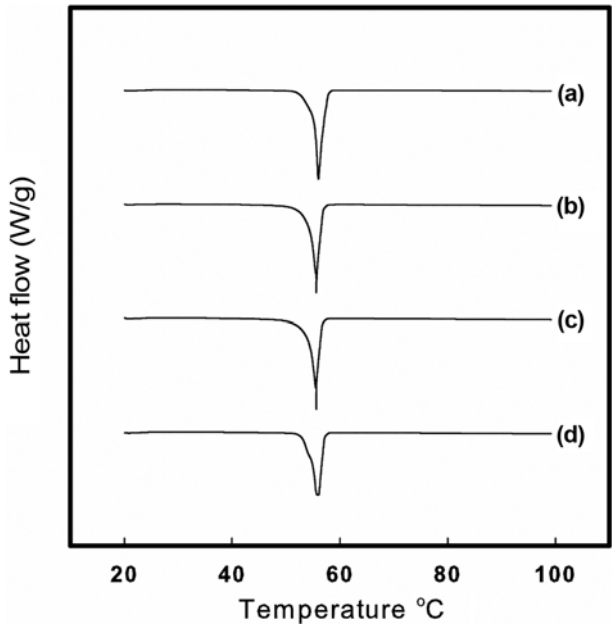

Fig. 7. DSC curve of PBSA (a), dry SLP (b), dry SLP containing dispersant (c), and dry SLP containing $CaCl_2$ (2 : 0.1) (d).

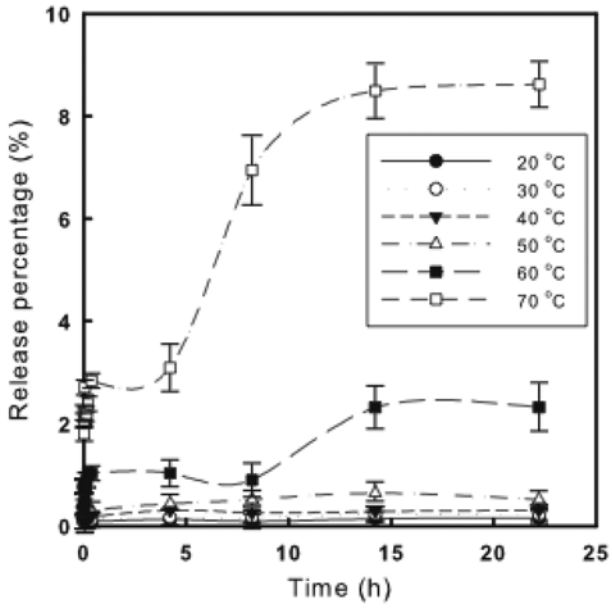

Fig. 8. Release of Ca^{2+} from SLP at 20 °C (●), 30 °C (○), 40 °C (▼), 50 °C (△), 60 °C (■), and 70 °C (□) for 22 hr.

4. Differential Scanning Calorimetry

Fig. 7 shows the DSC curve of PBSA, dry SLP, dry SLP containing dispersant and dry SLP containing $CaCl_2$ (2 : 0.1). PBSA exhibited an endothermic peak around 56 °C. PBSA used in the present study is composed of stearic acid/palmitic acid (38/62, w/w). The melting point of stearic acid and that of palmitic acid is reported to be 69.3 °C and 62.6 °C, respectively. The endothermic peak around 56 °C is believed to be the melting point of the mixture of the fatty acids. Due to the different chain length, they can interfere with their own crystalline structure by each other. Accordingly, the melting point of PBSA could be lower than that of each fatty acid. Since the melting point of PBSA was observed at one certain temperature, it can be said that PDSA is the molecularly homogeneous mixture of SA and PA. The melting point of dry SLP was almost the same as that of PBSA. It means that, during the melting and the solidifying of PBSA which are required for the preparation of SLP, the crystalline structure of the fatty acid was not altered. In addition, Tween20 was used as an emulsifier for the preparation of SLP, so it would be at the interface of SLP/water. In this circumstance, even though the amount of Tween 20 was significant (the weight ratio of PBSA to Tween 20 was 2 : 0.2), the emulsifier would hardly affect the crystal structure of the fatty acid core of SLP. Dry SLP containing dispersant also exhibited its melting point at the same position as SA did, indicating that dispersant (Tween 80/Span 80, 35/65 (w/w)) had no effect on the crystalline structure of PBSA. Tween 80 could migrate to the oil/water interface during the emulsification due to its relatively high HLB number (16); however, Span 80 can be dissolved in the molten PBSA due to its relative low HLB number (4.3) and affect the crystalline structure of the fatty acid core of SLP. Nevertheless, the amount of Span 80 seemed to be too small (the weight ratio of PBSA to Span 80 was 2 : 0.013, which is the ratio in the final formulation of SLP containing $CaCl_2$ (2 : 0.1)) to affect the crystal structure of the solid fatty acid. The melting point of dry SLP containing $CaCl_2$ (2 : 0.1) was found at almost the same position as that of PBSA. $CaCl_2$ is not dissolved in the lipid matrix of SLP, and it is enveloped in SLP as particles but not as molecules. Thus, there will be no effect of $CaCl_2$ on the crystalline structure of the solid PBSA.

5. Release of Ca^{2+} from SLP

Fig. 8 shows the release of Ca^{2+} from SLP at 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C for 22 hr. When the temperature of release medium was 20-50 °C, no appreciable amount was released. The matrix of SLP containing $CaCl_2$ (2 : 0.1) will be in a solid state at those temperatures because the melting point was found around 56 °C (Fig. 7). $CaCl_2$ particles would be stuck in the solid matrix and it would hardly have a chance to contact the surrounding water. When the temperature of release medium was 60 °C, the release degree increased slowly for the first 14 h and then no appreciable release was observed during the rest of the period, and the maximum release degree was about 2.3%. Since 60 °C is higher than the melting point of the SLP containing $CaCl_2$ (2 : 0.1), the matrix of the lipid particle will be in a liquid state. Accordingly, $CaCl_2$ particles can move freely and they will have the chance to contact the surrounding water, leading to the release. The release degree will depend on the number of $CaCl_2$ particles which can reach the surrounding water, and the frequency of the particles to contact water. Because 60 °C is just above the melting point of the matrix of lipid particle, the molten lipid will be somewhat viscous. In this circumstance, $CaCl_2$ particles near oil/water interface could contact water but not the particles in the middle part of molten oil droplet. In addition, the frequency of the particles to contact the surrounding water would be relatively low due to the viscous molten lipid. When the temperature of release medium was 70 °C, the release degree increased in a saturation manner and the release profile resembled that observed at 60 °C. However, the release degree at a given time

was much higher than that obtained at 60 °C. The viscosity of molten lipid at 70 °C will be lower than that at 60 °C. Hence, $CaCl_2$ particles at the higher temperature will move more freely and they will have more chance to contact the surrounding water, causing a more extensive release. Due to the lower viscosity of molten lipid at 70 °C, $CaCl_2$ particles in the deeper part of molten lipid droplet could reach the water/oil interface. In addition, the frequency of the particles to contact the surrounding water would also be higher. These may explain why the degree release was higher at the higher temperature.

Why is melting-induced calcium chloride release needed? Multivalent ions such as Ca^{2+}, Mg^{2+}, and Al^{3+} can cross-link alginate, a negatively charged polysaccharide, through electrostatic interaction [16,17]. Calcium chloride is usually used as a cross-linking agent for the preparation of alginate hydrogel bead (a few millimeters in diameter). The alginate bead is prepared by dropping the aqueous solution of alginate into aqueous solution of calcium chloride. The cross-linking takes place so fast that the droplets are solidified into beads immediately after they are dropped into calcium chloride solution. The surface of droplet will be cross-linked first, and then the interior of the droplet will be. For the cross-linkage throughout droplets, Ca^{2+} should diffuse into the droplet; thus the beads formed are usually stirred for a few hours. However, there is a problem with the interior cross-linking when the bulky lump of alginate hydrogel (e.g., when the dimension is 10-30 cm) is prepared, because it takes a long time for Ca^{2+} to diffuse into the lump and cross-link the interior. One of the strategies to circumvent this problem is to use calcium chloride-loaded SLP as a thermo-sensitive cross-linking particle. Calcium chloride-loaded SLP can be homogeneously dispersed in aqueous solution of alginate without cross-linkage of the polysaccharide. If the mixture is heated to a temperature higher than lipid melting point, calcium chloride will be dissolved out of SLP. As a result, the cross-linking will take place everywhere in the alginate solution at the same time. Therefore, calcium chloride-loaded SLP can be used as thermo-sensitive cross-linking particle for the preparation of bulky alginate hydrogel.

CONCLUSION

$CaCl_2$-loaded SLPs were successfully prepared by a melt/emulsification/solidification method. The diameter of $CaCl_2$-loaded SLP was 10-150, and the lipid particles in 50 μm-150 μm were the most frequently found. The specific loading of $CaCl_2$ in SLP was calculated to be 0.036 mg/mg. The surface of SLP was rough on the SEM photo possibly because $CaCl_2$ particles embedded at the lipid/water interface would have been dissolved in water phase, leaving voids on the surface of the particle. An EDS spectrum of $CaCl_2$-loaded SLP confirmed that the unenveloped $CaCl_2$ was completely removed by dialysis. Neither dispersant nor Tween 20 or $CaCl_2$ had little effect on the melting point of SLPs, indicating that they hardly disturb the crystalline structure of the fatty acid particle. No appreciable amount of Ca^{2+} was released out of SLP for 22 h from 20-50 °C, which is below the melting point of SLP (around 56 °C). However, an appreciable amount of Ca^{2+} was released above the melting point, and the maximum release degree at 60 °C and 70 °C was about 2.3% and 8.7%, respectively, during the same period. The matrix of the lipid particle was in a liquid state above the melting point, so $CaCl_2$ particles can move freely and they will have the chance to contact the surrounding water, leading to the release. $CaCl_2$-loaded SLP developed in the present study could be used as thermo-sensitive cross-linking particle for the preparation of bulky alginate hydrogel.

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