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Regular Article

# Benign preparation of aqueous core poly lactic-co-glycolic acid (PLGA) microcapsules

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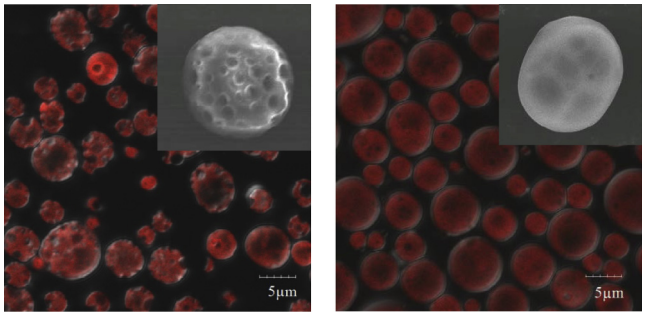
## GRAPHICAL ABSTRACT




Colloidosome-like PLGA microcapsules

Smooth shell PLGA microcapsules

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## ABSTRACT

Poly lactic-co-glycolic acid (PLGA) has attracted considerable attention as a polymer for drug delivery carriers. However, the hydrophobic property of PLGA often leads to the use of harmful organic solvents and poor encapsulation efficiency of hydrophilic materials. To our knowledge, a preparation method of aqueous core PLGA microcapsules without using harmful organic solvents has not been proposed. In this study, we attempted to establish an encapsulation technique of hydrophilic materials in aqueous core biodegradable and biocompatible PLGA microcapsules using vegetable oil as a continuous phase. As a result, the temperature of the oil/water mixture was required to be above the glass transition temperature. In this condition, two different types of morphology were prepared. When the water volume was below the solubility limit, PLGA microcapsules with a smooth shell were formed. In contrast, when the water volume was above the solubility limit, colloidosome-like microcapsules with PLGA nanoparticles assembled at the interface were formed. The obtained microcapsules were then heated at the glass transition temperature. The result is that aqueous core PLGA microcapsules with a smooth shell were prepared using plant oil as a continuous phase. Rhodamine B used as a hydrophilic model encapsulant, was successfully encapsulated in the PLGA microcapsules.



## 1. Introduction

Poly lactic-co-glycolic acid (PLGA) has attracted considerable attention as a polymer for drug delivery carriers because PLGA is

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biocompatible, biodegradable as well as Food and Drug Administration (FDA) approved [1–5]. When PLGA carriers are prepared, PLGA is often dissolved in a water immiscible, volatile organic solvent (dichloromethane is commonly used), because PLGA is a hydrophobic polymer [1,6]. The organic solvents are usually harmful. Consequently, the presence of residual solvent in the carrier and emission of volatile organic compounds to the environment are disadvantages of the method [7]. In addition, the hydrophobic property of PLGA can encapsulate hydrophobic materials, whereas it leads to poor encapsulation efficiency of water-soluble materials [1]. Aqueous core microcapsules with a thin shell, suitable for encapsulation of hydrophilic materials, are useful because they can protect the encapsulant from the surroundings and control the release of the encapsulant [8–10]. To our knowledge, the preparation of aqueous core PLGA microcapsules without the use of harmful organic solvents has not been proposed, although various preparation methods of PLGA carriers have been reported [11–18]. Aqueous core-PLGA shell microcapsules were prepared by internal phase separation from acetone-water in mineral oil emulsion [16]. Alginate-PLGA microparticles with a gel-core/hydrophilic polymer shell were prepared by a double emulsion based solvent evaporation method using dichloromethane as a solvent [11]. In contrast, PLGA micro/nanospheres, although not capsules, were prepared using non-toxic organic solvents (dimethyl carbonate) in droplet-based microfluidic platforms [18]. We have reported the preparation of aqueous core polymer microcapsules stabilized by polymer particles, called colloidosomes, using safe and low cost plant oil as the continuous phase [19–22]. If the oil dissolves PLGA, the preparation of aqueous core capsules by the precipitation of PLGA at the water in oil emulsion interface is expected.

In this study, we attempted to establish an encapsulation technique of hydrophilic encapsulants in aqueous core biodegradable and biocompatible PLGA microcapsules, without using harmful chemicals, allowing their use in biological applications. A single emulsion process was applied and vegetable oil was used as a safe solvent to dissolve the PLGA. Rhodamine B was used as a model hydrophilic encapsulant and was also used as a fluorescent dye, for detection of the water phase. If the processing time is short, surfactants to stabilize emulsions are not needed due to the viscosity of vegetable oil. The effect of solution temperature was investigated because the flexibility of the polymer depends on the temperature relative to the glass transition temperature (Tg) [23–28].

## 2. Materials and methods

### 2.1. Materials

Vegetable oil was purchased from Sainsbury's (United Kingdom). PLGA (Resomer® RG 502 H, lactide:glycolide = 50:50, molecular weight = 7000–17,000, glass transition temperature = 42–46 °C) and Coumarin 6 were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water with a resistivity of 18.2 MΩ cm was produced from a Millipore water purification system. Polyvinyl alcohol (PVA; polymerization degree = 500) was purchased from Nacali Tesque (Kyoto, Japan). Rhodamine B, polysorbitan monolaurate (Tween 20), and ethanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). All chemicals were used without further purification.

### 2.2. Solubility of water and PLGA in vegetable oil

A desired amount of water or PLGA was dissolved in 4 mL of vegetable oil using an ultrasonic homogenizer (Sonifier 250A, Branson, Danbury, CT) for 60 s. The solution was then transferred to a cuvette, and the absorbance and temperature of the solution was measured using a spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan) at a wavelength of 500 nm and a digital thermometer (TM-300, As one, Osaka, Japan). In addition, the temperature of the solution as a function of the emulsification time by the ultrasonic homogenizer, was measured using the thermometer.

### 2.3. Preparation of PLGA microcapsules

A typical experimental procedure to prepare PLGA microcapsules is shown in Fig. 1. Two mg of PLGA was dissolved in 4 mL of vegetable oil and the solution kept at a constant temperature. Then, 5 or 10 μL of water containing dissolved Rhodamine B (0.5 mg/mL) was added to the solution and the mixture was emulsified using the ultrasonic homogenizer for 20 or 40 s. Then, the emulsified solution was allowed to stand at room temperature. After the emulsion had cooled to room temperature, an equal volume of 2% (w/w) PVA aqueous solution was added, to transfer the generated particles from the oil to the water phase and the solution was mixed gently with a tube rotator (As one) at 60 rpm for 10 min. The generated particles were harvested by centrifugation at 8000 rpm for 5 min and washed three times with 2% (w/w) PVA aqueous solution to remove the oil. Finally, the obtained particles were dispersed in water and heated at 44 °C. The experimental conditions are summarized in Table 1.

### 2.4. Characterization

Fluorescent intensities (excitation/emission wavelengths 530/595 nm) of the added water and the washing solutions were measured using a microplate reader (SpectraFluor, Tecan, Crailsheim, Germany) and the encapsulation efficiency of Rhodamine B was estimated.

The morphology of the obtained particles was observed using a confocal laser scanning microscope (CLSM; FV-1000D, Olympus, Tokyo, Japan), an atomic force microscope (AFM; MFP-3D-BIO-J, Oxford Instruments Asylum Research, Santa Barbara, CA), and a field emission scanning electron microscope (FE-SEM; JSM-6700F, JEOL, Tokyo, Japan).

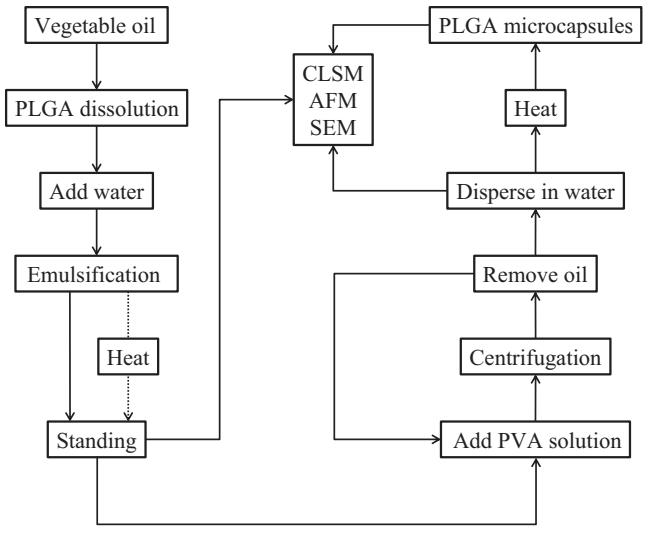



**Fig. 1.** Typical experimental procedure to prepare PLGA microcapsules.

**Table 1**
Experimental conditions.

| Sample | Initial temperature (°C) | Adding water (μL) | Mixing time (s) | Final temperature (°C) |
|---|---|---|---|---|
| A | 2.1 | 5 | 40 | 38.1 |
| B | 24.2 | 5 | 20 | 38.5 |
| C | 24.2 | 5 | 40 | 48.8 |
| D | 48.1 | 5 | 20 | 53.5 |
| E | 2.1 | 10 | 40 | 38.1 |
| F | 24.2 | 10 | 20 | 38.5 |
| G | 24.2 | 10 | 40 | 48.8 |
| H | 48.1 | 10 | 20 | 53.5 |

The fluorescent images and differential interference contrast of the obtained particles were observed using the CLSM with an oil-immersion objective lenses 100x of N.A. = 1.40 (UPLSAPO 100XO, Olympus). The CLSM observation conditions were as follows: Rhodamine B for the water phase (excitation/emission wavelengths 543/560–600 nm) and Coumarin 6 for the oil phase (excitation/emission wavelengths 473/510–520 nm). In addition, the median diameter of PLGA microcapsules was analyzed by measuring over 200 particles using the CLSM.

The surface of the obtained particles was imaged using the AFM integrated with an inverted optical microscope (Eclipse TE2000, Nikon, Tokyo, Japan) in tapping mode in an air-conditioned laboratory (24 ± 2 °C). AFM imaging was performed using a silicon cantilever probe (OMCL-AC200TS, Olympus, Tokyo, Japan) with a nominal spring constant of 9 N/m at a scan speed of 0.2 Hz and 1024 pixels per line scan.

The surface of the obtained particles was also imaged using a nano-suit method [29]: The harvested particles were dispersed in 1% (v/v) Tween 20 aqueous solution. The sample was then mounted on an aluminum stub with conductive carbon tape and the remaining solution with the sample was removed using clean dry filter paper. The plasma-treated Tween 20 film was prepared using a plasma cleaner (PDC-32G, Harrick Plasma, Ithaca, NY, USA) operated at the middle level under reduced air pressure for 5 min. The sample was introduced into the FE-SEM directly without further treatment and was observed in high-vacuum mode at 10 kV.

## 3. Results and discussion

The amount of water or PLGA that can be dissolved in 4 mL of the vegetable oil was firstly determined to choose the best experimental conditions. The solubility of water in vegetable oil is temperature dependent and knowledge of this is crucial in fabrication of PLGA microcapsules through a dissolution - reprecipitation process. When water was dissolved using the ultrasonic homogenizer for 60 s, the temperature of the solution increased from room temperature to about 56 °C. When the solution was allowed to stand at room temperature, the dissolved water precipitated. The absorbance and the temperature of the solution were monitored and the temperature when the absorbance increased was detected and plotted in Fig. 2A. It was confirmed that the volume of dissolved water increased with an increase in temperature. When the volume of added water was above 8 μL, the solution remained cloudy after emulsification. This is because the non-dissolved water formed a water in oil emulsion. In contrast, when the PLGA was fully dissolved in vegetable oil, the absorbance of the solution did not change after cooling to room temperature. A block of transparent PLGA was found to be precipitated at the bottom of the cuvette when the amount of added PLGA was above 25 mg. This means that the solubility of PLGA in 4 mL of the vegetable oil at room temperature is around 25 mg. When the ultrasonic homogenizer was used to emulsify the water/oil mixture, the temperature of the mixture increased with the emulsification time. It is important to know the solution temperature during the preparation method. Fig. 2B shows the relationship between the temperature of the vegetable oil and the emulsification time when the initial temperature was 2, 24, and 48 °C. The glass transition temperature is an important factor because the flexibility of the polymer is changed at the glass transition temperature [24]. This is marked on Fig. 2B. Four experimental conditions, marked as red circles in Fig. 2B, were selected to investigate the effect of the emulsification time (20/40 s) and the final temperature (under/over Tg) on the preparation of PLGA microcapsules. In addition, two water volumes (5/10 μL) were selected, to investigate the effect of the dissolution state of the water. At the final temperature selected in this study, 5 μL of water is completely dissolved, whereas 10 μL of water forms a water in oil emulsion (w/o).

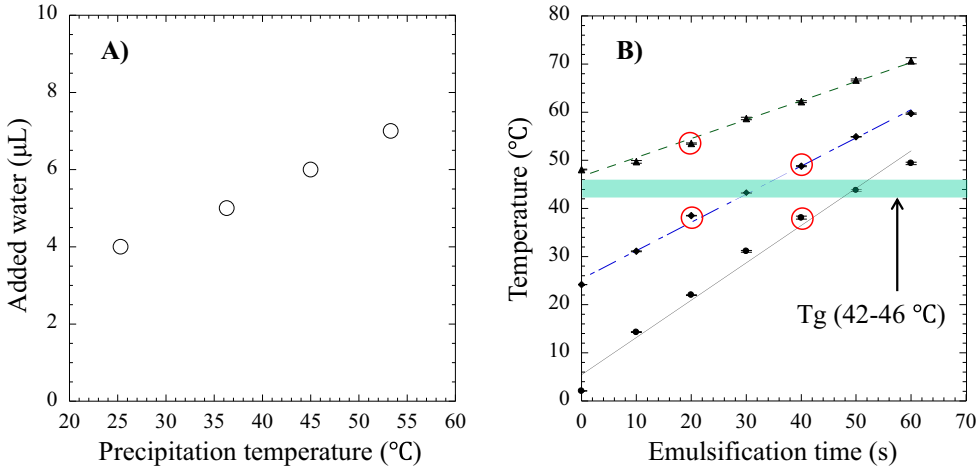



**Fig. 2.** (A) Solubility of water in 4 mL of vegetable oil and (B) Relationship between the solution temperature and emulsification time using ultrasonic homogenizer.

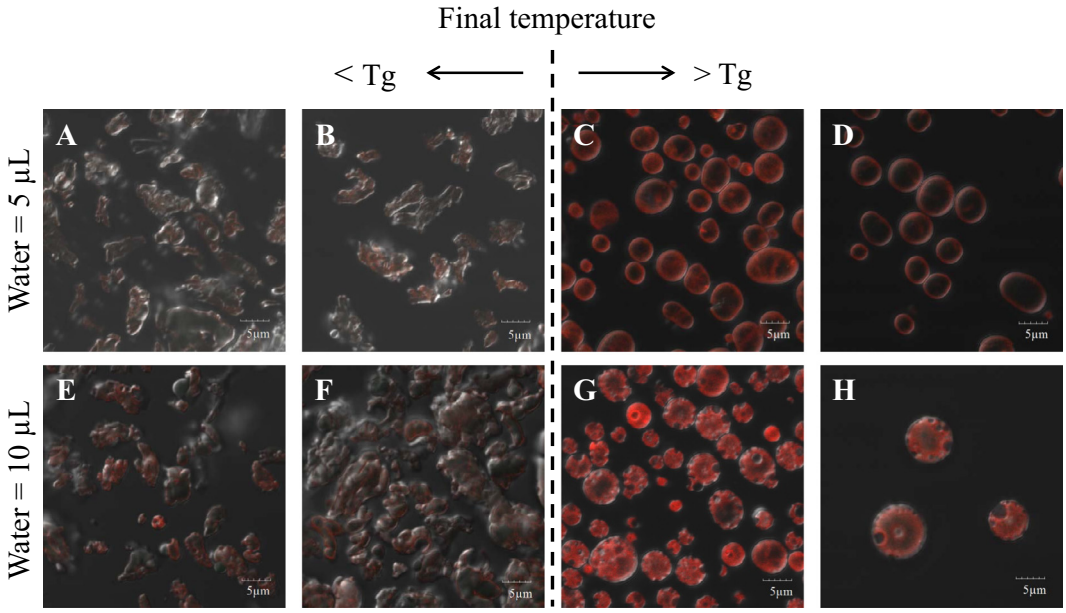



**Fig. 3.** Confocal microscope images of the obtained particles dispersed in water after washing. Experimental conditions: The letters of the alphabet on each image correspond to the sample of the experimental conditions listed in Table 1.

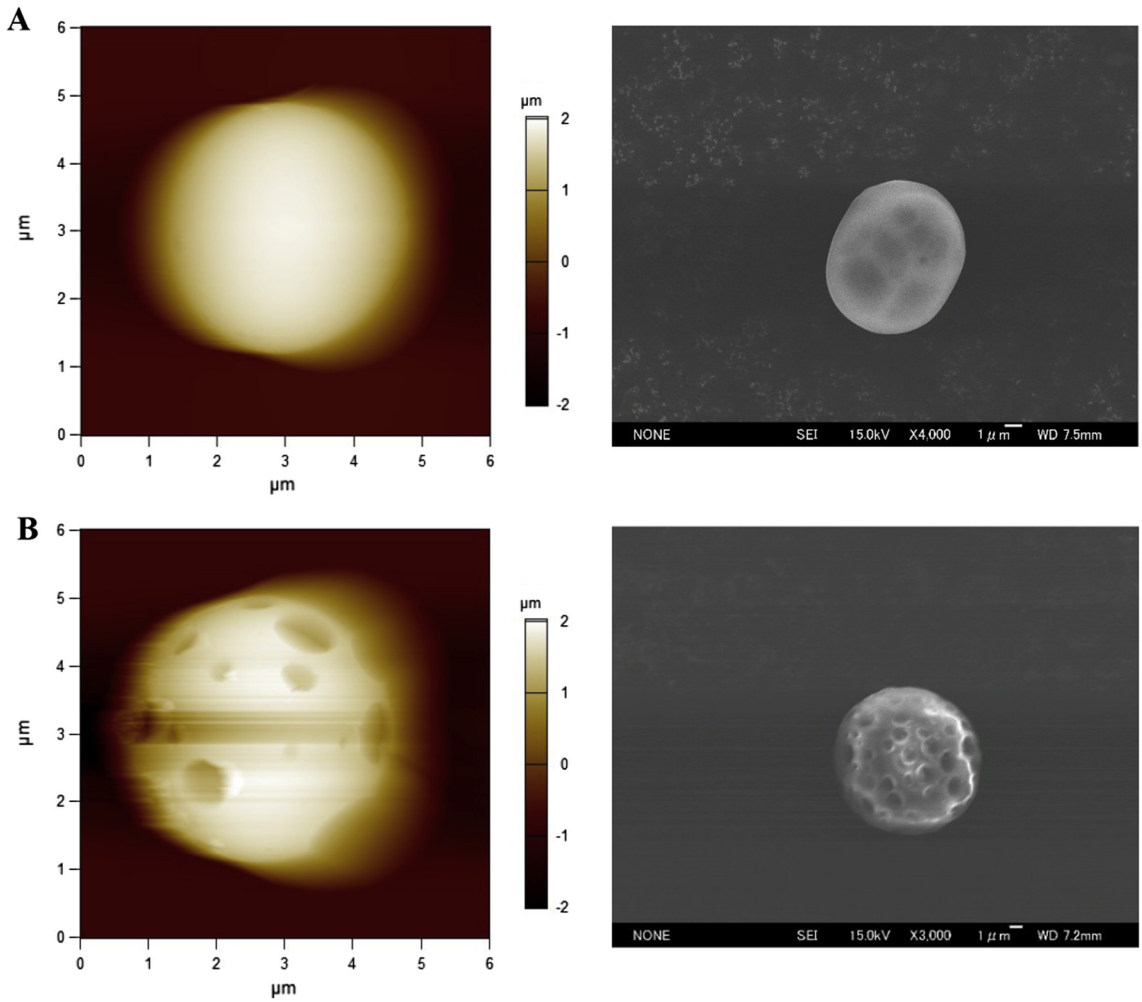



**Fig. 4.** Atomic force microscope images and scanning electron microscope images: (A) PLGA microcapsule shown in Fig. 3C and (B) colloiodosome-like PLGA microcapsule shown in Fig. 3G.

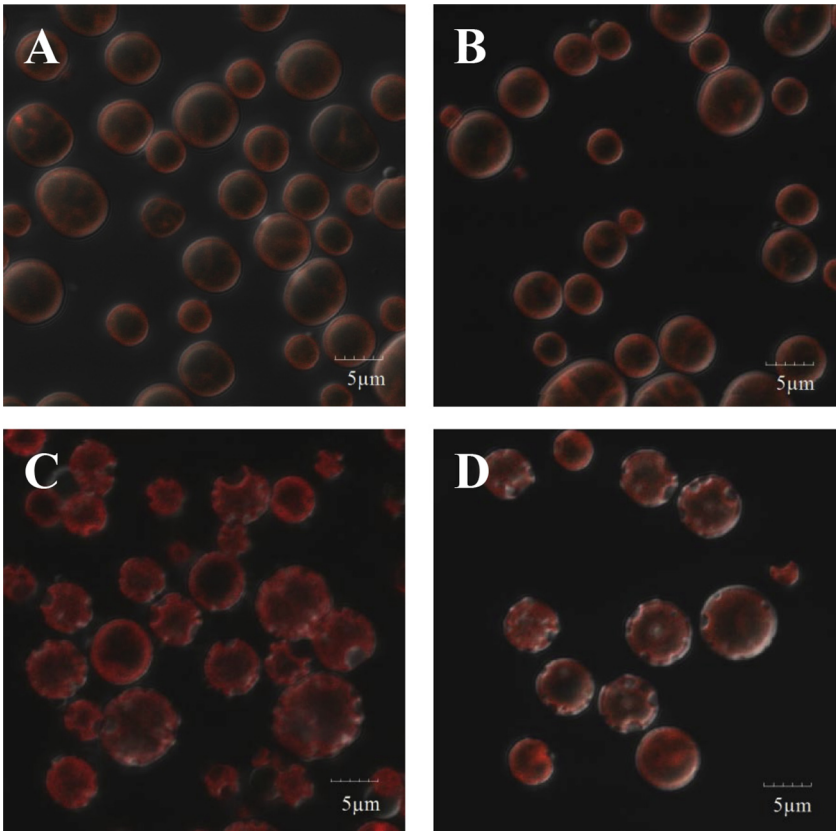
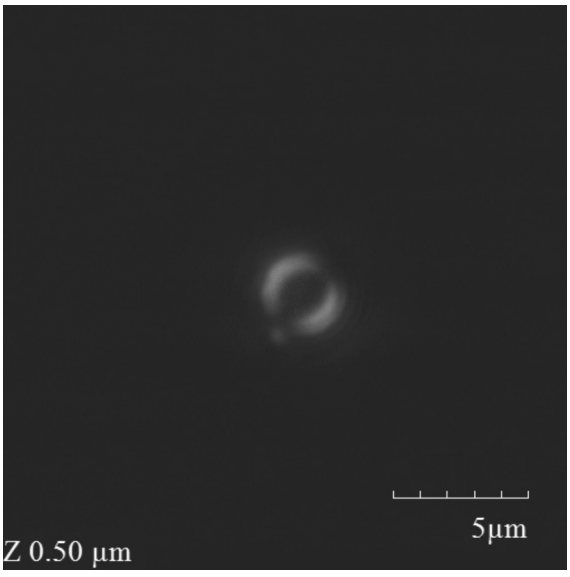



**Fig. 5.** Confocal microscope images of the obtained particles dispersed in water when the emulsified samples were heated at 44 °C for 10 min before cooling to room temperature at the experimental conditions of (A) Sample A, (B) Sample B, (C) Sample E, and (D) Sample F.

There are numerous experimental conditions to investigate and we found that temperature relative to Tg and the water volume relative to the saturation concentration were the most important The different experimental conditions are summarized in Table 1. The final temperature of the oil/water mixture solution after emulsification was controlled by the starting temperature and mixing time.

### 3.1. Low water volume

Fig. 3A–D show typical CLSM images of the obtained particles dispersed in water after washing with PVA solution, when the water volume was 5 μL. The letters on each image correspond to the experimental conditions listed in Table 1. The obtained particles were found to be amorphous when the final temperature of the solution was below the glass transition temperature (Fig. 3A and B). This is because the flexibility of PLGA polymer was low. In contrast, PLGA microcapsules with a median diameter of about 5.8 μm were formed when the final temperature was above the glass transition temperature (Fig. 3C and D). The three-dimensional movie image of the microcapsule in Video S1 of the Supporting Information confirmed the generated particles were microcapsules with a thin shell. The surface of the microcapsules was observed using AFM and SEM, as shown in Fig. 4A. These microscopic images revealed that the surface of the PLGA microcapsules was smooth. This result indicates that the shell of the PLGA microcapsule was formed by surface nucleation of PLGA on the oil/water interface not assembly of particles.




**Video S1.** 3D image of the PLGA microcapsule shown in Fig. 3C.

For the experimental conditions of Samples A and B the morphology of the precipitates were amorphous as shown in Fig. 3A and B. For these samples the solutions after emulsification was allowed to stand at 44 °C for 10 min before cooling to room temperature to check the effect of solution temperature on the morphology. CLSM images of the obtained particles dispersed in

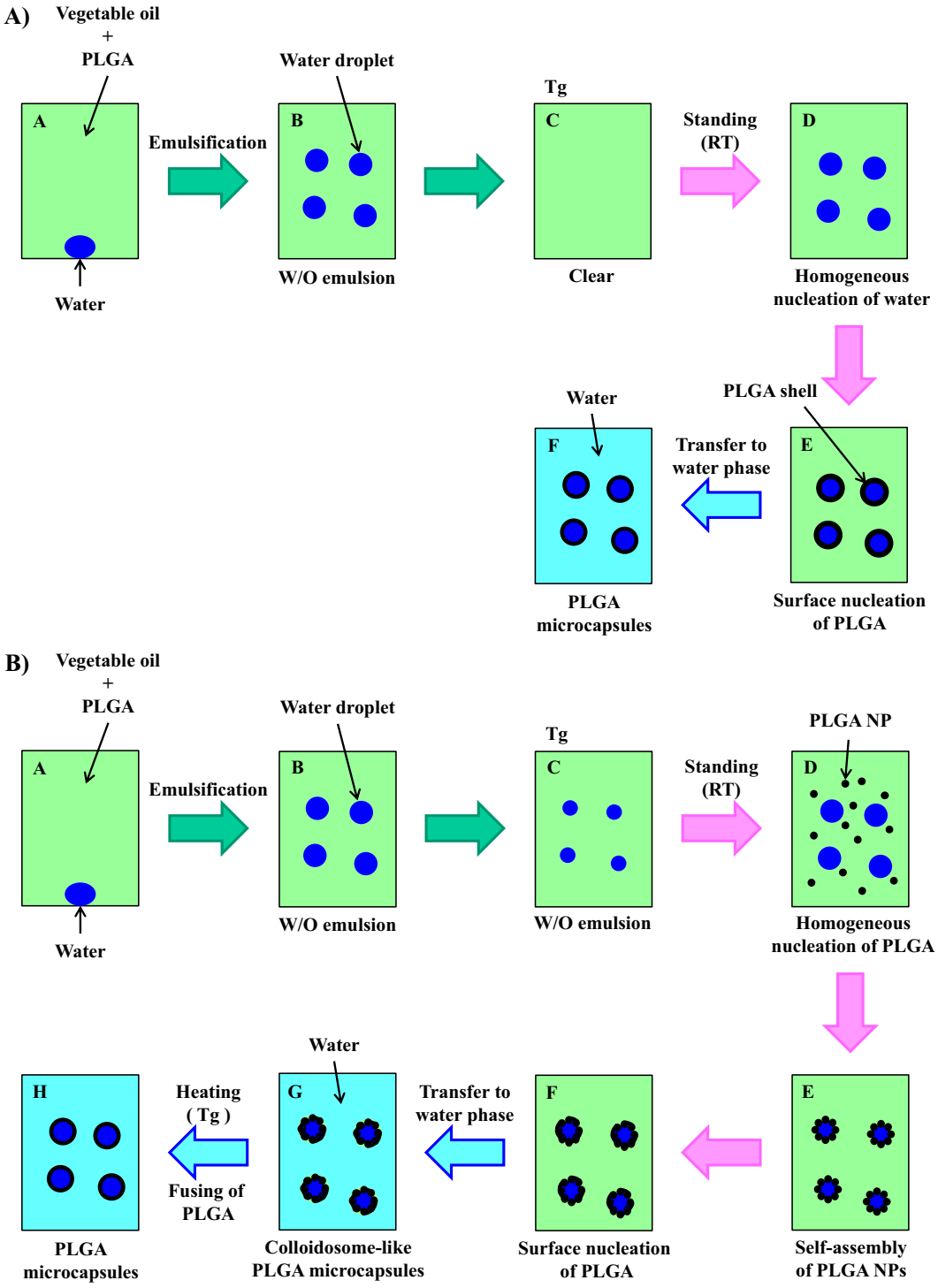



**Fig. 6.** Possible preparation mechanisms of aqueous core PLGA microcapsules: (A) Low water volume and (B) high water volume.

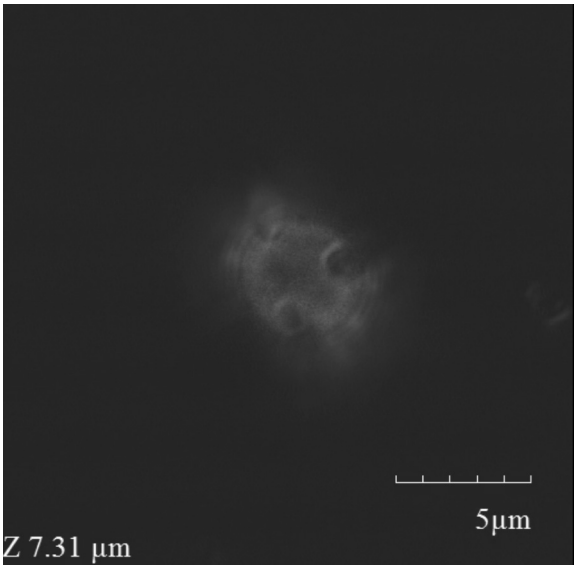
water after washing are shown in Fig. 5A and B. PLGA microcapsules were successfully prepared by heating the solution above the glass transition temperature. These results indicate that the temperature of the oil/water mixture was required to be above the glass transition temperature to form the PLGA microcapsules.

Based on the experimental results, a possible preparation mechanism of the aqueous core PLGA microcapsules is shown in Fig. 6A. When the water was added to the vegetable oil with dissolved PLGA, one big water droplet was formed at the bottom of the vessel (stage A). The solution was emulsified using the ultrasonic homogenizer and a water in oil emulsion was formed (stage B). When the temperature of the solution increased, the solution became clear (stage C). This means that the water and PLGA are either fully dissolved or some may remain dispersed as very small emulsion droplets in the vegetable oil. After emulsification, the solution was allowed to stand at room temperature. With a decrease of solution temperature, emulsions were firstly generated by homogeneous nucleation and micron sized water droplets were formed by their coalescence and/or heterogeneous nucleation (stage D), followed by surface nucleation of PLGA at the water/oil interface (stage E). The result is aqueous core microcapsules with a shell of PLGA. Finally, the PLGA microcapsules were transferred to a water phase

(stage F). We know however, that these microcapsules may not be suitable for the encapsulation of biological materials because the water is mostly if not completely dissolved in the oil during the preparation.

### 3.2. High water volume

Fig. 3E–H show the typical CLSM images of the obtained particles dispersed in water after washing, when the water volume was 10 μL (above the solubility limit). The three-dimensional movie image of Fig. 3G is shown in Video S2 of the Supporting Information. Irrespective of the emulsification time and volume of added water, the obtained particles were found to be amorphous when the final temperature of the solution was under the glass transition temperature (Fig. 3E and F). In contrast, colloidosome-like microcapsules with a median diameter of about 7.1 μm, where PLGA nanoparticles were assembled at the water/oil interface, were formed when the final temperature was above the glass transition temperature (Fig. 3G and H). The AFM and SEM images (Fig. 4B) revealed that the surface of the colloidosome-like microcapsule was pitted with craters. This is indicative of the self-assemble of PLGA nanoparticles and surface nucleation and/or fusing of PLGA at the interface.




**Video S2.** 3D image of the colloidosome-like PLGA microcapsule shown in Fig. 3G .

For Samples E and F the solutions were allowed to stand at 44 °C for 10 min before cooling to room temperature. CLSM images of the obtained particles, dispersed in water after washing, are shown in Fig. 5C and D. Colloidosome-like PLGA microcapsules were successfully prepared by heating the emulsified solution above the glass transition temperature. These results indicate that the morphologies of the obtained microcapsules depend on the water volume. In addition, in the case of Sample F, the solution was allowed to stand at 44 °C for 30 min and 50 °C for 10 min. As shown in Fig. 7, the morphology of the obtained microcapsules did not change when the heating temperature became higher or the heating time became longer. This means that the homogeneous nucleation of PLGA occurred under 44 °C and the generated PLGA nanoparticles were assembled at the surface of the water droplets

Based on the experimental results, a possible preparation mechanism of the aqueous core PLGA microcapsules is shown in Fig. 6B. After the temperature of the solution increased during emulsification, the PLGA dissolved solution cooled to room temperature. With a decrease of the solution temperature, homogeneous nucleation of PLGA occurred in the oil phase and the PLGA nanoparticles and water droplets coexisted (stage D). Then, the PLGA nanoparticles self-assembled on the water/oil interface (stage E), followed by the surface nucleation of PLGA at the interface and the colloidosome-like PLGA microcapsules with a rough surface were formed (stage F). Finally, the colloidosome-like microcapsules were transferred to the water phase (stage G), and were heated at the glass transition temperature. The final result is aqueous core PLGA microcapsules with a smooth shell (stage H).

To prepare PLGA microcapsules with a smooth shell, the colloidosome-like PLGA microcapsules dispersed in vegetable oil or water were heated at 44 °C. The resulting CLSM images are shown in Fig. 8. The morphology of the colloidosome-like microcapsules was found to be changed to a smooth shell by heating in water for 10 min (Fig. 8A). However, part of the microcapsules with a rough shell remained after the colloidosome-like microcapsules were heated in the vegetable oil for 60 min (Fig. 8B). The reason for this discrepancy is likely to be that the glass transition temperature of the polymer depends on the dispersion medium [24]. This means that the fusing of PLGA nanoparticles self-assembled at the interface was hard to achieve at 44 °C. Thus, we believe that the colloidosome-like microcapsules with a rough surface were formed not by fusion, but the surface nucleation of PLGA after the self-assembly of PLGA nanoparticles. Finally, the encapsulation efficiency of Rhodamine B in the case of Samples C and G were 95.8% and 96.0%. This result indicates that Rhodamine B used as a model hydrophilic encapsulant was successfully encapsulated in the PLGA microcapsules via a single emulsion process, using a safe vegetable oil as the continuous phase.

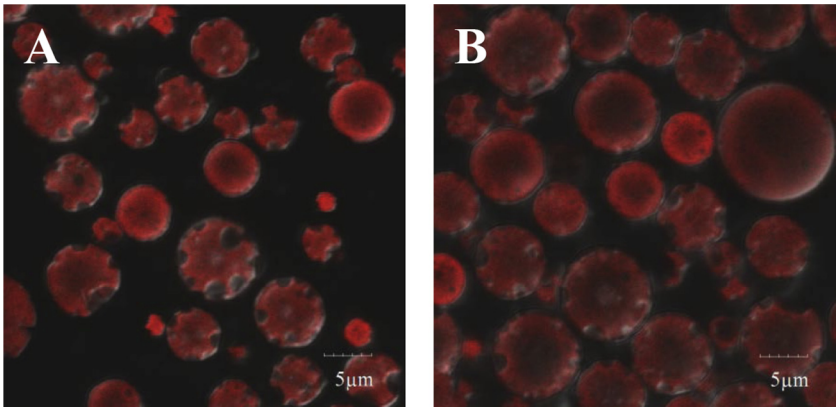



**Fig. 7.** Confocal microscope images of the obtained particles dispersed in water when the final temperature and the heating time after emulsification was changed from under the glass transition temperature for 10 min to (A) 44 °C for 30 min and (B) 50 °C for 10 min before cooling to room temperature at the experimental condition of Sample F.

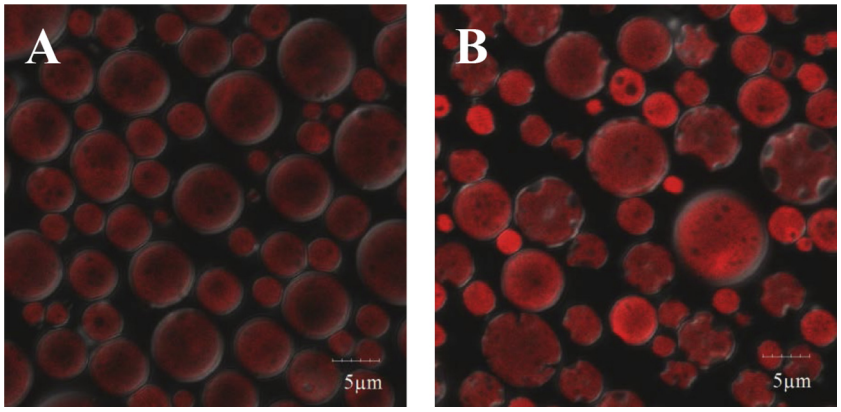



**Fig. 8.** Confocal microscope images of the obtained particles after heating the colloidosome-like microcapsules at 44 °C in (A) water for 10 min and (B) vegetable oil for 60 min.

## 4. Conclusions

Poly lactic-co-glycolic acid (PLGA) shell microcapsules for encapsulation of hydrophilic materials are made via a single emulsion process. We emphasize that the method outlined in this paper does not use any harmful chemicals and this is beneficial when compared to the myriad of preparation techniques available [1–7]. Vegetable oil was used as a safe solvent for dissolution of PLGA and two different morphologies were prepared depending on the amount of water added. When the volume of added water was below the solubility limit in vegetable oil, PLGA microcapsules with a smooth shell were formed. However, this type of microcapsule may not be suitable for encapsulation of biological materials because the water is completely dissolved in the oil during preparation. In contrast, when the water volume was above the solubility limit, colloidosome-like PLGA microcapsules with nanoparticles assembled at the emulsion interface were formed. Subsequently, the microcapsules were transferred to the water phase, and heated. To achieve smooth shells the temperature of the solution needed to be above the glass transition temperature of PLGA. Finally, a model hydrophilic molecule was encapsulated within the smooth shell aqueous core PLGA microcapsules.

Thus, we have successfully established a benign encapsulation technique of hydrophilic materials in aqueous core biodegradable and biocompatible PLGA microcapsules using vegetable oil as a continuous phase.

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