Materials Research Express

**PAPER**

Monodisperse albumin particles fabricated by membrane emulsification using anodic porous alumina

**Takashi Yanagishita, Reina Asami and Hideki Masuda**

Department of Applied Chemistry, Tokyo Metropolitan University, Minamiosawa, Hachioji, Tokyo 192-0397, Japan

E-mail: yanagish@tmu.ac.jp

**Keywords:** ordered anodic porous alumina, membrane emulsification, monodisperse particles

**Abstract**

We obtained monodisperse albumin particles by membrane emulsification using ordered anodic porous alumina with uniform-sized holes. The particle size could be controlled by adjusting the hole size in the alumina emulsification membrane using the linear relationship between the size of particle and the hole. We loaded a fluorescent dye into the albumin particles by adding it to the dispersed phase used for the emulsification. These monodisperse albumin particles with controlled size have potential for use in various applications, in particular, as drug carriers.

**Introduction**

The preparation of monodisperse fine particles has attracted increasing interest owing to their potential applications, for example, as drug carriers, and sensors, and in cosmetics [1–5]. As the performance of the functional devices depends on the size and uniformity of fine particles, it is necessary to accurately control the size of monodisperse fine particles to optimize the property of the devices. Various techniques for the fabrication of nanoparticles, including liquid phase synthesis, template process, and spray pyrolysis, have been reported so far [6–10]. However, difficulty remains in preparing fine particles with uniform and controlled sizes by these methods. We previously obtained monodisperse fine particles by membrane emulsification using an ordered anodic porous alumina formed by the anodization of Al in an acidic electrolyte [11, 12]. In membrane emulsification, monodisperse droplets are fabricated by injecting a dispersed phase into a continuous phase through uniform-sized holes [13]. High-throughput preparation of monodisperse solid particles can also be achieved through solidification of the droplets using appropriate species in the dispersed phase for membrane emulsification. An important advantage of this technique is that the nanoparticle size can be easily controlled by adjusting the hole size of the membrane. In addition, by changing the species in the dispersed phase, this technique enables the fabrication of monodisperse particles of various materials, including polymers and metal oxides. If monodisperse fine particles composed of biocompatible materials can be produced by this process, the obtained particles can be expected to be utilized for various applications including drug carriers for the drug delivery systems.

In the present study, we describe the fabrication of monodisperse protein particles by membrane emulsification using ordered anodic porous alumina. For the dispersed phase, we used an aqueous solution containing albumin, which is a typical water-soluble protein. Monodisperse solidified albumin particles were then obtained by heat treatment of the emulsion droplets. The obtained albumin particles are promising as drug carriers owing to their satisfactory biological compatibility.

**Experimental**

Figure 1 shows a schematic of membrane emulsification. In this study, we use ideally ordered anodic porous alumina as the emulsification membrane, which was prepared by a previously reported pretexturing process of Al [14]. Before pretexturing, an Al sheet was polished electrochemically in a mixture of perchloric acid and
ethanol (1:4 in volume ratio) at 0.1 mA/cm² for 4 min. Pretexturing was carried out by pressing a Ni mold with an ordered convex array of a 400 nm period on the Al substrate. Anodization of the pretextured Al was performed under a constant voltage of 160 V in a mixture of 0.25 M phosphoric acid and 0.1 M oxalic acid at 0 °C for 30 min. For the preparation of the alumina through-hole membrane, two-layer anodization using concentrated sulfuric acid was adopted [15–17]. The sample was anodized in concentrated (16 M) sulfuric acid at 160 V for 1 h to form a highly soluble alumina layer at the bottom of the first anodized alumina layer. The through-hole membrane was obtained by the selective dissolution of this alumina layer using a mixture of chromic acid and phosphoric acid. The hole size in the membrane was adjusted using 10 wt% phosphoric acid.

To form monodisperse emulsion droplets in this technique, it is important to avoid wetting of the membrane by the dispersed phase. Therefore, we hydrophobized the alumina through-hole membrane using n-tetradecylphosphonic acid. The membrane was soaked in ethanol containing 1.4 g l⁻¹ n-tetradecylphosphonic acid at 50 °C for 16 h. The membrane was then heat treated at 100 °C for 1 h. After the hydrophobic treatment, the membrane was attached to the holder used for the membrane emulsification using epoxy resin. For membrane emulsification, phosphate-buffered saline containing 20 wt% bovine serum albumin (general grade, pH 7.0, Nacalai Tesque, Japan) was used as the dispersed phase, and kerosene containing 2 wt% Span 80 and 1 wt% CR-310 was used as the continuous phase. The dispersed phase was extruded into the continuous phase through the uniform-sized holes in the alumina membrane under pressurized N₂. The obtained emulsion droplets were heated at 100 °C for 2 h to form solidified monodisperse albumin particles. We observed the albumin particles using optical microscopy (BX51, Olympus) and scanning electron microscopy (SEM; JSM6700F, JEOL).

Results and discussion

The optical microscopy image in figure 2(a) shows the uniform-sized emulsion sized droplets obtained by membrane emulsification using ordered anodic porous alumina with a hole size of 230 nm. Figure 2(b) shows an SEM image of the albumin particles obtained through solidification of the droplets by heat treatment. It is noteworthy that the spherical shape of the droplets of uniform size was maintained even after the solidification. Size distribution histograms of the emulsion droplets and solidified particles are shown in figure 2(c). For this histogram, the diameters of 100 droplets and 100 particles were measured from the optical microscopy and SEM images. The average diameters of the droplets and solidified particles were 1.5 and 1.1 μm, respectively. The difference in the diameters is attributed to volume shrinkage during drying.

In membrane emulsification, the particle diameters can be controlled by adjusting the hole size of the membrane. Figures 3(a)–(c) show the albumin particles formed using ordered anodic porous alumina with hole diameters of 100, 140, and 180 nm, respectively. The diameter of the alumina holes was adjusted by etching in 10 wt% phosphoric acid at 30 °C for 0, 30, or 60 min. Uniform-sized albumin particles of 270, 470, and 810 nm, respectively, were observed in the SEM images. Figure 3(d) shows the relationship between particle size and hole size of the alumina membrane. The diameter of the albumin particles decreased linearly with decreasing hole diameter. This result means that monodisperse albumin particles with a desired diameter can be obtained by adjusting the hole size of the alumina membrane.
Figure 4(a) shows the SEM images of the monodisperse albumin particles obtained using an alumina membrane with a 200 nm hole period and 55 nm hole size. The corresponding histogram in figure 4(b) shows a narrow size distribution with an average diameter of 160 nm. This value is in good agreement with the linear relationship in figure 3(d). Thus, we conclude that the obtained particle diameter is dependent only on the hole size regardless of the hole periods.

The SEM images in figure 5 show the effect of emulsification pressure on the particle size. In this experiment, ordered anodic porous alumina with a 155 nm hole diameter was used as the emulsification membrane. In emulsification using this membrane, we observed the formation of emulsion droplets at pressures above 80 kPa. At pressures of 80, 100, and 250 kPa (figures 5(a)–(c)), there was no significant change in the particle size and the relative standard deviation. However, at 300 kPa (figure 5(d)), the size of the albumin particles increased, and the...
Figure 3. (a)–(c) SEM images of the ordered porous alumina emulsification membrane and the obtained albumin particles; the hole sizes in the anodic porous alumina were 100, 140, and 180 nm, respectively. The relative standard deviations of the obtained particles were 20, 16, and 19%, respectively. (d) Relationship between the hole size in the anodic porous alumina and the diameter of the obtained albumin particles.
relative standard deviation also increased. This means that the application of excessive pressure should be avoided in membrane emulsification. Figure 5(e) shows the relationships of the average particle size and flow rate of the dispersed phase with the emulsification pressure. As mentioned above, the particle sizes were unchanged until 250 kPa, while the flow rate increased gradually with pressure. (This means that the number of droplets increased with pressure in this region). The size and the flow rate increased abruptly above 250 kPa, and the uniformity of the sizes deteriorated at this pressure. This is presumably due to the surface of the membranes becoming partially wetted by the dispersed phase at this pressure, which accelerated the flow rate. The abrupt increase in the flow rate prevented the formation of isolated uniform-sized droplets, resulting in the deterioration of the size uniformity.

Figure 6 shows the results of loading fluorescent dye to the monodispersed albumin particles. In this experiment, the dispersed phase having 0.001 wt% calcein was used for the emulsification. The SEM image shown in figure 6(a) confirms that uniform-sized albumin particles were obtained even when the dispersed phase containing a fluorescent dye was used. In the fluorescence microscopy image in figure 6(b), green fluorescence can be observed from the fine particles, indicating that our process enables the loading of the fluorescent molecules to the dispersed phase. This process will contribute to the preparation of drug carriers that require size uniformity and size controllability.

Conclusions

We fabricated monodisperse emulsion droplets containing albumin by membrane emulsification using an anodic porous alumina membrane. Solidified albumin particles with uniform sizes were obtained by subsequent heat treatment of the emulsion. The diameter of the albumin particles could be controlled by changing the hole size in the alumina membrane. We also loaded a fluorescent dye to the particles by adding it to the dispersed phase during emulsification. We believe that these monodisperse albumin particles have potential for use in various applications, including drug carriers.
Data availability statement

No new data were created or analysed in this study.
Figure 6. (a) SEM image and (b) fluorescence microscopy image of monodisperse albumin particles containing calcein molecules. The relative standard deviation of the obtained particles was 9%.

ORCID iDs

Takashi Yanagishita  https://orcid.org/0000-0002-6079-9294

References

[1] Wang C, Cheng L, Lin Y, Wang X, Ma X, Deng Z, Li Y and Liu Z 2013 Adv. Func. Mater. 23 3077
[2] Sun T, Zhang Y S, Pang B, Hyun D C, Yang M and Xia Y 2014 Angew. Chem. Int. Ed. 53 12320
[3] Howes P D, Chandrawati R and Stevens M M 2014 Science 346 53
[4] Zanganeh S et al 2016 Nat. Nanotechnol. 26 986
[5] Wissing S A and Müller R H 2003 Int. J. Pharm. 254 65
[6] Zhang N and Xu Y 2013 Chem. Mater. 25 1979
[7] Hang P et al 2014 J. Am. Chem. Soc. 136 8307
[8] Zhang J, Chaker M and Ma D 2016 J. Colloid Interface Sci. 489 138
[9] Hübner J et al 2017 Chem. Mater. 29 399
[10] Mueller R, Määder L and Pratsinis S E 2003 Chem. Eng. Sci. 58 1969
[11] Yanagishita T, Fujimura R, Nishio K and Masuda H 2010 Langmuir 26 1516
[12] Yanagishita T, Maejima Y, Nishio K and Masuda H 2014 RSC Adv. 4 1538
[13] Nakajima T, Shimizu M and Kukizaki M 1991 Key Eng. Mater. 61 513
[14] Masuda H, Yamada H, Satoh M, Asoh H, Nakao M and Tamamura T 1997 Appl. Phys. Lett. 71 2770
[15] Yanagishita T and Masuda H 2015 Electrochim. Acta 184 80
[16] Yanagishita T, Kato A and Masuda H 2017 Jpn. J. Appl. Phys. 56 035202
[17] Yanagishita T, Ozaki M, Kawato R, Kato A, Kondo T and Masuda H 2020 J. Electrochem. Soc. 167 163502