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Preparation of a mono-dispersed protein stabilized in an oil-in-water emulsion by a multi-staged membrane extrusion method

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Abstract

Multi-stage membrane extrusion (ME), a process that extrudes a premixed emulsion prepared by a conventional method through a porous membrane for several repeated cycle, was introduced to prepare an oil-in-water (O/W) emulsion. A uniform-size and fine emulsion is produced using this method: this consumes less energy, a key point in maintaining the structure of the emulsifier employed in the emulsion system. In the present work, a hexadecane emulsion stabilized by bovine serum albumin (BSA) was initially produced by a sonicator before extrusion through a polycarbonate membrane. After that, the properties of the resulting emulsion were explored by measuring the oil content after extrusion, the mean diameter of oil droplets, and the adsorption of BSA at the oil-water interface. The multi-stage ME was processed at various permeate fluxes in the range of 0.07-3.8 $Ls^{-1}m^{-2}$ for 1 to 3 repeated cycles and it was demonstrated that a third extrusion at a permeate flux lower than 2 $Ls^{-1}m^{-2}$ was successful in producing an emulsion with a smaller and more uniform-sized of oil droplets compared to the emulsion before extrusion.

Keywords**:** *bovine serum albumin, multi-stage membrane emulsification, polycarbonate membrane, droplet size distribution, and protein adsorption.*

1. Introduction

Oil-in-water (O/W) emulsions are widely found in a processing of many products, such as food, pharmaceuticals, and cosmetics. Controlling emulsion stability is important to provide desirable characteristics and required shelf life for emulsified products. The size and distribution pattern of oil droplets dispersed in a bulk phase play a crucial role for emulsion stability. Low emulsion dispersibility tends to be observed in systems containing large-sized and poly-dispersed oil droplets due to an enhancement of drop aggregation (McClement, 2004; Gutiérrez, Rayner & Dejmek, 2009). The conventional emulsification methods, e.g., rotor-stator homogenizer, colloid mill, and sonicator, always provide a significantly poly-disperse emulsion. Some instruments, e.g., high-pressure homogenizers, might provide a mono-disperse emulsion with high-energy consumption which leads to emulsifier structure damage, especially when proteins are employed to stabilize the emulsion (Scherze, Marzilger & Muschiolik, 1999). During the past decade, membrane emulsification (ME) has been developed for the utilization in many industries including food, pharmaceuticals, and so on (Valdisavljevic & Williams, 2005). ME is an emulsification process which can produce an emulsion with a narrower droplet size distribution by using less energy compared to a conventional method. In the present work, a multi-stage ME has been focused on because it could effectively produce a uniform size emulsion with a simple and easy experimental set up (Ribeiro, Rico, Badolato & Schubert, 2005; Surh, Jeong & Valdisavljević, 2008; Trentin, Ferrando, López, & Güell, 2009; Trentin, Güell, López & Ferrando, 2010; Valdisavljević, Shimizu & Nakashima, 2004). To operate the multi-stage ME, the premix emulsion is initially prepared by a conventional method before it is passed through a porous membrane for several repeated cycles. This process results in a breaking up of oil droplets into fine particles. With this application, a more uniform-sized emulsion is obtained with low energy input which is important to maintain the emulsifier structure (Valdisavljević, Shimizu & Nakashima, 2004).

In the present work, an emulsion was prepared by using bovine serum albumin (BSA) as the emulsifier. To produce the multi-stage ME emulsion, a polycarbonate membrane was used. The polycarbonate membrane, a hydrophilic membrane suitable for use in O/W emulsion systems (Valdisavljević & Williams, 2005), is generally employed to manufacture potable water, wine, and dairy products. The objective of this work is to establish suitable conditions for the

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multi-stage ME process by providing a small and uniform-sized emulsion. The premix emulsion was prepared and filtered through a membrane with various permeate fluxes. To evaluate the effect of ME extrusion on the emulsion properties, oil content, oil droplet mean diameter, droplet size distribution pattern, and adsorption of BSA at the oil-water interface were examined.

2. Materials and methods

2.1 Materials

BSA was purchased from Sigma Chemical (St. Louis, Mo) and used as received without further purification. n-Hexadecane from Wako Pure Chemical (Osaka, Japan) was employed in the oil phase to produce an oil-in-water (O/W) emulsion. To prepare an aqueous buffer solution pH 7, disodium hydrogen phosphate 12-water ($Na_2HPO_4.12H_2O$) and sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$) from Wako Pure Chemical were used. Guaranteed grade NaN_3 from Kishida Chemicals (Osaka, Japan) was added as an anti-microbial agent. For the multi-stage ME, a polycarbonate membrane of 3 μm pore size (Isopore^{TM}, Millipore, Ireland) was used. The effective membrane diameter was 13 mm giving an effective area of 133 mm^2. The membrane was set in a stainless holder (Millipore, Ireland).

2.2 Experimental set-up

The multi-stage ME was performed as in Figure 1. Firstly, BSA solution (1 g/L) was prepared in a 10 mM buffer solution (pH 7, containing 0.02% NaN_3) and mixed with hexadecane by sonication (Sonifier 250, Branson Sonic Power Co., USA) operated at 40% output for 90 s. The oil content of the premix emulsion was 35% (v/v). Next, the premix emulsion was filtered through the membrane at various constant permeate flux (J) rates aiming to determine the conditions that will provide a uniform-sized emulsion. The premix emulsion was loaded into a plastic syringe (volume capacity 3 mL) and squeezed out through the membrane at the various J in the range of 0.07-3.8 $Ls^{-1}m^{-2}$; $J = V/(t\ A)$, where V is the volume of emulsion, t the time used for the total volume to pass through the membrane, and A the effective area of the membrane. The multi-stage ME was repeated up to three times using the same membrane. The extrusion was processed in two directions, controlled by a 3-way valve. The arrows show the direction of extrusion in each repeated cycle (Figure 1).

2.3 Oil content in the extruded emulsion

The percentage of oil content in emulsion, R, was estimated by precisely measuring density; $R = 100[(\rho - \rho_1)\rho/(\rho_2 - \rho_1)\rho_2]$, where ρ, ρ_1, and ρ_2 are the density of the emulsion sample, aqueous phase, and oil, respectively. The densitometry was performed using a U-tube oscillating density meter (DMA 5000, Anton Paar GmbH, Graz) at 25±0.001 ˚C.

2.4 Droplet size determination

The volume-weighted mean diameter, d_{43}, for oil droplets in the emulsion was estimated utilizing a laser diffraction particle size analyzer (SALD-3000, Shimadzu, Kyoto); $d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$, where n_i is the number of droplets in the ith range of sizes, the mean diameter of which is d_i. d_{43} is sensitive to the presence of large oil drops and effectively indicates the degree of drop aggregation. All samples were triplicate measured and the mean d_{43} value was reported. The distribution of the droplet size was also considered in determine the effects of extrusion.

2.5 Adsorption amount of BSA around oil droplets

The surface density of the adsorbed BSA at the oil-water interface was estimated as follows: the emulsion was centrifuged to separate oil droplets using a dispersion stability analyzer (LUMifuge116, L.U.M. GmbM, Germany) which can accelerate phase separation of the emulsion up to 1200 times faster than under normal gravity conditions. The preliminary tests confirmed that the centrifugation at 2000 rpm for 1 h was enough to completely separate oil drops to form a cream layer above the aqueous serum phase without evidence of oil drop coalescence. After the centrifugation, the serum phase was taken out to quantify the amount of unabsorbed BSA. The concentration of BSA was estimated through the *o*-phthaldialdehyde (OPA) assay (Fayle, Healy, Brown, Reid, Gerrard & Ames, 2001) using a fluorescence spectrophotometer (FP-6300, Jasco, Tokyo): the excitation and emission wavelengths were 340 nm and 455 nm, respectively. The surface density of adsorbed BSA at the oil-water interface, Γ, was calculated as follows; $\Gamma = V_{aq}(C_{int}-C_{ser})/V_{oil}S$, where V_{aq} and V_{oil} are the volume of aqueous and oil phases, C_{int} initial BSA concentration before the emulsification, C_{ser} BSA concentration in the serum phase after the centrifuge (unadsorbed BSA), and S is the total interfacial area of oil drops dispersed in emulsion samples which was estimated by the particle size analyzer.

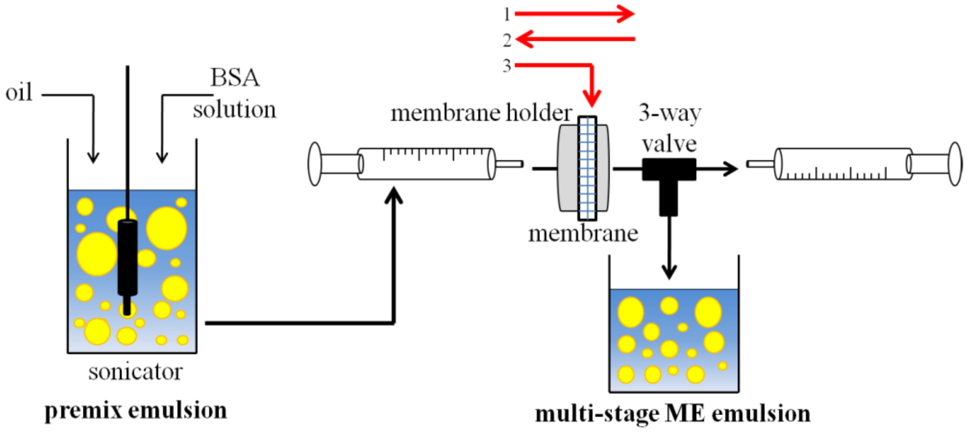


Figure 1 Experimental setup for multi-stage ME

3. Results and discussion

3.1 Effects of membrane extrusion on oil content and drop size of emulsion

The multi-stage ME was performed at the permeate flux, *J*, of 0.16 and 3.8 $Ls^{-1}m^{-2}$. The oil content, *R*, and the mean oil droplet diameter, d_{43}, after *n*-time membrane extrusion are shown in figure 2. With the increase of *n*, *R* was reduced for both the extrusion rates. For the emulsion size, different behavior was observed depending on *J*. At *J* of 0.16 $Ls^{-1}m^{-2}$, the droplet diameter decreased gradually from ca. 10 µm to 6 µm with the extrusion cycle. The same tendency of droplet size reduction during extrusion has already been reported (Surh, Jeong & Vladisavljević, 2008; Trentin et al., 2009; Trentin et al., 2010). At high *J*, 3.8 $Ls^{-1}m^{-2}$, on the contrary, droplet size continuously increased with the repeated extrusion to ca. 40 µm after the third process.

The ME process caused the oil droplets of 10-µm mean diameter in the emulsion to be pressed towards the 3-µm pore size membrane. The oil drops slowly passed through the membrane pore deforming their shape while the small water molecules could promptly permeate the membrane. In the final stage of extrusion, some oil drop parts remained over the membrane as a concentrated emulsion (cream). This led to the reduction of oil content for the emulsions after extrusion. Actually, the cream phase was seen on the membrane after a third extrusion at *J* = 0.16 $Ls^{-1}m^{-2}$. It has been mentioned that in a highly concentrated cream phase, oil droplets were deformed to a plant-cell-like hexagonal structure leading to a stretching of the protein film (van Aken, 2002).

If the protein film is stretched above a critical value by an external stress (from the pressure applied to the extrusion in this case), film rupture and oil drop coalescence will occur resulting in an oil phase separation (van Aken, 2002). In this experiment, the separation of oil was found for the ME emulsion extruded at $3.8Ls^{-1}m^{-2}$.

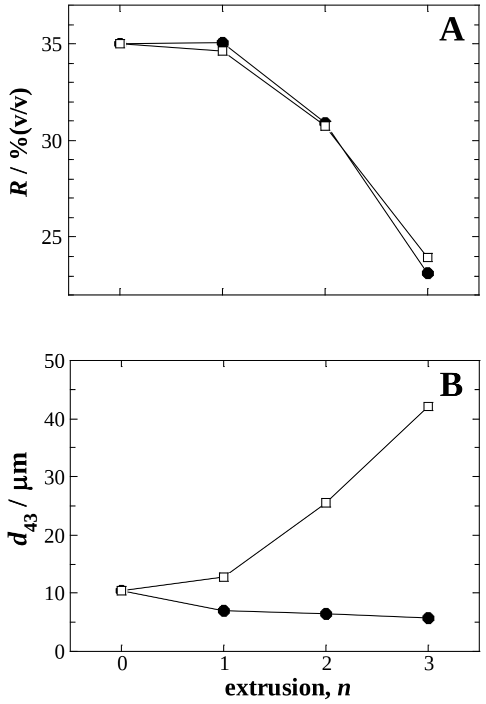


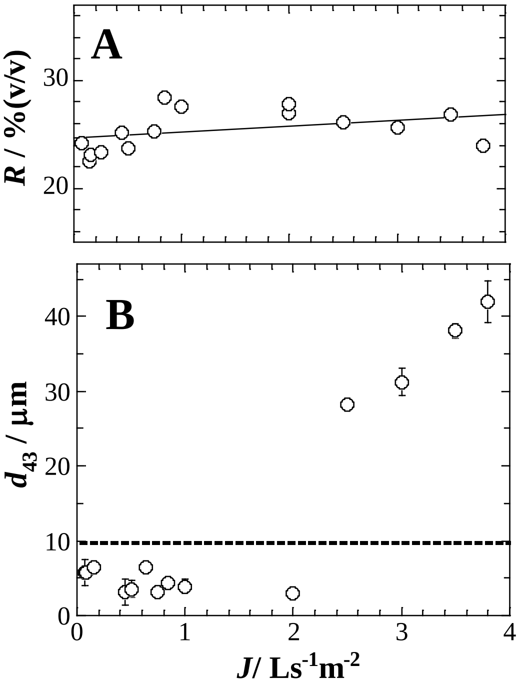
Figure 2 Oil content, *R*, (**A**) and volume-mean oil droplet diameter, d_{43}, (**B**) for emulsions after the *n*-time extrusion at the permeate flux of 0.16 (●) and 3.8 (□) $Ls^{-1}m^{-2}$.

To clarify the mechanism of ME emulsion size depending on *J*, the multi-stage ME was prepared at various *J* in the range of 0.07-3.8 $Ls^{-1}m^{-2}$ as described below.

3.2 Effects of permeate flux on oil content and droplet size of emulsion

Figure 3 shows *R* and d_{43} as a function of *J* and the distribution of oil droplet size for the multi-stage ME emulsions at some *J*. Slightly higher *R* was observed at high *J*. For the emulsion size, a third extrusion at the *J* below 2 $Ls^{-1}m^{-2}$ was able to diminish the emulsion size from ca. 10 μm to 5 μm. At the *J* higher than 2 $Ls^{-1}m^{-2}$, however, the emulsion size drastically increased as shown in figure 2B. This result suggests that the threshold *J* leading to the drop size growth was ca. 2 $Ls^{-1}m^{-2}$. The droplet size distribution pattern indicating the poly-modal curve had the two major peaks around 5 and 40 μm for the emulsion before extrusion (Figures 3C). After the third extrusion, the distribution curve changed to unimodal-like (Figure 3D and 3E). It has been reported that although the ME process could modify the emulsion system to be smaller-sized and unimodal, some large oil drops were always detected in the ME emulsions stabilized by BSA (Trentin et al., 2010) and lecithin (Surh, Jeong & Valdisavljević, 2008). When the extrusion was performed at 3.8 $Ls^{-1}m^{-1}$ (Figure 3F), the evidence of drop coalescence, suggesting that the extrusion at too high *J* leads to coalescence and apparently is not suitable to prepare fine ME emulsions.

Figure 3D (3E) shows only one predominant peak around 6 μm (3 μm) after the extrusion at 0.16 (0.75) $Ls^{-1}m^{-2}$ which approximately corresponds to one of the major peaks of the original emulsion (Figure 3C). This result suggests that oil drops with 40-μm diameter in the original emulsion were disrupted down to several-μm drops during the extrusion. To permeate the membrane, the large oil drops had to be deformed to enter the pores, and the shear stress, as well as the friction between oil drops and pore walls, leads to the disruption of deformed oil drops (Valdisavljević, Shimizu & Nakashima, 2004).



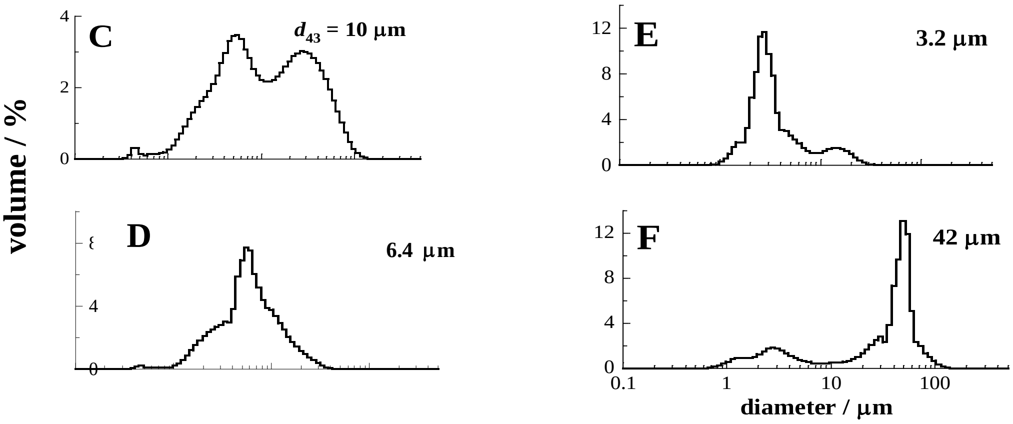


Figure 3 Permeate flux (*J*) dependence on *R* (**A**), d_{43} (**B**), and droplet size distribution patterns of the emulsions before extrusion (**C**) and after the third extrusion by the *J* of 0.16 (**D**), 0.75 (**E**), and 3.8 (**F**) $Ls^{-1}m^{-2}$. The broken line in B indicates d_{43} of the emulsion before extrusion.

At a higher permeation flux (above 2 Ls^{-1} m^{-2} in the present case), extreme drop disruption and a concomitant increase of oil-water interfacial area are expected (Valdisavljević, Shimizu & Nakashima, 2004; Ribeiro et al., 2005). Because of the intensively increased interfacial area, the surface density of adsorbed BSA at the oil-water interface should be lowered significantly leading to less protection against coalescence between oil drops. If the emulsifier in the bulk cannot adsorb the newly produced oil-water interface, a coalescence of oil droplets will promptly occur resulting in an increased emulsion size (Trentin et al., 2010). To confirm this, the adsorbed amount of BSA at the oil-water interface for the ME emulsions prepared at various *J* was estimated, as described in the next section.

3.3 Adsorption of BSA at the oil-water interface

The adsorption amount of BSA at the oil-water interface was explored measuring the BSA concentration in the serum, C_{ser}, and the surface density of adsorbed BSA, Γ (see Figure 4). Before the extrusion, C_{ser} was ca. 80 mgL^{-1}, and Γ was 1.9 mg m^{-2} which was compared to the reported value for BSA adsorption at the air-water interface (2 ± 0.2 mg m^{-2}) (Lu, Su & Thomas, 1999). At *J* below 0.2 Ls^{-1}m^{-2}, there was no evidence of BSA desorption from the interface by the ME; C_{ser} and Γ were almost the same as the emulsion before the extrusion. The extrusion at *J* in the range 0.2-2 Ls^{-1}m^{-2} led to a detachment of BSA from the oil-water interface, and was suggested by the increase (decrease) of C_{ser} (Γ). When *J* was increased to above 2 Ls^{-1}m^{-2}, C_{ser} was still higher than the initial value and a gradual increase of Γ with *J* up to 1.9 mg m^{-2} was confirmed .

According to the results in Figure 3B, small droplets were produced by the extrusion in the range of *J* of 0.2-2 Ls^{-1}m^{-2} suggesting the production of a large interfacial area. The lowered Γ observed in this condition may be due to a slow adsorption of BSA to the newly produced interface compared to the drop disruption process. At *J* above 2 Ls^{-1}m^{-2} where a higher degree of drop disruption and a larger interface area creation occur, a bare interface should be present due to the slow adsorption rate of BSA. After that, oil drops would coalesce and increase their size to minimize the total interfacial area. Then, BSA adsorption might proceed to effectively cover the interfacial area resulting in the increased Γ of 1-2 mg m^{-2}.

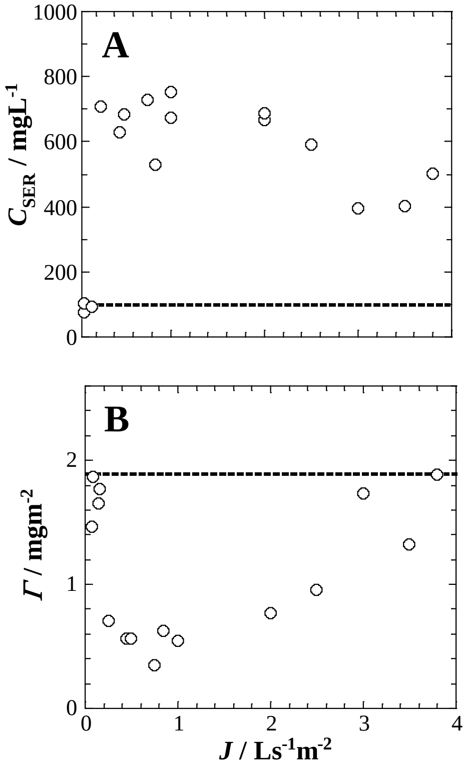


Figure 4 Permeate flux, *J*, dependence on the BSA concentration in the serum, C_{SER}, (**A**) and the surface density of adsorbed BSA at the oil-water interface, Γ, (**B**) for the third ME emulsions. The broken lines indicate C_{SER} and Γ of the emulsion before extrusion.

4. Conclusion

The present work shows that the multi-stage ME was successful in modifying the emulsion to a smaller and more uniform-sized system compared to the emulsion produced by a conventional method. According to the oil droplet size and distribution patterns of the emulsions, there was evidence of drop disruption after the extrusion. To permeate through the membrane, a large oil droplet had to be deformed to enter the pore inlet and drop disruption occurred due to the shear stress and the friction between oil drops and pore walls. The disruption of the deformed oil drop led to a decreasing of the droplet size and concomitant increasing of the interfacial area. If the pressure applied to extrude the emulsion was too high, an extreme drop disruption and intensively increased interfacial area can be expected. This might lead to a presence of a bare interface with the effect of promoting a coalescence of oil droplets. This work suggests that the threshold of permeate flux leading to an emulsion size increase was 2 Ls^{-1}m^{-2}.

Therefore, third extrusion by the *J* lowered than 2 $Ls^{-1}m^{-2}$ is capable of producing a uniformed and fine emulsion. Considering the adsorption of BSA on oil drop surfaces, extrusion by the *J* of 0.2-2 $Ls^{-1}m^{-2}$ led to lowered surface density of adsorbed BSA at the oil-water interface which was expected because of the slow rate of BSA adsorption to the newly produced interfacial area when compared to the drop disruption rate. As the *J* increased up to 2 $Ls^{-1}m^{-2}$, however, a gradual development of surface density of adsorbed BSA with *J* up to 1.9 mg m^{-2} was observed.

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