Microfiltration and Stabilization of Egg Yolk Phospholipid Emulsions by a Microporous Glass Membrane

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ABSTRACT: A microporous glass membrane with a narrow-range pore size was applied for the microfiltration of egg yolk phospholipid emulsions. The oil-in-water and water-in-oil emulsions were successfully filtered using the membrane without any coalescence of oil droplets or the breakdown of the emulsions. The filtrated oil-in-water emulsion was stable for at least 6 wk when stored at 5°C. The results obtained suggest that the technique would be valuable for the precise filtration of emulsions for food uses as well as intravenous fat and/or drug carrier emulsions, and offer the stabilization of the emulsions. *JAOCS 74,* 1255–1258 (1997).

KEY WORDS: Egg yolk phospholipid, emulsion, emulsion stability, microfiltration, microporous glass membrane.

Formation and stabilization of emulsions are of great importance in industrial application such as the food, pharmaceutical, cosmetic, chemical, and textile industries where the safety and stability of emulsion products are considerations: for example, an intravenous emulsion, which could be used for parenteral nutrition or drug delivery system (1,2), and perfluorocarbon emulsions which could be used as blood substitutes (3). On the other hand, the toxicity and medical effect of the emulsifier on the body must be taken into consideration (4,5). Thus, egg and soy phospholipids are usually used as natural emulsifiers and have been proven to be very useful in the preparation of drugs and cosmetics. The physicochemical properties of phospholipid emulsions have been studied using various oil phases and various sources of phospholipids (6–10). However, there are unsolved problems regarding the stability of phospholipid emulsions because of their low interfacial adsorptivity compared to other artificial emulsifiers.

An emulsion is considered a dispersion of one liquid in another, a two-phase system from the thermodynamic point of view. Since the emulsion is thermodynamically unstable, flocculation and coalescence immediately occur after emulsification. Generally, the stability of an emulsion depends upon the emulsifying agent, droplet size, net charge, and mechanical and physical properties of the adsorbed film (11). The size distribution of emulsified droplets is the most important parameter in characterizing any emulsions. Stability and resistance to creaming, rheology, chemical reactivity, and physiological efficiency are influenced by both relative size and size distribution (12). Optimal particle design and strict size control are important factors for the preparation of stable emulsions, and also for discovering novel dispersions which contain solid or liquid particles. The emulsions made by conventional instruments, such as colloid mills, toothed discs, dispersing machines and high-pressure homogenizers, show considerable polydispersion where droplet size distribution is usually between 0.1 and 100 μm. A new emulsifying technique, called membrane emulsification, has been proposed by Nakashima *et al*. (13). This membrane is made from a microporous glass membrane with a uniform range pore size distribution. It was reported that the technique is valuable for the production of stable simple and multiple emulsions for food uses as well as intravenous fat and/or drug carrier (14–16). This membrane also is expected as a filtration element of emulsions because of its unique structure of the membranes. Some methods have already been proposed to filter or concentrate emulsions by using ultrafiltration or reverse osmosis (RO) membranes (17). However, there are many unsolved problems in connection with the microfiltration of micro- or submicroemulsions using these methods. Little information is available on the microfiltration of emulsions using the microporous glass membrane. In this study, the authors propose that a microporous glass membrane is a valuable tool for the precise filtration and stabilization of emulsions.

MATERIALS AND METHODS

Materials. Soybean oil was purchased from Sigma Chemicals (St. Louis, MO). Egg yolk phosphatidylcholine and lysophosphatidylcholine, 98% pure, were kindly provided by Q.P. Corporation (Tokyo, Japan) and were used without further purification. Polyglycerol esters of polycondensed ricinoleic acid (PGCR) were obtained from Sakamoto Yakuhin Co. Ltd. (Osaka, Japan). Other chemicals were obtained from Sigma. Microporous glass membrane made with Al_2O_3-SiO_2 type glasses (hydrophilic membrane) was purchased from Ise Chemical Corp. (Tokyo, Japan) and was 0.36–1.36 μm in diameter, 0.5 μm in thickness, and 230 mm in length. A hy-

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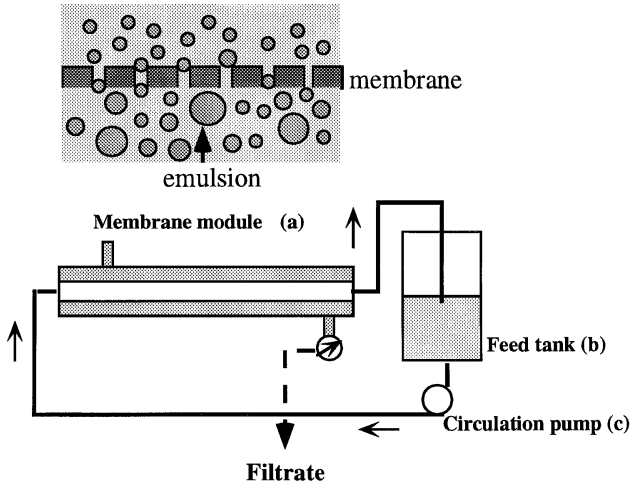

FIG. 1. Composition of the microfiltration apparatus of emulsions: membrane module (a), feed tank (b), and the circulation pump (c).

drophobic membrane was prepared according to the method described previously (18).

Microfiltration of emulsions. Microfiltration of emulsions by the microporous glass membranes was done as follows: Polydispersed oil-in-water (O/W) and water-in-oil (W/O) type emulsions were prepared by homogenizing soybean oil and water solution containing 1.0% lysophosphatidylcholine and 5.0% glucose for O/W emulsion or 5.0% glucose solution and soybean oil containing 0.5% phosphatidylcholine and PGCR for W/O emulsion, respectively, using a high-speed homogenizer (Polytron PT 2000; Brinkmann, Mississauga, Canada) at 24,000 rpm for 3 min. These polydispersed emulsions were passed through suitable hydrophilic or hydrophobic membranes to remove the larger droplet size of emulsions using the same apparatus as shown in Figure 1. This system consists of the membrane module (a), feed tank (b), and the circulation pump (c). The protocol for emulsion filtration is also shown in this figure. The microporous membrane was prewetted with the continuous phase, prior to the start of the membrane microfiltration, i.e., the hydrophilic membranes for O/W emulsions were fully wetted with the water phase and the hydrophobic membranes for W/O emulsions were fully wetted with the soybean oil phase. Wetting of the membranes was effectively conducted by immersing the microporous membrane in the continuous phase and degassing it under vacuum, while subjecting it to ultrasound for 30 min. The membrane was then carefully attached to the membrane module. Changes in the droplet size distribution were monitored by light scattering, using a Mastersizer X (Malvern Instruments, Lin, Malvern, United Kingdom). This particle analyzer was also used to assess the stability of emulsion during aging. All data are expressed as volumetric percentage.

RESULTS AND DISCUSSION

Microfiltration of emulsions. Figure 2 shows the change in droplet size distribution of O/W and W/O emulsions made by a homogenizer after microfiltration by the membrane and the photomicrographs of the filtrated emulsions. The O/W emulsion composed of 1.0% lysophosphatidylcholine and 20% soybean oil showed a polydispersed phase with two spread peaks (Mean diameters are 3.5 and 10.6 μm.). This emulsion was passed through a hydrophilic membrane (average pore size: D_m = 5.2 μm) to remove the larger droplets. The larger droplets (i.e., >6.0 μm) were successfully removed without any coalescence and breakdown of the droplets. The W/O emulsion, composed of phosphatidylcholine, PGCR and soybean oil stabilized with a dispersion, also showed two spread peaks droplet distribution. The mean diameters of emulsions were 1.1 and 2.8 μm, respectively. A hydrophobic membrane (D_m =1.4 μm) was selected to remove the droplets greater than 1.5 μm using the same procedures described above. The dispersed W/O emulsion was successfully microfiltered by the porous glass membranes without forming any coalescences. Attempts in filtering emulsions using membranes such as ultrafiltration or RO membranes have been used to condense or separate emulsions (17). However, these methods are limited by available solvents, heat resistance, and mechanical intensity. The regulation of particle size of the emulsions made from phospholipids is required in order to define their applications in cosmetic or medical uses (19). However, it has been very difficult to design monodispersion emulsions by conventional instruments. Fujita *et al.* (20) reported that particles larger than 6 μm in fat emulsions can cause serious side effects such as emboli. Recently, a simple preparation method was reported for simple and multiple emulsions stabilized with egg yolk phospholipids (16). Higashi *et al.*(15) applied the membrane emulsification technique for lipid microdroplet of monodisperse and reported its effectiveness to treat patients with hepatocellular carcinoma. The present microfiltration method of phospholipid emulsions would offer the practical advantage for pharmaceutical or cosmetic applications by selecting the suitable membrane with narrow particle size distribution.

Stability of the filtrated O/W emulsion. Figure 3 shows the changes of the filtrated O/W emulsion which was passed through the hydrophilic membrane (D_m = 5.2 μm) after storage for 6 wk at 5°C. The size distribution of the emulsion (mean diameter = 3.62 μm) was not affected by aging. Slight creaming was observed, but no coalescence and breakdown of the droplets were seen during the storage. The emulsion exhibited a sharp droplet distribution from 1.21 to 4.6 μm (mean diameter = 3.88 μm). On the other hand, the nonfiltrated O/W emulsion made by the disperser was skewed to larger droplet sizes during aging due to coalescence and breakdown, i.e., mean droplet size changed from 4.37 to 8.47 μm and the distribution of the droplet size ranged from 0.8 to 56.4 μm. Stabilization of emulsions is important technology with applications in the chemical industry, in the formulation of drugs and in food production. Stability of emulsions is influenced by various factors, e.g., viscosity, an energy barrier, and the van der Waals interaction (21). The stability of oil droplets by phospholipids is affected by the phospholipids

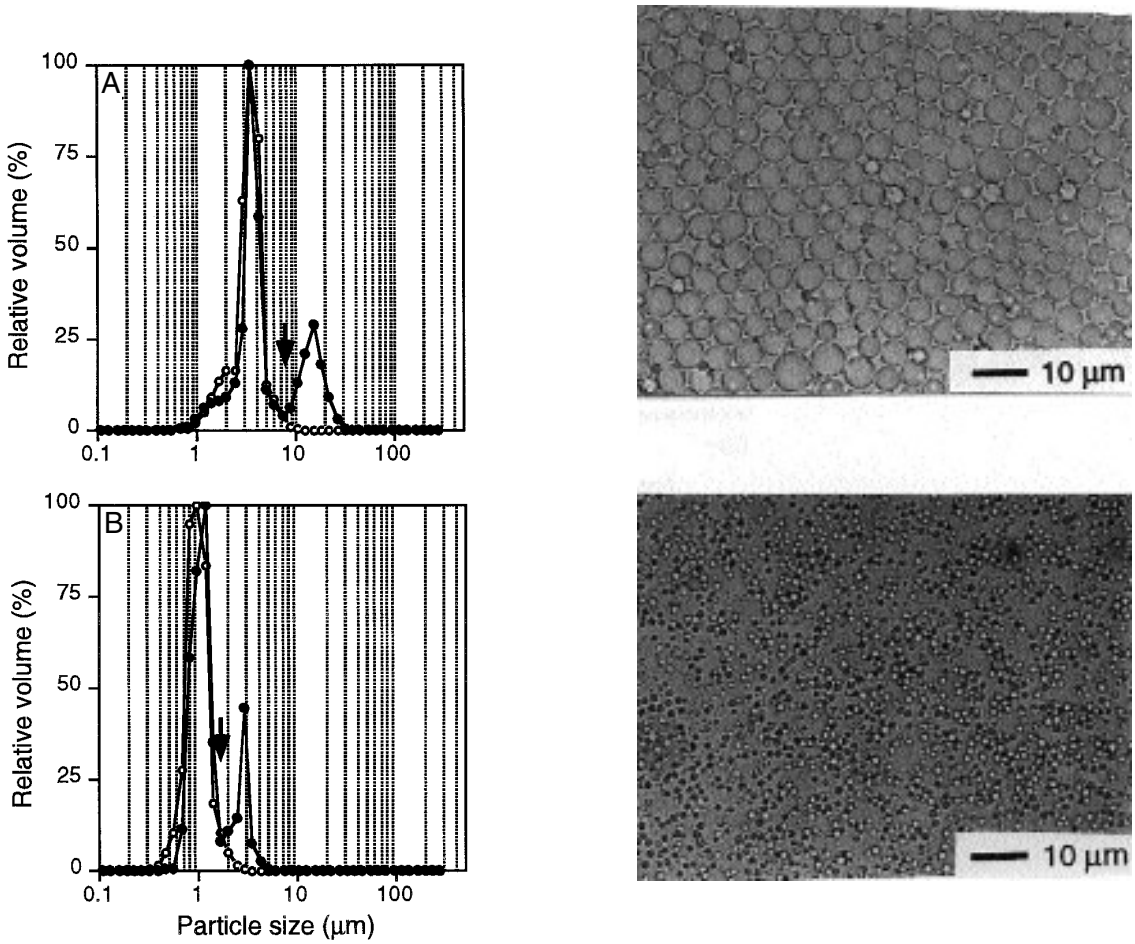

FIG. 2. Changes in particle size distribution and photomicrographs of oil-in-water (O/W) (A) and water-in-oil (W/O) (B) emulsions after the treatment of microfiltration by porous glass membranes: (●): before filtration , (○): after filtration. The arrows show the pore size of the membranes used here.

composition. The addition of ionic lipids such as phosphatidic acid and phosphatidylinositol led to increased stability of the droplets (8). Cholesterol reduced the degree of dispersion of phosphatidylcholine–triglyceride emulsions, but enhanced the phospholipid emulsion stability (7). The droplet size of phospholipids emulsions depended on their compositions; subsequently, smaller droplets are formed in the order of lysophosphatidylcholine, phosphatidylcholine, phosphatidic acid, sphingomyelin, and phosphatidylethanolamine (22). The present data indicated the dispersity of emulsion droplets is also an important parameter in stabilizing phospholipid emulsion as well as droplet size. In conclusion, microfiltration using the size-controlled porous glass membrane would appear to be a most effective method for controlling the particle size of intravenous fat emulsions and/or drug carrier emulsions as well as for the stabilization of emulsions.

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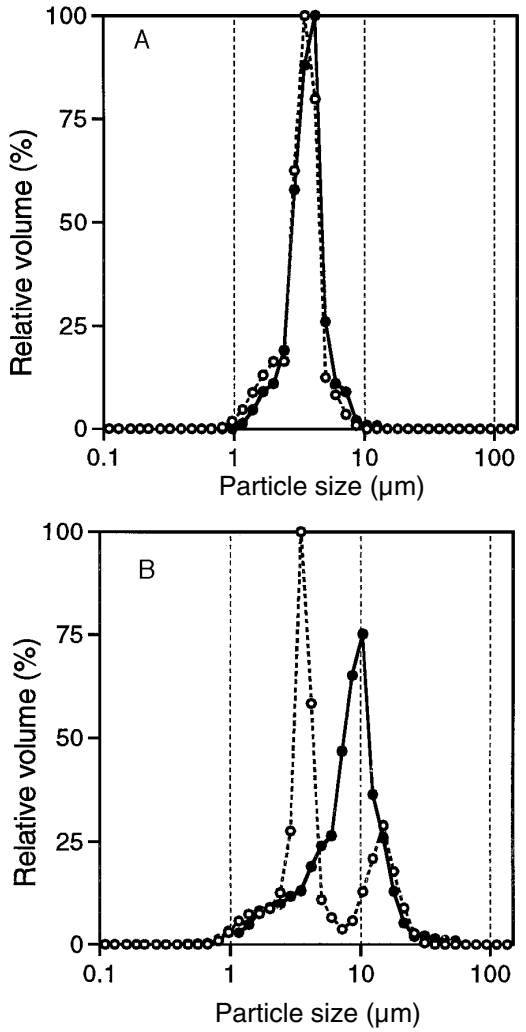

FIG. 3. Changes in particle size distribution of filtrated O/W emulsion (A) and polydispersed O/W emulsion (B) containing 1.0% lysophosphatidylcholine and 20% soybean oil. (○): Stored for 0 day, (●): stored for 6 wk at 5°C. See Figure 2 for abbreviation.

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