Food Sci. Technol. Res., **7** (4), 300–302, 2001

Note

Preparation of W/O/W Emulsions at Low Emulsifier Concentrations

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Received March 7, 2001; Accepted August 1, 2001

W/O/W emulsions were prepared using low concentrations of emulsifiers in the oily and outer aqueous phases, and the formation of the emulsions were evaluated by the encapsulation efficiency of a fluorescent marker in the inner aqueous phase. W/O/W emulsions were produced even at low emulsifier concentrations, although the standard deviation of the encapsulation efficiency immediately after preparation became larger as the emulsifier concentration decreased. Long-term stability of the emulsions with no emulsifier was unsatisfactory, although emulsions with a lipophilic emulsifier only in the oily phase were stable for 30 days with a decrease in the encapsulation efficiency of less than around 5%. Emulsifier species also significantly affected the formation of W/O/W emulsions.

Keywords: W/O/W emulsion, low emulsifier concentration, encapsulation efficiency

W/O/W emulsions are appreciated as useful tools in pharmaceutical industries to carry hydrophilic bioactive substances into human bodies (Jager-Lezer *et al*., 1997) and are drawing increasing attention from food manufacturers for use in the production of functional food products (Matsumoto, 1986). Many research studies have been carried out on the production of W/O/W emulsions and, in most of them, two or more species of emulsifiers, usually lipophilic and hydrophilic types, were added to the oily and outer aqueous phases in the first and second steps of homogenization during the two-step emulsification procedure. Our previous study suggested that low concentrations of hydrophilic emulsifiers in the outer aqueous phase led to better stability of the resultant W/O/W emulsion, independent of the species of counterpart lipophilic emulsifiers in the oily phase (Musashino *et al*., 2001). In this study, we investigated the emulsifier concentrations in the oily and outer aqueous phases optimal for preparation of a W/O/W emulsion, focusing on the proportion of emulsion formation immediately after preparation and the stability of the resultant emulsion at lower emulsifier concentration ranges.

Materials and Methods

Materials Oleic acid and soybean lecithin were purchased from Wako Pure Chemical Industries, Osaka. Span 80 (sorbitan monooleate) was obtained from Aldrich Chemical Company, WI. Lipophilic emulsifiers, Sunsoft 818DG (tetraglycerin polyricinoleate) and 818SX (hexaglycerin polyricinoleate), were supplied by Taiyo Kagaku, Mie. PTSA (1,3,6,8-pyrenetetrasulfonic acid, tetrasodium salt), which was a fluorescent marker loaded into the inner aqueous phase, was purchased from Molecular Probes, OR. It was reported (Tokgoz *et al*., 1996) that the marker was stable and that an emission intensity of more than 90% was recorded after one week of storage at 20˚C under direct sunlight. All the chemicals were of the highest grade available and were used without further purification.

Preparation of the W/O/W emulsion A multiple emulsion was prepared through the two-step emulsification procedure reported previously (Musashino *et al*., 2001). The inner aqueous phase (0.9 ml) containing PTSA at a concentration of 1.0×10^{-4} mol/*l* as a fluorescent marker and the oily phase (2.1 ml) which was oleic acid containing a lypophilic emulsifier were homogenized with a rotor-stator type blender (Physcotron NS-50, Nichion Irika, Tokyo) for 2 min to form a W/O emulsion. The outer aqueous phase with or without a hydrophilic emulsifier (3 ml) was then added to the W/O emulsion and homogenized again for 1 min to obtain a W/O/W emulsion. The blender was operated at rotation intensities of graduation 60 (corresponding to ca. 22,000 rpm) and 40 (ca. 10,000 rpm) for the first and second emulsification steps, respectively. A portion of the W/O/W emulsion (1 ml) was sampled immediately after preparation for PTSA concentration analysis. Alternatively, the resultant W/O/W emulsions were, in some cases, pipetted into several test tubes (0.5 ml each) and stored at room temperature without stirring. The emulsions were intermittently analyzed to follow the change in PTSA encapsulation efficiency with time.

Evaluation of the emulsion stability The PTSA encapsulation efficiency, β, was employed as a measure of W/O/W emulsion stability (Musashino *et al*., 2001):

$$\beta=\frac{M_0-M_t}{M_0}\times100\,[\%] \qquad (1)$$

where M_0 is the total amount of PTSA over the whole system, and M_t is the amount of PTSA leaked into the outer aqueous phase at time t.

To quantify PTSA in the outer aqueous phase, a multiple emulsion was diluted 40 times with distilled water, centrifuged at 7,500 rpm for 10 min at 10˚C, and its lower phase was then filtered through a membrane filter (cellulose acetate, pore size = 0.45 μm, Advantec, Tokyo) to eliminate W/O emulsions. The PTSA concentration of the sample was measured by a spectrofluorophotometer (RF-1500, Shimadzu Corp., Kyoto) at excitation and emission wavelengths of 374 and 404 nm, respectively.

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Results and Discussion

Effect of lecithin concentration in the outer aqueous phase on preparation of a W/O/W emulsion Soybean lecithin was added to the outer aqueous phase of a W/O/W emulsion as a hydrophilic emulsifier. Four kinds of edible emulsifiers, hexaglycerin polyricinoleate (Sunsoft 818SX), tetraglycerin polyricinoleate (Sunsoft 818DG), soybean lecithin, and Span 80, were added to the oily phase at a fixed concentration of 10% (w/v) except for Span 80 (20% (w/v)), and W/O/W emulsions were prepared at a variety of lecithin concentrations in the outer aqueous phase. In the case of Span 80, W/O/W emulsions were not obtained over the whole range of lecithin concentration tested (0–1% (w/v) in the outer aqueous phase); the emulsion was phase-inverted during the second emulsification step to produce a thick W/O emulsion. Other emulsifiers added to the oily-phase produced W/O/W emulsions successfully, and the PTSA encapsulation efficiency of these resultant emulsions immediately after preparation is shown in Fig. 1. Combinations of Sunsoft 818SX or 818DG in the oily phase and lecithin in the outer aqueous phase yielded W/O/W emulsions with high average PTSA encapsulation efficiency of more than 95% at every lecithin concentration. However, the standard deviations of the average value among three runs became larger along with a decrease in the emulsifier concentration in the outer aqueous phase. Even when no emulsifier was added to the outer aqueous phase, multiple emulsions with high PTSA encapsulation efficiency and small standard deviation value were formed. This might be attributable to the stabilization effect of the oily phase-outer aqueous phase interfacial membranes by the lipophilic emulsifier added in the oily phase or to the emulsification ability of oleic acid itself which was used as the oily phase. Although adding lecithin both in the oily and outer aqueous phases as an emulsifier also produced W/O/W emulsions, these emulsions phase-inverted in a few hours to W/O emulsions.

Effect of oily phase emulsifier concentration The above experiments showed that W/O/W emulsions were producible even in the absence of an emulsifier in the outer aqueous phase. We then investigated the influence of emulsifier concentration in the oily phase on W/O/W emulsion formation with no addition

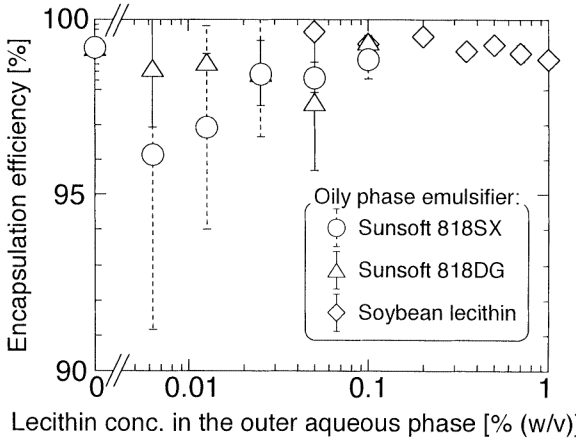

Fig. 1. Effect of soybean lecithin concentration in the outer aqueous phase of a W/O/W multiple emulsion on the encapsulation efficiency of PTSA in the inner aqueous phase immediately after preparation. The volumetric ratio of the inner aqueous/oily/outer aqueous phases = 3/7/10 (v/v/v). The concentration of the emulsifiers added in the oily phase, Sunsoft 818SX (○ with dashed-line error bars), Sunsoft 818DG (△ with solid-line error bars), and soybean lecithin (◇), was fixed at 10% (w/v). Symbols indicate the mean ±SD (n=3). Error bars for soybean lecithin and for Sunsoft 818SX and 818DG at the lecithin concentration of 0% are hidden behind the symbols.

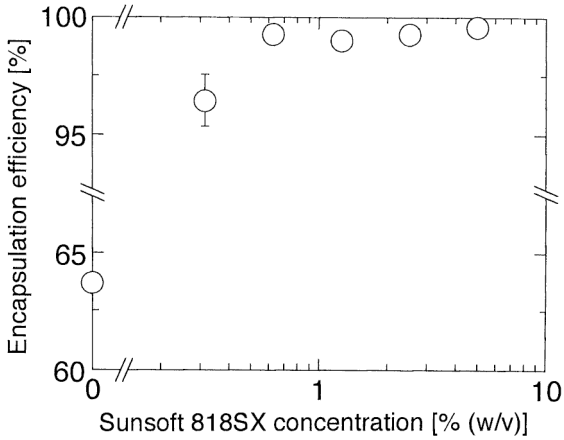

Fig. 2. Relationship between the PTSA encapsulation efficiency of a W/O/W emulsion immediately after preparation and Sunsoft 818SX concentration in the oily phase. No emulsifier was added to the outer aqueous phase during the second homogenization step.

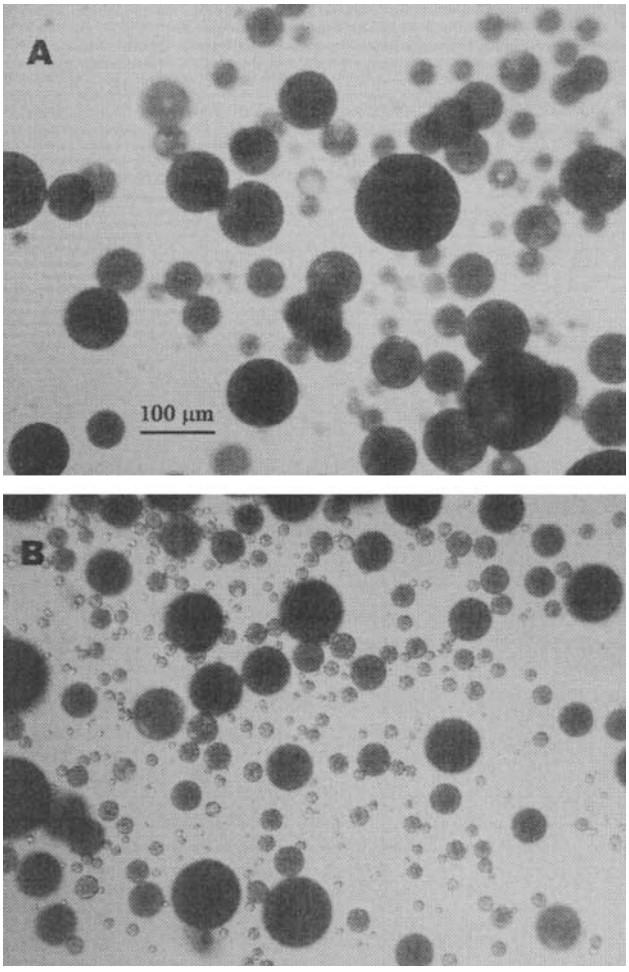

Fig. 3. Micrographs of a W/O/W emulsion stored at room temperature for (A) 1 and (B) 24 h. In the preparation of the emulsion, Sunsoft 818SX was added to the oily phase at a concentration of 2% (w/v) and no emulsifier was added to the outer aqueous phase.

of an emulsifier in the outer aqueous phase. Figure 2 illustrates the PTSA encapsulation efficiency of the W/O/W emulsions immediately after preparation using Sunsoft 818SX as the oily phase emulsifier. At a Sunsoft 818SX concentration range higher than around 0.5% (w/v), W/O/W emulsions of fairly high PTSA encapsulation efficiency were obtained with a small standard deviation value even with no emulsifier in the outer aqueous phase. The standard deviation of the PTSA encapsulation efficiency, however, increased at lower concentrations of Sunsoft 818SX. No addition of emulsifier in either the oily or outer aqueous phase resulted in the greatest deviation which protruded from the figure frame. In these emulsions, creaming or oiling off was also observed from immediately after preparation, implying low stability of the resultant emulsions with time. The reason for the occasional success in W/O/W emulsion preparation without any emulsifier can again be ascribed to the emulsification ability of oleic acid. The long-term stability of the W/O/W emulsion was also tested for emulsions with no emulsifier in the outer aqueous phase. The decrease in PTSA encapsulation efficiency was around 5% during 30-day storage at room temperature when Sunsoft 818SX was added to the oily phase at a concentration of 2% (w/v) (data not shown). Figure 3 shows the micrographs of the emulsions stored at room temperature for 1 and 24 h. No significant change in appearance was observed.

Thus, W/O/W emulsions were producible in the short run even at a low emulsifier level, but the emulsifier contributed to long-term stability of the resultant emulsions. The emulsifier levels in the oily and outer aqueous phases, therefore, should be selected on the basis of whether the resultant emulsions will be stored for a while before consumption or will be consumed or further processed immediately after preparation. The emulsifier species also affected the feasibility of the emulsion to phase inversion.

Acknowledgements This study was supported by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN), Japan. Sunsoft 818 SX and 818 DG were supplied by Taiyo Kagaku.

References

Jager-Lezer, N., Terrisse, I., Bruneau, F., Tokgoz, S., Ferreira, L., Clausse, D., Seiller, M. and Grossiord, J.L. (1997). Influence of lipophilic surfactant on the release kinetics of water-soluble molecules entrapped in a W/O/W multiple emulsion. *J. Control. Release*, **45**, 1–13.

Matsumoto, S. (1986). Preparation of W/O/W emulsions with a view to possible food applications. *Hyomen*, **24**, 381–392 (in Japanese).

Musashino, K., Hasegawa, Y., Imaoka, H., Adachi, S. and Matsuno, R. (2001). Preparation of W/O/W multiple emulsions with polymers in the outer aqueous phase. *Food Sci. Technol. Res.*, **7**, 78–83.

Tokgoz, N.S., Grossiord, J.L., Fructus, A., Seiller, M. and Prognon, P. (1996). Evaluation of two fluorescent probes for the characterization of W/O/W emulsions. *Int. J. Pharm.*, **141**, 27–37.