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Factors Affecting the Properties of Ethanol-in-Oil Emulsions

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Factors affecting the rheological properties and stability of ethanol-in-oil (E/O) emulsion were studied. Emulsions prepared with lower ethanol concentration exhibited higher apparent viscosity, smaller droplet size and narrower droplet size distribution. However, decreasing ethanol concentration may cause a reduction in the amount of emulsified ethanol, facilitating partial separating out of ethanol. The effect of the degree of polymerization of polyglycerol esters of oleic acid on E/O emulsions was investigated with decaglycerol esters of oleic acid (MO-750), hexaglycerol esters of oleic acid (MO-500), and tetraglycerol esters of oleic acid (MO-310). The efficiencies of the emulsifying agents were evaluated by measuring their interfacial activity and ability to stabilize E/O emulsions. Although no significant differences in the interfacial tension values were recognized, the stability of the emulsions increased with the degree of polymerization of the emulsifying agent. Besides sunflower oil, soybean oil and olive oil can also be used to prepare stable E/O emulsions. The characteristics of the emulsions were determined.

Keywords: ethanol-in-oil emulsion, polyglycerol esters of oleic acid, apparent emulsion viscosity, turbidity, droplet size distribution

Rapid developments in related technologies have significantly enhanced the capacity to synthesize and purify functional compounds and to screen new drug compounds. Emulsion has proven to be an effective carrier system for functional compounds and drugs. However, the major difficulty in applying functional compounds or drugs containing emulsions is the poor solubility of the compounds (Collins-Gold *et al.*, 2000). Drugs or functional compounds can be classified into three types according to their solubility: water-soluble, oil-soluble, and water- and oil-insoluble compounds (Washington, 1996). Oil-soluble compounds are normally formulated by dissolving the compound in the oil prior to emulsification. Although water-soluble compounds are not good candidates for emulsion formulations (Washington, 1996; Buszello & Müller, 2000), their modification can provide lipophilic prodrugs or drug derivatives that can be located in the oil phase of the emulsion (Buszello & Müller, 2000). The case of compounds having poor solubility in water and oil is much more complex, and special formulation strategies are required. Therefore, development of suitable drug delivery systems or functional emulsions is especially important. Up to the present, there have been basically two types of drug emulsion: those in which the oil-soluble drug is already dissolved in the oil phase before production and those in which the drug is dissolved in a commercial fat emulsion, usually with the aid of organic solvents such as ethanol or dimethyl acetamine shortly before application (Lucks *et al.*, 2000). The latter runs the risk of drug precipitation in the aqueous phase or the breakdown of the carrier emulsion, which restricts its commercial application. The use of emulsions containing ethanol as the polar phase seems to be the best way to overcome these drawbacks.

Although alcohol-containing emulsions have been of considerable research interest in the past decade, attention has concentrated on protein-stabilized emulsions (Dickinson & Golding, 1998; Agboola & Dalgleish, 1996; Burgaud & Dickinson, 1990). Systematic studies of alcohol-containing emulsions stabilized by small molecular surfactants are totally lacking. As the first step of an attempt to develop such an emulsion carrier system for insoluble or poorly soluble functional compounds or drugs, we have successfully prepared a stable model E/O emulsion system (Xu *et al.*, 2000). The emulsion contains 95% ethanol as the dispersed phase, sunflower oil as the continuous phase, and a nonionic surfactant MO-750 as the emulsifying agent. We have shown that, besides being a promising system for functional and drug delivery emulsions, the E/O emulsion is worthy of fundamental investigation (Xu *et al.*, 2001a). Although MO750 has a limited effect on the interfacial tension at the ethanol-oil interface, it plays an important role in stabilizing an E/O emulsion. The surface-active stabilization driving mechanism (i.e. the *Gibbs-Marangoni effect*) (Borwankar *et al.*, 1999) is not applicable here to explaining the long-term stability of E/O emulsions because MO-750 is dissolved in the dispersed phase in preparation of the emulsion.

Generally, amphiphilic molecules or their micelles and aggregates, form monolayers or multilayers inside interfacial films, conferring stability on emulsions (Dickinson & Golding, 1998, Opawale & Burgess, 1998, Bergeron, 1999, Borwankar *et al.*, 1999). Small-angle X-ray scattering (SAXS) has revealed that MO-750 formed aggregates in the oil phase, leading to the assumption that the formation of a layered structure of MO-750 aggregates inside the interfacial film confers stability on E/O emulsions (Xu *et al.*, 2001a). The ethanol content greatly influences the stability of oil-ethanol emulsions, probably by affecting the formation of the interfacial film (Xu *et al.*, 2001b). E/O emul-

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sions prepared with an MO-750 concentration within a range of 0.1 to 1wt% were stable, indicating that the interfacial films formed were effective in protecting the droplets against coalescence and Ostwald ripening; however, an E/O emulsion prepared with a 5wt% MO-750 concentration was very unstable. The instability at high MO-750 concentration is probably due to irregular structuring formed inside the interfacial film, weakening the strength of the film (Xu *et al.*, 2001b). Detailed investigation of the effect of ethanol concentration on the properties of E/O emulsions has not yet been carried out. This kind of investigation is necessary, since ethanol concentration may affect the solubility of a drug, and thus influence the efficiency of a drug delivery system. In this paper, we examined the influence of ethanol concentration, the degree of polymerization of polyglycerol esters of oleic acid and vegetable oil type on the rheological properties (droplet size, size distribution and apparent viscosity), and the stability of E/O emulsions.

Experimental

Materials High-oleic sunflower oil (oleic acid content >90%) was supplied by Nippon Lever Co., Ltd., Tokyo. Olive oil, soybean oil, dehydrated ethanol (99.5%) and 95% ethanol (containing by volume 95% ethanol and 5% water) were purchased from Wako Pure Chemical Industries, Ltd., Osaka. We prepared 85% ethanol by adding a suitable volume of pure water to 99.5% ethanol. Polyglycerol esters of oleic acid were kindly provided by Sakamoto Yakuhin Kogyo Co., Ltd., Osaka. The structure of these polyglycerol esters is shown in Fig. 1.

Preparation of emulsions In this study, we focused on E/O emulsions with an average droplet diameter of several micrometers, falling within the size range of many industrial emulsion products. The homogenization conditions were adjusted to ensure that the desired droplet size was obtained. MO-750 is barely soluble in ethanol and oil at room temperature, and is predominantly ethanol-soluble at high temperatures, therefore, MO-750 was first dissolved in ethanol at 60°C. After the ethanol solution containing MO-750 was cooled to room temperature, the oil phase was added and homogenized with a homogenizer (Polytron® PT3000, KINEMATICA AG, Lucerne, Switzerland) at 3000 rpm for 10 min. The space between the neck of the vial and the rotating shaft was sealed to prevent evaporation during homogenization. After emulsification, samples were placed in vials tightly sealed with double caps to prevent ethanol evaporation, and these were kept at room temperature. All the samples were prepared in triplicate for measurement.

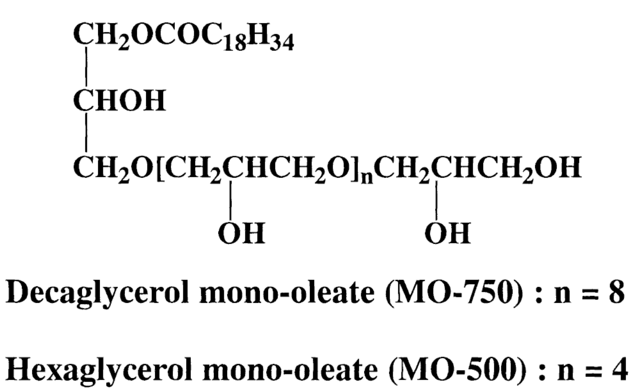

Fig. 1. Structures and commercial names of polyglycerol esters of oleic acid.

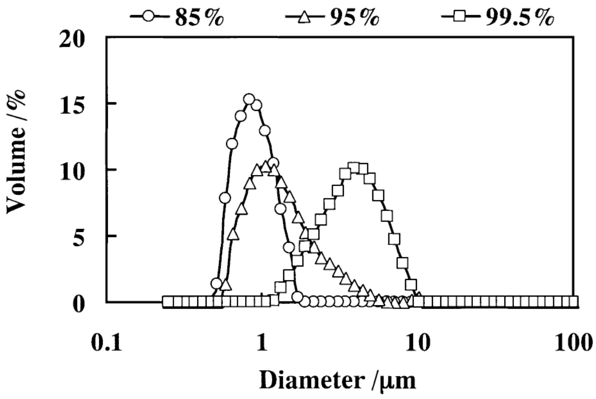

Fig. 2. Effect of ethanol concentration on the droplet size distributions of E/O emulsions stabilized by 1wt% MO-750 at an ethanol phase content of 10 wt%.

Measurement of apparent emulsion viscosity Apparent viscosities of emulsions were measured at 25°C with a microviscosimeter from Haake (Karlsruhe, Germany). Measurements were repeated five times per sample, and mean values were calculated.

Measurement of turbidity Turbidity was measured with a UV/VIS/NIR spectrophotometer (model V-570, JASCO Corporation, Tokyo) at 500 nm. Details of the principle of the measurement are described elsewhere (Pearce & Kinsella, 1978; Liu *et al.*, 1999; Reddy & Fogler, 1981). Short light path length quartz cells with screw caps were used.

Measurement of interfacial tension Interfacial tensions were measured at 25°C using the pendant drop method with a full automatic interfacial tensiometer (Model PD-W, Kyowa Interface Science CO., Ltd., Asaka). Every sample measurement was replicated at least ten times to ensure reproducibility. The mean values were used for data analysis.

Measurement of droplet size and size distribution We used a laser diffraction particle size analyzer for highly turbid samples (SALD-200V-ER-HC, Shimadzu Co., Kyoto) to measure droplet size and size distribution of the emulsions. Samples were placed in the narrow space between two specially made glass slides, preventing multiple scattering effects and obviating the need for dilution, with its attendant negative effects. Measurements were repeated at least three times per sample, and mean values were calculated.

Results and Discussion

Effect of ethanol concentration The effect of ethanol concentration on the rheological properties and stability of E/O emulsions was investigated with MO-750 as the stabilizer. In preliminary experiments, we found that the solubility of MO-750 decreased with ethanol concentration. MO-750 can be dissolved completely in 95% ethanol, but is difficult to dissolve in aqueous ethanol solutions where the ethanol concentration is lower than 75%. Since high water content of ethanol solution decreases the

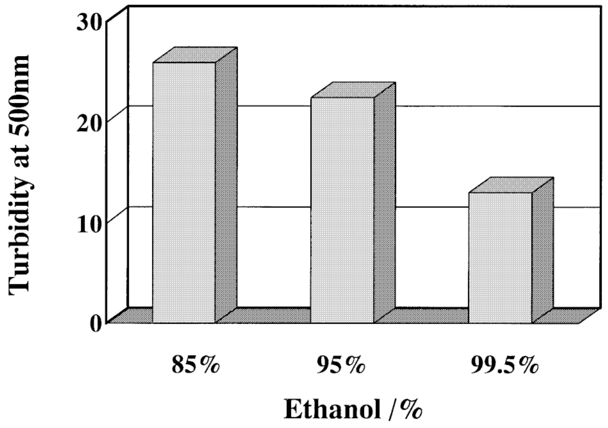

Fig. 3. Effect of ethanol concentration on the turbidity of E/O emulsions stabilized by 1wt% MO-750 at an ethanol phase content of 10wt%.

effectiveness of incorporation of poorly soluble compounds, the experiments were carried out with 85%, 95% and 99.5% ethanol.

E/O emulsions with a lower ethanol concentration exhibited a narrower size distribution and a small mean volume droplet diameter (Fig. 2). The mean volume droplet diameters we obtained for these emulsions were 0.8 μm with 85% ethanol, 1.3 μm with 95% ethanol and 3.3 μm with 99.5% ethanol. According to the Mie theory for light scattering by dispersed spherical particles, the interfacial area of an emulsion is twice its turbidity (Pearce & Kinsella, 1978). An emulsion with a lower ethanol concentration has a larger interfacial area and thus a higher turbidity (Fig. 3). However, a very thinly separated ethanol layer was observed on the top of the emulsified layer in the emulsion prepared with 95% ethanol within the first several days of storage, which also became much more apparent in the case of 85% ethanol. After that, phase separation did not proceed further; the bottom emulsified layer remained stable, which was confirmed by following the turbidity change with time in preliminary experiments. In contrast, the emulsion prepared with dehydrated ethanol showed good stability, and no phase separation was observed. It seems that there are maximum emulsified volumes for aqueous ethanol, which decrease with reduction in ethanol concentration (i.e., increasing water content). As a result, droplets initially coalesce until the emulsified volume reaches its maximum. In diesel fuel-ethanol hybrids, the presence of a very small amount of water caused phase separation and movement of the ethanol and water to the top of the mixture (Schwab & Pryde, 1984). It has been reported that polyglycerol esters of oleic acid formed lamellar liquid crystal with water (Guo *et al.*, 2000). The actual mechanism of partial phase separation caused by the presence of a small amount of water in E/O emulsions remains unknown. Besides ethanol content, which significantly influenced the formation and stability of E/O emulsions (Xu *et al.*, 2001b), it was shown here that the ethanol concentration might affect E/O emulsion stability in a more complex way. The effect of ethanol concentration on the apparent viscosity of E/O emulsions is shown in Fig. 4, demonstrating that the apparent viscosity of these emulsions decreased with the increase in ethanol concentration. This is attributable to the higher ethanol concentration having greater ability to reduce the apparent viscosity of an emulsion.

Effect of the degree of polymerization of emulsifying agents In a screening test with a number of commercially available food emulsifying agents in our previous study (Xu *et al.*, 2000), we found that sorbitan fatty acid esters (Span 40, 60, 80, and 85), polyglycerol fatty acid esters (PO-500, PO-310, DAO-750) and lecithin were poor stabilizers for E/O emulsions. Stable E/O emulsions could be prepared with polyglycerol esters of oleic acid (MO-310, MO-500 and MO-750). However, the E/O emulsions obtained showed different stabilities. For E/O emulsion prepared with MO-310, MO-500 and MO-750 at an ethanol weight fraction of 0.2, ethanol and oil completely separated within one month for MO-310, within two months for MO-500 and within five months for MO750. These three emulsifying agents differ mainly in the degree of polymerization of glycerol, i.e. tetraglycerol ester of oleic acid, hexaglycerol ester of oleic acid and decaglycerol ester of oleic acid have 4, 6 or 10 glycerol groups (Fig. 1). To verify how the degree of glycerol polymerization in the emulsifying agents affects their emulsification efficiency and the properties of E/O emulsions, we first measured the interfacial activity of MO-310, MO-500 and MO-750 at the ethanol/oil interface.

No significant reductions in interfacial tension at 95% ethanol/

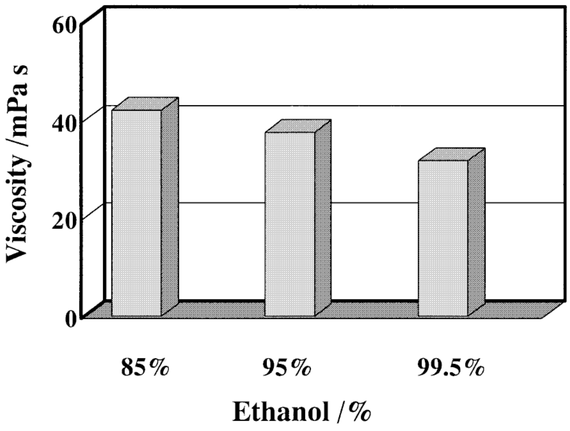

Fig. 4. Effect of ethanol concentration on the apparent viscosity of E/O emulsions stabilized by 1wt% MO-750 at an ethanol phase content of 10wt%.

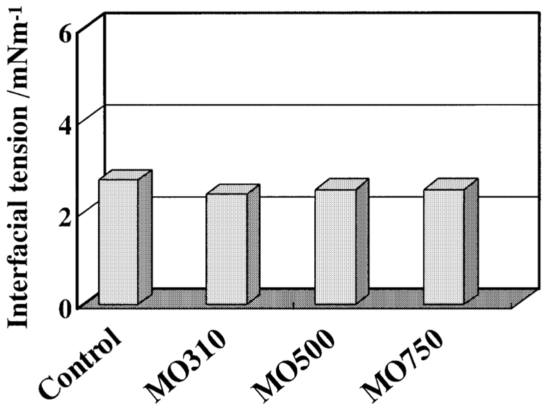

Fig. 5. Effect of the degree of polymerization of the emulsifying agents on their interfacial activities in a 95% ethanol-oil system. The concentration of emulsifying agent was 1wt%.

oil interfaces were observed in the presence of the emulsifying agents (Fig. 5). The interfacial tension values obtained were almost the same, regardless of the degree of polymerization, indicating that the degree of glycerol polymerization did not affect their interfacial activity in the ethanol-oil system. Based on the similar interfacial performance and structural properties of these emulsifying agents, it is reasonable to presume that E/O emulsions prepared with MO-310 and MO-500 share the same stabilization mechanism as MO-750. That is, the layering structure of the surfactant aggregates formed inside the interfacial films stabilizes the emulsions. Since the hydrophobic moieties of these surfactants are the same, the hydrophilic characteristics of the emulsifying agents depend on the number of glycerol groups and will increase with the glycerol group number. A more hydrophilic emulsifying agent is more prone to form aggregates in oil. Furthermore, the size of the unaggregated surfactant molecule affects the size of its aggregates. This in turn will influence the effective thickness of the layering structure inside the interfacial film. On this basis, it was supposed that an emulsifying agent having a higher degree of polymerization would form thicker/stronger interfacial films; consequently, an emulsion prepared with such an emulsifying agent would be more stable.

In general, if an emulsion is stable, the interfacial area and the turbidity of the emulsion do not change with time. The stabilities of E/O emulsions were evaluated by monitoring turbidity over a period of time. No significant change in the turbidity of the emulsion prepared with MO-750 was observed within the period studied (Fig. 6). Although the initial turbidities of the emulsions prepared with MO-500 and MO-310 were almost the same, the turbidity decrease with MO-500 was faster than that with MO-310. This agrees with the assumption that a more stable emulsion could be prepared with an emulsifying agent having a greater degree of polymerization. We can conclude that although the degree of polymerization did not greatly affect the interfacial activities of the emulsifying agents in an ethanol-oil system, it does impart different stability to E/O emulsions. The interfacial activity of an emulsifying agent is thus seen to be of lesser importance in an ethanol-oil system for comparing the ability to stabilize E/O emulsions. An inverse effect of polymerization degree of nonionic surfactants (ethoxylated alcohols) on the micelle size and on interfacial thickness stability was reported by Nikolov *et al.* (1990). Specifically, they found that the micelle size in aqueous solution and the interfacial thickness stability decreased with increase in the degree of ethoxylation of the surfactants. This can be explained by noting that a surfactant with a higher degree of ethoxylation has a greater hydrophilicity since the ethylene oxide groups contribute hydrophilicity to the surfactants. Greater hydrophilicity increases the solubility of the surfactant in water and thus is detrimental to the formation of micelles in water. This results in a decrease in micelle size and interfacial thickness, and hence less stability of the interfacial film. These results are supportive of the hydrophilicity/hydrophobicity of a surfactant playing a very important role in the stability of the interfacial film formed by the structure of micelles/aggregates, and this affects the stability of an emulsion.

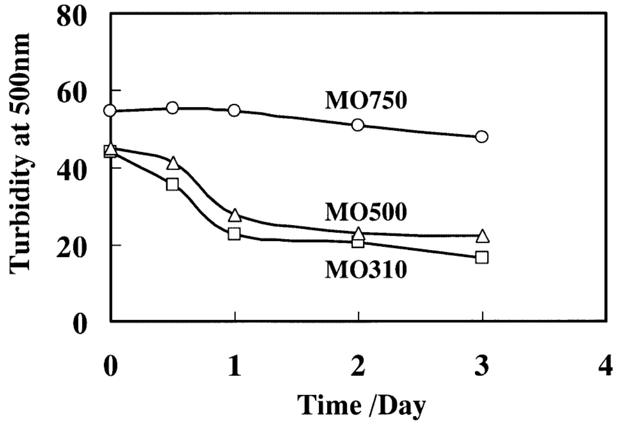

Fig. 6. Changes in the turbidity of E/O emulsions prepared with MO-310, MO-500, and MO-750 at an ethanol (95%) content of 10wt%. The concentration of emulsifying agent was 1wt%.

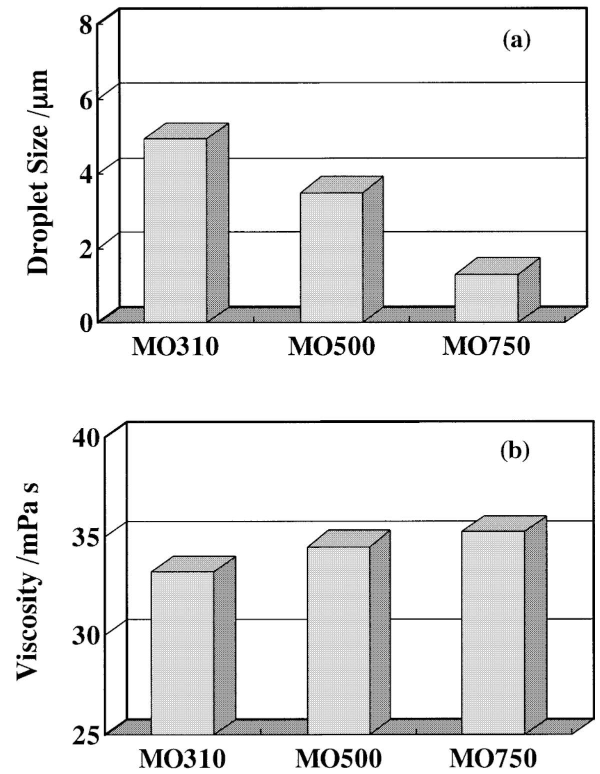

Fig. 7. Effect of the degree of polymerization of the emulsifying agents on the droplet size (a) and viscosities (b) of E/O emulsions containing 10wt% ethanol (95%) and 1wt% emulsifying agent.

The droplet sizes of E/O emulsions prepared with MO-310, MO-500 and MO-750 tended to decrease with increase in the degree of polymerization (Fig. 7a). The average volume diameters obtained were 5.0 μm for MO-310, 3.5 μm for MO-500 and 1.3 μm for MO-750. It is generally accepted that smaller droplets will be formed in a system with a lower interfacial tension. Since there are no significant differences in the interfacial activity of the three emulsifying agents in an ethanol-oil system, it was expected that there would be no difference in the average droplet sizes obtained for the emulsions. It appears that the experimental result contradicts the expectation. The inconsistency between the interfacial activity of a surfactant and the droplet size obtained was also reported in an O/W emulsion stabilized with a commercially available soyasaponin (SC) (Gohtani & Yamano, 1999).

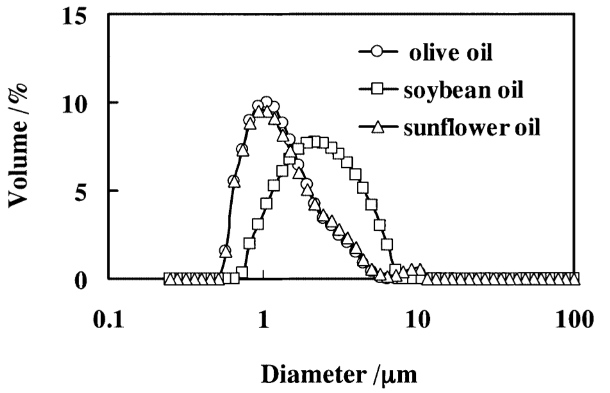

Fig. 8. A comparison of the droplet size distributions of E/O emulsions prepared with sunflower oil, soybean oil, and olive oil. Ethanol (95%) content was 10wt%; the concentration of emulsifying agent was 1wt%.

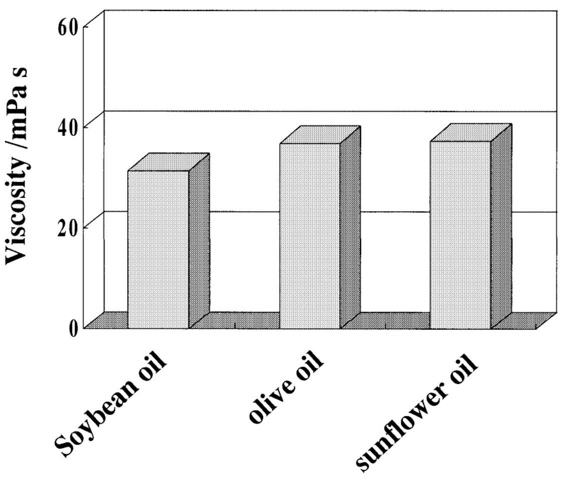

Fig. 9. Apparent viscosity of E/O emulsions prepared with sunflower oil, soybean oil, and olive oil. Ethanol (95%) content was 10wt%; the concentration of emulsifying agent was 1wt%.

Even though, of the emulsifying agents studied, SC showed the strongest interfacial activity at a water/oil interface, the emulsion obtained had the largest average diameter. It was demonstrated that the weak interfacial film formed by SC was insufficient to stabilize the emulsion and coalescence took place immediately after emulsification. The authors emphasized that the major role of the interfacial activity of an emulsifying agent is to improve emulsification efficiency, not to stabilize emulsions, where the stability of emulsions depends greatly on the strength of the interfacial film. The significance of the amphiphilic molecules in maintaining the strength of an interfacial film was also emphasized by other researchers (Opawale & Burgess, 1998). It was pointed out that for long-term stability against coalescence and consequent phase separation, the ability of a surfactant to impart strength to the interfacial film is more important than its effect on interfacial tension. Since the strength of interfacial films decreased with the degree of polymerization of the emulsifying agent, the emulsion prepared with an emulsifying agent of lower degree of polymerization had a rapid coalescence rate, leading to an increased average droplet diameter, which was confirmed by the turbidity experiments (Fig. 6). Furthermore, we examined the relationship between the degree of polymerization of the emulsifying agents and apparent emulsion viscosities. The apparent viscosity of the emulsions did increase slightly with increase in the degree of polymerization of the emulsifying agents (Fig. 7b), indicating that the emulsifying agents mainly stabilized the E/O emulsions by forming rigid interfacial films.

Effect of vegetable oil type It was reported (Lucks *et al.*, 2000) that several kinds of vegetable oils have been used to formulate nutritional emulsions and drug delivery emulsions. Therefore, it is necessary to investigate the possibility of making E/O emulsions with other kinds of vegetable oils. We chose two widely used vegetable oils, soybean oil and olive oil to prepare E/O emulsions with MO-750 as the stabilizer and found that stable E/O emulsions could be prepared with these oils. The average volume droplet diameters obtained were 2.2 μm for soybean oil emulsion and 1.2 μm for olive oil emulsion. A comparison of the droplet size distributions of the emulsions is presented in Fig. 8. The droplet size distribution of olive oil emulsion was similar to that of sunflower oil emulsion, whereas the droplet size distribution of soybean oil emulsion showed a slight shift towards a larger droplet size. As shown in Fig. 9, the apparent viscosity values of sunflower oil emulsion and olive oil emulsion were almost the same, and were greater than that of soybean oil emulsion. Further experiments are required to clarify what causes the difference in emulsion properties between soybean oil and the two other oils.

In conclusion, we demonstrated that the ethanol concentration influenced the properties of E/O emulsions. Average droplet size and width of droplet size distribution increased with ethanol concentration, whereas the apparent emulsion viscosity decreased with the increase in ethanol concentration. Increasing water content may reduce the amount of emulsified ethanol, facilitating the separating out of a part of the ethanol phase. Even though the interfacial activity did not significantly depend on the degree of polymerization of the emulsifying agent in the ethanol-oil system, the stability of E/O emulsions was significantly improved as the degree of polymerization of the emulsifying agent increased. Besides sunflower oil, stable E/O emulsions can also be prepared with soybean oil and olive oil.

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