Formation and Investigation of Microemulsions Based on Jojoba Oil and Nonionic Surfactants

M. Shevachman[a], **A. Shani**[a,*], **and N. Garti**[b,*]

[a]Department of Chemistry, Ben-Gurion University of the Negev, Be'er-Sheva 84105, Israel, and
[b]Casali Institute of Applied Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

ABSTRACT: The properties of jojoba oil make it uniquely suited as a raw material for the cosmetics industry. Water-based, thermodynamically stable preparations of jojoba oil are essential in many formulations. New microemulsions were prepared based on jojoba oil and different nonionic surfactants, namely polyoxyethylene-(ethylene oxide)$_{10}$-oleyl alcohol (Brij 96V) and ethoxylated sorbitan esters (Tweens). The effects of the surfactants and of primary alcohols as cosurfactants on the isotropic regions of the phase diagram were elucidated. It was found that, up to a certain cosurfactant chain length, the isotropic region expanded considerably as chain length increased. The size of the isotropic region also increased as a function of the ethylene glycol content of the aqueous phase in microemulsions based on ethoxylated alcohol but shrank when ethylene glycol was included in microemulsions prepared with ethoxylated sorbitan esters. Secondary structural phase transitions from water-in-oil to bicontinuous and to oil-in-water structures (as determined by measuring conductivity and viscosity) were found to be related to jojoba oil content. Dynamic light scattering and small angle X-ray scattering studies established that incorporation of jojoba oil into Brij 96V micelles caused micellar transformation from elongated to spherical droplets and a decrease in the aggregation number.

Paper no. J10835 in *JAOCS 81*, 1143–1152 (December 2004).

KEY WORDS: Brij 96V, cosurfactants, droplet shape, ethoxylated sorbitan esters, ethylene glycol, *Jojoba oil*, medium-chain alcohols, *microemulsion*, solubilization, structural transformation.

Jojoba liquid wax (also called jojoba oil) is a stable, highly lipophilic, non-toxic "oily" material obtained from the seeds of the desert plant jojoba (*Simmondsia chinensis*) (Fig. 1). This unique oil differs from common vegetable oils and animal fats in that it is composed mainly (97%) of linear long-chain esters, which are characteristic components of waxes. More than 80% of these are esters of C_{18}-, C_{20}-, C_{22}-, and C_{24}-chain monounsaturated alcohols and acids (1).

Jojoba wax resists oxidation even at elevated temperatures and can remain chemically unchanged for years. Owing to its excellent ability to penetrate the skin and high resistance to oxidation, jojoba wax is widely used as a component in cosmetics. Its derivatives have potential applications in a variety of commercial fields, including pharmaceuticals and lubrication (1). Numerous recent studies have demonstrated the feasibility of incorporating jojoba as an oil phase in formulas containing active compounds to enhance the efficacy of topical drugs (2,3).

$(Z,Z)\text{-}CH_3(CH_2)_7CH{=}CH(CH_2)_mCOO(CH_2)_nCH{=}CH(CH_2)_7CH_3$

Jojoba oil

m = 7, 9, 11, 13
% 11, 71, 14, 1

n = 8, 10, 12, 14
% 1, 44, 45, 9

FIG. 1. Averaged molecular structure of jojoba oil.

The growing interest in microemulsions (ME) for cosmetic and pharmaceutical applications is linked to their physicochemical properties—thermodynamic stability, spontaneous formation, clear appearance, low viscosity, and high solubilization capacity (4). A particularly attractive possibility is that of using ME to enhance the efficacy of molecularly solubilized, hydrophobic, biologically active compounds by providing a readily controlled, stable medium for the solubilized components, together, perhaps, with protection against degradative reactions (5).

The use of alcohols as cosurfactants in the preparation of ME has a long history. Alcohols are added to ionic surfactants to compensate for the salting out effect. In the case of nonionic surfactants, the role of the alcohol is typically to balance their affinity for water and oil and thereby lower the interfacial tension between the oil phase and the water phase, which is often too high for commercial surfactants.

The properties of ME are largely determined by two parameters—the spontaneous curvature (H_0) of the surfactant film, which is the curvature that the amphiphilic film strives to attain, and the elasticity of the interfacial monolayer, described by the bending moduli κ and $\bar{\kappa}$ (these values show how difficult it is to deform the amphiphilic film) (6–8). When H_0 is positive, the surfactant film is convex toward the water and promotes the ME of an oil-in-water formation (O/W) ME. If H_0 is close to zero, then flat domains are formed (such as bicontinuous structures and lamellar liquid crystals). When H_0 is negative, the surfactant film is convex toward the oil, favoring water-in-oil (W/O) ME. The spontaneous curvature of the surfactant film is affected by the nature of the surfactant and the length of the alcohol chain (8). Alcohol can modify the spontaneous curvature of the surfactant film when its penetration into the interface is important enough to increase interface flexibility (alcohol as cosurfactant), or it can change the polarity of the polar and apolar phases (alcohol as cosolvent).

---

*To whom correspondence should be addressed.
E-mail: garti@vms.huji.ac.il; ashani@bgumail.bgu.ac.il

As is well documented in the literature, an increase in the flexibility of the surfactant film is obtained by adding alcohols with different chain lengths. In addition, we added polyol to the aqueous phase to decrease its hydrophilicity. We studied the effects of polyols and of different alcohol types and contents in two very different types of surfactants, one with a high molecular volume, which forms loose ("open", not tightly packed) interfaces (Tween), and the other with a small molecular volume, which forms tightly packed interfaces (Brij). Our aim was to establish prerequisites for preparing new nonionic microemulsions based on jojoba oil and incorporating permitted cosmetic and pharmaceutical components. ME droplet sizes and shapes were correlated with the character of the surfactant packing as well as with the effects of polyol and alcohol on the interface.

To the best of our knowledge, this contribution represents the first extensive study of ME based on jojoba oil. We present phase diagrams and microstructure investigations for nonionic systems with three, four, and five components. Particle sizes and aggregation numbers were determined using light-scattering experiments in diluted regions to avoid particle interaction and interpenetration.

EXPERIMENTAL PROCEDURES

Materials. Polyoxyethylene-10EO-oleyl alcohol (Brij 96V) where EO-ethylene oxide, was purchased from ICI Specialty Chemicals (Essen, Germany). Ethoxylated sorbitan esters (Tween 20, ethoxylated-20EO-sorbitan monolaurate; Tween 60, ethoxylated-20EO-sorbitan monostearate; and Tween 80, ethoxylated-20EO-sorbitan monooleate) were obtained from Sigma Chemical Co. (St. Louis, MO). Crude jojoba oil with an iodine value of 80.9 was a gift of Jojoba Israel (Kibbutz Hatserim, Israel), and it was used without further purification. The alcohols ethanol (analytical), 1-butanol (CP), 1-pentanol (CP), and 1-hexanol (purum, ≥98%) were purchased from Fluka (Fluka Chemie, Buchs, Switzerland). 1-Heptanol (CP) and 1-octanol (CP) were produced by Frutarom (Haifa, Israel) and used without further purification. Chemically pure (CP) ethylene glycol (EG) was obtained from BDH (Poole, United Kingdom). Water was double distilled.

Sample preparation. Pseudo-ternary phase diagrams were constructed in the following way: Mixtures of surfactant, oil, and alcohol (weight ratio of oil/alcohol was kept at 1:1) were titrated with water, at room temperature, to the solubilization limit, which was defined as the transition from the monophasic region to a polyphasic region or to a birefringent phase. The transition to a two-phase system could be detected visually, being marked by the appearance of cloudiness. Liquid crystal phases were identified using crossed polarizers.

Solubilization parameters. Two suitable solubilization parameters were used in this work: the maximal amount of solubilized water, denoted W_m (9), and the total isotropic monophasic area, denoted A_T (10). W_m was determined by titration of the oil phase with water (or water-diol mixture), as previously reported (9). Relative error was estimated as ±2% for calculated W_m (wt%) and ±0.5% for calculated A_T (%).

Conductivity measurements. Conductivity measurements were performed on a Bench Conductivity meter (model HI 8819 or HI 8820; Hanna Instruments, Padova, Italy).

Rheological behavior. Rheological behavior was examined using a CSL-50 R&D-grade rheometer (TA Instruments GmbH, Alzenau, Germany). Steady shear viscosity and oscillatory shear deformation were determined. The samples were thermostated at 25°C using a model 9100 thermostat from PolyScience, Division of Preston Industries, Inc. (Niles, IL).

Small-angle X-ray scattering (SAXS) measurements. X-ray scattering experiments were performed using Ni-filtered Cu Kα radiation (0.154 nm) from a Philips sealed tube X-ray generator that operated at a power rating of up to 1.36 kW. X-radiation was further monochromated and collimated by means of a single Franks mirror and a series of slits and height limiters and measured by a linear position-sensitive detector. The samples were inserted into 1.5-mm quartz capillaries. The temperature was maintained at 25 ± 1°C.

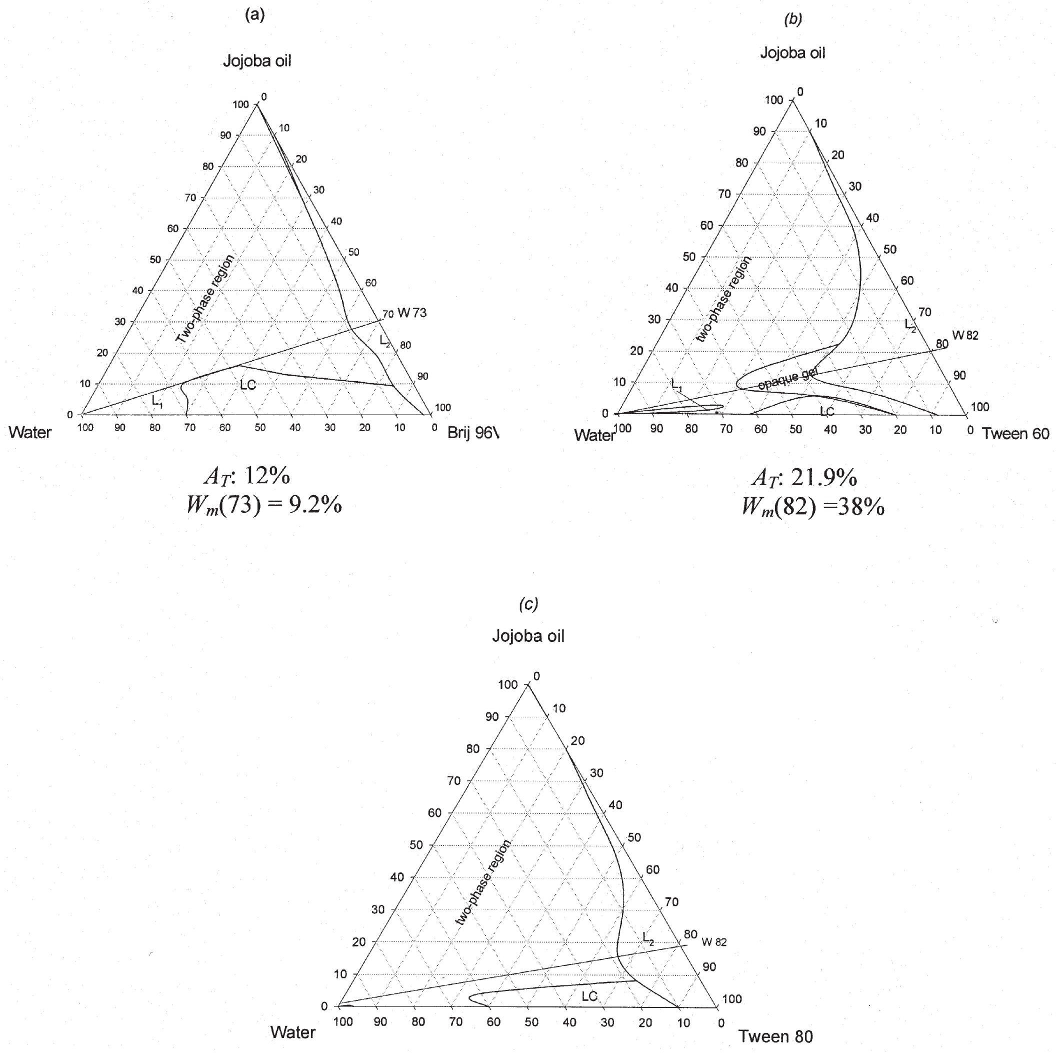
Dynamic light scattering (DLS). Light scattering experiments were performed using an argon ion laser (model 165 Spectra Physics; with a wavelength of 514.5 nm). The temperature was maintained at 25 ± 1°C. The correlation functions were derived from the signals determined in digital self-correlation with 72 channels (B12030 AT; Brookhaven Instruments, Holtsville, NY). The hydrodynamic radii of the particles were obtained from the Stokes-Einstein equation, $D = k_BT/6\pi\eta\ R_H$, where R_H is the hydrodynamic droplet radius, D the diffusion coefficient, η the solvent viscosity, T the temperature (K), and k_B the Boltzmann constant. Aggregation numbers of micelles were calculated from micellar weight.

Cryo-transmission electron microscopy (TEM) experiments. Samples were prepared as previously described (10). The thin film of the ME over the grid was vitrified in liquid ethane. The grid was transferred under liquid nitrogen to a cold-stage (Model 626; Gatan, Inc., Warrendale, PA), which was introduced into the electron microscope (JEOL 2000FX), which was operated at 100 kV in the conventional TEM mode. The working temperature was below −160°C.

RESULTS AND DISCUSSION

Effect of surfactants on formation of jojoba oil microemulsions. The phase diagram for the three-component system [jojoba oil/Brij 96V/water] is presented in Figure 2A. It consists of restricted regions of W/O (L_2) and O/W ME (L_1), a liquid crystal (LC) area, and a very large two-phase region. Replacing the ethoxylated alcohol with the ethoxylated sorbitan ester Tween 60 (Fig. 2B) significantly enlarged the isotropic regions, producing a greater L_2 area and therefore a greater total monophasic area (A_T = 21.9 vs. 12%). Among the Tweens, Tween 20, being the most hydrophilic of the surfactants, solubilized the least amount of oil (A_T = 2.5%; data not shown). One can clearly see that the phase diagrams for jojoba oil with Tween 60 and Tween 80 (Figs. 2B and 2C) have larger W/O ME regions as compared with the system based on Brij 96V. On the other hand, only Brij 96V (Fig. 2A) formed a substantial O/W ME region with jojoba.

It is well known that the solubilization process is affected



A_T: 12%
$W_m(73) = 9.2\%$

A_T: 21.9%
$W_m(82) = 38\%$

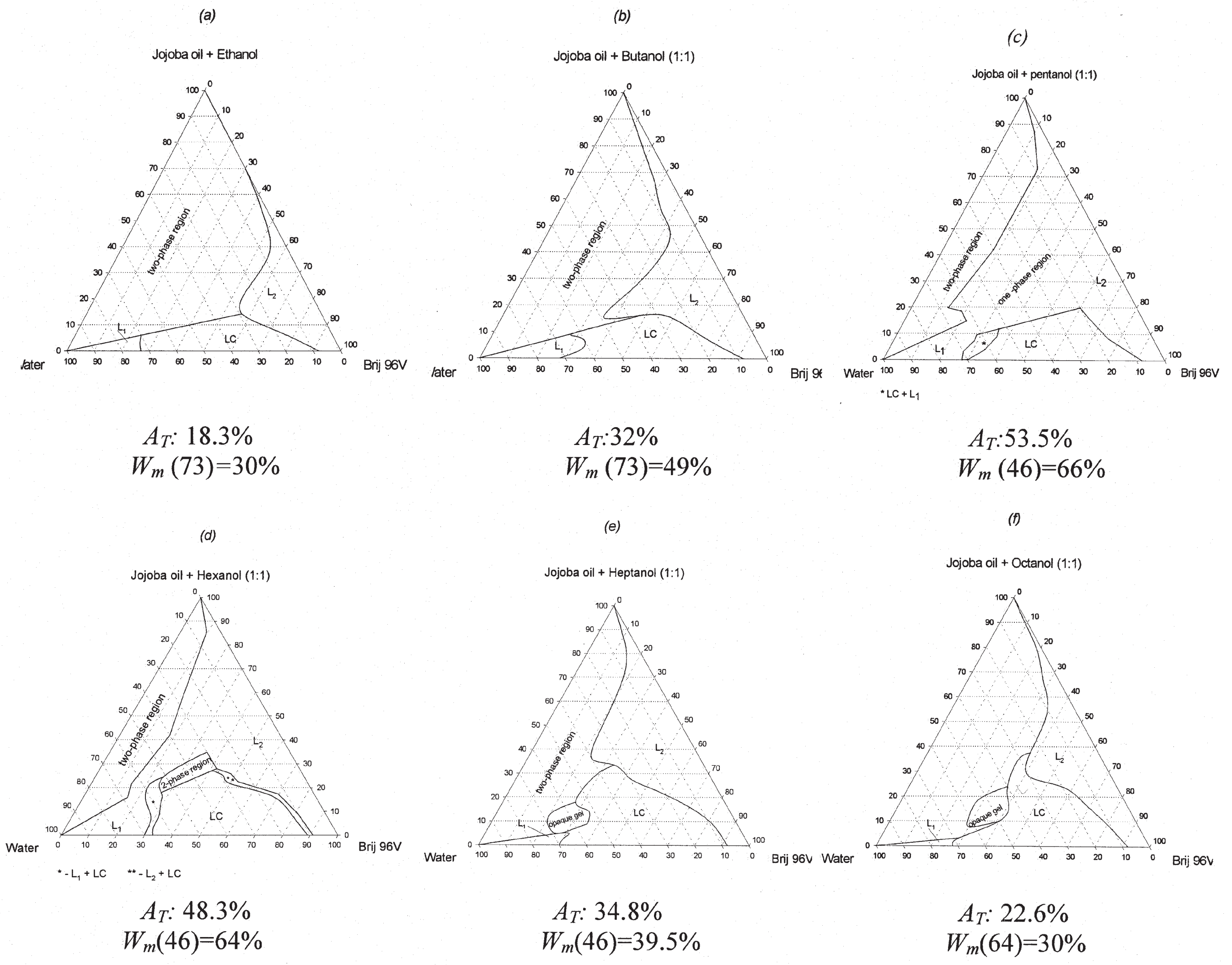
A_T: 12%
W_m (82)=17%

FIG. 2. Phase diagrams of ternary systems, at 25°C, containing jojoba oil and water plus (A) Brij 96V; (B) Tween 60; and (C) Tween 80. L_2 and L_1 denote water-in-oil (W/O) and oil-in-water (O/W) microemulsions, respectively; LC denotes liquid crystalline area. A_T, total isotropic monophasic area; W_m, maximal amount of solubilized water.

by the chemical structure of the surfactant and by the nature of the oil. Recent studies (11,12) suggest that the molecular volume of the oil may be more important than its polarity in determining phase behavior. The capacity of a surfactant to solubilize jojoba oil strongly depends on the ability of the jojoba oil molecules to penetrate into the hydrophobic part of the surfactant film. Therefore, the solubilization capacity is mainly determined by the volume occupied by the jojoba molecules. Jojoba oil has a high M.W. and is a simple ester of two long-chain fatty components (a fatty alcohol and a FA). It contains two double bonds (*cis*-configuration) that cause folding of the chain. We may therefore suppose that, compared with simple alkane, jojoba molecules occupy a relatively large molecular volume fraction at the interface.

Because of their bulky head groups, Tween 60 and Tween 80 pack more loosely at the interface and allow better penetration of oil chains into the surfactant tails, resulting in the formation of a relatively large area of W/O ME (L_2) and small area of O/W ME as compared with Brij 96V. The L_2 area in the system formed with Tween 60 is larger than that in the system formed with Tween 80. This difference in phase behavior may be attributed to the hydrophobic part of Tween 80, which, like Brij 96V, comprises a *cis*-double bond in the alkyl chain, presumably causing folding of the chain and allowing a smaller amount of jojoba oil to penetrate.

It has been established (13) that the aggregation number of micelles is influenced by the length of the hydrophobic group, l_c, the volume of the hydrophobic group, V_H, and the cross-sectional area of the hydrophilic head group, a_0. Since the hydrophobic part is $C_{18:1}$ in the case of Brij 96V and Tween 80 and $C_{18:0}$ in



(a) A_T: 18.3% W_m (73)=30%

(b) A_T:32% W_m (73)=49%

(c) A_T:53.5% W_m (46)=66%

(d) A_T: 48.3% W_m(46)=64%

(e) A_T: 34.8% W_m(46)=39.5%

(f) A_T: 22.6% W_m(64)=30%

FIG. 3. Pseudo-ternary phase diagrams of the system [jojoba oil/Brij 96V/water] at 25°C with different alcohols: (A) ethanol; (B) butanol; (C) pentanol; (D) hexanol; (E) heptanol; and (F) octanol. For abbreviations see Figure 2.

the case of Tween 60, the main factor responsible for the observed differences in solubilization must be the head group. The aggregation number of surfactant molecules decreases as the cross-sectional area of the hydrophilic group increases. In the Brij 96V system, in which the head groups occupy a relatively small area at the interface, jojoba oil solubilization was better than in the systems with ethoxylated sorbitan esters. Of the nonionic surfactants studied, Brij 96V was therefore judged to be most suitable for the preparation of O/W ME with jojoba oil.

Effect of short- and medium-chain alcohols on the phase behavior of the Brij 96V ($C_{18:1}E_{10}$) system. Alcohols are known to act as cosurfactants (14). Alcohols with short or medium chain length were therefore added to the oil phase to improve the solubilization of water in jojoba oil and of jojoba oil in the aqueous phase. The phase diagram for jojoba oil and Brij 96V in the presence of ethanol as cosurfactant is presented in Figure 3A. The addition of ethanol had the effect of increasing the total monophasic area [A_T = 18.3%, W_m (82) = 30%] as compared with the system without cosurfactant [A_T = 12%, W_m (73) = 9.3%] (Fig. 2A). Replacing the ethanol by butanol (Fig. 3B) further enlarged the A_T region and reduced the two-phase region [A_T = 32%, W_m (73) = 49%]. Pentanol as cosurfactant (Fig. 3C) produced the largest L_2 and L_1 isotropic regions [A_T = 53.5%, W_m (46) = 66%]. It is interesting to note that as the hydrophobicity of the added alcohol increased, so maximal water solubilization was obtained in a region richer in oil. The more hydrophobic alcohols were less effective in forming an ME. ME prepared with heptanol (Fig. 3E) and octanol (Fig. 3F) exhibited reduced solubilization capacity.

It is interesting to note that for microemulsions based on oils with a shorter alkyl chain (such as *n*-octane or *n*-dodecane), the maximal ME isotropic area is registered in the presence of short-chain alcohols (11).

The observed improvement in oil and water solubilization can be attributed to partitioning of the alcohol between the oil and the aqueous phase. Short-chain alcohols such as ethanol

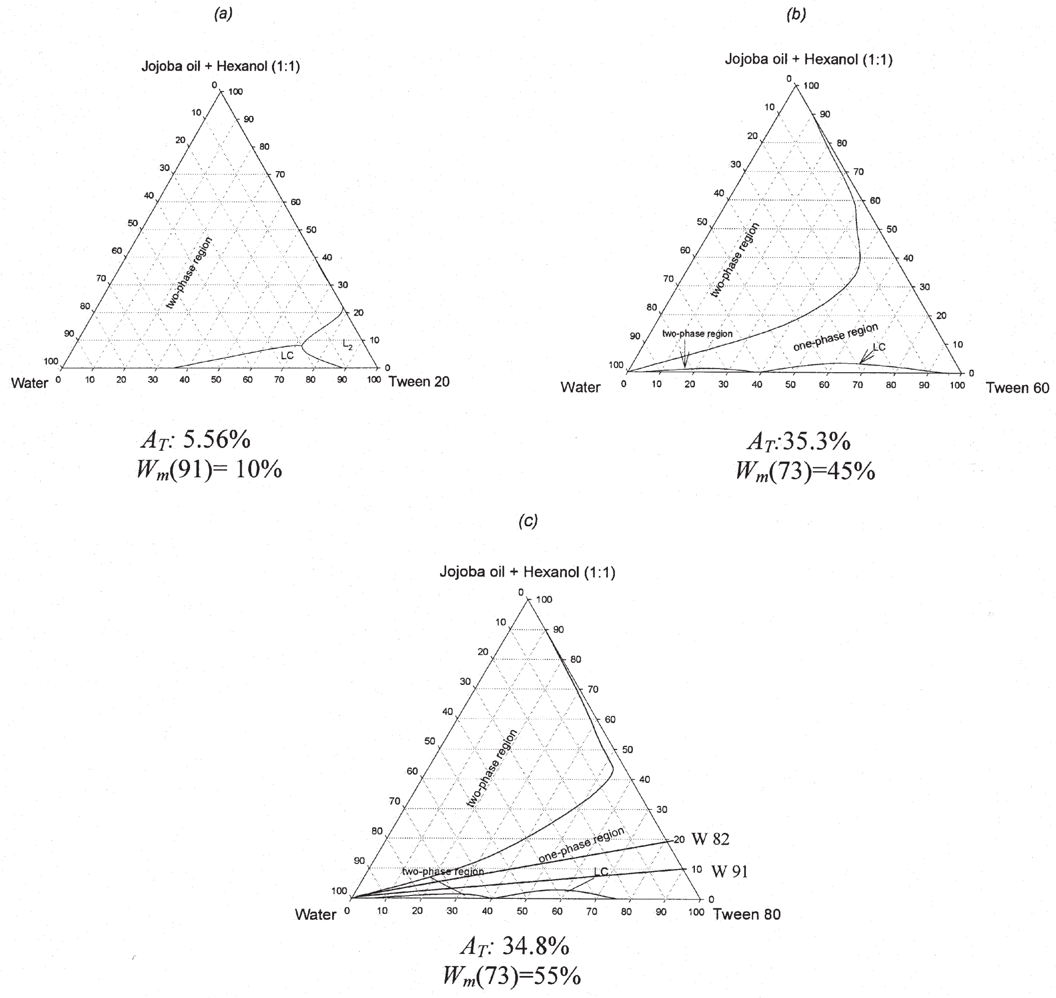
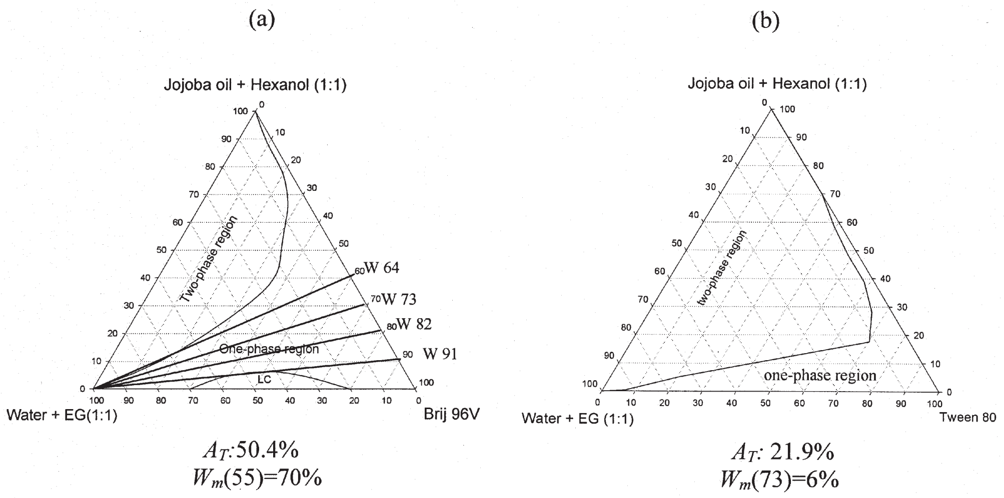


FIG. 4. Phase-ternary phase diagrams of microemulsions made with ethoxylated sorbitan esters and hexanol as cosurfactant (A) Tween 20; (B) Tween 60; (C) Tween 80 at 25°C. For abbreviations see Figure 2.

are relatively soluble in water (and sparingly soluble in jojoba oil), and therefore there is no strong force that drives the alcohol molecules to migrate to the interface and enlarge the interfacial area. Medium-chain alcohols (butanol, pentanol, and hexanol) have better solubility in jojoba oil but are less soluble in water, and thus they penetrate to some extent into the hydrophobic core and decrease its hydrophobicity (as cosolvents). However, the total isotropic area of the system decreased when heptanol and octanol were included, probably due to the growth of attractive interactions between hydrophobic chains; these particular alcohols have good solubility in jojoba oil and tend to migrate completely into the oil phase, and therefore they do not participate in the construction of interface. Maximal solubilization was found in the presence of pentanol.

Effect of hexanol on the phase behavior of systems based on ethoxylated sorbitan esters (Tweens). The effect of hexanol on systems formed from ethoxylated sorbitan esters, such as Tween 20 (ethoxylated-20EO sorbitan monolaurate), Tween 60 (ethoxylated-20EO-sorbitan monostearate), or Tween 80 (ethoxylated-20EO-sorbitan monooleate), was examined. The phase diagram for Tween 20 microemulsions was only slightly altered by the inclusion of hexanol ($A_T = 5.6\%$ in the presence of hexanol vs. $A_T = 5\%$ in its absence; see Fig. 4A). This result can be attributed to the high hydrophilicity of the surfactant (hydrophilic-lipophilic balance = 16.7).

The systems comprising Tween 60 or Tween 80 with hexanol (Figs. 4B and 4C) differed significantly in their phase behavior from Brij 96V with the same cosurfactant (Fig. 3D). Typical U-type ME were formed with an uninterrupted and continuous transition from one kind of ME (W/O) to another (O/W). The isotropic region extended all the way from the oil-rich region to the water corner, and the LC area shrank dramatically. Because the bilayered surfactant structures had been destroyed, an isotropic region of microemulsion with "diluting channels" was formed. The phase diagrams for the pseudoternary systems [jojoba oil/hexanol/Tween 60/water/] (Fig. 4B) and [jojoba oil/hexanol/Tween 80/water/] (Fig. 4C) clearly show the dilution lines W82 and W91 reaching the water cor-



A_T:50.4%
$W_m(55)$=70%

A_T: 21.9%
$W_m(73)$=6%

FIG. 5. Phase diagram of (A) [jojoba oil/hexanol/Brij 96V/water/EG] and (B) (jojoba oil/hexanol/Tween 80/water/EG), at 25°C. EG, ethylene glycol; for other abbreviations see Figure 2.

ner. Hexanol penetrated effectively to the interfacial film and increased interfacial film elasticity by reducing the bending moduli of this film.

Effect of EG on the phase behavior of the Brij 96V ($C_{18:1}E_{10}$) *and ethoxylated sorbitan ester systems.* EG is the shortest water-soluble polyol. EG was dissolved in an aqueous phase at a water/EG weight ratio of 1:1. In the phase diagram for the system containing Brij 96V as surfactant (Fig. 5A), the isotropic area was a little over 50%, slightly larger than in the absence of EG (Fig. 3D). The area of W/O ME decreased in the oil-rich region, whereas the O/W ME phase expanded. The most significant influence of EG on the Brij 96V-based phase diagrams was in the liquid crystal phase area, which was substantially reduced. The diluting channels from W/O to O/W ME are clearly seen. Dilution lines W64, W73, and W82 reach the water corner of the phase diagram.

It is known (15) that diols act mainly as cosolvents in the aqueous phase. They modify the structure of the aqueous phase by breaking the hydrogen-bonding network and interacting strongly with the water. The polyol/water interactions influence the solubility parameter of the solvent. The solubilization parameter increases in EG/water mixtures, resulting in improved solubility of the surfactant monomers and thus a higher critical micelle concentration (CMC). However, it is evident that additives such as EG have a "salting in" effect similar to that of short-chain alcohols and that they therefore play a role as cosurfactants (16). The change induced in the liquid crystal area of the system (jojoba oil/hexanol/Brij 96V/water/EG) by the addition of EG can be interpreted in terms of the properties of the interfacial film: The LC area is destabilized in favor of ME formation, indicating an increase in film flexibility. A certain portion of the EG content apparently becomes incorporated in the surfactant layer as a cosurfactant, leading to increased interfacial fluidity.

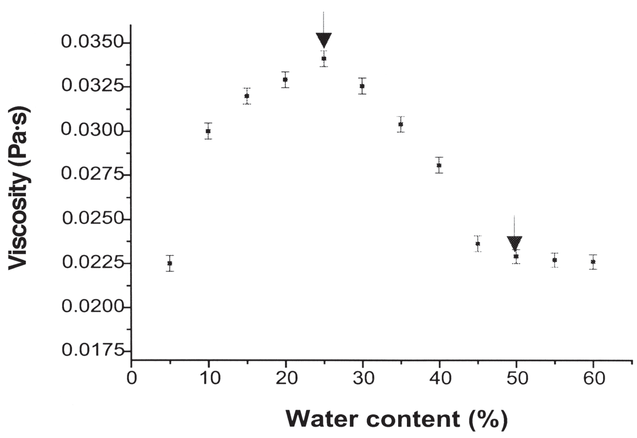


FIG. 6. Viscosity of four-component system [jojoba oil/hexanol/Brij 96V/water] along dilution line W46 at 25°C. Error bars represent SD, n = 3. Arrows indicate deflections in measurements (see text).

When a polyol/water mixture was used instead of pure water in the continuous phase, the microemulsions became more resistant to temperature fluctuations, a phenomenon also observed in previous investigations (16). This effect has important implications for pharmaceutical and cosmetic preparations.

The phase diagram for the Tween 80 system with EG is shown in Figure 5B. Here, although the liquid crystal area has been obliterated, the isotropic area has shrunk relative to the same system without the diol (Fig. 4C). The phase diagram in Figure 4C shows about 50% more total monophasic area (A_T = 34.8 vs. 21.9%). The maximal amount of water was significantly less in the presence of EG [W_m (73) = 6% vs. W_m (73) = 55% without EG]. A similar pattern was observed in the case of the Tween 60 system. Replacing EG by propylene glycol caused a further decrease in monophase formation (results not shown).

The shrinkage of the isotropic region that occurred when EG was added to Tween-based systems was due to the change in polarity, which affected solubilization of the jojoba oil by the ethoxylated sorbitan ester system.

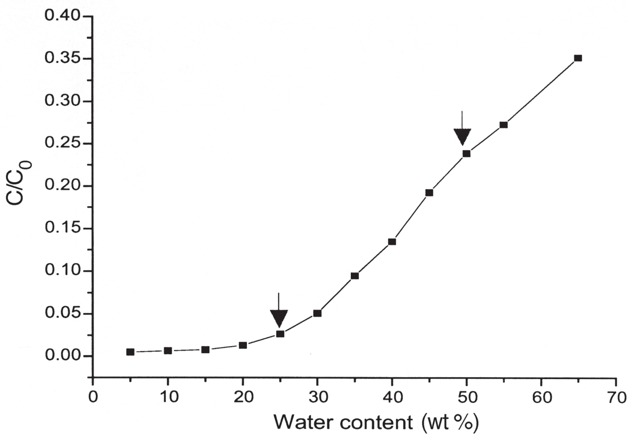


FIG. 7. Normalized conductivity (C) of the system [jojoba oil/hexanol/Brij 96V/water] along dilution line W46, as a function of water content (wt%) at 25°C. Arrows indicate deflections in measurements; see text.

The microstructure of the system [jojoba oil/hexanol/Brij 96V/water] was studied along dilution lines W46 and W73. The W46 line is the line along which maximal water solubilization was detected (65%). Dilution line W73 passes through the W/O ME area, the liquid crystal area, and a significant O/W ME area that continues up to the water corner of the phase diagram.

Microstructure investigations along dilution line W46. (i) Rheological behavior. Viscosity proved a useful tool for elucidating secondary structural changes of the system. Figure 6 shows the results of viscosity measurements along dilution line W46. As expected, the samples examined along this dilution line exhibit Newtonian flow behavior (viscosity is independent of the shear rate). At first, viscosity increases gradually with increasing water content, but later two major deflection points are clearly seen. After a maximum value is reached at ϕ_W = 0.25, viscosity declines, subsequently flattening out at ϕ_w = 0.50.

At low water contents the interactions between the dispersed particles are negligible and the microemulsion consists of isolated ‘hard sphere-like’ globules dispersed in the continuous oil medium (17). The initial rise in viscosity with increasing water content is attributable to the growth of dispersed droplets and is due to the formation of clusters drawn from adjacent globules. The decline in viscosity at $\phi_w > 0.25$ is related to the first structural change. Transition from a W/O ME to bicontinuous structures has taken place. After 50% of the water has been added to the system, a second transition occurs, corresponding to bicontinuous transition to O/W ME.

(ii) Conductivity measurements. Measuring conductivity is a useful way of obtaining information about micellar interactions and transitions. W/O ME, as might be expected, have a low conductivity, 10^{-9}–10^{-7} $\Omega \cdot \text{cm}^{-1}$. When water is added, the conductivity rises sharply over a narrow range and then remains practically unchanged, conveniently maintaining a level that is considerably higher than before the transition. Conductivity was measured along dilution line W46 of the system (jojoba oil/hexanol/Brij 96V/water), the line along which maximal water solubilization was detected (65%) (Fig. 7).

A sharp increase in conductivity was registered on transition from swollen reverse micelles to the bicontinuous phase ($\phi_w = 0.25$). At $\phi_w = 0.5$ a second transition took place, attributable to the bicontinuous structural transformation to O/W ME. The conductivity results are in good agreement with the viscosity measurements.

(iii) SAXS measurements. The SAXS spectra of ME exhibit a single broad maximum at $q \neq 0$, followed by a monotonic decrease of the scattering intensity $I(q)$ at large values of the wave vector amplitude q [$q = (4\pi/\lambda)\sin\theta$, where 2θ is the scattering angle and $\lambda = 1.54$ Å for Cu radiation]. The scattering pattern was fitted to the expression derived by Teubner and Strey (18):

$$I(q) = \frac{1}{a_1 + c_1 q^2 + c_2 q^4} + b \qquad [1]$$

where a_1, c_1, c_2, and b are constants. Such a functional form is convenient for the fitting of spectra.

Equation 1 corresponds to a real space correlation function of the form

$$\gamma(r) = \sin kr/kr\, e^{-r/\xi} \qquad [2]$$

The correlation function describes a structure with periodicity d ($d = 2\pi/k$) damped as a function of the correlation length ξ. d and ξ are related to the constants in Equation 1 by the following expressions (18):

$$d = \left[\frac{1}{2}\left(\frac{a_1}{c_2}\right)^{1/2} - \frac{c_1}{4c_2}\right]^{-1/2} \qquad [3]$$

$$\xi = \left[\frac{1}{2}\left(\frac{a_1}{c_2}\right)^{1/2} + \frac{c_1}{4c_2}\right]^{-1/2} \qquad [4]$$

From k and ξ, Chen and Chang (19) derived an empirical parameter that characterizes bicontinuous microemulsions for ionic systems, $k\xi$. From experiments on ionic systems in the middle range with surfactant weight fractions of less than about 15%, it was found that $1.5 < k\xi < 2$. These values characterize disordered sponge-like bicontinuous structures. When surfactant concentration increases (as in our case), $k\xi$ can exceed the above limits, and then the structure is termed ordered bicontinuous, i.e., alternating sheets of oil and water separated by surfactant film (20).

Figure 8 shows the microemulsion periodicity d (Fig. 8A) and ME correlation length ξ (Fig. 8B) as a function of water volume fraction. As expected, the distance between micelles (d) increases in a monotonic manner with dilution. The correlation length increases as well. When water is located at the micellar core, the surfactant aggregates swell upon dilution, resulting in an increase in the order parameter (growth of ξ). When water is part of the bulk phase, additional dilution should reduce the order of the system and therefore ξ should decrease.

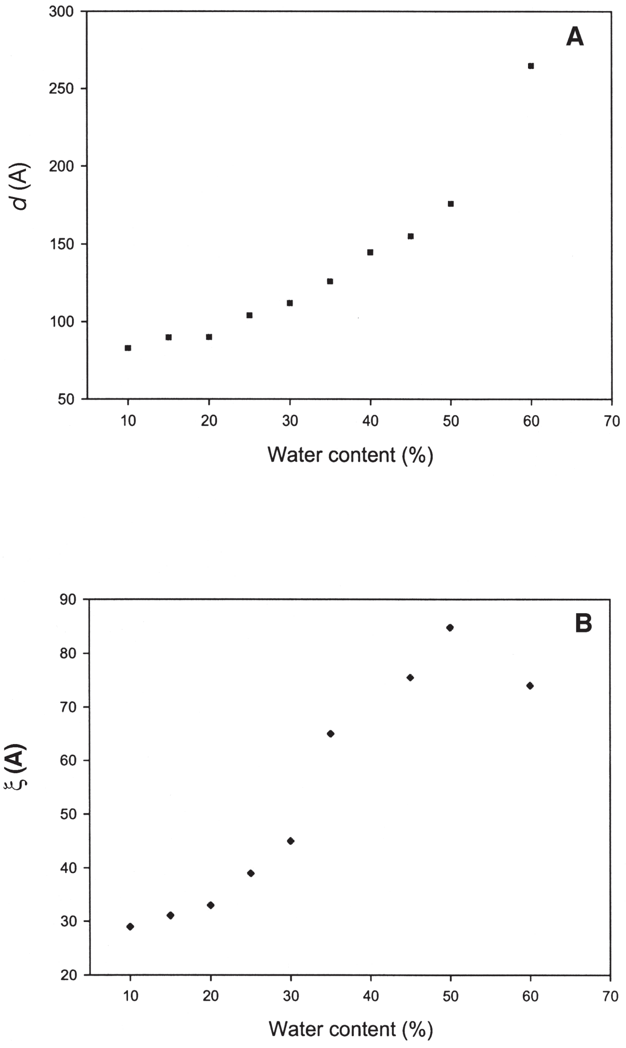


FIG. 8. Dependence of microemulsion periodicity *d* (A) and correlation length ξ (B) on water content along dilution line W46.

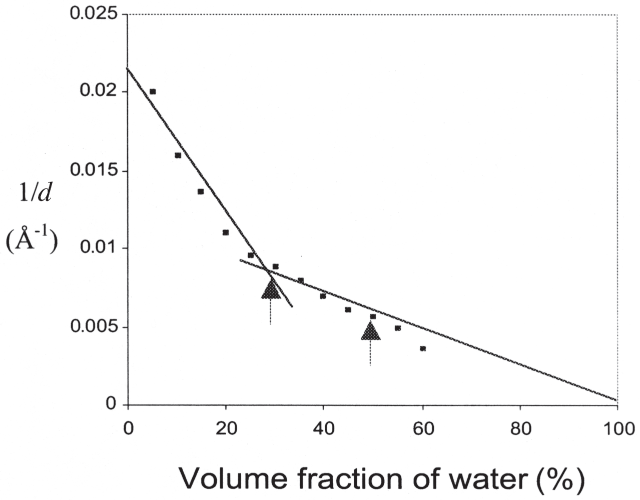


FIG. 9. Dependence of 1/*d* on volume fraction of water along dilution line W46, where d represents distance between micelles. Arrows indicate deflections in measurements; see text.

The effect is clearly detected at water contents >50%. Therefore, we can conclude that microstructural transition to O/W ME occurs at this point.

Graphs plotting 1/*d* vs. water content (ϕ_w) can provide information about the dimensionality of swelling along the dilution line. Figure 9 shows this dependence for dilution line W46. Swelling with water can be described by Equation 5 (21):

$$1/d = 1/d_0 (1 - \phi_w) \qquad [5]$$

where *d* denotes the periodicity and d_0 the thickness of the (surfactant + oil + alcohol) layer. If the swelling is one-dimensional, the graph of 1/*d* vs. ϕ_w should be linear, with the intercept on the abscissa at $\phi_w = 1$. Isotropic systems that obey this equation have been characterized as "local lamellar structures." Such behavior is distinctive to dilution line W46 at $0.25 < \phi_w < 0.5$. The values calculated for $k\xi$ lie somewhat beyond this range (larger than 2), which means that ordered bicontinuous structures have been formed in this region. Similar behavior was obtained in an earlier study for a system composed of dodecane/pentanol/$C_{12}EO_8$ along dilution line W55 (22).

At $\phi_w > 0.5$ the system is no longer a bicontinuous microemulsion, and the microstructure of the system has undergone transformation to O/W microemulsion. These results are consistent with the viscosity and conductivity measurements.

Microstructure investigations along dilution line W73. (i) Viscosity measurements. Viscosity measurements were performed for the system [jojoba oil/hexanol/Brij 96V/water] with and without EG along dilution line W73 (Figs. 10A and B). In the presence of EG, an isotropic one-phase microemulsion was found to extend along this dilution line from the surfactant–oil mixture to the water corner, uninterrupted by phase separation or liquid crystals (Fig. 5A). Maximal viscosity was registered at $\phi_w = 0.30$, although EG caused drastic changes in this dilution line, with destruction of liquid crystals.

(ii) SAXS measurements. From analysis of the X-ray experiments, the sequence of phases along dilution line W73 was as follows: W/O ME ⇒ L_α ⇒ O/W ME. After ~25% of the water had been added to the system [jojoba oil/hexanol/Brij 96V], liquid crystals began to appear alongside the L_2 ME. At $\phi_w = 0.30$ a lamellar liquid crystal area with a characteristic pattern was formed (ratio of spacing of first two reflections 1:2). At $\phi_w = 0.55$ the liquid crystal phase coexisted with L_1 ME. After 62% of the water had been added, an isotropic O/W ME phase extending up to the water corner of the phase diagram was formed.

(iii) DLS examination of the O/W region. The hydrodynamic radii of diluted O/W ME droplets in a system based on Brij 96V, as obtained from DLS measurements, are shown in Table 1. As expected, increasing the oil content caused an increase in particle sizes. The table shows that the micelles at 99 wt% water contained very minor quantities of solubilized oil and cosolvent, yet were slightly larger than those in the micellar solution of surfactant alone (45 and 40 Å, respectively).

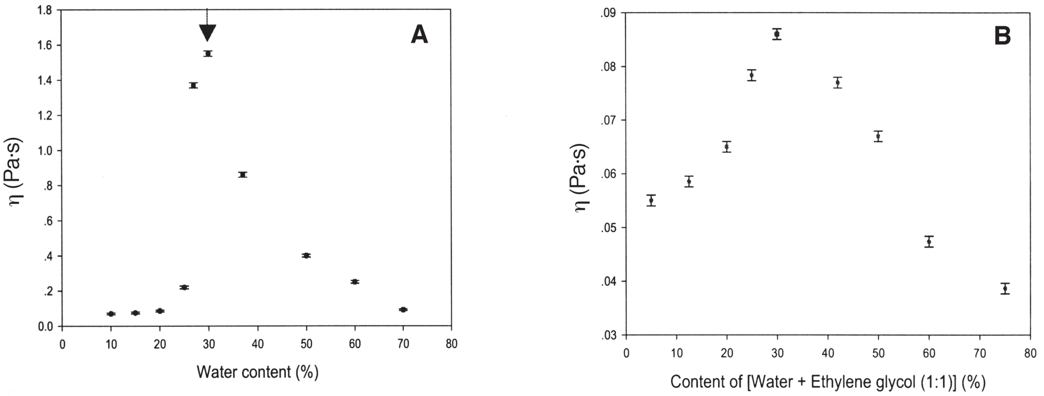


FIG. 10. Viscosity measurements of the (A) four-component system [jojoba oil/hexanol/Brij 96V/water] and (B) five-component system [jojoba oil/hexanol/Brij 96V/EG/water] along a dilution line W73 at 25°C. Error bars represent SD, $n = 3$; arrow in (A) represents deflection in measurement; see text.

The droplets were somewhat larger when the composition contained less surfactant and more oil. The sizes of the droplets at 99 wt% water content were respectively, 45, 55, and 65 Å along dilution lines W91 (the richest in surfactant), W82, and W73 (the poorest in surfactant), reflecting the role of both oil and surfactant in organizing micellar/droplet size (swelling) and shape. The average micellar radius at 99 wt% of water along dilution line 73 was significantly smaller than that of microemulsion droplets containing larger amounts of oil. Radii of 65, 60, and 55 Å were measured for 99, 95, and 90 wt% water, respectively. This growth is linked to the interparticle interactions occurring in more concentrated microemulsions when the oil content increases. The droplets swell and their motion slows.

Droplet size decreased significantly in the presence of EG (droplet size 40 Å vs. 55 Å without EG). Israelachvili *et al.* (23) proposed an explanation based on packing parameter for micelles in water based on surfactant geometry. The surfactant packing parameter $V_H/l_c a_0$ (where V_H is the volume of the hydrocarbon tail, a_0 the cross-sectional area of the hydrophilic head group, and l_c the critical chain length) is affected by the presence of a cosolvent or cosurfactant that can pack together with the surfactant. On addition of EG to the system, the surface area of the hydrophilic group increases, causing a decrease in the packing parameter. This in turn leads to an increase in the mean curvature of the surfactant film and a decrease in droplet diameter. Another explanation for the smaller droplet size in the presence of the diol is that, because the EG-hydrocarbon interfacial tension is much smaller than the water-hydrocarbon interfacial tension, there is less interfacial free energy in EG solutions (24). This situation is responsible for the smaller aggregation numbers of the aggregates formed in the presence of EG and hence for the smaller micellar size.

The aggregation number (N_{aggr}) of the micelles formed by Brij 96V in water (present in Table 1) is substantially larger than N_{aggr} in the system containing jojoba oil and hexanol. Surfactants with a long hydrophobic chain ($C_{18:1}$) such as Brij 96V form asymmetric (elongated) micelles with a N_{aggr} number in water. When large oil molecules such as jojoba oil are incorporated into these elongated micelles, they become spherical. The aggregation number of micelles incorporating jojoba oil is therefore significantly smaller than that of empty surfactant micelles.

As expected, the N_{aggr} of micelles containing jojoba oil increases as the oil content increases, until it reaches the same value as for empty micelles (compare 99 wt% water along W73 with 1 wt% surfactant water solution in Table 1). However, in this case the micelles are not elongated but rather spherical (as shown by cryo-TEM images). As expected, the N_{aggr} of micelles obtained in the presence of EG was significantly smaller than that obtained without the diol.

Warisnoicharoen *et al.* (25) found that, contrary to other surfactants, Brij 96V ($C_{18:1}E_{10}$) incorporates high molecular volume oils such as TG (soybean oil or Miglyol 812) more readily

TABLE 1
Hydrodynamic Radii (Å) and Micellar Aggregation Number of the System [Jojoba Oil/Hexanol/Brij 96V/Water] as Determined by Dynamic Light Scattering Experiments[a]

	1 wt% surfactant in water solution	99 wt% of water along W91	99 wt% of water along W82	99 wt% of water along W73	95 wt% of water along W73	90 wt% of water along W73	90 wt% of (w.+EG) along W73
R_H	40 ± 5	45 ± 5	55 ± 5	65 ± 10	60 ± 10	55 ± 5	40 ± 5
N_{aggr}	310 ± 25	190 ± 20	230 ± 20	290 ± 25	—	—	100 ± 15

[a] R_H, hydrodynamic droplet radius; N_{aggr}, micellar aggregation number; W91, W82, W73, dilution lines 91, 82, and 73, respectively; EG, ethylene glycol.

than it does low molecular volume oils such as ethyl butyrate or ethyl caprylate. The reason for this is that, even in small amounts, large oil molecules cause deformation of the elongated $C_{18:1}E_{10}$ micelles to spherical ME droplets. Incorporation of oils with a small molecular volume does not produce such a transformation (at relatively low concentrations). From the N_{aggr} numbers and the shapes determined by DLS and cryo-TEM measurements, it appears that the micelles formed by Brij 96V in the presence of jojoba oil are spherical (in a diluted regime)—not elongated like the micelles formed by the surfactant alone. Therefore, we may conclude that it is the incorporation of jojoba oil in the Brij 96V micelles that causes the transformation from an elongated to a spherical shape.

ACKNOWLEDGMENTS

The authors are grateful to Professor Yesha'yahu Talmon (Technion, Haifa) for the cryo-TEM investigations and to Dr. Ellen Wachtel (Weizman Institute) for the help in interpretations of the scattering experiments results.

REFERENCES

1. Shani, A., The Struggles of Jojoba, *CHEMTECH 25 (5)*: 49–54 (1995).
2. Scwarz, J.S., M.R. Weisspapir, A. Shani, and S. Amselem, Enhanced Anti-inflammatory Activity of Diclofenac in Jojoba Oil Submicron Emulsion Cream, *J. Appl. Cosmetol. 14*:19–24 (1996).
3. El-Laithy, H.M., and K.M.F. El-Shaboury, The Development of Cutina Lipogels and Gel Microemulsion for Topical Administration of Fluconazole, *AAPS Pharm. Sci. Tech. 3(4)*: E35 (2002).
4. Malmsten, M., Microemulsions, in *Surfactants and Polymers in Drug Delivery*, Drugs and Pharmaceutical Sciences, edited by J. Swarbrick, Marcel Dekker, New York, 2002, Vol. 122, pp. 133–159.
5. Chiu, Y.C., and W.L. Yang. Preparation of Vitamin E Microemulsion Possessing High Resistance to Oxidation in Air, *Colloids Surf. 63*:311–322 (1992).
6. Strey, R., Microemulsion Microstructure and Interfacial Curvature, *Colloid Polym. Sci. 272*:1005–1019 (1994).
7. Leung, R., and D.O. Shah. Solubilization and Phase Equilibrium of Water-in-Oil Microemulsions I. Effect of Spontaneous Curvature and Elasticity of Interfacial Films, *J. Colloid Interface Sci. 120*:321–329 (1987).
8. Gradzielski, M., Effect of the Cosurfactant Structure on the Bending Elasticity in Nonionic Oil-in-Water Microemulsions, *Langmuir 14*:6037–6044 (1998).
9. Garti, N., A. Aserin, S. Ezrahi, and E. Wachtel, Water Solubilization and Chain Length Compatibility in Nonionic Microemulsions, *J. Colloid Interface Sci. 169*:428–436 (1995).
10. Regev, O., S. Ezrahi, A. Aserin, N. Garti, E. Wachtel, E.W. Kaler, A. Khan, and Y. Talmon, A Study of the Microstructure of a Four-Component Nonionic Microemulsion by Cryo-TEM, NMR, SAXS, and SANS, *Langmuir 12*:668–74 (1996).
11. Malcolmson, C., C. Satra, S. Kantaria, A. Sindhu, and M.J.. Lawrence, Effect of the Nature of the Oil on the Incorporation of Testosterone Propionate into Nonionic Oil-in-Water Microemulsions, *J. Pharm. Sci., 87*:109–116 (1998).
12. Warisnoicharoen, W., A.B. Lansely, and M.J. Lawrence, Nonionic Oil-in-Water Microemulsions: The Effect of Oil Type on Phase Behavior, *Int. J. Pharm. 198*:7–27 (2000).
13. Rosen, M.J., Micelle Formation by Surfactants, in *Surfactants and Interfacial Phenomena*, 2nd ed., John Wiley & Sons, 1989, p. 114.
14. Strey, R., and M. Jonstromer. Role of Medium-Chain Alcohols in Interfacial Films of Nonionic Microemulsions, *J. Phys. Chem. 96*:4537–4542 (1992).
15. Kahlweit, M., G. Busse, and B. Faulhaber, Preparing Nontoxic Microemulsions with Alkyl Monoglucosides and the Role of Alkanediols as Cosolvents, *Langmuir 12*:861–862 (1996).
16. Garti, N., A. Yagmur, M.E. Leser, V. Clement, and H.J. Watzke, Improved Oil Solubilization in Oil/Water Food Grade Microemulsions in the Presence of Polyols and Ethanol, *J. Agric. Food Chem. 49*:2552–2562 (2001).
17. Gradzielski, M., and H. Hoffman, Rheological Properties of Microemulsions, in *Handbook of Microemulsion Science and Technology*, edited by P. Kumar, K.L. Mittal, Marcel Dekker, New York, 1999, pp. 161–192.
18. Teubner, M., and R. Strey, Origin of the Scattering Peak in Microemulsions, *J. Chem. Phys. 87*:3195–3200 (1987).
19. Chen, S.H., S.L. Chang, and R. Strey, Simulation of Bicontinuous Microemulsions: Comparison of Simulated Real-space Microstructures with Scattering Experiments," *J. Appl. Cryst. 24*:721–731 (1991).
20. Chen, S.H., S.L. Chang, R. Strey, J. Samseth, and K. Mortensen, Structural Evolution of Bicontinuous Microemulsions, *J. Phys.Chem. 95*:7429–7432 (1991).
21. Cabos, C., P. Delord, and J.C. Marignan, Local Lamellar Structure in Dense Microemulsions, *J. Phys. Rev. B 37*:9796–9799 (1988).
22. Ezrahi, S., E. Wachtel, A. Aserin, and N. Garti. Structural Polymorphism in a Four-Component Nonionic Microemulsion, *J. Colloid Interface Sci. 191*: 277–290 (1997).
23. Israelachvili, J.N., D.J. Mitchell, B.W. Ninham, Theory of Self-Assembly of Lipid Bilayers and Vesicles, *Biophys. Biochim. Acta 470*:185–201 (1977).
24. Nagarajan, R., and C.C. Wang, Theory of Surfactant Aggregation in Water/Ethylene Glycol Mixed Solvents, *Langmuir 16*: 5242–5251 (2000).
25. Warisnoicharoen, W., A.B. Lansely, and M.J. Lawrence. Light-Scattering Investigations on Dilute Nonionic Oil-in-Water Microemulsions, *AAPS PharmSci. 2(2)*:E12 (2000).

[Received April 2, 2004; accepted December 3, 2004]