Solubilization of Lycopene in Jojoba Oil Microemulsion

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ABSTRACT: The unique properties of jojoba oil make it an essential raw material in the manufacture of cosmetics. New, totally dilutable U-type microemulsions of water, jojoba oil, alcohols, and the nonionic surfactant polyoxyethylene-10EO-oleyl alcohol (Brij 96V) have been formulated recently. Here, these microemulsions are shown to be capable of solubilizing lycopene, a nutraceutical insoluble in water and/or oil, much more effectively than the solvent (or a solvent and surfactant blend) can dissolve them. In water-in-oil (W/O) and oil-in-water (O/W) microemulsions with 10 and 90 wt% water, respectively, the normalized maximal solubilization efficiency α is *ca.* 20-fold larger than its solubility. The solubilization capacity of the system is mainly surfactant-concentration dependent. The lycopene resides at the interfaces of the W/O and O/W microemulsions and engenders significant structural changes in the organization of the microemulsion droplets. In the absence of lycopene, the droplets are spherical; when lycopene is added, compaction of the droplets and formation of threadlike droplets are observed. On further addition of lycopene, the bridging effect wanes and the droplets revert to a spherical shape. The enhanced solubilization demonstrated for lycopene opens up new options for formulators interested in making liquid and transparent products for cosmetic or pharmaceutical uses.

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KEY WORDS: Brij 96V, jojoba oil, lycopene, microemulsion, solubilization.

Jojoba oil (see Scheme 1 for averaged molecular structure) has excellent skin penetration capability and high stability to oxidation, characteristics that make it a preferred ingredient in present-day cosmetics. Formulations containing jojoba or its chemical derivatives have potential applications in other areas as well, including pharmaceuticals (topical applications) and lubrication (1–3). At the same time, microemulsions offer features that are attractive for cosmetic and pharmaceutical applications, principally, thermodynamic stability, spontaneous formation, clear appearance, low viscosity, and high solubilization capacity (4,5). Microemulsions based on various types of oils have been reported, but to date none have included jojoba oil.

We recently developed the ability to make new types of oil concentrates composed of oil, hydrophilic nonionic surfactants, alcohol, and propylene glycol. These concentrates consist of reverse micelles. If water is added to the concentrates, water-in-oil

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

Jojoba oil

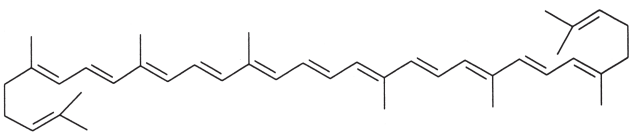
m = 7, 9, 11, 13	n = 8, 10, 12, 14
% 11, 71, 14, 1	1, 44, 45, 9

SCHEME 1

(W/O) microemulsions are formed. On further dilution with water, the structures that contain water in their core transform continuously and gradually into a bicontinuous phase (L_3), and at even higher water dilutions they transform into oil-in-water (O/W) microemulsions. These unique, self-assembled, nanosized liquid structures are actually U-type microemulsions, with characteristic water dilution channels that start at the oil–surfactant mixture line and connect to the water-rich corner of the phase diagram. In the course of our work, we learned to make such microemulsions based on jojoba oil as the oily phase.

Lycopene (Scheme 2) is an important antioxidant with a low solubility in both aqueous and oil phases. It has been used for years as a food colorant. Recent studies have established the antioxidative ability of lycopene and its potential usefulness in alleviating certain types of cancer (among them skin cancer) and chronic coronary heart disease (6,7). Owing to its antioxidative and antitumor activities, lycopene is an attractive compound for the cosmetic and pharmaceutical industries. Because of its poor solubility and low bioavailabity, lycopene is seldom used in cosmetic clear-water-based formulations.

In this study, we attempted to solubilize lycopene in our water-dilutable U-type microemulsions and to maximize the solubilization capacity of the microemulsion over a range of different dilutions. We were aware that the addition of medicinal compounds or natural ingredients with health benefits could be expected to affect the phase behavior of the system, and in particular that incorporation of lycopene could influence the microstructure of the system. The loading capacity of the microemulsions and the effect of lycopene on the spontaneous curvature and elasticity of the microemulsion were examined, as reflected in structural transitions within the isotropic phase.



SCHEME 2

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EXPERIMENTAL PROCEDURES

Materials. Polyoxyethylene-10EO-oleyl alcohol (Brij 96V) was produced by ICI Specialty Chemicals (Essen, Germany). Crude jojoba oil with an iodine value of 80.9 was a gift of Jojoba Israel (Kibbutz Hatserim, Israel) and was used without further purification. 1-Hexanol (purum, ≥98%) was purchased from Fluka (Buchs, Switzerland) and was used without further purification. Water was double distilled. Commercial lycopene was obtained from Lycored (Be'er-Sheva, Israel). The product is composed of 10 wt% of lycopene, the rest being tomato oleoresins. The product was used without further purification.

Sample preparation. Pseudo-ternary phase diagrams were constructed in the following way: The mixtures of surfactant, oil, and alcohol (the oil/alcohol weight ratio 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 by the appearance of cloudiness. Liquid crystal phases were identified using crossed polarizers.

Dynamic light scattering. Light-scattering experiments were performed using an argon ion laser (Model 165; Spectra Physics) with wavelength 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) of Brookhaven Instruments (Holtsville, NY). The hydrodynamic radii of the particles were obtained from the Stokes–Einstein equation, $D = k_B T/6\pi\eta R_H$, where R_H is the hydrodynamic droplet radius, D the diffusion coefficient, η the solvent viscosity, T the temperature, and k_B the Boltzmann constant. Aggregation numbers of micelles were calculated from micellar weight.

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, 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.

Cryo-transmission electron microscopy (TEM) experiments. Samples were prepared as previously described (8). The thin film of the microemulsion 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, operated at 100 kV in the conventional TEM mode. The working temperature was below –160°C.

Lycopene solubilization. Lycopene was heated in jojoba oil for 5 min to 120°C and cooled to *ca.* 50°C. Surfactant, alcohol, and water were added dropwise to obtain a single-phase microemulsion. The samples were cooled and stored at room temperature.

RESULTS AND DISCUSSION

Lycopene solubilization. A typical phase diagram for the system [jojoba oil, alcohol, water, and ethoxylated alcohol (Brij 96V)] is shown in Figure 1.

The solubilization of lycopene in a typical four-component system consisting of jojoba oil, hexanol, Brij 96V, and water was studied along water dilution line W73 (70 wt% surfactant and 30 wt% oil phase). Table 1 presents the solubilities (in ppm) of lycopene in the individual components of the microemulsion and compares them with its solubilization capacities in four different microemulsion compositions: 10 wt% water (representing a typical W/O microemulsion), and 80, 90, and 95 wt% water (representing some typical dilutions of O/W microemulsions). We used the term *maximum solubilization capacity* or *solubilization load (β value)* to designate the absolute solubilization (in ppm) with reference to total microemulsion weight. However, β does not give the real solubilization load, since it does not take into consideration the fact that any further dilution of the concentrate would cause the amount of oil to decrease, thereby reducing the solubility. We therefore adjusted this value to accord with the oil phase content, coining the term *solubilization efficiency (α value)* for the lycopene solubilization maxima adjusted for solubility in the current oil fraction. Similarly, to reflect the solubilization capacity of the interface, we adjusted the quantities solubilized (maximum load) to accord with solubility in all three major microemulsion components combined—jojoba oil, Brij 96V, and hexanol. We called this the *maximum solubilization efficacy (γ value).*

We see from Table 1 that α and γ of lycopene were much higher than its solubility. In the W/O microemulsion sample, α (amount of lycopene solubilized in W/O swollen reverse micelles, adjusted for oil amount) was nearly 20 times greater than the solubility in jojoba oil (14,650 vs. 750 ppm), whereas γ was more than 2.9 times this amount (2,180 vs. 750 ppm) and

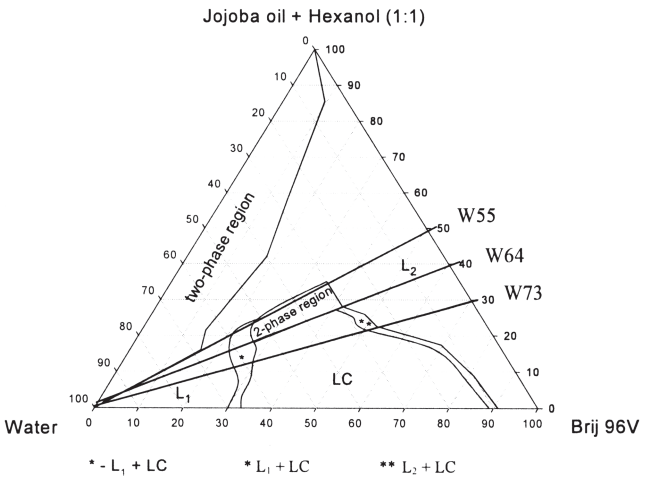

FIG. 1. Pseudo-ternary phase diagrams of the jojoba oil/hexanol/Brij 96V/water system at 25°C.

TABLE 1
Lycopene Solubility (ppm) in Individual Components of Microemulsion (ME), Solubilization Efficacy (ppm), and Solubilization Efficiency (ppm) in Systems Containing Jojoba Oil, Brij 96V, Water, and Hexanol Along Dilution Line W73 at 25°C

System	Jojoba oil	Hexanol	Brij 96V	Water	W/O ME, 10% water	O/W ME, 80% water	O/W ME, 90% water	O/W ME, 95% water
Solubility	750	830	770	≤10	—	—	—	—
γ^a	—	—	—	—	2,180	940	1,950	2,250
α^b	—	—	—	—	14,650	6,350	13,000	15,000

[a]Solubilization efficacy γ—solubilization capacity values adjusted for the (oil + surfactant + hexanol) content.
[b]Solubilization efficiency α—solubilization capacity values adjusted for the oil content. The estimated experimental error for the solubility/solubilization data is *ca.* 2%.

nearly 2.9 times larger than lycopene solubility in Brij 96V (770 ppm). Also, the amount of the solubilized lycopene is much higher than its solubility in hexanol (830 ppm). Similarly, in the O/W region, solubilization of lycopene was much larger than its solubility in jojoba oil or its solubilization in a mixture of the major components. It is easy to see that the value of α in the O/W emulsion containing 95% water is 20 times larger than the solubility in jojoba oil, which reveals a remarkable improvement in lycopene solubilization along the interfacial film of the microemulsion system.

As noted earlier, O/W microemulsions containing lycopene and based on jojoba oil with Brij 96V can be further diluted with water without triggering phase separation. This feature has important practical implications, since eventual pharmaceutical or cosmetic applications are likely to be water-based and to require a range of degrees of dilution.

Table 2 presents results from an additional set of experiments in which the water content was kept constant at 65 wt% (representative cosmetic formulation), but the proportion of surfactant in the oil/surfactant mixture was varied. The proportion of surfactant in the total oil phase along dilution lines W55 and W73 (Table 2) was, respectively, 50 and 70 wt%. It is clear that the surfactant enhanced solubilization efficiency: Solubilization efficiency (α) increased from 1,740 to 3,400 ppm as the surfactant content rose from 50 to 70%, respectively, whereas the (oil + hexanol) content decreased. The effect is not purely additive, since the increase in solubilization represents a twofold increase for a relatively modest increase in surfactant content. We may conclude that solubilization is largely surfactant dependent. This is confirmed by the behavior of the γ values (Table 2): Solubilization efficacy (γ) remained constant, indicating (i) that alcohol does not contribute much to solubilization of the lycopene, and (ii) that solubilization of lycopene is dependent on surfactant concentration.

An interesting effect was observed along dilution line W73 of O/W microemulsions based on jojoba oil and Brij 96V: Solubilization efficiency with respect to lycopene increased as the water content of the system rose (see Fig. 2A). A photograph of samples of O/W microemulsions containing different amounts of lycopene is given in Figure 2B; the samples were collected along dilution line W73. The red color of the rightmost sample is due to the higher concentration of solubilized lycopene.

Thus, the amount of solubilized lycopene adjusted to the jojoba oil content increases with water dilution. This result is surprising: We would have expected solubilization to decrease as the interface becomes convex toward the oil and as it becomes hydrophilic. In an earlier study we examined food-grade microemulsions with D-limonene in the oil phase as a medium for solubilizing phytosterols (9) and lutein (10). The latter are lipophilic molecules containing hydroxyl or carboxyl groups capable of interacting with the hydrophilic head groups of the surfactant or the alcohol. Phytosterols have one hydroxyl group on their sterol skeleton, whereas free lutein (in its nonesterified form) consists of one (mono-) or two (di-) carboxylic group(s) at the end of the carotenoid chain. We found that these nutraceuticals were progressively desorbed from the interface upon dilution with water, resulting in a significant decrease in solubilization in the O/W microemulsion as compared with the W/O microemulsion. However, we also observed (11) that, contrary to expectation, the solubilization of lycopene increased with dilution. The present findings are in good agreement with

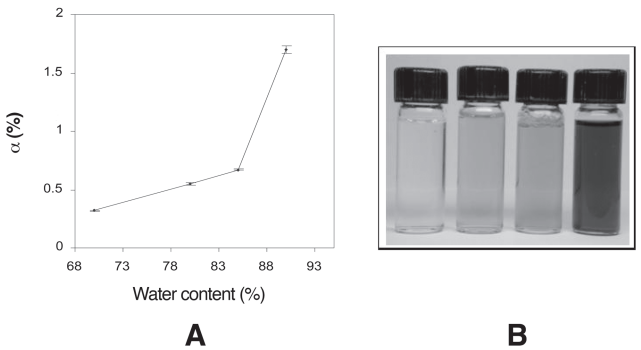

FIG. 2. Solubilization efficiency α with respect to lycopene along dilution line W73 of the oil-in-water microemulsion at 25°C (A) and a photograph of samples containing 0, 3, 4, and 10 mg of solubilized lycopene per gram of jojoba oil (B).

TABLE 2
Lycopene Solubilization (ppm) Along Different Dilution Lines at Constant Water Content (65 wt%) at 25°C

System	W55	W64	W73
Solubilization efficacy, γ	≥508	508	507
Solubilization efficiency, α	1,740	2,540	3,400

TABLE 3
Influence of Solubilized Lycopene on Droplet Diameter (Å) of O/W Microemulsions, Based on SAXS and DLS Experiments[a]

System	Particle diameter (Å)
80% water	110 ± 5
80% water + 5 mg lycopene per g jojoba oil	100 ± 5
95% water	110 ± 10
95% water + 4.5 mg lycopene per g jojoba oil	100 ± 10
99% water	130 ± 20
99% water + 4.5 mg lycopene per g jojoba oil	90 ± 20

[a]O/W, oil-in-water; SAXS, small-angle X-ray-scattering; DLS, dynamic light-scattering.

our previous observations, but their interpretation remains elusive. One possible explanation is related to interfacial packing: Lycopene, because of its extremely low solubility, may prefer the interface over the water or the oil phases, resulting in an unexpected growth in solubilization capacity at high dilutions. An alternative explanation might be that the geometrical configuration of lycopene is better accommodated at the hydrophilic interface than in the relatively flat, more hydrophobic interface of the bicontinuous region; accommodation between surfactant tails might be preferred over solvation because the bulky jojoba oil tails find it difficult to penetrate the surfactant tails. In addition, one should bear in mind that the number of lycopene molecules in an O/W microemulsion is very small—our estimates suggest that there are more than 100 molecules of surfactant for every molecule of lycopene, so lycopene hardly affects the interface and can freely reside there.

Influence of solubilized lycopene on the microstructure of microemulsions. Results of SAXS and dynamic light-scattering experiments are presented in Table 3. Surprisingly, solubilization of lycopene in O/W microemulsions led to a decrease rather than an increase in droplet size.

To help elucidate the influence of lycopene on the microstructure of our O/W microemulsions, we performed a cryo-TEM study of diluted samples. Cryo-TEM images of samples containing 95 wt% of water without and with lycopene are shown in Figure 3. The empty swollen micelles [with small amounts (*ca*. 2.5%) of oil and alcohol in each droplet] are spherical and noninteracting (Fig. 3A). However, as the images clearly reveal, even minor amounts of lycopene suffice to transform the spheroid swollen micelles into well-ordered threadlike micelles that interact strongly with each other (Fig. 3B). Upon further addition of lycopene (e.g., 13 mg of lycopene per gram of jojoba oil, α = 13,000 ppm), the threadlike micelles (containing only *ca*. 1.5% of oil and 1.5% of alcohol) revert to spherical droplets similar in structure and shape to the empty droplets, but slightly smaller.

It is not clear why minor amounts of lycopene should so strongly affect the size and the shape of the droplets. A simple calculation reveals that at 95% water along dilution line W73, the oil content is 1.5 wt% and solubilized lycopene amounts to 4,000 ppm per gram of oil (or 1% from the oil). For an aggregation number of *ca.* 200, every micelle will have ~2 molecules of lycopene dangling at its interface (since the solubility of lycopene in oil or water is negligible). The threadlike effect could be due to the lycopene molecules serving as a sinter or bridge between the micelles and connecting them into threadlike, elongated necklaces, much as many ethoxylated surfactants (such as ethoxylated alcohols) are known to do (8). Moreover, lycopene incorporated into the microemulsion represents only 10% of the tomato oleoresin mixture. Therefore, in practice, the microemulsion also contains the 90% of oleoresins, which may affect the microstructure of the microemulsion.

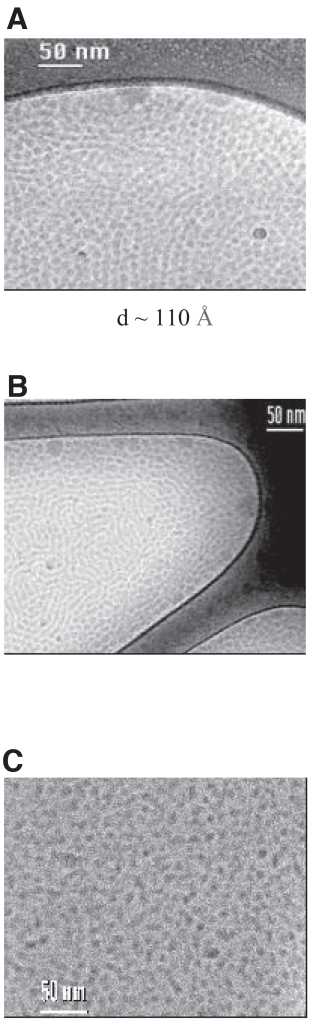

FIG. 3. Cryo-transmission electron microscopy images of vitrified (jojoba oil/hexanol/Brij 96V/water) microemulsion: (A) microemulsion with 95% water; (B) same microemulsion with 4 mg of lycopene per gram of jojoba oil; and (C) same microemulsion with 13 mg of lycopene per gram of jojoba oil.

When a larger amount of lycopene is added to the same sample (to reach α = 13,000 ppm), the micelles revert from threadlike to spherical. This high concentration of lycopene (4% of the oil) causes a more concave curvature toward the oil (elongated micelles are transformed into spherical). The same effect on micellar form was observed (Garti, N., unpublished data) upon incorporation of 2% poly(propylene oxide) into a system containing 4 mg of lycopene per gram of jojoba oil.

Very little work has been done on the influence of solubilized compounds on the phase behavior and microstructure of microemulsions. Spernath *et al*. (11) studied the influence of solubilized lycopene on a nonionic microemulsion based on ethoxylated sorbitan esters (Tweens); self-diffusion NMR experiments revealed that lycopene, which was incorporated along the interfacial film, affected both the microstructure and the composition in the region in which transformation from W/O to bicontinuous and from bicontinuous to O/W microstructure occurred. von Corswant and Thoren (12) found that solubilization of water-insoluble felodipine (a drug) in a bicontinuous microemulsion based on soybean PC changed the polarity of the oil phase but failed to trigger any transformation, whereas incorporation into the same system of a sparingly water-soluble drug—*(R)*-N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl] arginamide (BIBP3226)—caused a transition from bicontinuous to O/W microstructure.

When the amount of lycopene reaching the surfactant film was augmented, a more concave curvature toward the oil was obtained (elongated micelles became spherical). The explanation for this is that lycopene, which is sparingly soluble in the oil, prefers the concave curvature toward the oil [we noted a similar phenomenon in a previous investigation (11)]; it splits the threadlike micelles into at least two smaller spherical micelles, in the same manner as a hydrophobic polymer.

To recapitulate, the capacity of the reversed micelles of concentrates based on ethoxylated alcohol, alcohol, and jojoba oil to solubilize lycopene is very much greater than the ability of pure jojoba oil to dissolve it. Such concentrates can be diluted with water to form W/O microemulsions, which upon further dilution transform into a bicontinuous phase and finally into an O/W microemulsion without exhibiting phase separation or release of the solubilized lycopene. The solubilization efficiency of the microemulsion is very high. Maximal solubilization decreases with dilution, but solubilization values adjusted (normalized) for the oil content of the given microemulsion reveal an increase in lycopene interfacial solubilization upon dilution. Solubilization is mainly surfactant-concentration dependent and is less dependent on alcohol content. Empty droplets of O/W are spherical at water contents above 95%, but in the presence of lycopene they become compacted to form threadlike necklace-type droplets. Further addition of lycopene weakens the bridging effect, causing the droplets to revert to a spherical shape. Addition of lycopene also induces some changes in curvature.

The formulation developed in this study has total dilution capabilities that could serve as a basis for the incorporation of significant lycopene concentrations in water–clear cosmetic preparations.

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