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Solubilization Patterns of Lutein and Lutein Esters in Food Grade Nonionic Microemulsions

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Lutein, a naturally occurring carotenoid, is widely distributed in fruits and vegetables and is particularly concentrated in the *Tagetes erecta* flower. Epidemiological studies suggest that a high lutein intake (6 mg/day) increases serum levels that are associated with a lower risk of cataract and age-related macular degeneration. Lutein can either be free or esterified (myristate, palmitate, or stearate). Both are practically insoluble in aqueous systems, and their solubility in food grade solvents (oils) is very limited, resulting is low bioavailability. To improve its solubility and bioavailability, lutein was solubilized in U-type food grade microemulsions based on ethoxylated sorbitan fatty acid esters, glycerol, *R*-(+)-limonene, and ethanol. Some of the main findings are as follows: (1) reverse micellar and W/O compositions solubilized both luteins better than an O/W microemulsion, and maximum solubilization is obtained within the bicontinuous phase; (2) free lutein is solubilized better than the esterified one, in the W/O microemulsions, whereas the esterified lutein is better accommodated within the O/W microemulsion; (3) vegetable oils decrease the solubilization of free lutein; (4) glycerol and alcohol enhance the solubilization of both luteins; (5) solubilization is surfactant-dependent in all mesophase structures, but its strongest effect is in the bicontinuous phase.

KEYWORDS: Lutein; microemulsion; solubilization; nonionic emulsifier

INTRODUCTION

Microemulsions are interesting, alternative drug-delivery vehicles for improved bioavailability, due to their simple industrial production, easy sterilization, relative simplicity, inexpensive preparation, thermodynamic stability, and capacity to solubilize water-insoluble lipophilic drugs, vitamins, and other nutrients (*1*).

Many new nutritional additives with health benefits have lately been used in the form of tablets, capsules, etc., in powdered form.

There is increasing evidence that the macular pigments, carotenoids lutein (**Figure 1a**) and zeaxanthin, play an important role in the prevention of age-related macular degeneration, cataracs, and other blinding disorders. The carotenoids are situated in the macula (*macula lutea*, yellow spot) between the incoming photons and the photoreceptors and have maximum absorptions at 445 nm for lutein and 451 nm for zeaxanthin. As a result, lutein and zeaxanthin can function as a blue light filters (400−460 nm). The blue light enters the inner retinal layers, thereby causing the carotenoids to attenuate its intensity. In addition to the protection abilities of the macula against blue wavelength damage, these carotenoids can also improve visual acuity and scavenge harmful reactive oxygen species that are formed in the photoreceptors (*2*−*4*).

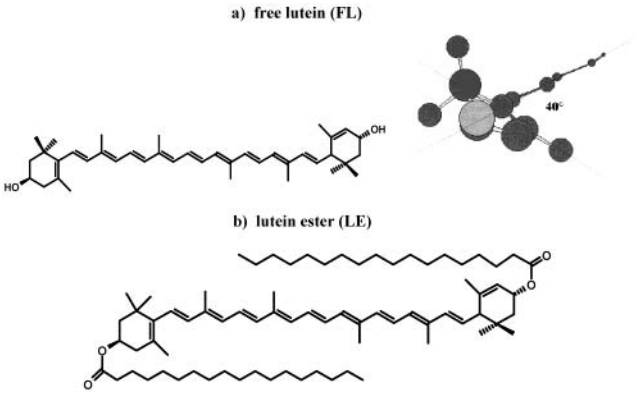

Figure 1. (a) Molecular structures and three-dimensional arrangement of free lutein and (b) molecular structures of lutein ester.

With aging, some of the eye antioxidant suppliers are diminished and antioxidant enzymes are inactivated. This action appears to be related to the accumulation, aggregation, and eventual precipitation in lens opacities of damaged proteins. The results of this sequence of events are eye disorders (*5*).

To increase our understanding of the potential benefits of carotenoids in general and lutein in particular, it is important to obtain more insight into their bioavailability and the factors that determine their absorption and bioavailability (**Figure 2**).

Bioavailability is defined as "the fraction of an ingested nutrient that is available for utilization in normal physiological functions or for storage". Published information on the bio-

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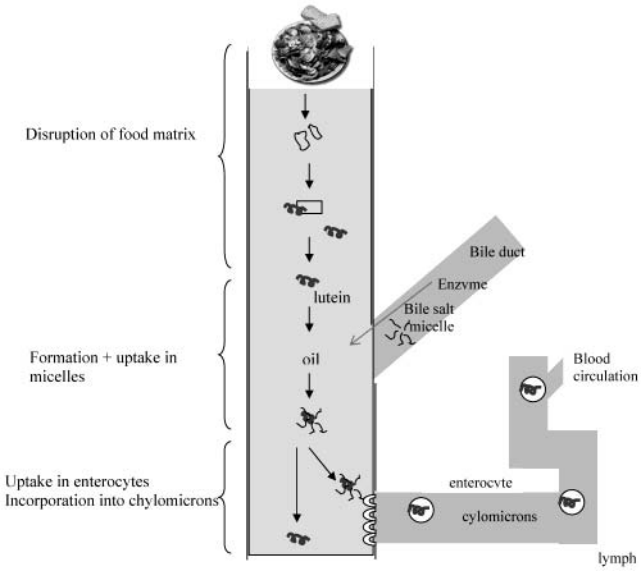

Figure 2. Steps of carotenoid absorption.

availability of carotenoids is based mainly on the measurement of carotenoids in serum or plasma after ingestion (*6*).

Factors that may interfere with the rate of each of the absorption steps will affect the overall bioavailability of the ingested carotenoids.

Herbst et al. found that lutein diester (LE) (**Figure 1b**) shows greater bioavailability than free lutein (FL), which suggests that the human body's digestive tract is efficient in cleaving lutein esters. Therefore, esterified lutein in food may be equally or better bioavailable than free lutein (*6*).

In our present study we explored the "solubilization capacity" and "solubilization capability" of different microemulsion formulations for both lutein and lutein diesters (of fatty acids) and have stressed the differences between the two. Because it is not obvious which of the two is more bioavailable when used without a supportive vehicle, the difference in bioavailability might even be greater once each of them, or their mixture, is formulated in a microemulsion as a transport vehicle.

The matrix in which the carotenoid is incorporated strongly affects the bioavailability. The relative bioavailability of lutein from a diet supplemented with a variety of vegetables is 67% and that from spinach is 45% (*7*). The bioavailability of lutein appears to be lower from green leafy vegetables than from other vegetables. It may be attributed to its entrapment and complexing to proteins in chloroplasts and within cell structures. Such entrapment may not only be physical (matrix effect) but also molecular. It was also found that cooking increases the bioavailability of carotenoids, possibly because of the softening or disruption of plant cell walls and the disruption of the carotenoid−protein complexes. Studies on other carotenoids have shown that intake within, or together with, vegetable oils improved the bioavailability (*7*, *8*).

It is, therefore, important to provide new possible liquid vehicles for improved bioavailability and for possible incorporation of the lutein into aqueous-based drinks or foods.

Microemulsions consist of an aqueous phase, a lipophilic phase, and a surfactant. Cosurfactant and cosolvent are required to form so-called Winsor-IV microemulsions (or U-type, L-phase microemulsions) that can be diluted from the oil-rich side to the aqueous-rich corner smoothly without visual phase separation (**Figure 3**).

The simplest representation of a dilute microemulsion is the "droplet model". The formulations consist of a low percentage

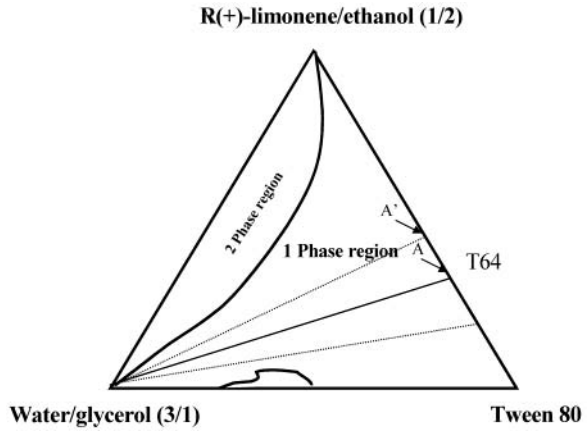

Figure 3. Phase diagrams and dilution lines of a system composed of *R*-(+)-limonene/EtOH (1:2 w/w) as the oil phase, Tween 80 as the emulsifier, and water/glycerol (3:1 w/w) as the aqueous phase. Dilution line 64 is of 60 wt % surfactant and 40 wt % oil phase.

of oil or water in the internal phase, solubilized by a surfactant film, cosurfactant, and cosolvent. When larger quantities of oil or water are present, a bicontinuous structure is formed in which water and oil should be separated by an interfacial layer (*1*).

A U-type, nonionic microemulsion L-phase, based on five components (oil, short-chain alcohol, water, polyol, and surfactant), was previously prepared in our laboratory and served as the basis for this study (*9*). The short-chain alcohol and polyol induced the formation of both W/O and O/W microemulsions. The transition from W/O microemulsion into O/W happens gradually and continuously without any phase separation. After preparing the microemulsion, we solubilized lutein and lutein esters, extracted from marigold flower (*10*, *11*).

The aim of this study is to explore the ability of the L-phase, Winsor-IV food grade microemulsions to solubilize free lutein and lutein diester. Phase diagrams have been constructed, and the guest molecules (free and esterified lutein) were solubilized.

EXPERIMENTAL PROCEDURES

Materials. The reagents used were *R*-(+)-limonene (98%) and Tween 80 [polyoxyethylene (20) sorbitan monooleate], purchased from Sigma Chemical Co. (St. Louis, MO). Ethanol (EtOH) and glycerol were obtained from Frutarom (Haifa, Israel). Free lutein (L-OH) (low purity, 20 wt % of free lutein in corn oil; high purity, powdered compound containing a minimum of 75 wt % of free lutein, ∼10 wt % waxes, and 2−8 wt % zeaxanthins) was obtained from Kemin (Des Moines, IA). Lutein ester (oleoresin with 13.4 wt % lutein ester) was obtained from Inexa C.A. (Quito, Ecuador). All components were used without further purification. The water was double-distilled.

Phase Diagrams. Five-component systems were described in pseudo-ternary phase diagrams (as reported recently, at 25 °C) (*9*). Stock solution of water and glycerol at a constant weight ratio of 3:1 was made. The ethanol/oil weight ratio was held constant at 1:2. Mixtures of surfactant/oil phase (ethanol and oil) or mixtures of surfactant/aqueous phase (water and glycerol) were prepared in culture tubes, sealed with viton-lined screw caps at predetermined weight ratios of oil phase to surfactant, or aqueous phase to surfactant, and kept in a 25 ± 0.3 °C water bath. Microemulsion areas were determined in phase diagrams by titrating either the oil/surfactant phase or aqueous phase/surfactant mixtures with the aqueous phase or the oil phase, respectively. All samples were vigorously stirred. The samples were allowed to equilibrate for at least 24 h before they were examined. In all samples tested, evaporation loss was negligible. The different phases were determined, using ocular and optical (crossed polarizers) methods. Every sample, which remained transparent and homogeneous after vigorous vortexing, was considered as belonging to a monophasic area in the

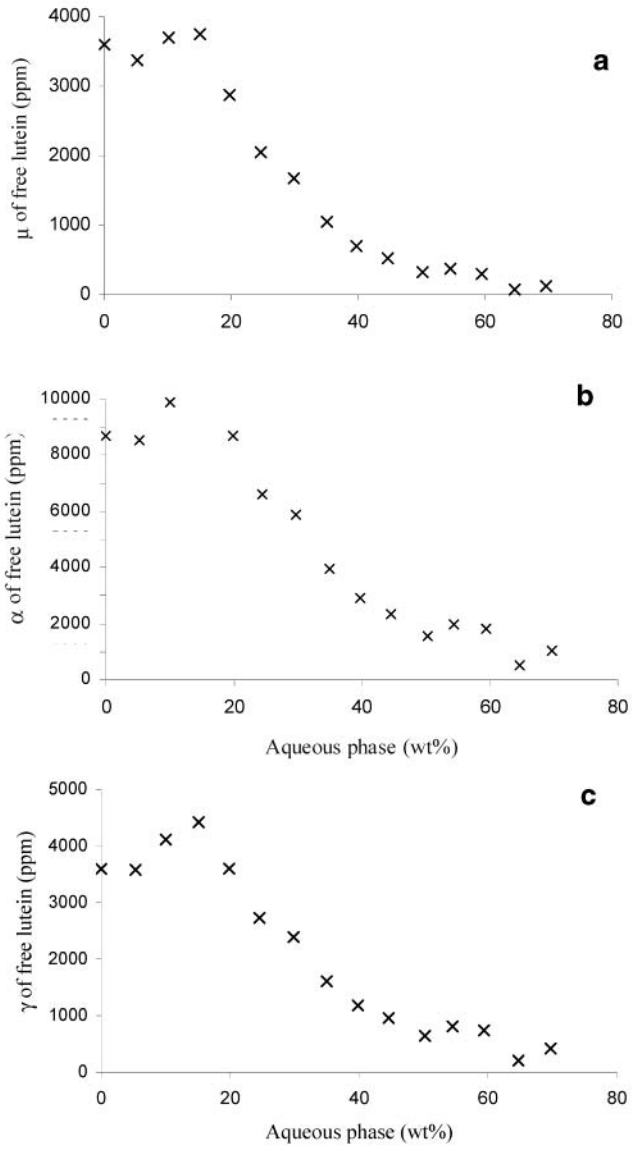

Figure 4. (a) Maximum solubilization capacity of free lutein (20 wt %), μ (ppm), in microemulsion, (b) solubilization capacity, α (mg of free lutein normalized to the amount of oil phase at each dilution point tested), and (c) total solubilization capacity, γ (amount of solubilized FL normalized to the total amount of oil, alcohol, and surfactant). All three parameters were plotted against the aqueous phase content along dilution line 64 at 25 °C.

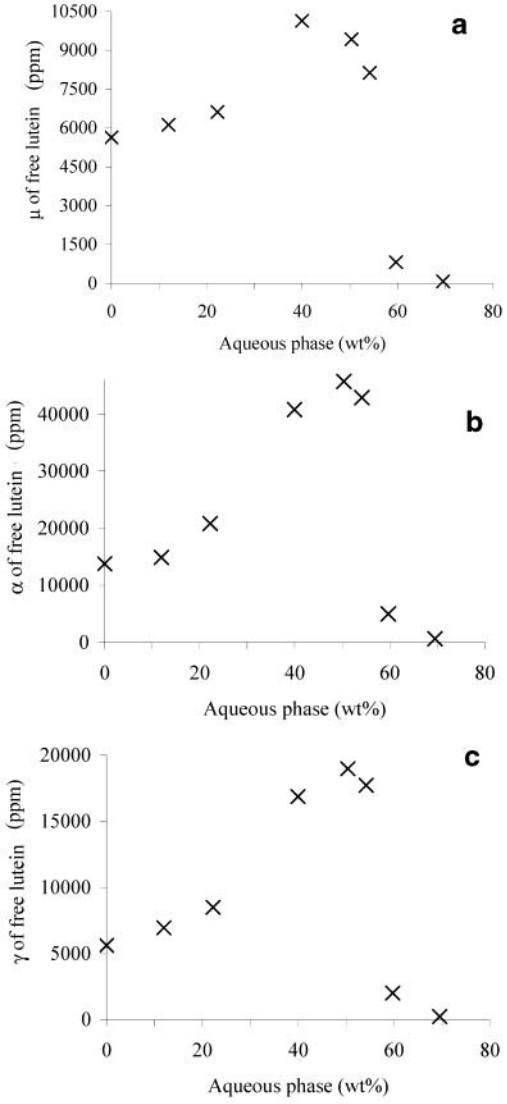

Figure 5. (a) Maximum solubilization capacity of free lutein (75 wt %), μ (ppm) in microemulsion, (b) solubilization capacity, α (mg of free lutein normalized to the amount of oil phase at each dilution point tested), and (c) total solubilization capacity, γ (amount of solubilized FL normalized to the total amount of oil, alcohol, and surfactant). All three parameters were plotted against the aqueous phase content along dilution line 64 at 25 °C.

phase diagram. The accuracy in the location of the phase boundaries is within 4 wt %.

Solubilization Measurements. Free lutein or lutein ester was added to an empty microemulsion composed of *R*-(+)-limonene, ethanol, glycerol, water, and surfactant. The composition, which includes the slurred lutein, was heated to 70°C for 10 min. The samples, once trasparent, were cooled and stored at 25°C. Samples that remained transparent for at least 10 days were considered to be microemulsions.

RESULTS AND DISCUSSION

The main advantage of a microemulsion, as a nutraceutical vehicle for human intake, is the enhanced solubilization of lipophilic nutrients, such as lutein. However, the phase behavior of a microemulsion might change after solubilization of the guest molecules into the core or at the interface of the vehicles and can cause phase separation, microstuctural changes, or droplet size increase (droplet swelling).

The effect of the aqueous phase dilution on the solubilization has been tested and is presented using three different definitions reflecting the significance of the findings. In the first presentation the "solubilization capacity", which represents the maximum solubilization (in wt %) of the guest molecule, per given formulation, was plotted against the aqueous phase content (μ) along dilution line 64 (**Figures 4a**, **5a**, and **6a**). Such presentation of the results has "application value" (important to technologists or formulators), because it demonstrates how much "total nutraceuticals can be solubilized per preparation". Such presentation is somewhat scientifically misleading if one wishes to evaluate the "solubilization capacity efficiency" of the guest molecule, because previous data presentation does not take into consideration the aqueous dilution factor along the dilution line. To compare the oil phase solubility of lutein to its "effective solubilization capacity" in the microemulsion, we used a different term, which is "the amounts (wt %) of solubilized lutein per weight content of the oil phase in the microemulsion", which was defined as the "solubilization efficiency" and denoted α

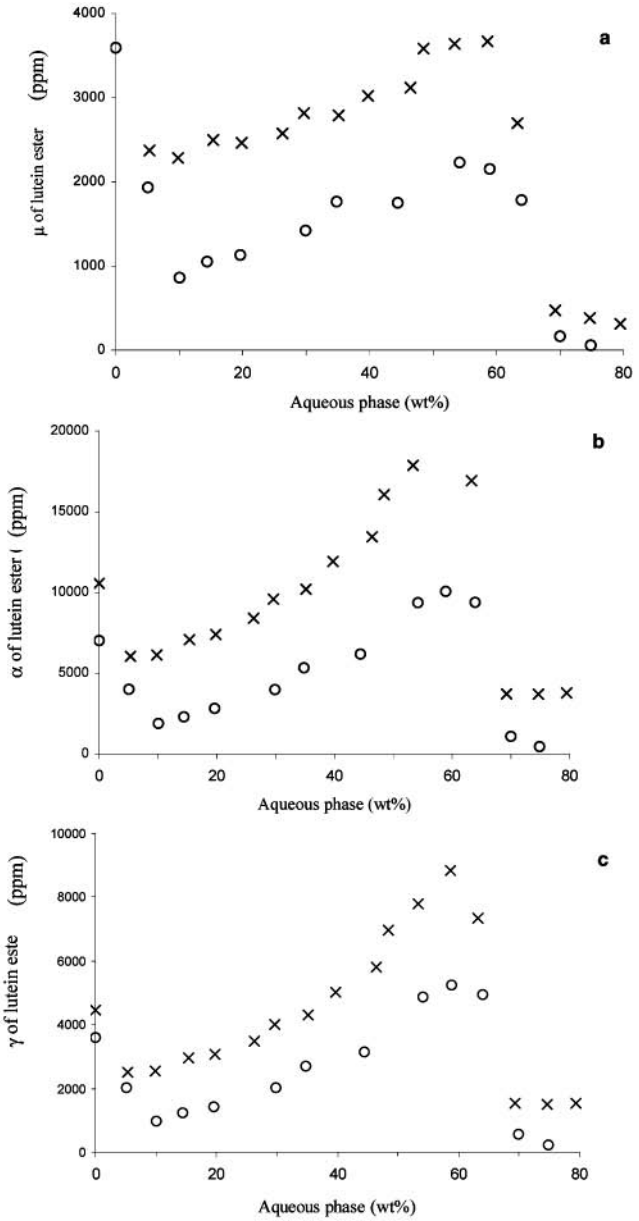

Figure 6. (a) Maximum solubilization capacity of lutein ester, μ (ppm) in microemulsion, (b) solubilization capacity, α (mg of lutein ester normalized to the amount of oil phase at each dilution point tested), and (c) total solubilization capacity, γ (amount of solubilized LE normalized to the total amount of oil, alcohol, and surfactant). All three parameters were plotted against the aqueous phase content along dilution lines 55 (○) and 64 (×) at 25 °C.

(**Figures 4b**, **5b**, and **6b**). This presentation compares the "solubility power" of the core solvent to the "solubilization capacity" of the microemulsion. The "amount of solubilized guest molecule against the total oil and surfactant phases content" (γ) was also plotted (in the third format of presentation, **Figures 4c**, **5c**, and **6c**), which reflects the total solubility core and interfacial solubilization capacity.

To better understand the role of the surfactant in the solubilization efficiency, one can plot the maximum weight percent of solubilization of lutein against the oil and surfactant content. The value γ is therefore the total "core solubility" and "interfacial solubilization".

The qualitative trends are similar in the three modes of presentation, but quantitatively there are some differences reflecting the meaning of each presentation mode.

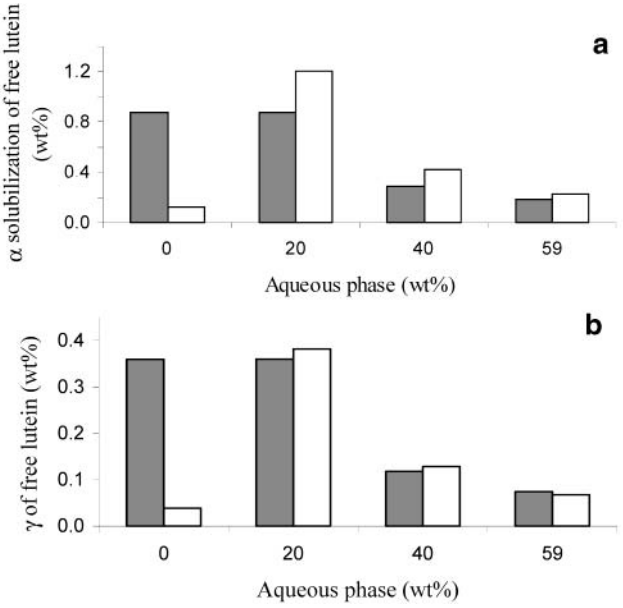

Figure 7. (a) Solubilization capacity, α (wt % of FL normalized to the amount of oil phase at each dilution point tested), and (b) total solubilization capacity, γ (wt % of solubilized FL normalized to the total amount of oil, alcohol, and surfactant). Parameters were plotted against the aqueous phase content along dilution lines 64 (shaded bars) and 73 (open bars) at 25 °C.

Table 1. Solubility of Free Lutein and Lutein Ester in the Microemulsion Components: *R*-(+)-Limonene, EtOH, Tween 80, Glycerol, Water, *R*-(+)-Limonene/EtOH (1:2 w/w), and *R*-(+)-Limonene/Tween 80 (1:3 and 1:5 w/w) (70 wt % of Aqueous Phase at 25 °C)

microemulsion component	free lutein (ppm)	lutein ester (ppm)
R-(+)-limonene	1200	6950
Tween 80	950	130
ethanol	550	50
water	<10	<10
glycerol	<10	<10
R-(+)-limonene/ethanol 1:2	4600	2550
R-(+)-limonene/Tween 80 1:3	3100	5300
R-(+)-limonene/Tween 80 1:5	2450	2200

Solubilization Measurements. Figure 3 shows a phase diagram that includes the isotropic areas, the components used to form the U-type microemulsion dilutable with an aqueous phase from a reverse micelles side to a W/O microemulsion, bicontinuous phase, and O/W microemulsion. The details are described in our previous studies (*9*).

1. Free Lutein (20 wt % in Corn Oil). The solubilization capacity of free lutein, along two dilution lines, 64 and 73, corresponding to oil/alcohol/surfactant weight ratios of 1:2:4.5 and 1:2:7, respectively, is demonstrated in **Figures 4a** and **7**.

One must note that the "reverse micellar concentrate", L_2 (point A in dilution line 64, **Figure 4a**), can solubilize 3500 ppm of lutein as compared to 1200 ppm in *R*-(+)-limonene, 2500 ppm in *R*-(+)-limonene/Tween 80 (1:5), and 4600 ppm in *R*-(+)-limonene/EtOH (1:2) (**Table 1**). It seems, therefore, that the surfactant, together with the alcohol (Tween 80) that is needed to form the reverse micelles, suppresses the solubility of the free lutein, mainly because it brings into the interface (consumes) part of the alcohol. As a result, less free alcohol is available in the *R*-(+)-limonene continuous phase, manifesting itself in a lower solubility/solubilization capacity of lutein.

When an aqueous phase is added to the system, water-in-oil droplets are formed and covered by surfactant and alcohol. The

aqueous phase consists of a water/glycerol mixture. The glycerol reduces the hydrophilicity of the aqueous phase and enhances the oil penetration within the interface.

Up to ~20 wt % aqueous phase the solubilization remains practically constant despite the dilution (~3500 ppm in microemulsion and ~9900 ppm based on oil phase). This suggests that the interface, which becomes richer in alcohol/surfactant, can solubilize some lutein that under these conditions will be less soluble in the continuous phase (less alcohol and less surfactant). Note that free lutein is somewhat hydrophilic, and its solubility is higher in the two sites (in the surfactant phase and in the oil/alcohol phase) than in each of them separately.

Further dilution, from 20 to ~70 wt % aqueous phase, causes significant solubilization suppression (reduction to 100 ppm in microemulsion and to 200 ppm based on surfactant and oil phase). At ~20 wt % aqueous phase, in the presence of excess alcohol, it seems that the aqueous phase is slowly enriched with alcohol (because the surfactant needs less alcohol as the water flattens). A more realistic aqueous phase content (effective aqueous phase content) is ~35 wt % (based on half of the alcohol migrating to the aqueous phase). At this aqueous phase content (35 wt % calculated) the microemulsion undergoes a phase transition into a bicontinuous phase. The interface is less susceptible to the solubilization of hydrophilic lutein, and its capacity drops. The solubilization suppression is greater than the dilution factor.

The bicontinuous phase gradually inverts into an O/W microemulsion (droplet phase), and the lutein, which is not well accommodated at the convex interface, results in very low solubilization, below its solubility in the oil phase or even below its solubility in *R*-(+)-limonene alone. It seems (and it will be further stressed in the SD-NMR results) that the *R*-(+)-limonene participates, in part, at the O/W interface, which leads to a lower free oil content in the O/W microemulsion. Similarly, the surfactant (hydrophilic nature) is efficiently consumed by the interface and is no longer present in the core of the microemulsion.

The main conclusions from these partial findings are that (1) reverse micelles dissolve/solubilize lutein mostly at the continuous oil phase and in part at the surfactant interfacial layer; (2) the bicontinuous phase solubilizes the lutein partially, to a limited extend, at the interface, but its dissolution capacity is significantly reduced; and (3) lutein accommodation at the O/W microemulsion interface is more difficult (almost impossible). The oil content is restricted and, as a result, the maximum solubilization is very low.

2. Free Lutein (75% in Oleoresin). It is known that triglycerides (vegetable oils), due to their high molecular volume fraction, are difficult to accommodate at the interface of both W/O and O/W microemulsions. Therefore, lutein of high purity (less vegetable oil) is somewhat better solubilized in any of the microemulsion surfaces (W/O, bicontinuous, O/W). **Figure 5a** demonstrates the solubility/solubilization capacity of the "free lutein of high purity".

One must note that the "reverse micellar concentrate", L_2, consisting of oil/alcohol and surfactant at a 1:2:4.5 weight ratio (0% aqueous phase), can solubilize 5600 ppm of lutein or 13000 ppm when calculated on the basis of the oil phase (**Figure 5a**,**b**). This is higher than its solubility in each of the components. Despite the fact that free lutein dissolves better in the oil/EtOH mixture than in the oil alone (**Table 1**), the microemulsion, even though poor in alcohol (low weight ratio) in the continuous phase, can solubilize more free lutein of high purity, suggesting a strong interfacial dependency.

An improved solubilization is observed once water is added (up to 40 wt % of aqueous phase) despite the dilution effect. This suggests that the interface, which becomes richer in alcohol/surfactant, can solubilize some lutein that under these conditions will be less soluble in the continuous phase (less alcohol and less surfactant).

Once W/O droplets start to invert into a bicontinuous phase (40 wt % aqueous phase), the free lutein is better accommodated at the interface and its solubilization capacity increases significantly (up to 10000 ppm based on the microemulsion and 17000 ppm based on the oil phase plus surfactant) (**Figure 5a**,**c**).

It can be seen that the main significant differences between the "high-purity lutein" (75 wt %) and the "low-purity lutein" (20 wt %) are within the bicontinuous phase, where the purer lutein is much better accommodated and solubilized at the interface than the low-purity one. High-purity lutein is solubilized by 10-fold more than that expected in vegetable oils.

Once the droplets invert into O/W, a strong decrease occurs, similar to the effect noted with the low purity of free lutein.

Lutein solubilization was tested along an additional dilution line (richer in surfactant, dilution line 73). From **Figure 7** one can see, as expected, that the L_2 reverse micelles are richer in surfactant and, therefore, less alcohol is needed to construct the micelles and the surfactant. Furthermore, alcohol excess will migrate to the continuous oil phase and will reduce the lutein solubility in the oil.

Once water is added, the lutein is gradually consumed by the interface, and the solubilization of the lutein in the surfactant-rich composition is greatly enhanced. This suggests, again, that once the W/O microemulsion droplets grow and are distorted, and the droplets invert into bicontinuous phase, the system solubilization becomes surfactant-dependent.

Upon full inversion into O/W droplets, the advantage of solubilization is lost again. At the O/W region, no significant differences between the surfactant-rich and surfactant-poor systems exist (solubilization capacities along dilution lines 64 and 73 are similar).

3. Lutein Diester (LE). The lutein diester used in this study is dispersed in its own oleoresin and not in vegetable oil. All calculations are adjusted to the lutein ester content. Similarly to the lutein solubilization, in these experiments we have defined four different solubilization regions.

In the reversed micellar region, L_2 (dilution lines 55 and 64) (point A′ in the phase diagram, **Figure 3**), 4000 ppm of LE is solubilized (**Figure 6a**), whereas its solubility in the *R*-(+)-limonene/alcohol (1:2) mixture is 2500 ppm and in *R*-(+)-limonene alone the solubility reaches 7000 ppm. The solubilization in the surfactant phase is very low (130 ppm), whereas in mixtures of *R*-(+)-limonene/surfactant at 1:3 and 1:5 (line 64) the solubilities are 5300 and 2200 ppm, respectively (**Table 1**). These findings are in good agreement with the expected solubility of a very hydrophobic (lipophilic) compound of lutein diester, which is highly soluble in the oil phase, and its solubility drops greatly when polar components ared added to the system (alcohol or Tween 80).

In the micellar system, the solubilization is slightly dependent on the oil phase composition, which again suggests that LE does not fit geometrically at the interface.

Very high solubilization levels are observed (**Figure 6**) once some aqueous phase is added. The high solubilization is attributed to the increase in droplet size and larger interfacial number of sites available at the interface. Once W/O droplets start to invert into a bicontinuous structure, the lutein is better

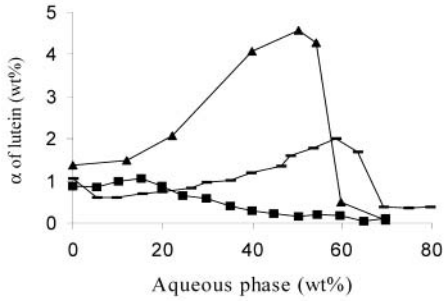

Figure 8. Maximum solubilization capacity of free lutein (20 wt %) (▲), free lutein (75 wt %) (■), and lutein ester (—), α (mg of nutraceutical normalized to the amount of oil phase at each dilution point tested). Parameters were plotted against the aqueous phase content along dilution line 64 at 25 °C. The lines serve only as guides to the eye.

accommodated at the interface and its solubilization capacity increases significantly (up to 2000 and 4000 ppm in lines 55 and 64).

A strong solubilization decrease in LE occurs when the droplets invert from a bicontinuous structure into O/W droplets. The LE accommodation is slightly reduced (restricted) due to its lipophilic nature.

Solubilization along dilution line 64 shows significantly higher solubilization contributions due to the fact that more surfactant is present on the account of the oil and the alcohol. Despite the fact that LE dissolves better in the oil than in the oil/surfactant mixture, the microemulsion based on higher surfactant weight ratio solubilizes more LE than calculated (maximum solubilization of 8800 ppm in line 64, based on surfactant and oil phase, compared to 5200 ppm in line 55), suggesting a strong interfacial surfactant dependency.

To stress the differences between the lutein and lutein ester along the aqueous phase dilution, the solubilization capacities were normalized against the oil phase [*R*-(+)-limonene + alcohol] content for each composition. **Figure 8** shows the "solubilization efficiency" of the microemulsion.

It is clearly seen that in the aqueous-poor (up to 30 wt %) region (of W/O) the lutein is solubilized better than LE. The free lutein should not be predispersed in vegetable oil because it might lose its solubilization capacity. In the bicontinuous area the free lutein solubilization is strongly suppressed by the vegetable oil. Its solubilization improves only if it is dispersed in oil, structurally consisting of straight hydrophobic tails similar to the aliphatic nature of the *R*-(+)-limonene. The LE shows somewhat better solubilization (although in absolute terms it is still very limited) in the O/W microemulsion (>60 wt %) in comparison to the free lutein.

4. Glycerol Effect. Glycerol is part of the aqueous phase and, thus, theoretically it is not expected to participate at the interface and should not affect the solubilization of the free lutein or the LE. However, glycerol imparts a flattening effect on the interface of the W/O microemulsion and converts the system into a bicontinuous phase. It can be clearly seen that at low water content (20 wt %) glycerol does not affect the solubilization capacity of the lutein (**Figure 9**). At 40 wt % aqueous phase, a bicontinuous structure is formed and glycerol has a pronounced solubilization effect. At a 3:1 w/w ratio the microemulsion has the highest isotropic areas (*9, 12*) and also the highest solubilization capacity (of water) along the selected dilution line (W_T). These microemulsions are capable of solubilizing the highest lutein concentrations (10130 ppm) in comparison to those that did not contain glycerol (8900 ppm).

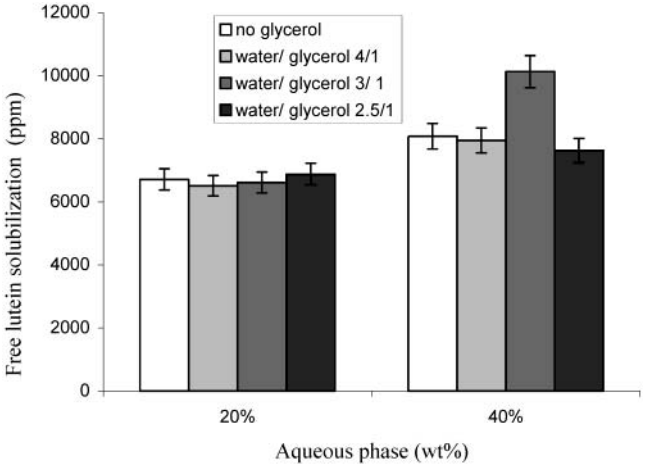

Figure 9. Maximum solubilization of free lutein in a system composed of *R*-(+)-limonene/EtOH (1:2 w/w) as the oil phase, Tween 80 as the emulsifier, and different ratios of water/glycerol as the aqueous phase. Parameters were plotted against the aqueous phase content along dilution line 64 at 25 °C.

Table 2. Maximum Solubilization of Free Lutein and Lutein Ester in a System Composed of *R*-(+)-Limonene/EtOH (1:2, 1:3, and 1:4 w/w), as the Oil Phase, and Tween 80, as the Emulsifier, and Water/Glycerol (3:1 w/w), as the Aqueous Phase[a]

	R-(+)-limonene/EtOH ratio		
	1:2	1:3	1:4
LE, ppm	470	1520	950
FL, ppm	125	390	400

[a] Parameters were plotted against the aqueous phase content along dilution line 64 at 25 °C.

5. Alcohol Effect. **Table 2** shows the effect of alcohol in the O/W microemulsion (areas where the solubilization is low). Alcohol enhances the solubilization of both the free and esterified luteins. At a 1:3 ratio [*R*-(+)-limonene/ethanol] the alcohol effect is at its optimum, whereas at lower and higher alcohol contents its effect is more moderate. It seems that the competitive adsorption dictates its behavior. The lutein and the LE must be accommodated at the interface in order to detect high solubilization amounts (above its solubility within the core or at the continuous phase). Such improved accommodation will occur at flat interfaces (bicontinuous) or if the alcohol or glycerol will allow the guest molecule to penetrate within the interface.

The results obtained from the solubilization measurements are not trivial and could not be easily predicted from the chemical structure and/or lipophilicity of the lutein and LE. Almost any component in the microemulsion has a significant role in enhancing or suppressing the solubilization capacity of the core of the microemulsion and its interface.

When the core has no water (reverse micelles), the free lutein and lutein ester are easily accommodated at the core and at the interface, imparting efficient solubilization. Once water is present at the core of the microemulsion, the solubilization depends on the nature of the interface and the interplay of the other components (alcohol, glycerol) and their relative concentration at the interface and the continuous oily phase. The excess alcohol migrates to the oil phase and reduces the solubility of the LE, whrtrsd the free lutein is less affected.

At the bicontinuous phase the situation seems to be very complex and difficult to predict.

Once the O/W interface is formed in the presence of the hydrophilic emulsifier, both molecules are easily accommodated at the interface or within the core, and the solubilization is gradually reduced to almost zero, which means that lutein will cause phase separation and turbidity in the system or will crystallize out.

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