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Self-Diffusion Nuclear Magnetic Resonance, Microstructure Transitions, and Solubilization Capacity of Phytosterols and Cholesterol in Winsor IV Food-Grade Microemulsions

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Microemulsions are of growing interest to the food industry as vehicles for delivering and enhancing solubilization of natural food supplements with nutritional and health benefits. The incorporation of molecular phytosterols, cholesterol-lowering agents, in food products is of great interest to the food industry. In this work is demonstrated the use of water dilutable food-grade microemulsions consisting of ethoxylated sorbitan ester (Tween 60), water, *R*-(+)-limonene, ethanol, and propylene glycol as vehicles for enhancing the phytosterols solubilization. Phytosterols were solubilized up to 12 times more than the dissolution capacity of the oil [*R*-(+)-limonene] for the same compounds. The solubilization capacity of phytosterols and cholesterol along a dilution line in a pseudo-ternary phase diagram [on this dilution line the weight ratio of *R*-(+)-limonene/ethanol/Tween 60 is constant at 1:1:3] was correlated to the microstructure transitions along the dilution line. Structural aspects were studied by self-diffusion NMR spectroscopy. The ability of phytosterols to compete with cholesterol for penetration into bile salt micelles in the gut may be limited to rich aqueous systems (O/W microemulsion).

KEYWORDS: Cholesterol; ethoxylated sorbitan esters; food microemulsion; nonionic surfactants; phytosterols; phytostanols; *R*-(+)-limonene; SD-NMR; solubilization capacity

INTRODUCTION

Improving the bioavailability of natural food supplements with nutritional and health benefits (nutraceuticals) is of a great interest to the food industry.

The growing interest in microemulsions as vehicles for food formulations arises mainly from the advantages of their physicochemical properties (*1*). Microemulsions can solubilize large amounts of lipophilic and hydrophilic food additives, and they can serve as microreactors that enhance reaction efficiency and allow selective extraction. This has attracted the attention of scientists and technologists (*1*, *2*). Oil-in-water (O/W) microemulsions open the prospect of enhancing the solubility of hydrophobic vitamins, other nutrients, and flavors. This is of particular interest, as it can provide a well-controlled way for incorporating active ingredients and may protect the solubilized components from undesired degradative reactions (*1*).

An elevated serum cholesterol level is a well-known risk factor for coronary heart disease (*3*). Most strategies for lowering serum cholesterol require dietary restrictions or the use of drugs. The prospect of lowering cholesterol levels by consuming foods fortified with natural phytonutrients is considered to be much more attractive (*3*).

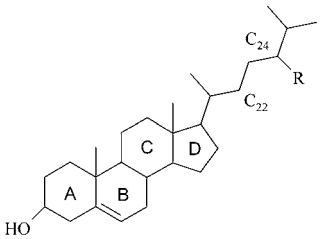

Figure 1. Molecular structure of cholesterol and some abundant phytosterols (R = H − cholesterol; R = CH_2CH_3 − β-sitosterol; R = CH_2CH_3 and additional double bond at C_{22} − stigamsterol; R = CH_3 − campesterol; R = CH_3 and additional double bond at C_{22} − brassicasterol).

Phytosterols (plant sterols) are steroid alcohols. Their chemical structure resembles human cholesterol, as can be seen in **Figure 1**. Sterols are made up of a tetracyclic cyclopenta[*a*]-phenanthrene ring system and a long flexible side chain at the C-17 carbon atom. The four rings have *trans* configurations, forming a flat α system (*4*, *5*). Moreover, the sterols create planar surfaces, at both the top and bottom of the molecules, because the 20*R* conformation is preferred in the side chain.

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This allows for multiple hydrophobic interactions between the rigid sterol nucleus (the polycyclic component) and the membrane matrix (*4*, *6*, *7*). Only the side chains of the various sterols are different. These minor differences result in major differences in their biological functions.

Growing interest in phytosterols as important food ingredients started after Peterson et al. (*8*) reported that the addition of soy sterols to a cholesterol-enriched diet prevented an increase of the plasma cholesterol level. This effect significantly reduced the incidence of atherosclerotic plaque in chick aorta (*8*). Since then, numerous clinical investigations have indicated that administration of phytosterols to human subjects reduces the total plasma cholesterol and low-density lipoprotein (LDL) cholesterol levels (*9*, *10*). Because of their poor solubility and limited bioavailability, high doses (up to 25 g/day) were required to have a noticeable effect. For this reason, and also after the appearance of "statin" drugs, phytosterols were abandoned as cholesterol-lowering agents (*3*).

The renewed interest in phytosterols as cholesterol-reducing agents came after Miettinen et al. (*11*) discovered that phytostanols (hydrogenated phytosterols), fatty acyl ester derivatives (stanol esters), could be readily incorporated into fatty foods, such as margarines. The major drawbacks of these products are derived from the fact that they are esterified with fatty acids, their ballast groups double their molecular weights, and they have to be dissolved in a fatty matrix (margarine or oil). The consumption of 23−50 g/day of triglyceride is needed to obtain the recommended daily intake of phytosterols (*12*). Recently, Ostlund et al. (*12*) showed that 300 mg/day of stanols administered in lecithin micelles can reduce cholesterol levels by 37%. This possibility of introducing phytosterols is applicable only in capsules and cannot be used in water-based foods because the phospholipid micelles are not dilutable with water in water-continuous systems.

The exact mechanism by which phytosterols inhibit the uptake of dietary and endogenous cholesterol is not completely understood. One theory suggests that cholesterol in the presence of phytosterols precipitates in a nonabsorbable state (*3*). A second theory suggests that cholesterol is displaced by phytosterols in the bile salt and phospholipid-containing mixed micelles, preventing its absorption (*3*).

The enhanced phytosterols solubilization in O/W microemulsions is believed to enhance its bioavailability and to maximize its absorption in human tissues. This is due to the fact that the droplet size is in the range of several nanometers.

As was demonstrated in our previous study (*13*), the solubilization capacity and efficiency of lycopene are affected by microstructure transitions from water-in-oil (W/O) to bicontinuous and from bicontinuous to oil-in-water (O/W). In the present study we are report these effects on the solubilization capacity and efficiency of phytosterols and cholesterol.

The objectives of the present study are to explore the ability of our unique dilutable food-grade microemulsions to solubilize phytosterols, to investigate the correlation between solubilization capacity of the phytosterols and the microemulsion microstructure transitions, and to study the influence of solubilized phytosterols compared to cholesterol on the microemulsion microstructure.

MATERIALS AND METHODS

Materials. Tween 60 [polyoxyethylene (20) sorbitan monostearate] was of commercial grade and purchased from Sigma Chemical Co. (St. Louis, MO). *R*-(+)-limonene (98%) was also supplied by Sigma Chemical Co. Ethanol (EtOH) was obtained from Frutarom (Haifa, Israel). Propylene glycol (PG; 1,2-propanediol) was purchased from BDH (Poole, U.K.). Cholesterol was purchased from Sigma Chemical Co. Phytosterols were obtained from ADM Nutraceuticals (Decatur, IL). All components were used without further purification. The water was double distilled.

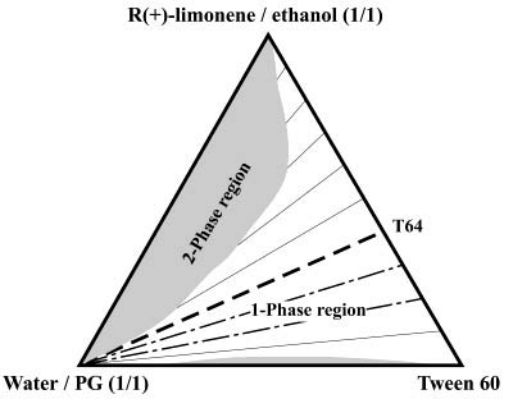

Figure 2. Pseudo-ternary phase diagram (25 °C) of water/PG/*R*-(+)-limonene/ethanol/Tween 60 system with a constant weight ratio of water/PG (1:1) and a constant weight ratio of *R*-(+)-limonene/ethanol (1:1). Solubilization of sterols was studied along dilution line T64 [on this dilution line the weight ratio of *R*-(+)-limonene:ethanol:Tween 60 is constant at 1:1:3].

Phase Diagram. The five-component system was constructed (at 25 °C) as recently reported (*14*). This system was described on pseudo-ternary phase diagrams (**Figure 2**).

Solubilization Measurements. The solubilized material and *R*-(+)-limonene were heated at 110 °C for 15 min. The water, PG, EtOH, and surfactant were then added dropwise to obtain a single-phase clear microemulsion with the desired composition. Finally, the samples were cooled and stored at 25 °C. Samples that remained transparent for at least 10 days were considered to be microemulsions.

Self-Diffusion Measurements. NMR measurements were performed at 25 °C on a Bruker DRX-400 spectrometer with BGU II gradient amplifier unit and a 5 mm BBI probe equipped with a *z*-gradient coil, providing a *z*-gradient strength (g) of up to 55 G cm^{-1}. The self-diffusion coefficients were determined using bipolar-pulsed field gradient stimulated spin−echo (BPFG-SSE). This work utilized bipolar-gradient pulses as described by Wu et al. (*15*) for reduced eddy current ring down.

Experiments were carried out by varying g and keeping all other timing parameters constant. The self-diffusion coefficient (D) is given by

$$I = \frac{I_0}{2} e^{-R(t)-(\gamma G\delta)^2 D(\Delta-\delta/3)} \quad (1)$$

where I is the measured signal intensity, I_0 is the signal intensity for $g = 0$, γ is the gyro magnetic ratio for the ^{1}H nucleus, δ is the gradient pulse length, Δ is the time between the two gradients in the pulse sequence (and hence defines the diffusion time), and $R(t)$ is a constant that takes into account nuclear relaxation. Because in our experiments $R(t)$ is constant, we do not consider it further. Typical experiments used a Δ of 100 ms, a δ of 8 ms, and g values from 1.7 to 32.3 G cm^{-1} in 32 steps.

RESULTS AND DISCUSSION

Dilution of a micellar solution consisting of *R*-(+)-limonene plus EtOH as the oil phase and polyoxyethylene (20) sorbitan monostearate (Tween 60) as the surfactant with an aqueous phase composed of water and PG caused gradual microstructure transitions, as we demonstrated in our previous study (*13*). The solubilization capacity (SC) and solubilization efficiency (α) of phytosterols and cholesterol along dilution line T64 were

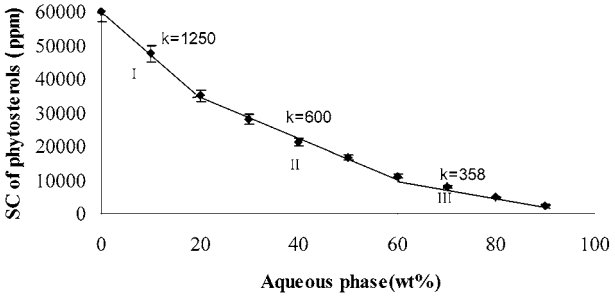

Figure 3. Solubilization capacity (SC) curve of phytosterols along the dilution line T64 at 25 °C. The three regions along the curve are (I) W/O microstructure, (II) bicontinuous microstructure, and (III) O/W microstructure.

determined (see **Figure 2**). On this dilution line the weight ratio of *R*-(+)-limonene/ethanol/Tween 60 is constant at 1:1:3.

A. Phytosterol Solubilization. The SC of phytosterols in a micellar solution containing surfactant and oil phase (at a 6:4 weight ratio, respectively) is 60000 ppm (6 wt %). As can be seen from **Figure 3**, the SC of phytosterols decreases as the aqueous phase concentration increases. In a microemulsion containing 90 wt % aqueous phase, the SC is only 2400 ppm, that is, a decrease of 96% in the SC of phytosterols.

A possible explanation for these dramatic decreases in the SC of phytosterols could be related to the locus of solubilization. In systems free of water the locus of solubilization is at the micelle interface. As an aqueous phase is introduced, water-in-oil (W/O) swollen micelles are formed, and the hydrophilic OH groups of the phytosterols are oriented toward the aqueous phase, thus causing the molecules to insert themselves between the surfactant hydrophobic chains. This change in the locus of solubilization causes a decrease in solubilization at the interface. Suratkar and Mahapatra (*16*) observed a similar change in the locus of solubilization of phenolic compounds in SDS micelles.

The decrease in solubilization capacity as the aqueous phase concentration increases may be also attributed to microstructure transformations. The structural transformation from W/O via bicontinuous to O/W microstructure forces the phytosterols to solubilize between the hydrophobic amphiphilic chains, which is a less preferable location, thus causing a decrease in the SC.

Closer examination of the solubilization patterns in **Figure 3** reveals three regions of solubilization. The regions differ by their slope (k). A sharp slope ($k = 1250$) can be seen in the first region (0−20 wt % aqueous phase). In the second region (20−60 wt % aqueous phase) a more moderate slope is observed ($k = 600$) that reflects a decrease of >50% compared with the slope of the first region. In the third region (>60 wt % of aqueous phase) the slope is very moderate ($k = 360$), a decrease of 40% compared to the slope in the second region.

In our previous study (*13*) we correlated the changes in SC of lycopene along dilution line T64 to the microstructure transformation. Changes in the microstructure were detected by monitoring the diffusion coefficient of the water and oil [*R*-(+)-limonene]. The same technique was applied in this study.

To evaluate the self-diffusion data in terms of microstructure, the relative diffusion coefficient (D/D_0) of the two solvents was calculated (*17*). Relative diffusion coefficients were obtained by dividing the water and oil diffusion coefficients (D^W and D^O, respectively) in the microemulsion by the diffusion coefficient of water in the pure aqueous phase (D_0^W) and of oil in the neat phase (D_0^O). It is well documented (*17*) that if the D/D_0 values of water and oil differ by >∼1 order of magnitude, discrete particles of the slowly diffusing solvent exist, whereas

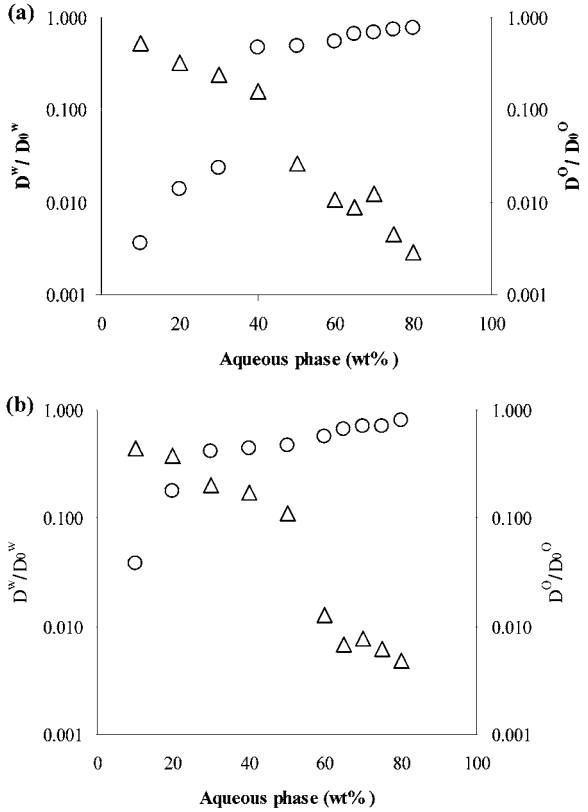

Figure 4. Relative diffusion coefficient of water (○) and *R*-(+)-limonene (△) in microemulsions without (a) and with (b) phytosterols, as calculated from SD-NMR results at 25 °C. D_0^W, the diffusion coefficient of water, was measured in a solution containing water/PG (1:1) and determined to be 55.5×10^{-11} m^2 s^{-1}. D_0^O, the diffusion coefficient of pure *R*-(+)-limonene, was determined to be 38.3×10^{-11} m^2 s^{-1}.

if the D/D_0 of the oil and water are of the same order of magnitude, a bicontinuous structure is suggested.

Figure 4 shows the relative diffusion coefficients of water and *R*-(+)-limonene in "empty" microemulsions (a) and microemulsions solubilizing phytosterols (referred to as phytosterols microemulsion) (b), as a function of the aqueous phase concentration (w/w). One can clearly see that the absence or presence of phytosterols makes little difference. It can also be seen that in phytosterol microemulsions the value is easily interpreted, whereas the behavior of empty microemulsions is somewhat more difficult to explain because gradual changes take place. The formation of discrete particles is more pronounced in the water-diluted systems in the presence of phytosterols than in the empty microemulsions.

Systems solubilizing phytosterols (**Figure 4b**) containing up to 20 wt % aqueous phase probably have a discrete W/O microstructure, because the relative diffusion coefficients of water and *R*-(+)-limonene differ by >1 order of magnitude. Phytosterol microemulsions containing 20−60 wt % aqueous phase seem to have a bicontinuous microstructure, as the relative diffusion coefficients of water and *R*-(+)-limonene are of the same order of magnitude. Finally, phytosterol microemulsions containing >60 wt % of aqueous phase exhibit an O/W microstructure, as the relative diffusion coefficients of water and *R*-(+)-limonene differ by >1 order of magnitude.

It seems that the borders between the three different regions in the SC curve (**Figure 3**) are an indication of the microstructure transition along the dilution line. The three regions in the SC curve correspond to the microstructure transformations as indicated by the relative diffusion coefficient curve (**Figure 4b**).

Table 1. Phytosterol Solubility at 25 °C

medium	phytosterol solubility (ppm)
R-(+)-limonene	25000
Tween 60	25000
ethanol	<10
water	<10
propylene glycol (PG)	<10
R-(+)-limonene/Tween 60 (4:6)	150000

The general behavior trend of the diffusion coefficients is similar for microemulsions with or without phytosterols, but the transition points from one microstructure to the other are different. **Figure 4** indicates that the phytosterol solubilization influences the transition from W/O to bicontinuous to O/W microstructure. In empty microemulsions a distinct region of W/O microstructure is formed between 0 and 30 wt % (**Figure 4a**) of aqueous phase, whereas in phytosterol microemulsions the formation of such a region is more diffusive. In phytosterol microemulsions containing 20 wt % of aqueous phase (**Figure 4b**) the relative diffusion coefficients of water and *R*-(+)-limonene are within the limit of 1 order of magnitude. This implies that in phytosterol microemulsions containing 20 wt % of aqueous phase the microstructure is gradually transformed from W/O to a bicontinuous microstructure. Discrete W/O droplets will be formed only in a system containing <20 wt % of aqueous phase. In empty microemulsions the formation of a true bicontinuous microstructure occurs at a much later stage when it contains a minimum 30 wt % of aqueous phase (**Figure 4a**). In the phytosterol microemulsions the bicontinuous microstructure exists over a wide region of aqueous phase content (20−60 wt % of aqueous phase) (**Figure 4b**). Antalek et al. (*18*) have also detected a transformation from W/O to bicontinuous microstructure by a self-diffusion (SD)-NMR technique. The point of the formation of a bicontinuous microstructure in our empty microemulsion (30 wt % of aqueous phase) seems to correspond to the "brake point" found by Antalek et al. in AOT-based microemulsions, which seem to be completely different.

The transition from bicontinuous to O/W microstructure in phytosterol microemulsions is sharp. It occurs when the system contains 60 wt % of aqueous phase (**Figure 4b**). In empty microemulsions this transition occurs more gradually (**Figure 4a**).

It seems that the phytosterols have a strong effect on the spontaneous curvature of the micelles. As a result, the interface curvature decreases at lower water concentration. This effect is more pronounced in the presence of phytosterols than in empty micelles or in the presence of lycopene as demonstrated in our previous study (*13*).

Solubilization Efficiency (α). To compare the solubility of phytosterols in *R*-(+)-limonene to their solubilization in a microemulsion, one must normalize the amount of phytosterols to the amount of *R*-(+)-limonene in the microemulsion (solubilization efficiency designated α).

Table 1 shows the solubility of phytosterols in the microemulsion components. Note that the phytosterol solubilization efficiency in a reverse micelle system is 6 times (150000 ppm) higher than the solubility of phytosterols in *R*-(+)-limonene (25000 ppm). This enhanced solubilization is remarkable, yet swollen micelles have no practical value without the capability of being diluted by water without decomposition, because many of the final food applications will be in aqueous environments. Moreover, such micellar mixtures cannot be diluted with any type of oil phase [including *R*-(+)-limonene] and, therefore, are not practical for oil-continuous phase applications as well. It is essential, therefore, to construct micellar concentrates capable of being diluted in both oil and water phases. The microemulsions described in this paper are unique in those properties.

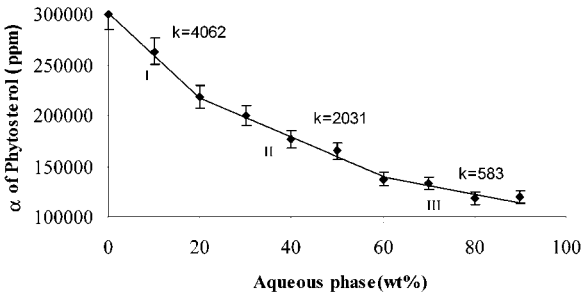

Figure 5. Solubilization efficiency (α) of phytosterols along the dilution line T64 at 25 °C. The three regions along the curve are (I) W/O microstructure, (II) bicontinuous microstructure, and (III) O/W microstructure.

Figure 5 and **Table 1** show α values as a function of the aqueous phase content. The α value of phytosterols in microemulsion is much higher than that of phytosterols in *R*-(+)-limonene. The increase of solubilization efficiency suggests that phytosterols are incorporated at the interface. One should note that the α value of phytosterols microemulsions containing >60 wt % of aqueous phase is lower than the α value of reverse micellar systems. The advantage of these microemulsions is revealed by their dilution capability by the aqueous phase. The solubilization efficiency (α) curve can be divided into three regions as was shown for the SC curve. Changes in the slope of the α curve are better seen than changes in the SC curve.

In the first region (0−20 wt % of aqueous phase) the curve slope (*k*) is 4060. In the second region (20−60 wt % of aqueous phase) the slope (*k*) is only half the slope of the first region ($k = 2030$). In the third region (>60 wt % of aqueous phase) the slope is 3.5 times smaller than the slope of the second region ($k = 583$).

The regions on the α curve (**Figure 5**) correspond to the regions on the relative diffusion coefficient curve, with transitions occurring in the microstructure along the dilution line (**Figure 4b**).

B. Solubilization of Cholesterol versus Phytosterols. One of the mechanisms suggested for the ability of phytosterols to lower cholesterol absorption is that of competitive solubilization. The phytosterols compete with the cholesterol on the solubilization location in the bile salt micelles, which transfer them through the gut membrane to the blood stream (*3*).

The study of the solubilization of cholesterol versus phytosterols in these model systems may shed light on this theory.

Solubilization Capacity. The SC of cholesterol in a micellar solution containing surfactant and oil phase at a 6:4 weight ratio is 80000 ppm (8%). The SC of cholesterol decreases with increasing aqueous phase concentration (**Figure 6**) like that of phytosterols. Again, we can distinguish three different regions along the SC curve. In the first region (0−30 wt % of aqueous phase) (**Figure 6**) the SC of cholesterol remains almost unchanged (a decrease of 12%). In the second region (30−50 wt % of aqueous phase), a sharp decrease from 68000 to 15000 ppm in SC occurs. In the third region (50−90 wt % of aqueous phase), a more moderate decrease from 15000 to 1700 ppm is observed.

As we have demonstrated, these different regions of the SC curve are also detected by SD-NMR. In the first region (**Figure 7a**, 0−30 wt % of aqueous phase), the microstructure is of a

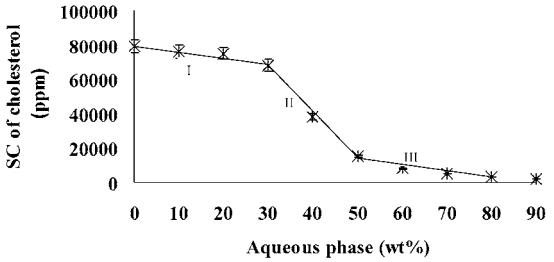

Figure 6. Solubilization capacity (SC) of cholesterol along the dilution line T64 at 25 °C. The three regions along the curve are (I) W/O microstructure, (II) bicontinuous microstructure, and (III) O/W microstructure.

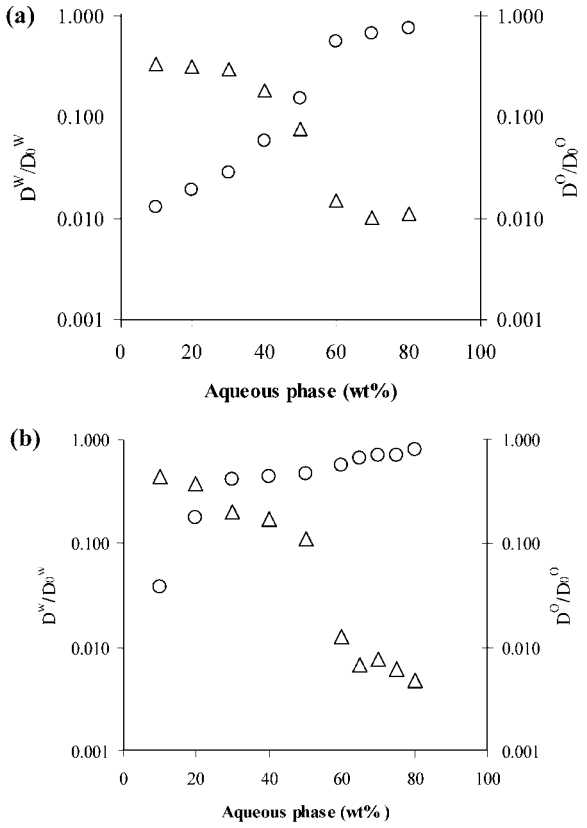

Figure 7. Relative diffusion coefficient of water (○) and *R*-(+)-limonene (△) in cholesterol-containing microemulsions (a) and phytosterol microemulsions (b) as calculated from SD-NMR results at 25 °C. D_0^W was measured in a solution containing water/PG (1:1) and determined to be 55.5×10^{-11} m^2 s^{-1}. D_0^O, the diffusion coefficient of pure *R*-(+)-limonene, was determined to be 38.3×10^{-11} m^2 s^{-1}.

W/O nature. In the second region (30−50 wt % of aqueous phase) a bicontinuous microstructure is formed, whereas in systems containing >50 wt % of aqueous phase an O/W microstructure is obtained.

Changes in behavior along the dilution line can be associated with the interactions between the cholesterol and the hydrophilic headgroups. In the reverse micellar system, the cholesterol molecules are solubilized between the surfactant hydrophobic chains, close to the hydrophilic parts of the surfactant. The OH group of cholesterol has a good interaction with the hydrophilic part of the surfactant. A W/O microstructure is being formed as water is being added to the system. The water interacts with the polar parts of surfactant molecule; thus, fewer cholesterol molecules can interact with the polar part of the surfactant molecule. As more water is added to the system (>30 wt % of aqueous phase) a bicontinuous microstructure is being formed.

Table 2. Expected Solubilization in Microemulsion as a Result of Cholesterol Dissolution in *R*-(+)-Limonene versus Experimental Results

aqueous phase wt %	expected dissolution of cholesterol (ppm)	phytosterol solubilization (ppm)
0	140	790
10	126	760
20	112	750
30	98	680
40	84	380
50	70	150
60	56	80
70	42	50
80	28	30
90	14	15

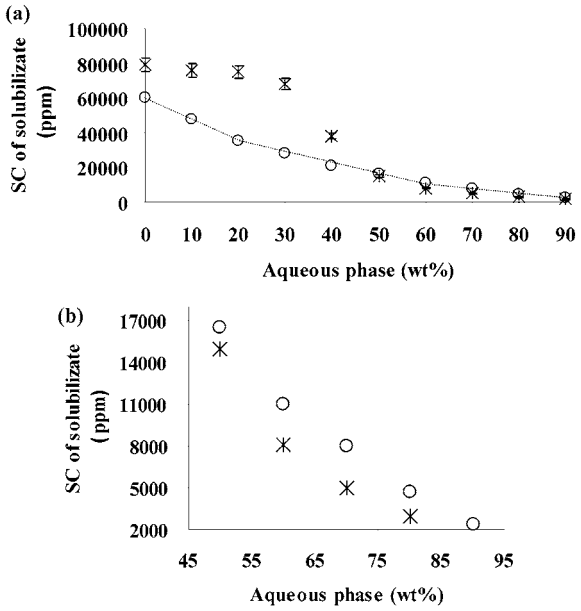

Figure 8. Solubilization capacity (SC) of cholesterol (×) and phytosterols (○) along the dilution line T64 at 25 °C: (a) 0–90 wt % of aqueous phase; (b) 50–90 wt % of aqueous phase.

The cholesterol molecules are forced to solubilize between the hydrophobic parts of the surfactant close to the oil phase, as the polar headgroups of the surfactant fully interact with the water molecules. Thus, a sharp decrease in SC of cholesterol occurs.

Cholesterol dissolution in *R*-(+)-limonene is 7 wt %. The SC of cholesterol in O/W microemulsion containing >60 wt % of aqueous phase is mostly due to cholesterol dissolution in *R*-(+)-limonene, as can be seen in **Table 2**. Thus, changes in the SC of O/W microemulsions that contain cholesterol are dependent on the amount of oil.

When comparing the solubilization capacity of phytosterols and cholesterol, one can easily see the difference, especially in the W/O and bicontinuous microemulsions. The SC of cholesterol is 25−55% higher than phytosterols' SC in systems containing up to 40 wt % of aqueous phase (**Figure 8a**). Increasing the aqueous phase concentration to >50 wt % causes an inversion in the SC behavior of cholesterol and phytosterols. The phytosterols' SC is higher by 10−35% than that of cholesterol in microemulsions containing >50 wt % of aqueous phase (**Figure 8b**). Thus, in order for phytosterols to block cholesterol incorporation (by competition mechanism) into bile salt micelles effectively, one can use O/W microemulsions. These results also suggest that the direct incorporation of phytosterols into oil-based foods (such as margarine) will be

less effective than if used in microemulsion as a vehicle or in water-based systems.

Comparing the relative diffusion coefficient of water and *R*-(+)-limonene in cholesterol and phytosterols containing microemulsions along the T64 dilution line reveals a different influence on the microstructure.

The presence of phytosterols induces the formation of a bicontinuous microstructure (20 wt % of aqueous phase), indicating that phytosterol solubilization affects the spontaneous curvature of the amphiphilic film (**Figure 7b**). On the other hand, cholesterol solubilization does not affect the spontaneous curvature of the interface, thus forming a bicontinuous microstructure at 40 wt % aqueous phase concentration as in the empty microemulsion (**Figure 7a**).

Conclusions. We have demonstrated the use of food-grade microemulsions as vehicles for solubilizing phytosterols.

Phytosterols were solubilized in the microemulsions up to 12 times more than their solubility in *R*-(+)-limonene.

Phytosterols solubilization along any dilution line is microstructure-dependent. Solubilized phytosterols influenced both the microstructure and the compositions at which the transformations from W/O to bicontinuous to O/W microstructures occurred. The presence of phytosterols increases the spontaneous curvature of the interface.

Phytosterols and cholesterol influence the microstructure of the microemulsion differently. In contrast to phytosterols, cholesterol does not have any influence on the spontaneous curvature of the interface. It seems that the preferable locus of phytosterols solubilization is at the interface of the micelles, whereas the preferable locus of cholesterol is between the hydrophobic chains of the surfactant molecule but closer to the hydrophilic heads. This difference in the locus of solubilization may be the cause for the differences in the microemulsion microstructure.

The solubilization capacity of phytosterols is higher than that of cholesterol only in microemulsion systems containing >50 wt % of aqueous phase. Thus, it seems that phytosterols may successfully compete with cholesterol on the entrance into bile salt micelles in the gut only if they are introduced in a water-based microemulsion.

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