Stabilized Nanoparticles of Phytosterol by Rapid Expansion From Supercritical Solution Into Aqueous Solution

Submitted: May 18, 2004; Accepted: September 8, 2004.

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ABSTRACT

The basic objective of this work was to form stable suspensions of submicron particles of phytosterol, a water-insoluble drug, by rapid expansion of supercritical solution into aqueous solution (RESSAS). A supercritical phytosterol/CO_2 mixture was expanded into an aqueous surfactant solution. In these experiments 4 different surfactants were used to impede growth and agglomeration of the submicron particles resulting from collisions in the free jet. The concentration of the drug in the aqueous surfactant solution was determined by high-performance liquid chromatography, while the size of the stabilized particles was measured by dynamic light scattering. Submicron phytosterol particles (<500 nm) were stabilized and in most cases a bimodal particle size distribution was obtained. Depending on surfactant and concentration of the surfactant solution, suspensions with drug concentrations up to 17 g/dm^3 could be achieved, which is 2 orders of magnitude higher than the equilibrium solubility of phytosterol. Long-term stability studies indicate modest particle growth over 12 months. Thus, the results demonstrate that RESSAS can be a promising process for stabilizing submicron particles in aqueous solutions.

KEYWORDS: supercritical fluid, submicron particles, water-insoluble drug, phytosterol, bioavailability.

INTRODUCTION

The poor solubility of pharmaceutical substances in aqueous solvents leads to a low bioavailability, which is one of the main problems in processing new applications. Because of poor solubility, IV injection of such drugs is often difficult. Moreover, the poor solubility is usually combined with low dissolution rates, which results in low drug absorption. However, the bioavailability can be improved by decreasing the particle size and thus increasing the surface-volume ratio, which leads to an increasing solubility.[1,2] In the pharmaceutical industry, several conventional techniques (eg, milling and grinding, spray-drying, freeze-drying, high-pressure homogenization, ball and air jet milling) have been used for particle size reduction. The disadvantages of using these techniques are thermal and chemical degradation of the product, broad particle size distribution, and cumbersome solids handling.[3,4] To overcome these obstacles, the production of submicron particles using supercritical fluids is gaining in importance in areas such as material science and pharmaceutical technology as supercritical fluids are characterized by several advantages.

Until now, there have been several methods for the formation of small organic particles. The primary techniques for particle formation based on supercritical fluids are rapid expansion of supercritical solutions (RESS), particle generation from gas saturated solution (PGSS), and gas antisolvent (GAS). Based on minor variations of the GAS process, different techniques including aerosol supercritical extraction system (ASES), precipitation with a compressed antisolvent (PCA), supercritical antisolvent (SAS), and solution enhanced-dispersion by supercritical fluids (SEDS) are in use.[5] More details about particle design using supercritical fluids can be found in a very comprehensive survey, written by Jung and Perrut.[6]

Various publications show that the RESS process enables the micronization of thermally labile materials and the formation of particles of less than 500 nm in diameter.[7-14] The RESS process uses the high solvating power of supercritical fluids. After loading the supercritical fluid with the solute, an extremely fast phase change from the supercritical to the gas-like state takes place during the expansion in the supersonic free jet. This phase change leads to high supersaturation and subsequently to particle formation. Since the solvent is a dilute gas after expansion, the RESS process offers a solvent-free final product.[15,16] The improvement of the bioavailability of the RESS-produced griseofulvin has been verified by dissolution experiments according to the Stricker model. The dissolution rate of griseofulvin produced by RESS is about 2-fold higher than the common micronized material.[11,12] Modeling results suggest that it should be possible to form particles smaller than 50 nm in diameter.[17,18] The difficulty in achieving these small particle sizes is most likely due to growth and agglomeration during collisions in the subsonic free jet. Until now, much work was done on producing particles of pure solutes by RESS, but only a few studies investi-

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Table 1. Molecular Weight, Critical Micelle Concentration, and Micelle Size of the Surfactants Investigated*

Surfactant Type	Molecular Weight(g/mol)	cmc (wt.-%)	Micelle Size(nm)
Tween 80	1310	0.0013-0.0016	5-20
SLS	288	0.24	≤5
Solutol HS15	-	0.005-0.02	7-20
Lutrol F68	7680-9510	≈0.37-0.46	2-6

*cmc indicates critical micelle concentration; and SLS, sodium lauryl sulfate.

gated the stabilization of submicron particles in surfactant solutions.[6] However, some experimental investigations indicate that this process enables the formation of stable suspensions of submicron particles.[4,10,12,14,19-22]

Recently, Young et al[19] demonstrated the ability to form stable suspensions of submicron particles of cyclosporine A by rapid expansion from supercritical to aqueous solution (RESAS). In this investigation, suspensions with very high payloads (up to 54 g/dm^3) have been achieved with a mean diameter of 500 nm and particle size distributions ranging from 40 to 920 nm, using various surfactant systems based on phospholipid vesicles.[19] In addition, the experiments demonstrate that the obtained particle size distribution is influenced by the nozzle size, and therefore CO_2 flow rate, and by drug concentration. In a former investigation, Young et al demonstrated the ability of Tween 80 to stabilize 400 to 700 nm cyclosporine A particles at concentrations as high as 38 g/dm^3.[4]

The feasibility to stabilize submicron phytosterol particles in aqueous surfactant solutions has been demonstrated by Türk et al.[10,12,14,20-22] In preliminary investigations, the nonionic surfactant Tween 80 and the anionic surfactant sodium lauryl sulfate (SLS) were chosen to impede growth and agglomeration of submicron phytosterol particles. In all cases, the pre-expansion temperature and pressure were 418 K and 25 MPa, respectively; the inner diameter of the nozzle was 50 µm, and the receiving solution was heated to a constant temperature of 298 K. In case of a 1.0 or 5.0 wt% Tween 80 solution, the smallest particles range from 10 to 60 nm; however, larger particles in the range of 62 to 200 nm and 620 to 1600 nm were observed. A similar result was obtained for phytosterol stabilized in either 0.22 or 1.1 wt% aqueous SLS solution. The smaller particles range from 7 to 40 nm and the larger particles range from 30 to 165 nm and 180 to 1100 nm.[12,14] In further experiments, the pre-expansion temperature and pressure were 388 K and 20 MPa, and the inner nozzle diameter was 35 µm. In case of a 1.0 or 5.0 wt% Tween 80 solution, the smallest particles range from 10 to 35 nm; larger particles in the range of 80 to 130 nm and 280 to 1100 nm were observed. A significant change in the particle size distribution was observed for both SLS solutions. The smaller particles range from 30 to 75 nm, and the larger particles range from 80 to 300 nm.[20] In these studies, drug loadings up to ≈6 g/dm^3 were achieved in the surfactant solutions.

The results summarized above indicate that the obtained particle size distribution depends on the flow-rate of the drug solution, the chemical properties of the surfactants, and the surfactant concentration. Based on these experiences, the influence of surfactant type, surfactant concentration, and flow-rate of the drug solution on particle size and long-term stability has been investigated in the present work.

Materials and Methods

Phytosterol was chosen as a model drug to produce aqueous suspensions of a water-insoluble drug and was obtained from Fluka Chemie GmbH, Taufkirchen, Germany. Phytosterol is present in foods and reduces intestinal cholesterol absorption. Structurally similar to cholesterol, phytosterol can be used therapeutically to lower cholesterol in human blood.[23] The composition of phytosterol—85% ß-sitosterol, 10% stigmasterol, and 5% campesterol—was measured by high-performance liquid chromatography (HPLC).[21,22]

CO_2 (AGA, Karlsruhe, Germany) was chosen as supercritical solvent because it is a nonflammable, inexpensive, and nontoxic solvent. Owing to the low critical temperature (T_C = 304 K, p_C = 7.38 MPa), supercritical CO_2 allows processing at moderate temperatures.

The anionic surfactant SLS was purchased from Carl Roth GmbH and Co, Karlsruhe, Germany, and the nonionic surfactant polyoxyethylene sorbitan monooleate (Tween 80), from Fluka Chemie GmbH. Poloxamer (Lutrol F68) and the nonionic surfactant polyethylene glycol-15-hydroxystearate (Solutol HS15) were provided by BASF AG, Ludwigshafen, Germany. The surfactants are approved for use as excipients in delivery formulation. The molecular weight and the critical micelle concentration (cmc) of the surfactants used in this study are shown in Table 1. For the surfactants investigated, the size of the micelles is in the range of 5 to 20 nm depending on the surfactant. All materials and solvents were of the purest grade available and were used without further purification.

The solubility of unprocessed phytosterol in the aqueous surfactant solutions of interest was determined from a saturated solution at 298 K. Excess drug was added to 50 cm^3 of each surfactant solution and allowed to equilibrate with stirring for 1 week at 298 K. The dissolved drug content was determined by the HPLC method described below by analyzing a filtrate of each saturated solution.

The drug concentration in the aqueous surfactant solution was measured by HPLC. All samples were analyzed using a Hewlett-Packard 1100 equipped with a PR 18-column (LiChrospher 100-5, 125 × 4 mm, Merck GmbH, Darmstadt, Germany). The mobile phase ratio was methanol-water 98:2,

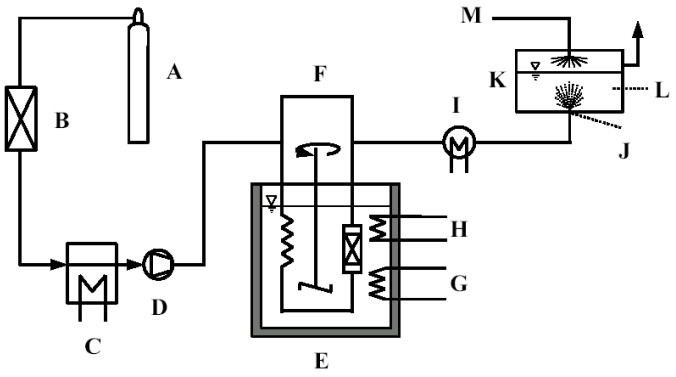

Figure 1. Schematic of apparatus used for the RESSAS experiments: (A) solvent; (B) column with molecular sieve; (C) cooling device; (D) pump; (E) extraction unit; (F) bypass; (G) heating; (H) cooling; (I) preheater; (J) capillary nozzle; (K) expansion chamber; (L) aqueous surfactant solution; and (M) N2 gas inlet.

and flow rate was 1 cm^3/min. Phytosterol was detected at 210 nm. As a rule, the time between the formation of the phytosterol suspensions and HPLC analysis was 1 to 3 days.

Dynamic light scattering (DLS) measurements were conducted to determine the size of the micelles and of the stabilized particles in terms of number-weighted and mass-weighted size distributions; the detection wavelength was 632.8 nm (ALV-Vertriebsgesellschaft mbH, Langen, Germany). Usually, particle size measurements were made within 2 days after the production of the suspensions.

The surfactants were selected with regard to the velocity with which surfactant molecules adsorb at interfaces and stabilize the particles by steric or electrostatic hindering. Therefore, measurements of the dynamic interfacial tension (DIT) were performed using a maximum bubble pressure tensiometer (Lauda GmbH and Co KG, Lauda-Königshofen, Germany).

Rapid Expansion of a Supercritical Solution Into Aqueous Solution

A schematic representation of the supercritical solution into aqueous solution (RESSAS) apparatus used to produce stable suspensions of submicron particles is shown in Figure 1. The apparatus is designed for experiments in the temperature range of 300 to 600 K and pressures up to 60 MPa. A more detailed description of the apparatus and the experimental procedure can be found elsewhere in literature.[9,14,24] In all cases, the gaseous CO_2 is condensed, subcooled, and pressurized to the desired pressure with a diaphragm pump. To minimize the unsteadiness of the flow and to accelerate thermal equilibrium, pure CO_2 flows through the thermostated bypass section into the thermostated high-pressure vessel and through the capillary nozzle into the expansion chamber. After achieving equilibrium, the bypass section is closed and the supercritical CO_2 flows through an extraction column packed with phytosterol. Then, the supercritical solution flows through a heated tube into a heated high-pressure vessel where the preexpansion temperature is measured with a thermocouple and the pre-expansion pressure is measured with a digital pressure gauge. In contrast to the RESS into air experiments, the supercritical CO_2 mixture is expanded through a heated capillary nozzle directly into the aqueous surfactant solution (50 cm^3). For most of the experiments, a nozzle with an inner diameter of D = 50 μm and a length of L = 50 μm was used. For comparison, a smaller nozzle (inner diameter of 35 μm, L/D = 1) was used for a few experiments. To bring the expanded solution, and hence the particles being formed, into rapid contact with the surrounding aqueous surfactant solution, the nozzle is located at the bottom of the expansion chamber (V = 0.6 dm^3). The temperature of the aqueous surfactant solution is measured by a thermocouple, which is immersed in the liquid. Unfortunately, depending on surfactant and concentration, the aqueous solution foamed more or less extensively. To suppress the foam produced during RESSAS, gaseous nitrogen was blown down into the expansion chamber ≈30 cm above the top of the foam.

Based on our experience with the encapsulation of phytosterol in low molecular weight polymeric matrices,[25] the experiments reported below were performed at the following process conditions: extraction temperature 323 K, pre-expansion temperature 388 K, pre-expansion pressure 20 MPa, nozzle temperature 398 K, and solution temperature 303 K.

Results and Discussion

Phytosterol Solubility

The influence of surfactant concentration on solubility in the surfactant solutions is shown in Table 2 and depicted in Figure 2. As a result of the solubility measurements, phytosterol is practically insoluble in the Lutrol F68 solutions investigated. For the other surfactants, the solubility of the drug increases with increasing surfactant concentration. At 298 K, the solubility of phytosterol is 0.025 g/dm^3 in a 1.0 wt% SLS solution and increases with increasing SLS concentration up to 0.1 g/dm^3 in a 5.0 wt% SLS solution. Similar results were obtained in case of the Tween 80 and Solutol HS15 solutions. In these solutions, the drug solubility increases from 0.02 ± 0.001 g/dm^3 at 1.0 wt% to 0.09 g/dm^3 at 5.0 wt%. In the concentration range considered, these values correspond to a relatively constant drug/surfactant ratio of 0.0019 g/g for both, Tween 80 and Solutol HS15, and of 0.0023 g/g for SLS. Thus, the solubility of phytosterol in SLS solutions is approximately 24% higher than in the respective Tween 80 and Solutol HS15 solutions.

Experimental data of the solubility of phytosterol in supercritical CO_2 are not available in literature. Therefore, the solubility of phytosterol in supercritical CO_2 was calculat-

Table 2. Solubility of Phytosterol in Various Surfactant Solutions at 298 K*

Surfactant Type	Surfactant Concentration (wt-%)	Phytosterol Solubility (g/dm³)	Drug/Surfactant Ratio (g/g)
Tween 80	0.1	0.0072	0.0072
	1.0	0.0215	0.0021
	2.0	0.0363	0.0018
	5.0	0.0904	0.0018
SLS	0.1	0.0186	0.0186
	1.0	0.0247	0.0025
	2.0	0.0513	0.0026
	5.0	0.1006	0.0020
Solutol HS15	0.1	0.0026	0.0026
	1.0	0.0191	0.0019
	2.0	0.0378	0.0019
	5.0	0.0880	0.0018
Lutrol F68	0.1	<0.001	-
	1.0	<0.001	-
	2.0	<0.001	-
	5.0	<0.001	-

*SLS indicates sodium lauryl sulfate.

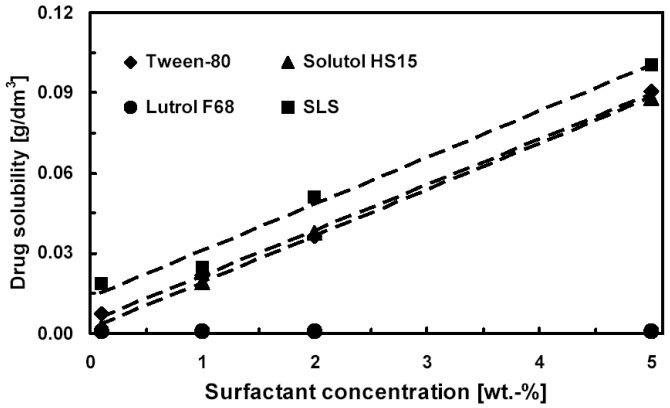

Figure 2. Influence of concentration on solubility of phytosterol in various aqueous surfactant solutions.

ed using the Peng-Robinson equation of state (PR-EOS) using the classical van der Waal's mixing and combination rules.[26] The binary interaction parameter in the attraction term of the PR-EOS has been fitted to experimental data of the melting point of phytosterol under CO_2 pressure. In these experiments, the solid-liquid-gas-line (SLG-line) was determined according to the first melting point method. It is shown in literature that this approach enables a reliable prediction of the solubility of solids in supercritical fluids.[27-30] The normal melting point of phytosterol was verified using differential scanning calorimetry (DSC) and was found to be 413 K.[30] With increasing CO_2 pressure, the melting temperature of phytosterol decreases continuously to 373 K at 26 MPa.[30] Applying the PR-EOS as described above leads to an equilibrium mole fraction of phytosterol in the supercritical mixture of 1×10^{-4} at the prevailing conditions (323 K, 20 MPa).

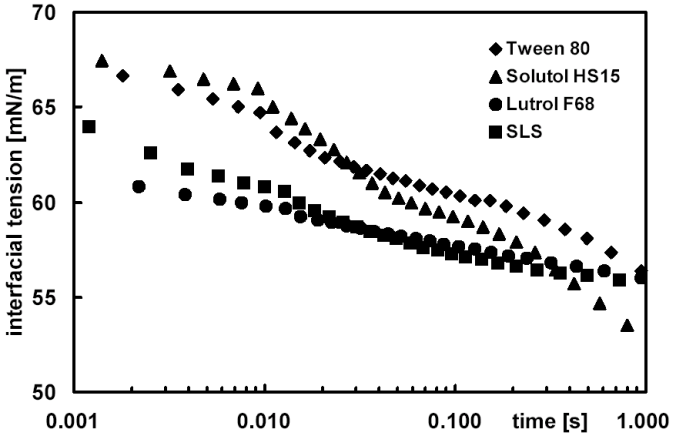

Figure 3. Interfacial tension versus time of a 0.1 wt% surfactant solution.

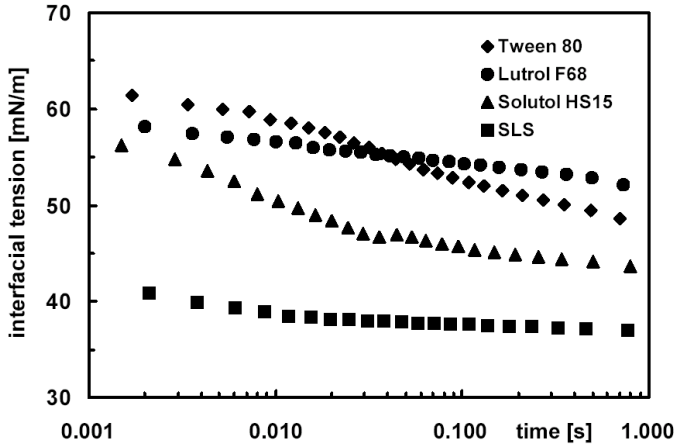

Figure 4. Interfacial tension versus time of a 1.0 wt% surfactant solution.

Dynamic Interfacial Tension

Typical dynamic interfacial tension (DIT)-curves of the surfactant solutions investigated are shown in Figure 3 and Figure 4 for a surfactant concentration of 0.1 and 1.0 wt%. In case of SLS and Lutrol F68 the lower concentration was below the cmc. For all surfactants investigated, a decrease of the interfacial tension with increasing surfactant concentration was observed. However, the behavior of Tween 80 and Solutol HS15 differs markedly from the behavior observed for Lutrol F68 and SLS. As shown in Figure 3, the interfacial tension decreases from 68 to 55 mN/m within 1 second for the former surfactants while in case of SLS a decrease from 64 to 59 mN/m and of Lutrol® F68 a decrease from 61 to 56 mN/m can be noticed. A similar result is obtained for the higher concentrated surfactant solutions (see Figure 4). Within 1 second, the interfacial tension decreases from 62 to 48 mN/m (Tween 80), 58 to 43 mN/m (Solutol HS15), 41 to 37 mN/m (SLS), and 58 to 52 mN/m (Lutrol F68).

These results indicate that the decrease of the interfacial tension with respect to time is relatively constant for each surfactant investigated. For both, Tween 80 and Solutol HS15, a

Table 3. Effect of Surfactant Type on Measured Particle Size Distribution of Phytosterol Particles Stabilized in 50 cm^3 of a 1.0 wt% Surfactant Solution at 303 K*

Surfactant Type	Particle Size (nm)	Yield (%)	Drug-Concentration (g/dm^3)	Drug/ Surfactant Ratio (g/g)
Tween 80†	12-22 160-360	95	10.9	1.1
Solutol HS15	22-41 60-540	43	17.1	1.1
SLS‡	30-55 80-220	71	5.6	0.5
Lutrol F68	42-54 190-380	5.5	2.4	0.14

*SLS indicates sodium lauryl sulfate.
†Spray time, 120 minutes.
‡1.1 wt%; d_N = 35 µm; spray time, 210 minutes.

significantly higher decrease of the interfacial tension was observed than for Lutrol F68 and SLS. However, it should be considered that at higher surfactant concentrations the lowest absolute values of the interfacial tension were obtained for SLS. As shown in Figure 4, the interfacial tension is reduced to 37 mN/m at an SLS concentration of 1 wt%.

Effect of Surfactant Type

In Table 3, the results of spraying a supercritical CO_2/phytosterol mixture into aqueous solutions are summarized. In all cases the surfactant concentration was 1.0 wt%, which is far above the cmc. Table 3 shows surfactant concentration, particle size distribution, yield, and the final drug concentration in the suspension as measured by HPLC. In each experiment, the concentration of phytosterol in CO_2 was known, and the flow rate through the nozzle was measured. Hence, the yield is the percentage of the recovered phytosterol, according to HPLC, relative to the amount of phytosterol sprayed into the solution. Figure 5 shows a typical particle size distribution of phytosterol particles measured by DLS. For all surfactants investigated, a bimodal particle size distribution was obtained and small phytosterol particles were stabilized, although with different particle sizes (see Figure 5).

As shown in Table 3, Tween 80 stabilizes small particles ranging from 12 to 22 nm as well as larger particles from 160 to 360 nm at a high drug concentration of 10.9 g/dm^3. Using Solutol HS15 led to a similar drug/surfactant ratio and a relatively broad particle size distribution. As shown in Figure 5, the smaller particles ranged from 22 to 41 nm and the larger ones ranged from 60 to 540 nm.

In case of SLS, a relatively narrow particle size distribution ranging from 30 to 55 nm and 80 to 220 nm at a concentration of 5.6 g/dm^3 was received. Therefore, it must be noted that a smaller nozzle (d_N = 35 µm) was used. As shown in Tables 4 and 5, increasing the diameter from 35 to 50 µm

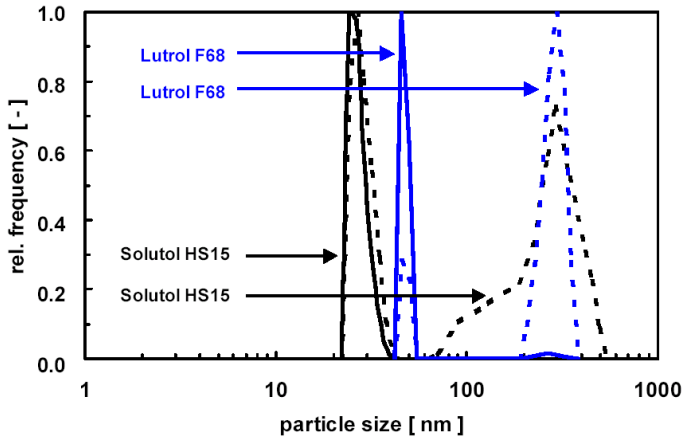

Figure 5. Particle size distribution of phytosterol particles measured by DLS. The particles were stabilized in an aqueous solution consisting of either 1.0 wt% Solutol HS15 or 1.0 wt% Lutrol F68. The full line holds for number-weighted size distribution and the dotted line for mass-weighted size distribution.

leads to a noticeable increase in drug concentration. According to the relatively high equilibrium solubility, these surfactants have a high affinity for phytosterol, resulting in a high yield and high drug concentration.

In contrast to the results discussed above, the use of Lutrol F68 results in a low drug concentration of 2.4 g/dm^3 and 2 narrow peaks. As shown in Figure 5, the smaller particles ranged from 42 to 54 nm and the largest ones, from 190 to 380 nm. The particle size distributions depicted in Figure 5 indicate that Solutol HS15 stabilizes the particles more effectively than Lutrol F68. The low yield and low drug concentration suggest that Lutrol F68 has a lower affinity for phytosterol, which is consistent with the very low equilibrium solubility.

Effect of Surfactant Concentration

The results of spraying a supercritical CO_2/phytosterol mixture into aqueous solutions with different surfactant concentrations are summarized in Table 4. The table shows surfactant concentration, particle size distribution, yield, and the final drug concentration in the suspension as measured by HPLC. Again, for all surfactants and concentrations investigated, a bimodal particle size distribution was obtained. With the exception of Lutrol F68, increasing the surfactant concentration results in a shift of the first peak toward smaller particles, while the second peak shifts slightly towards larger particles. In addition, the second peak becomes broader.

A typical example of this behavior is given in Figure 6. The figure shows the particle size distribution of phytosterol stabilized in an aqueous solution consisting of either 0.1 or 2.0 wt% Solutol HS15. In the case of the former solution, particles ranging from 29 to 35 nm and from 140 to 240 nm were stabilized. In the latter, higher concentrated solution, smaller

Table 4. Effect of Surfactant Concentration on Measured Particle Size Distribution of Phytosterol Particles Stabilized in 50 cm^3 Surfactant Solution at 303 K*

Surfactant Concentration (wt%)	Particle Size (nm)	Yield (%)	Drug-Concentration (g/dm^3)	Drug/Surfactant Ratio (g/g)
Solutol HS15, 0.1	29-35 140-240	17.5	6.3	4.3
Solutol HS15, 1.0	22-41 60-540	43	17.1	1.1
Solutol HS15, 2.0	13-22 70-770	23	10.0	0.28
Lutrol F68, 0.1	50-70 170-420	10	5.3	2.6
Lutrol F68, 1.0	42-54 190-380	5.5	2.4	0.14
Lutrol F68, 2.0	60-80 200-460	12	4.6	0.15
Tween 80, 0.5†	32-49 220-530	67	11.4	2.3
Tween 80, 1.0‡	12-22 160-360	95	10.9	1.1
Tween 80, 2.0†	8-15 200-670	57	9.9	0.5
SLS, 0.22§	40-80 100-300	51	4.5	2.0
SLS, 1.1‖	30-55 80-220	71	5.6	0.5

*SLS indicates sodium lauryl sulfate.
†Spray time, 180 minutes.
‡Spray time, 120 minutes.
§d_N = 35 μm; spray time, 240 minutes.
‖d_N = 35 μm; spray time, 210 minutes.

Table 5. Influence of Nozzle Diameter on Particle Size Distribution and on Drug Concentration

Exp No.	Surfactant Concentration (wt-%)	Spray Time (min)	Particle Size (nm)	Drug Concentration (g/dm^3)	Drug/Surfactant Ratio (g/g)
1	Tween 80, 0.5	180	32-49 220-530	11.4	2.3
2	Tween 80, 1.0	120	12-22 160-360	10.9	1.1
3	Tween 80, 2.0	180	8-15 200-670	9.9	0.5
4	Tween 80, 1.0*†	120	13-25 60-130 370-1150	6.0	0.6
5	Tween 80, 5.0*‡	210	7-13 80-140 280-810	4.1	0.08
6	Solutol HS15, 0.1	120	27-32 160-290	2.9	1.9
7	Solutol HS15, 0.1	240	29-35 140-240	6.3	4.3

*Nozzle diameter is 35 μm; L/D = 1.
†CO_2 flow rate is 3.6 g/min.
‡CO_2 flow rate is 5 g/min.

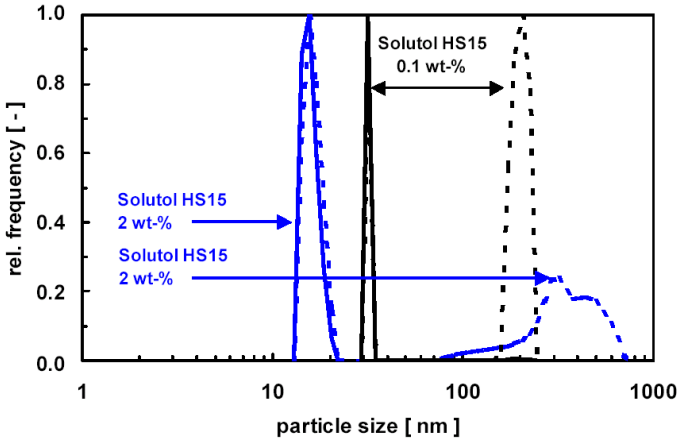

Figure 6. Particle size distribution of phytosterol particles measured by DLS. The particles were stabilized in an aqueous solution consisting of either 0.1 or 2.0 wt% Solutol HS15. The full line holds for number-weighted size distribution and the dotted line for mass-weighted size distribution.

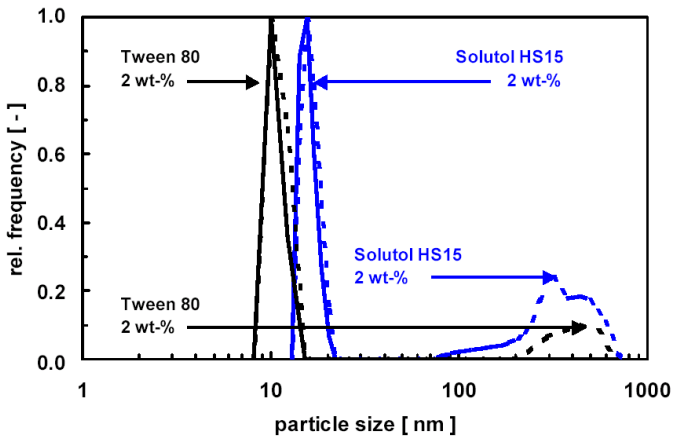

Figure 7. Particle size distribution of phytosterol particles measured by DLS. The particles were stabilized in an aqueous solution consisting of either 2.0 wt% Tween 80 or Solutol HS15. The full line holds for number-weighted size distribution and the dotted line for mass-weighted size distribution.

Table 6. Suspension Stability After Different Storage Times at 298 K*

Tween 80 Concentration (wt%)	Δ t (month)	Particle Size (nm)	Drug Concentration (g/dm^3)	Particle Size After Δ t (nm)
0.5	6	32-49 220-530	11.4	60-120 150-610
1.0	4	12-23 160-360	10.9	22-42 80-950
2.0	4	8-15 200-670	9.9	8-18 60-1370
5.0	12	7-13 80-140 280-810	4.1	18-27 80-910

*Δ t indicates change in time.

particles ranging from 13 to 22 nm and from 70 to 770 nm were stabilized. The particle size distributions depicted in Figure 6 indicate that the higher concentrated solution stabilizes the particles more effectively than the lower one. This result is mainly caused by the following 2 effects: (1) as shown in Figures 3 and 4, increasing the surfactant concentration leads to a decrease of the DIT, which enables a more rapid diffusion of the surfactant to the particle surfaces; and (2) increasing the surfactant concentration leads to increasing equilibrium solubility and therefore in a higher affinity for phytosterol.

In contrast to the results obtained for Solutol HS15, no obvious influence of surfactant concentration on particle size was observed for Lutrol F68. In the concentration range investigated, smaller particles from 40 to 80 nm and larger particles from 170 to 460 nm were obtained. Independent from surfactant concentration, a relatively small particle size distribution was obtained and the slightest foaming was observed. The low yield and relatively low drug concentration of 2.4 to 5.3 g/dm^3 indicate a low affinity for phytosterol, according to the low equilibrium solubility. Furthermore, as shown in Figures 3 and 4, increasing the surfactant concentration led to a comparatively small decrease in the DIT.

In case of Tween 80, the stabilization in a low concentrated solution led to particles ranging from 32 to 49 nm and from 220 to 530 nm at phytosterol concentrations of 11.4 g/dm^3. Similar to Solutol HS15, an increase of the surfactant concentration from 0.5 to 2.0 wt% results in a shift of the first peak toward smaller particles, while the second peak shifts slightly towards larger particles. In addition, the second peak becomes broader, as shown in Figure 7 and Table 6. However, at a surfactant concentration of 1.0 wt%, the second peak was narrower than for the lower and higher concentration. Among other reasons (see below), this result might be explained by the shorter spray time (see Table 5). The comparison with the results obtained for Solutol HS15 is shown in Figure 7. It is obvious that the first peak is shifted toward smaller and the second peak toward larger particles.

These results indicate that Tween 80 stabilizes the particles slightly more effectively than Solutol HS15. However, it seems that these differences are of minor importance, which is consistent with the similar equilibrium solubility of phytosterol in both surfactants.

Using SLS and increasing the surfactant concentration from 0.22 to 1.1 wt% results in a shift of the first and the second peak toward smaller particles at drug concentrations of 4.5 and 5.6 g/dm^3. In case of the lower concentrated solution, particles ranging from 40 to 80 nm and 100 to 300 nm were observed, while the higher concentrated solution led to particles stabilized ranging from 30 to 55 nm and from 80 to 220 nm. Although a smaller nozzle was used in these experiments, a relatively high drug/surfactant ratio was obtained. This result was mainly owing to the increasing equilibrium solubility corresponding to a higher affinity for phytosterol and to the decrease of the DIT, which enabled a more rapid diffusion to the particle surfaces.

Effect of Flow Rate and Spray Time

The effect of nozzle diameter on particle size of stabilized phytosterol particles is summarized in Table 5. In Experiments 1 to 3, 6, and 7, a nozzle with an inner diameter of 50 µm was used. At the prevailing preexpansion conditions such a nozzle produces a CO_2 flow rate of ≈8 g/min. For comparison, in Experiments 4 and 5 a nozzle with an inner diameter of 35 µm (≈3.6 g/min and 2.5 g/min, respectively) was used.

The results given in Table 5 show that the significantly higher flow rate always results in a bimodal particle size distribution and in obviously smaller stabilized particles. Since the higher flow rate leads to a higher turbulence, the smaller particle size distribution is the consequence of a more intense mixing of the surfactant solution inside the expansion chamber.

The results of Experiments 2 and 4 as well as 6 and 7 demonstrate a satisfactory agreement of these experiments. From the results of Experiments 6 and 7, it follows that at a comparatively low Solutol HS15 content, increasing the spray time increases the drug concentration without any influence on particle size distribution. On the contrary, Experiments 2 and 3 suggest that with increasing spray time, the particle size distribution become broader at Tween 80 concentrations of 1 and 2 wt%. It seems that the surfactant level is insufficient to stabilize the particles markedly above a drug concentration of ≈10 g/dm^3. This finding is confirmed by the comparison of the particle size distribution obtained for Solutol HS15. Increasing surfactant concentration led to a broader particle size distribution at a drug concentration of 10 or 17 g/dm^3. For cyclosporine A, Young et al reported a similar trend in particle size at drug concentrations above 10 g/dm^3. These authors observed a nearly linear increase of the mean particle diameter with drug concentration.[19] On the contrary, the results obtained for Lutrol F68 at low drug concentrations between 2.4 and 5.3 g/dm^3 showed no influence of drug concentration on particle size distribution.

The increase in particle size distribution with drug concentration may be caused by several factors. One reason is simply that the particle collision rate is directly proportional to the square of particle concentration.[10,19] Increasing spray time and drug concentration can lead to surfactant depletion in the aqueous solution, especially at low surfactant concentrations. Thus, fewer of the initial surfactant's molecules are available for the stabilization of newly generated particles.

Long-term Stability

In general, the time between the formation of the phytosterol suspensions and HPLC analysis was 1 to 3 days. All samples were stored at 298 K and analyzed after different time intervals by HPLC. In addition, the long-term stability of phytosterol particles stabilized in different Tween 80 solutions after different storage times was examined, and the results are shown in Table 6. Independent from drug concentration and storage time a bimodal particle size distribution was obtained for all samples. However, all samples showed a modest particle growth resulting on a broadening of the size distribution. The modest change in the size distribution was observed for the lowest and the highest concentrated solution. In summary, Table 6 shows that the produced suspensions experienced only a modest broadening of the size distribution, although the samples were stored at room temperature. In future studies, this deficiency will be overcome by storing the samples at 277 K and a nitrogen headspace.

Based on the results discussed above the following trends were observed:

Using Lutrol F68, Tween 80, and Solutol HS15, a bimodal particle size distribution was obtained by spraying a supercritical CO_2/phytosterol mixture through a 50-µm nozzle into a 50-cm^3 surfactant solution. In case of SLS, a bimodal size distribution was also obtained, although a 35-µm nozzle was used. This result can be explained by the relatively low DIT of SLS.

At constant surfactant concentration, particle size distribution and drug concentration is influenced by both equilibrium solubility of the drug and DIT. Except for Lutrol F68, increasing surfactant concentration results in a shift of the first peak toward smaller particles. This may be mainly the result of the decrease of the DIT, which enables a more rapid diffusion to the particle surfaces. As expected, the influence of surfactant concentration becomes more distinctive with increasing concentration. On the contrary, no significant influence of surfactant concentration on particle size distribution and drug concentration was observed in case of

Solutol HS15, which is consistent with the DIT and the negligible equilibrium solubility. Thus as a result, the first peak is mainly affected by the DIT of the surfactant.

Several effects may have caused the existence of the second peak including less mixing of the aqueous solution and therefore partial depletion of the surfactant solution, which resulted in particle growth within this region where the particles can be stabilized less effectively. As mentioned above, in all cases the aqueous solution foamed more or less extensively. Thus, particles could grow owing to their relatively long residence time in the foam. In addition, bubble collisions within the aqueous solution as well as within the foam may promote further particle growth. Thus, the comparison of the particle size distributions obtained for Solutol HS15 with the results for the other surfactants suggests that the broadness of the second peak is mainly the result of foaming. However, this point requires more detailed investigation.

At a given mass flow rate and a similar spray time, the comparison between Solutol HS15 and the other surfactants investigated shows that the achievable drug loading is mainly influenced by the equilibrium solubility and therefore by the strength of the drug-surfactant interaction in the aqueous solution.

CONCLUSION

By RESSAS, phytosterol particles less than 500 nm in diameter were stabilized in different surfactant solutions with drug concentrations up to 17 g/dm^3. In most cases, a bimodal size distribution was obtained and the smallest stabilized particles were one order of magnitude lower than produced by RESS into air. The broadness of particle size distribution and the drug concentration in the solution was influenced by surfactant type and surfactant concentration. The small particle size, the high phytosterol concentration (approximately 2 orders of magnitude higher than the equilibrium solubility), and the satisfying long-term stability demonstrates that RESSAS can be a promising process for stabilizing submicron particles in aqueous solutions.

ACKNOWLEDGEMENTS

The authors are grateful to Sonja Viereck and Claudia Lauer who performed parts of the experiments, Sylvia Keller (Universität Jena, Germany) for the determination of the phytosterol composition and Clemens Posten (Universität Karlsruhe, Germany) for the use of the DLS-device. Part of the project is supported by the Deutsche Forschungsgemeinschaft (DFG grant no. Tu 93/5-1, 5-2) and by the State of Baden-Württemberg, Germany, which is gratefully acknowledged.

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