RESSAS: A PROMISING TECHNOLOGY FOR IMPROVING SOLUBILITY OF POORLY WATER-SOLUBLE PHARMACEUTICALS

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Many pharmaceutical substances are insoluble or only slightly soluble in aqueous media and the application of oral or injectable drugs is often limited by the low bioavailability. Due to the fact, that the bioavailability of drugs depends on the velocity of dissolution and absorption, nanoscale particles are needed in order to maximize the surface area, and to enhance the bioavailability.
In recent years, we have used the RESS-process successfully to comminute water-insoluble drugs (Griseofulvin, Phytosterol, Ibuprofen). These experiments led to agglomerated particles in the range of 250 ± 50 nm depending on solvent, pre- and post-expansion conditions. To minimize agglomeration of the particles due to coagulation during the expansion process and to suspend the particles, we utilized RESSAS (Rapid Expansion of Supercritical Solutions into Aqueous Solutions) to produce stable aqueous suspensions of water-insoluble drugs. In these experiments Tween® 80, a nonionic surfactant and the anionic surfactant SDS was chosen to impede growth and agglomeration of the nanoscale drug particles. The concentration of the drug in the aqueous surfactant solution was measured by high performance liquid chromatography (HPLC) and the size of the stabilized particles was measured by dynamic light scattering (DLS). Independent of the pre-expansion conditions ultrafine Phytosterol particles (20 – 80 nm) were stabilized. Depending on surfactant and composition of the aqueous surfactant solution a bimodal particle size distribution was observed and suspensions with loadings up to 12 g/l could be achieved. Based on these promising results, the influence of various chemically different surfactants (Lutrol® F68 and Solutol® HS15, Tween® 80 and SDS) on particle size distribution and on payloads was investigated.

1 INTRODUCTION

Poorly soluble drugs are a general problem in pharmaceutical drug formation [1]. Typical problems associated with poorly soluble drugs are low bioavailability and erratic absorption. Attempts to increase the dissolution velocity and saturation solubility and thus solving the problem of the low bioavailability in oral or parental application of water-insoluble drugs are solubility enhancement by reducing the particle size. Traditional micronization techniques such as spray drying, emulsion solvent extraction, and processes based on high shear, for example, high-pressure homogenization, ball milling and air jet milling have certain drawbacks. Micronization with these techniques tends to broad particle size distributions in the range from 0,1 µm to approximately 25 µm and only a negligible amount being below 1 µm. Moreover, products can be denatured by exposure to high temperatures or residual solvent concentration. Hence, processing techniques that do not rely

on organic solvents, high temperatures and which can provide small particles with narrow size distributions are highly desirable [1-3]. Recent investigations show, that the rapid expansion of supercritical solutions (RESS) enables the micronization of thermally labile materials and the formation of particles of less than 500 nm in diameter. Depending on solvent, pre- and post-expansion conditions, the experiments led to particles in the range of 160 to 350 nm in diameter. These agglomerated particles consist of primary particles in the range of 50 to 150 nm [4-8]. There is, of course, the important limitation that such particles are very difficult to be included in solid dosage forms since they are hardly compressible. These facts lead to some important aspects for the further development of the RESS-process. The expansion from the supercritical state to atmospheric pressure reduces the solvent density and initiates intense nucleation. In the free jet, the particle formation steps include nucleation, condensation of solute molecules about the nuclei and coagulation of particles. In order to reduce the coagulation rate in the free jet, the rapid expansion from supercritical solution into aqueous solution (RESSAS) was applied [3,9,10]. In RESSAS, the supercritical mixture is expanded directly into an aqueous solution containing a surfactant. Theoretical calculations show that particles are formed in the size of 5 to 10 nm in the supersonic free jet [11]. The difficulty to achieve the theoretical particle size is likely due to growth and agglomeration during collisions in the free jet. To stabilize such small particles, the surfactant must be able to adsorb rapidly onto the particle surfaces as they precipitate and hinder growth in the free jet. Using surfactant solutions with a concentration above the critical micelle concentration the primary particles can be stabilized at an early stage. Here we focus on the influence of chemically different surfactants on the stabilization of nanoscale particles. Four different surfactants were used to stabilize particles in an aqueous solution to investigate the influence on particle size distribution and on payloads.

2 MATERIALS AND METHODS

2.1 Materials

CO_2 was chosen as supercritical solvent of interest since it is an environmentally benign solvent that is nonflammable, inexpensive and essentially non-toxic. Due to the low critical temperature (T_C = 304 K, p_C = 7,38 MPa), supercritical CO_2 allows processing at moderate temperatures.

Phytosterol was obtained from Fluka Chemie GmbH and was used without further purification. The composition of Phytosterol (85% ß-Sitosterol, 10% Stigmasterol and 5% Campesterol) was measured by High Pressure Liquid Chromatography (HPLC) [12]. The anionic surfactant SDS was purchased from Carl Roth GmbH & Co. and the nonionic surfactant Tween® 80 from Fluka Chemie GmbH. The tri-block-copolymer Lutrol® F68 and the nonionic surfactant Solutol® HS15 were provided from BASF AG.

HPLC in combination with UV-detection was used to quantify the drug concentration in the aqueous suspension. The column used for the HPLC was a RP18 (125x4mm). The mobile phase consisted of a mixture of 98% methanol and 2% water and the detection wavelength was 210 nm.

The mass weighted and number weighted particle size distribution of the particles in the suspension was determined by dynamic light scattering (DLS) with an ALV-HPPS from ALV-Laser GmbH. The wavelength of the HeNe-laser is 632,8 nm. Particle size measurements were made within 2 days after the production of the suspensions.

The surfactants were classified with regard to their stabilization properties by measuring the dynamic interfacial tension (DIT). These measurements were performed using a maximum bubble pressure tensiometer (Lauda GmbH & Co. KG).

2.2 EXPERIMENTAL

The RESSAS-experiments were carried out with the test plant depicted in Fig. 1. The equipment was designed for experiments in the temperature range from 300 to 600 K and for pressures up to 60 MPa [13]. In all experiments, the gaseous CO_2 is cleaned, sub-cooled, and pressurized to the desired pressure with a diaphragm pump. The supercritical CO_2 flows through an extraction column, packed with Phytosterol. In contrast to the RESS experiments, the supercritical mixture is expanded through a heated nozzle (L/D = 1, D = 50 µm) directly into the aqueous surfactant solution. The nozzle is located on the bottom of the expansion chamber and the aqueous surfactant solution temperature is measured by a thermocouple submerged in the liquid. To bring the expanded solution, and hence the particles being formed, in rapid contact with the surrounding area the nozzle is located approximately 2 cm below the surface of the aqueous surfactant solution. The expansion chamber can be used at pressures up to 1 MPa and temperatures up to 323 K. With a temperature-controlled heater, which is integrated in the bottom of the expansion chamber, the temperature can be held constant. To suppress and drain any foam produced during RESSAS, pressurized air (0,1 MPa to 1 MPa) is blown down on top of the foam. All experiments were performed at an extraction temperature of 323 K, a pre-expansion temperature of 388 K and a pre-expansion pressure of 20 MPa, resulting in a CO_2 flow-rate of 8 g/min.

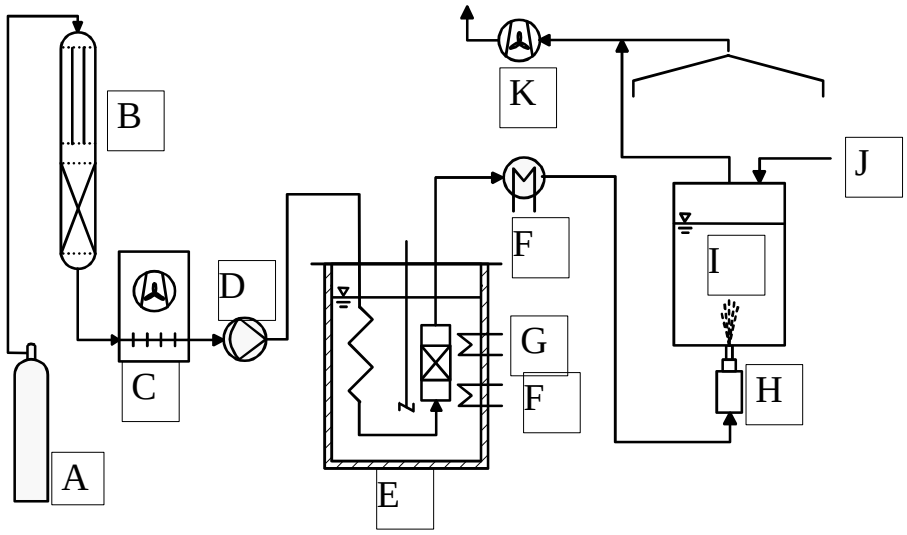

Figure 1: A, solvent; B, column with molecular sieve; C, liquefaction; D, diaphragm pump; E, thermostated extractor; F, heater; G, cooler; H, heated laser-drilled nozzle; I, expansion chamber with surfactant solution; J, pressurized air; K, vent.

3 RESULTS AND DISCUSSION

3.1 SOLUBILITY

First, the solubility of unprocessed Phytosterol in different aqueous surfactant solutions was determined from a saturated solution at 298 K. Excess drug was added to 50 ml of each surfactant solution and allowed to equilibrate with stirring for 1 week at 298 K. The dissolved drug content was determined by the above-mentioned HPLC method by analyzing a filtrate of each saturated solution. The goal of our RESSAS-experiments was to produce suspensions with a drug / surfactant ratio much higher as obtained from the equilibrium solubility in the surfactant solution. The influence of surfactant concentration on the solubility of Phytosterol in the surfactant solutions is shown in Fig. 2. As a result, Phytosterol is practically insoluble in the Lutrol® F68 solutions. For all the other surfactants investigated, the solubility of the drug increases with increasing surfactant concentration. In addition, the

solubility of Phytosterol in SDS solutions is about twofold higher than in respective Tween® 80 and Solutol® HS15 solutions.

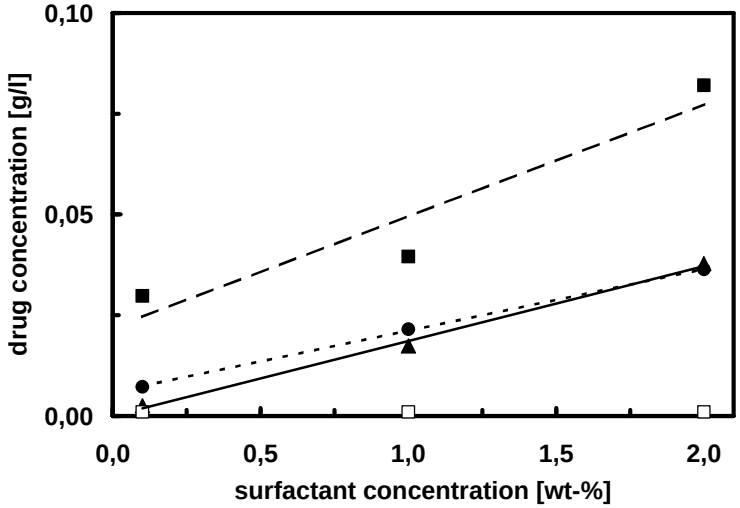

Figure 2: Equilibrium solubility of Phytosterol in various surfactant solutions at 298 K (■, SDS; ●, Tween® 80; ? , Solutol® HS15; □, Lutrol® F68).

3.2 DIT

Due to the amphiphilic behavior of the surfactants the molecules adsorb at interfaces resulting in the reduction of the interfacial tension. At a specific concentration, the so-called critical micelle concentration, the surface is covered maximal and the surface tension is minimal. Depending on surfactant and surfactant concentration additional surfactant molecules form different kind of micelles. The adsorption kinetic can be determined indirectly by measuring the change of the interfacial tension. The kinetic of the interfacial adsorption describes the velocity with which surfactant molecules adsorb at interfaces and stabilize them by steric or electrostatic hindering. Typical DIT-curves of Tween® 80 solutions are shown in Fig. 3. For all surfactants investigated, a decrease of the interfacial tension with increasing surfactant concentration was observed. In addition, the interfacial tension decreased markedly for surfactant concentrations above 0,5 wt-%. More details about the experiments and the obtained results can be found in literature [14].

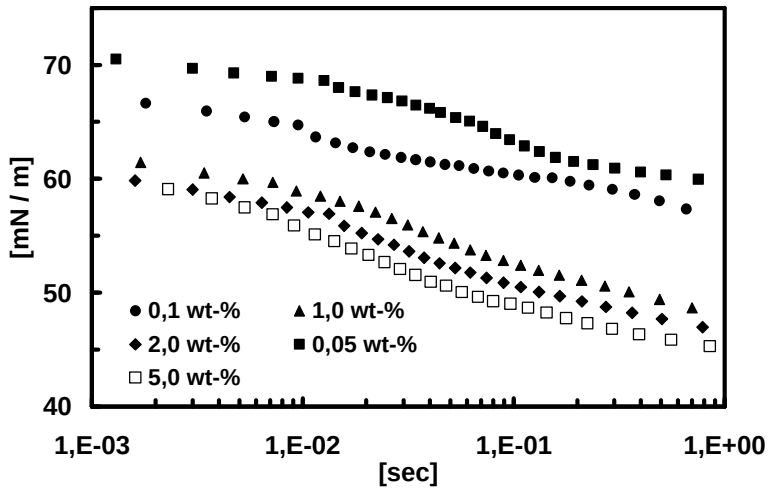

Figure 3: Interfacial tension against the age of the interface of Tween® 80.

3.2 DLS

As a control, the size of micelles in particle free surfactant solutions was determined by DLS. For all surfactants investigated, the micelle size is in the range of 2 – 20 nm. Table 1 summarizes the results of spraying a CO_2 / Phytosterol solution into different surfactant solutions. The table shows the surfactant concentration, the particle size distribution, and concentration of drug in the suspension determined by HPLC, and the equilibrium solubility. Dependent on surfactant and surfactant concentration, the drug concentration ranges from 4,5 to 17,1 g/l. As shown in Table 1, the smaller particles range from 12 to 80 nm and the larger particles are in the range from 70 to 770 nm. However, particle size distribution tends to a slightly broader distribution in the higher surfactant concentration in comparison to the lower surfactant concentration. The smaller particles demonstrate imposingly that the initially formed particles with very small particle size can be stabilized within the expanding jet without excessive particle growth due to agglomeration. In addition, Table 2 shows that the produced nanosuspensions are stable for at least 12 months with regard to particle size distribution.

Table 1: Effect of surfactant type and concentration on Phytosterol particles prepared by RESSAS for a stabilizing solution temperature of 298 K.

surfactant	surf. conc. [wt-%]	particle size [nm]	drug conc. [g/l]	eq. drug conc. at 298 K [g/l]
Solutol® HS15	0,1	29 – 35 140 – 240	6,3	0,003
Solutol® HS15	1	22 – 41 60 –540	17,1	0,02
Solutol® HS15	2	13 – 22 70 – 770	10,0	0,04
Lutrol® F68	0,1	50 – 70 170 – 420	5,3	< 0,001
Lutrol® F68	1	42 – 54 190 – 380	2,4	< 0,001
Lutrol® F68	2	60 – 80 200 – 460	4,6	< 0,001
Tween® 80	0,5	30 – 50 220 – 530	11,4	0,013
Tween® 80	1	12 – 22 160 – 360	10,9	0,021
SDS	0,22	40 – 80 100 – 300	4,5	0,03
SDS	1,1	30 – 55 80 - 220	5,6	0,045

4 CONCLUSIONS

In the present paper selected examples of ongoing experimental investigations have been presented. The feasibility to prepare stable nanosuspensions with different surfactants has been demonstrated. RESSAS-experiments lead to nanosuspensions with finely dispersed particles, which are stable over several months. The results demonstrate that the RESSAS-

process can be an efficient method for nanosuspension production. However, further work should result in better understanding of the underlying physical phenomena, and pointing out the relationship between the process conditions and product quality.

Table 2: Suspension stability after different storage times at 298 K.

Tween® 80 conc. [wt-%]	particle size [nm]	drug conc. [mg/ml]	Δ t [month]	particle size after Δ t [nm]
0,5	30 – 50 220 – 530	11,4	6	60 – 120 150 – 610
1	13 – 20 75 – 800	3,5	11	56 – 1130
1	12 – 22 160 – 360	10,9	4	22 – 44 80 – 950
2	8 – 15 35 – 70 230 – 550	3,0	6	8 – 24 70 – 1120
5	7 – 13 80 – 140 280 – 810	4,1	12	18 – 27 80 – 910

5 ACKNOWLEDGMENTS

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6 REFERENCES

[1] B. Subramaniam, R.A. Rajewski, K. Snavely; J. Pharm. Sci., 86, 8 (1997) 885.
[2] R.H. Müller, B.H.L. Böhm, M.J. Grau; Pharm. Ind., 61, 74 (1999) 175.
[3] T.J. Young, S. Mawson, K.P. Johnston, I.B. Hendriksen, G.W. Pace, A.K. Mishra; Biotechnol. Prog. 16 (2000) 402.
[4] M. Türk; J. Supercrit. Fluids, 15, 1 (1999) 79.
[5] B. Helfgen, M. Türk, K. Schaber; J. Powder Techn., 110 (2000) 22.
[6] M. Türk; professorial dissertation, Universität Karlsruhe (TH) (2001).
[7] M. Türk, P. Hils, B. Helfgen, R. Lietzow, K. Schaber; Part. Part. Syst. Charact., 19 (2002) 327.
[8] M. Türk, P. Hils, B. Helfgen, K. Schaber, H.-J. Martin, M.A. Wahl; J. Supercrit. Fluids, 22, 1 (2002) 75.
[9] M. Türk, R. Lietzow, P. Hils, K. Schaber: Chemical Engineering Transactions, 2 (2002) 621-626; edited by Alberto Bertucco, ISBN 88-900775-1-4.
[10] M. Türk; Chemie Ingenieur Technik 75, 6, (2003) 792.
[11] B. Helfgen, M. Türk, K. Schaber; J. Supercrit. Fluids 26, 3 (2003) 225.
[12] S. Keller, private communication.
[13] S. Cihlar; doctoral thesis, Universität Karlsruhe (TH) (2000).
[14] M. Türk, R. Lietzow; Proceedings of the International Congress for Particle Technology, PARTEC 2004, Nürnberg, March 16 - 18, 2004.