STABILIZATION OF PHARMACEUTICAL SUBSTANCES BY THE RAPID EXPANSION OF SUPERCRITICAL SOLUTIONS (RESS)

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ABSTRACT
Many pharmaceutical substances are often insoluble or only slightly soluble in aqueous media and the application of oral or injectable drug is often limited by its low bioavailability. A promising method to improve the bioavailability of pharmaceutical agents is the reduction of particle size by Rapid Expansion of Supercritical Solutions (RESS). The RESS-process enables the micronization of thermally labile materials and the formation of particles of less than 500 nm in diameter.
We recently demonstrated the potential of the RESS-process to comminute thermally labile substances and to control particle size, particle size distribution, and particle morphology. We have used the RESS-process successfully for the formation of nanoscale particles of thermally labile drugs (e.g. Griseofulvin, β-Sitosterol, Ibuprofen). Depending on solvent and pre- and post-expansion conditions, the experiments led to particle sizes in the range of 200 ± 50 nm. These agglomerated particles consist of primary particles in the range of 50 to 150 nm. 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.
In order to overcome this, and to prevent agglomeration of the primary particles, coating experiments where performed. Coating of nanoscale particles is of interest either for encapsulation or for functional purpose. Recently, we have demonstrated the potential of the RESS-process for the encapsulation of β-Sitosterol in low-molecular-weight-polymeric (Eudragit®) matrices. Another promising and alternative approach is the production of nano-suspensions. Based on our previous experiences we utilized RESSAS (Rapid Expansion of Supercritical Solutions into Aqueous Solutions) to produce stable aqueous suspensions of water-insoluble drugs (β-Sitosterol/Tween-80/H_2O) and (β-Sitosterol/SDS/H_2O).
A number of techniques were used for the characterisation of the particles. Dynamic light scattering (DLS) measurements were conducted to determine the size of the stabilized particles in terms of number-weighted, and mass-weighted size distributions. The size of the particles in the expansion chamber is measured online and in-situ with the Three-Wavelength-Extinction Measurement technique (3-WEM). A scanning electron microscope (SEM) was used to observe the morphology of the particle surface. Differential scanning calorimetry was used for physical characterization (melting point, heat of fusion) of the particles.

1. INTRODUCTION

An increasing number of newly developed pharmaceutical substances are poorly soluble in both aqueous and organic media. Thus, the application of oral or injectable drug is often limited by its insolubility, and the bioavailability of the drug is low compared to the initial dose and its toxicity threshold is close to the therapeutic dosage. The low bioavailability of

such drugs can be improved by reducing the size of the particles [1-3]. Until now, there are several methods for the formation of small particles of low volatile organic substances. The primary techniques for particle formation involving supercritical fluids are: RESS (Rapid Expansion of Supercritical Solutions), PGSS (Particle Generation from Gas Saturated Solution), and GAS (Gas Anti-Solvent). Based on minor variations of the GAS-process, different techniques, including Aerosol Supercritical Extraction System (ASES), Precipitation with a Compressed Anti-solvent (PCA), Supercritical Anti-Solvent (SAS) and Solution Enhanced-Dispersion by Supercritical fluids (SEDS), are now in use [4].

First of all, the goal of our investigations was to explore the process conditions for the formation of nanoscale drugs by RESS. It has been shown in our earlier publications that the RESS-process enables the micronization of thermally labile materials and the formation of particles of less than 500 nm in diameter [5-10].
In order to complete our experimental investigations, we utilized RESSAS (Rapid Expansion of Supercritical Solutions into Aqueous Solutions) to produce stable aqueous suspensions of water-insoluble drugs (β-sitosterol/Tween-80/H_2O) and (β-sitosterol/SDS/H_2O) [1-12].
In addition, we recently demonstrated the potential of the RESS-process for the encapsulation of β-sitosterol in low-molecular-weight-polymeric (Eudragit) matrices.

2. EXPERIMENTAL RESULTS

Typical results of our RESS experiments are summarised in Table 1. The experiments performed with benzoic acid, cholesterol, β-sitosterol, ibuprofen, and griseofulvin lead to particle sizes in the range of 170 - 330 nm. Some typical examples of the particles obtained from RESS-experiments are shown in Fig.1. The agglomerated particles show a spongy structure with a high surface area and consist of primary particles with a particle size between 50 nm and 150 nm.

Table 1: Particle size (3-WEM) of various solutes obtained from RESS-experiments. The nozzle temperature was ≈ 10 K above the respective pre-expansion temperature [5-10].

Solute	Pre-expansion conditions		Solvent	Particle size
Benzoic acid	348 – 418 K	20 – 30 MPa	CO_2	208 – 461 nm
Cholesterol	348 – 423 K	20 – 30 MPa	CO_2	192 – 253 nm
β-Sitosterol	348 – 423 K	20 – 30 MPa	CO_2	166 – 219 nm
Ibuprofen	308 – 318 K	10 – 20 MPa	CO_2	183 – 326 nm
Griseofulvin	348 – 418 K	20 – 30 MPa	CHF_3	193 – 323 nm

Our modelling results suggest that it should be possible to form particles smaller than 50 nm in diameter [13,14]. However, the difficulty to achieve these small particle sizes is most likely due to growth and agglomeration during collisions in the subsonic free jet. It is shown in some earlier investigations that a promising method to prevent particle growth is to spray the supercritical solution directly into an aqueous surfactant solution [3,8,10-12]. In these RESSAS experiments, the nozzle (inner diameter of 35 μm and a length of 50 μm) is submerged approximately 3 cm below the surface of the aqueous surfactant solution to ensure

a rapid contact of the expanded solution, and therewith the particles being formed, with the surrounding area. The anionic surfactant SDS (sodiumdodecylsulfate, M = 288.4 g/mol) and Tween-80 (polyoxyethylene sorbitan monooleate, M = 1310 g/mol) a non-ionic surfactant, were used to impede growth and agglomeration of β-sitosterol particles. To determine the size of the stabilized particles in terms of number-weighted and mass-weighted size distributions Dynamic Light Scattering measurements (DLS) were conducted.

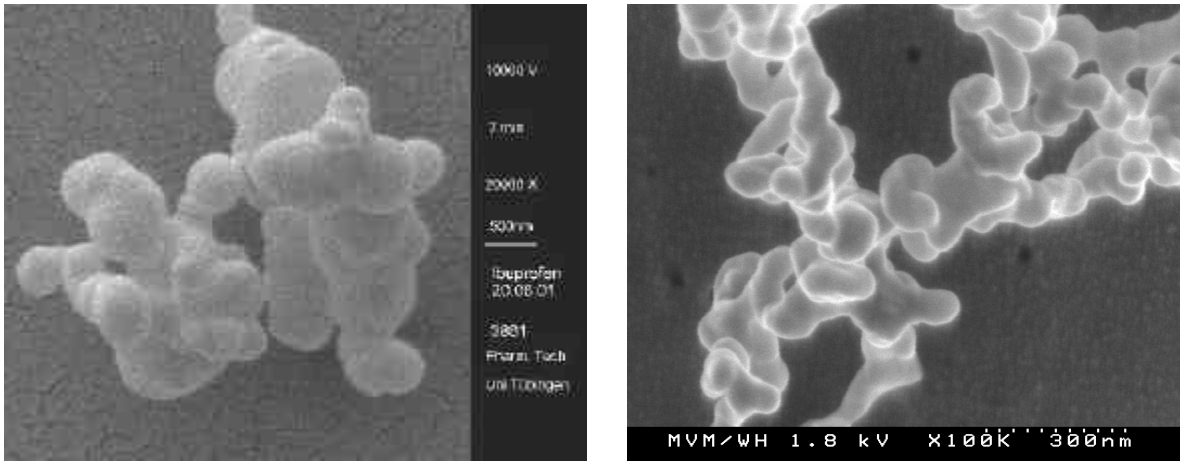

Fig. 1: SEM of ibuprofen (left) and of β-sitosterol (right) particles produced by RESS.

All RESSAS-experiments were performed at a pre-expansion temperature of 388 K, a pre-expansion pressure of 20 MPa and a solution temperature of 303 K. In the experiments performed with SDS, the aqueous solutions contain 0.22% or 1.1% (w/w) surfactant. In case of Tween-80, the aqueous solutions contain 1.0% or 5.0% (w/w) surfactant. The surfactant concentrations are higher than the critical micelle concentration of both SDS and Tween-80. Thus, as the base case, the clear aqueous surfactant solution were analysed by DLS. At both SDS concentrations investigated, the micelles were to small to scatter light and were not measurable by DLS. In contrast to SDS, micelles with a size of 7 to 14 nm in diameter were measured in both Tween-80 solutions. In Table 2 the results of our RESSAS-experiments are summarized.

Table 2: Effect of surfactant concentration on measured particle size distribution of the stabilized β-Sitosterol particles [11,12].

Surfactant concentration (w/w)	Particle size distribution [nm]		
1.0 % Tween-80	20 - 35	80 - 130	390 - 1100
5.0 % Tween-80	7 - 20	85 - 110	280 - 800
0.22 % SDS	40 - 75	110 - 300	
1.1 % SDS	30 - 55	80 - 200	

At all operating conditions investigated, very small β-sitosterol particles were obtained even though with different particle sizes. Also, an increase in surfactant concentration results in a decrease in the particle size for both surfactants. In case of Tween-80, the smallest particles range from 7 to 35 nm, however larger particles in the range from 80 to 130 nm and from 280 to 1100 nm are also determined. A similar result was obtained for SDS, the smaller particles range from 30 to 75 nm and the larger particles are in the range from 80 to 300 nm. In general, smaller particles where obtained in case of SDS when compared with Tween-80. The small 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 medical therapies the use of polymer-based drug delivery systems is of growing interest. Biodegradable polymers are commonly used as materials for drug delivery systems because their permeability and diffusivity can be modified and controlled. However much work was done on producing particles of pure solutes by RESS, but only a few studies investigated the simultaneous co-precipitation of two solutes [15-18]. Therefore, experiments have been performed with a preferred ethylacrylate-methylmethacrylate copolymer (Eudragit® L 100-55, Röhm Pharmaceuticals), and with a mixture of β-sitosterol/Eudragit (mass ratio = 10:1).
Fig. 2 shows schematically the apparatus used for these RESS-experiments. The apparatus enables experiments in the temperature range from 300 to 600 K and pressures up to 60 MPa [19,20]. In all experiments, the gaseous CO_2 is cleaned, 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 is expanded through a heatable capillary nozzle into the expansion chamber. After equilibrium, the bypass section is closed and the supercritical CO_2 flows through an extraction column, which is packed with the solute. In these experiments both solutes are thoroughly mixed and weighed out and then packed into one extraction vessel. Then the saturated supercritical solution flows through a heated tube into a thermostated high-pressure vessel where the pre-expansion temperature and the pre-expansion pressure is measured. The supercritical solution is expanded through a heatable capillary nozzle with an inner diameter of 50 µm and a length of 50 µm always down to atmospheric conditions (0.1 MPa, 300 K). The precipitated particles are measured in the expansion chamber online and in situ with the 3-Wavelength-Extinction Measurement technique (3-WEM). More details about the apparatus, the experiments, and the measurement technique can be found in literature [19-21]. A number of techniques were used for the characterisation of the particles. A scanning electron microscope (SEM) was used to observe the morphology of the particle surface. Differential scanning calorimetry was used for physical characterization (melting point, heat of fusion) of the particles.
In Figure 3 typical examples of pure β-sitosterol and of β-sitosterol/Eudragit particles are depicted. As mentioned above, the particles are usually agglomerated and show a spongy structure. In opposite to these results the RESS-experiments performed with a mixture of β-sitosterol/Eudragit lead to finely dispersed particles. This result qualitatively illustrates that the simultaneous co-precipitation of two solutes is a promising method to produce composite particles. These particles appear as a drug core encapsulated in a polymer coating. This result was confirmed by DSC analysis. The melting temperature and the heat of fusion of the micronized β-sitosterol was found to be 410.27 K and 53.6 J/g, which is clearly higher than those of the β-sitosterol/Eudragit mixture (402.39 K and 41.32 J/g).

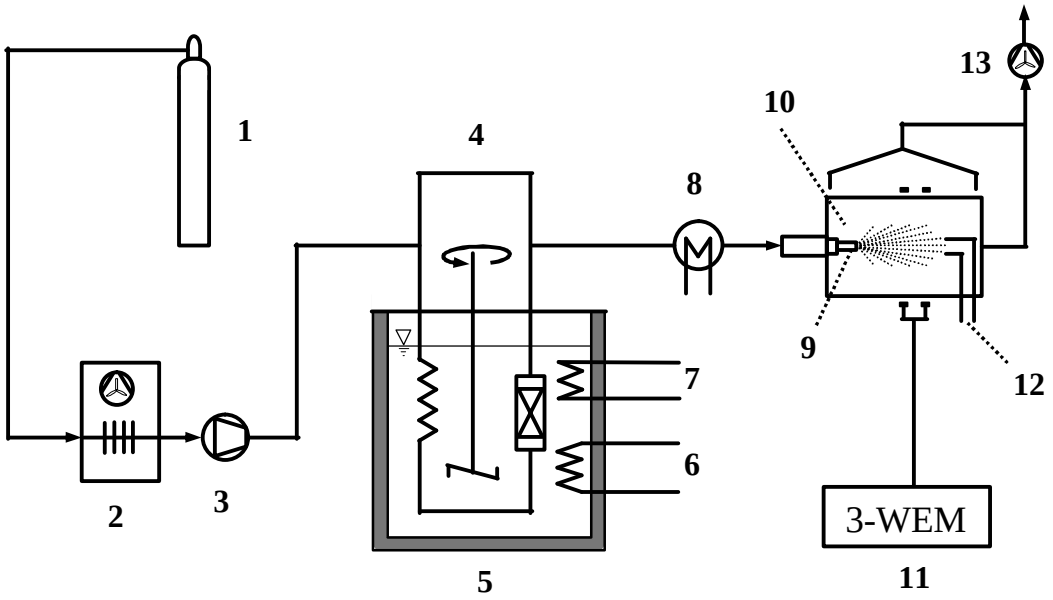

Fig. 2. Experimental apparatus for precipitation experiments: **1**, solvent; **2**, cooling device; **3**, pump; **4**, bypass; **5**, extractor unit; **6**, cooling; **7**, heating; **8**, pre-heater; **9**, capillary nozzle; **10**, expansion chamber; **11**, 3-WEM; **12**, sample for SEM; **13**, vent.

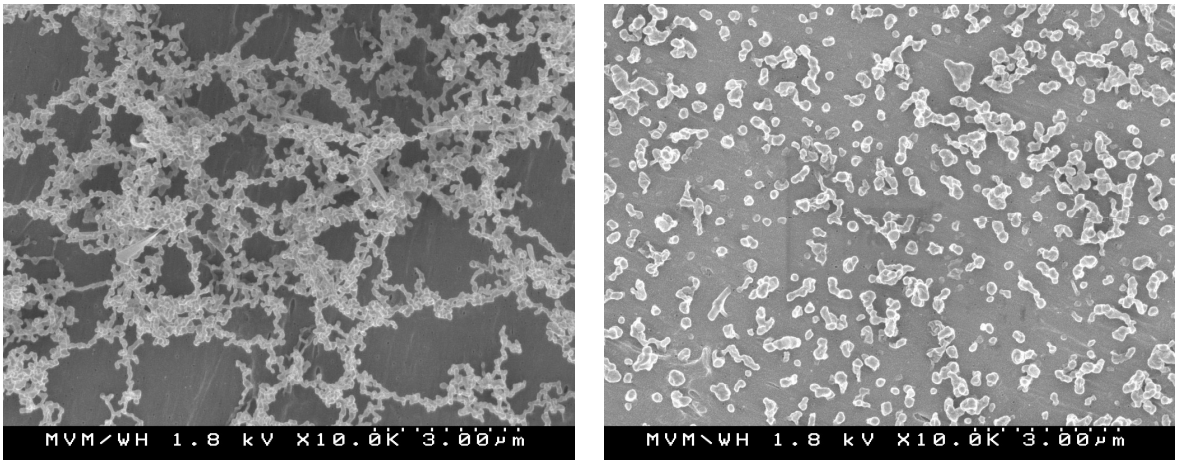

Fig. 3: SEM of pure β-sitosterol (left) and of β-sitosterol/Eudragit (right) particles produced by RESS.

CONCLUSION

In the present paper we have presented selected examples of ongoing experimental work. The results of our investigations can be summarised as follows: RESS-processing of pure solutes enables the formation of particles less than 500 nm in diameter. In addition, RESS enables the production of stable suspensions of nanoscale particles of β-sitosterol. Furthermore, we have shown that simultaneous co-precipitation of β-sitosterol/Eudragit lead to finely dispersed particles less than 500 nm in diameter. Thus, RESS is also attractive for the formation of polymer-based drug delivery systems.

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