Production and Stabilization of Submicron Organic Particles of Pharmaceutical Relevance by Rapid Expansion Processes

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Micronization of organic particles was performed by rapid expansion of supercritical solution into air (RESS) or into an aqueous solution (RESSAS). The influence of process conditions on the product was investigated by characterising the produced powder and suspension respectively with a variety of analysis methods. The mean particle size of the non-steroidal anti-inflammatory drug Naproxen was decreased from 15.2 µm (original material) to 0.7 µm applying RESS. A further reduction in size to 0.3 µm was possible with RESSAS using 0.4 wt-% PVP K25 as stabilizing agent. Naproxen concentrations up to 1.1 g/dm^3 could be achieved. The application of the surfactants Polydocanol and Tween®80 on the other hand lead to mean particle sizes of 13.7 µm and 7.8 µm in the suspension.

1. INTRODUCTION

The low solubility of organic substances in aqueous solutions is one of the main obstacles in the development of new pharmaceutical formulations. Examples are oral and intravenous drugs, because their application is often limited by the poor dissolution behaviour of the ingredients in aqueous media. By reduction of particle size and related increase of specific surface area it is possible to overcome this problem [1-8].

From former work it is known that the rapid expansion of supercritical solutions (RESS) is a promising possibility to produce submicron organic particles. In RESS experiments with different substances a mean particle size ≤ 1 µm was reached which leads to an improved dissolution behaviour in in-vitro test systems. However the expansion into gas phase results in coagulation and agglomeration of primary particles right after formation.

Therefore it is desirable to stabilize the particles directly in the free jet and convert them into the favoured pharmaceutical form. The rapid expansion of supercritical solutions into aqueous solutions (RESSAS), an upgrade of the RESS process, provides this opportunity. In this process the supercritical solution is directly expanded into an aqueous solution which contains a small amount of stabilizing agent such as a surfactant [8,9]. The feasibility to stabilize submicron Phytosterol, Salicylic acid, and Ibuprofen particles with a narrow size distribution in different surfactant solutions by RESSAS has been demonstrated by Türk and Lietzow [9-12]. More details about the obtained size of the stabilized particles and drug concentration in the suspension can be found in literature [8, 9].

In this paper we report on the use of RESS to produce submicron organic particles and the use of RESSAS to stabilize submicron particles in aqueous solutions. For this purpose the non-steroidal anti-inflammatory drug Naproxen was chosen as organic model substance. Naproxen is commonly used against pain, fever and inflammation.

Before performing RESS and RESSAS experiments the characteristics of Naproxen and the stabilizers PVP, Polydocanol and Tween®80 have been determined.

Solubility data for Naproxen in CO_2 have been reported in a former paper [13].

2. EXPERIMENTAL

2.1 Materials and methods

CO_2 (Linde; München, Germany) was chosen as supercritical solvent since it is a non-flammable, inexpensive, and non-toxic solvent. Due to the low critical temperature supercritical CO_2 (sc-CO_2) allows processing at moderate temperatures. The non-steroidal anti-inflammatory drug Naproxen was acquired from Sigma-Aldrich (Taufkirchen, Germany).

The non-ionic surfactant polyoxyethylene sorbitan monooleate (Tween®80) was purchased from Carl Roth (Karlsruhe, Germany). It is approved by the U.S. Food and Drug Administration for use as an excipient in either oral or parenteral delivery formulations [14]. Polyvinylpyrrolidone (PVP) was purchased from Carl Roth (Karlsruhe, Germany). Also known as additive with the E-number E1201 PVP is used as stabilizer in different food applications [15]. Furthermore PVP is used as binding agent in tablets and pharmaceutical applications because of its biological compatibility [16]. The third stabilizer Polydocanol, a non-ionic surfactant, was given by Kreussler (Wiesbaden, Germany).

Scanning Electron Micrographs (SEM) were made using a LEO 1530 scanning electron microscope (Carl Zeiss; Oberkochen, Germany). The samples were coated with a platinum layer of ~ 5 nm by means of a self-constructed sputter coater. The samples for SEM were deposited on a filter (47 mm Nucleopore Track-Etch Membrane, Polycarbonate, pore size 0.2 µm, Whatman PLC; Maidstone, UK).

Differential Scanning Calorimetry (DSC) was used for physical characterization (melting point and heat of fusion) of the unprocessed and the processed Naproxen. A DSC 204 Phoenix (Netzsch; Selb, Germany) was used to perform the measurements. The sample (~ 5 mg per run) was heated in an aluminium standard pan under a nitrogen gas flow of 20 cm^3/min. A heating rate of 5 K/min was used up to a maximum temperature of 473 K.

The powder X-ray diffraction (XRD) pattern of the unprocessed powder and the micronized substances were determined by using a Guinier instrument (model 642, adjustment base 601, mono-chromate Cu-K-α-radiation, λ = 1.54056×10 Å, Huber Diffraktionstechnik; Rimsting, Germany). The diffractograms have been recorded in a 2Θ range of $5° \leq 2\Theta \leq 60°$.

Particle size distributions (PSD) were determined with a Coulter L S230 and LS 13320 (Beckmann Coulter; Krefeld, Germany). This equipment allows the analysis of particles in a range of sizes between 0.04 and 2000 µm applying a laser diffraction method. Particle size measurements were made within 2 days after the production of the suspensions.

The solubility of the unprocessed Naproxen in the aqueous PVP K25, Polydocanol and Tween®80 solutions was determined from a saturated solution at 303 K. Excess drug was added to 40 cm^3 of each surfactant solution and allowed to equilibrate stirring constantly for 48 h at 303 K. The dissolved drug content was determined with high performance liquid chromatography (HPLC) from a filtrate of each saturated solution. The samples were analyzed using a LiChrospher column 100-5 RP18 (125 x 4 mm) (Agilent; Santa Clara, CA, USA) which was heated to 297 K. The flow rate of the mobile phase was set to 1 cm^3/min. Detection was carried out using a fluorescence detector Ex = 250 nm, Em = 410 nm. The HPLC analysis of the drug suspension was done within 1-3 days after production.

Polymer molar mass distributions (MMD) were measured via size exclusion chromatography (SEC) using the eluent N,N´-dimethyl acetamide (99 % pure) from Acros Organics (Geel, Belgium) containing 0.1 % LiBr (99%) from Sigma-Aldrich (Taufkirchen, Germany). The SEC set-up was calibrated using polystyrene standards (PSS) of low polydispersity [17].

The determination of equilibrium surface tension was carried out using a tensiometer DCAT 11 (DataPhysics; Filderstadt, Germany) with a Wilhelmy plate according DIN 53914.

2.2 Particle formation and particle stabilization

RESS and RESSAS experiments were carried out using the apparatus presented in various papers in detail [9-12]. Prior to the RESSAS experiments, the effect of pre-expansion temperature and pre-expansion pressure on the size and morphology of the precipitated Naproxen particles was studied. In these RESS experiments, a heatable 50 µm capillary nozzle (L/D = 10) was used for the expansion of the supercritical solution into an unheated ambient-pressure chamber. The pre-expansion pressure was maintained at the same pressure as the extraction unit. The Naproxen particles were precipitated inside the expansion chamber ($V = 22\ dm^3$) at atmospheric conditions. Samples for SEM-images were taken at a distance of 300 mm to the nozzle exit and deposited on a filter.

During the RESSAS experiments the supercritical CO_2/Naproxen mixture is expanded through a heated capillary nozzle (D = 50 µm, L/D=10) directly into the aqueous surfactant solution (usually 50 cm^3). 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. The temperature of the aqueous surfactant solution is measured by a thermocouple, which is immersed in the liquid. To suppress the foam produced during RESSAS, compressed air can be induced into the expansion chamber from above. More details about the apparatus and the experimental procedure can be found elsewhere in literature [9,12].

3. RESULTS AND DISCUSSION

3.1 Polymer molar mass distribution

Fig. 1 shows the MMD for the three different PVP samples. Additionally, the number averaged molar mass (M_N) and the polydispersity index (PDI = M_W/M_N) for these PVP samples are listed in Tab. 1.

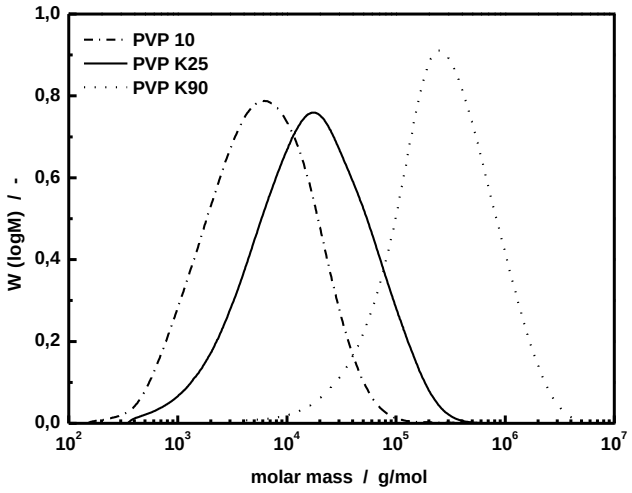

Fig. 1: Molar mass distribution of PVP 10, K25 and K90 [17].

Table 1: Molar mass distribution of the used polymers (PDI = M_W / M_N) [17].

Polymer	M_N / g/mol	PDI
PVP 10	3045	3.3
PVP K25	7641	4.1
PVP K90	137360	3.2

3.1 Solubility in aqueous surfactant solutions

The influence of PVP K25, Polydocanol and Tween®80 concentration on the solubility of Naproxen at 303 K is shown in Table 2. Except for PVP K25 the solubility of the substances investigated increases with increasing surfactant concentration. At 303 K, the solubility of Naproxen in pure water is 0.008 g/dm^3 (pH 4.8) which is in good agreement with the value, published in literature for 298.15 K and pH = 4 (0.0226 g/dm^3) [18]. While the solubility of Naproxen is ≤ 0.1 g/dm^3 in spite of increasing the PVP K25 concentration up to 5 wt-%, the Naproxen solubility increases with increasing concentration of Polydocanol and Tween®80 up to 2.55 and 2.76 g/dm^3 respectively. Similar trends were obtained in case of Ibuprofen, Salicylic acid and Phytosterol for the surfactant Tween®80 [9].

Table 2: Saturation solubility of unprocessed Naproxen in aqueous solutions of PVP K25, Polydocanol, and Tween®80 at 303 K.

	PVP K25		Polydocanol		Tween®80	
c_{Tensid} wt-%	$c_{Naproxen}$ g/dm^3	pH	$c_{Naproxen}$ g/dm^3	pH	$c_{Naproxen}$ g/dm^3	pH
0.0	0.008	4.8	0.008	4.8	0.008	4.8
0.5	< 0.02	4.8	0.29	4.5	0.16	4.7
1.0	-	4.2	0.56	4.6	0.42	4.8
1.5	-	4.0	0.88	4.5	0.67	4.8
2.0	< 0.02	4.0	1.2	4.6	0.85	4.8
3.0	< 0.02	4.0	1.81	4.5	1.41	4.9
4.0	0.04	3.9	2.32	4.6	1.75	4.9
5.0	0.07	3.9	2.55	4.6	2.76	4.9

3.2 Surface tension

As depicted in Fig. 2, Polydocanol and Tween®80 show surface active behaviour which results in the occurrence of a critical micelle concentration (CMC) at 0.048 g/dm^3 for Polydocanol and in a range from 0.013 g/dm^3 [19] to 0,034 g/dm^3 for Tween®80.

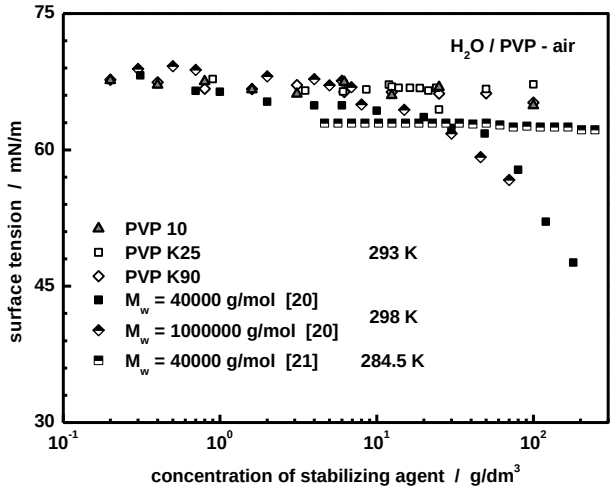

Fig. 2: Surface tension of aqueous PVP solutions against PVP concentration.

The surface tension of aqueous PVP solutions on the other hand is independent of the PVP concentration up to 100 g/dm^3 which is shown in Fig. 2. While Gabrielli et al. found the same behaviour for PVP of two different molar masses, Huang and Wang found a decrease in surface tension from ~ 20g/dm^3 [20,21].

3.3 Particle formation by RESS

3.3.1 Experiments

The first goal of our experimental investigations was to explore the process conditions for the formation of sub-micron Naproxen particles by RESS. These experiments were performed at an extraction temperature (T_E) of 313 K and a pre-expansion pressure ($p_0 = p_E$) of 20 - 30 MPa. In order to investigate the influence of the pre-expansion temperature (T_0) on the properties of particles produced by RESS, pre-expansion temperatures of T_0 = 323 K, 343 K, and 363 K were chosen at $p_0 = p_E$ = 20 MPa. As shown in Table 3 exemplarily, the mean particle size is 0.7 µm and no clear influence of pre-expansion temperature on particle size was observed. The increase of the pre-expansion pressure p_0 from 20 to 30 MPa at T_0 = 323 K has no influence on particle size. This result is in accordance with RESS experiments performed with CO_2/Cholesterol, CO_2/Phytosterol, CO_2/Ibuprofen, and CHF_3/Griseofulvin which lead to particle sizes in the range between 0.17 and 0.33 µm [8].

Table 3: Process conditions and particle sizes for RESS experiments (T_E = 313 K, $T_N = T_0 +$ 5 K, $p_E = p_0$, d_N = 50 µm) and PSD characteristics of original and processed Naproxen.

No.	T_0 K	p_0 MPa	$x_{15,3}$ µm	$x_{50,3}$ µm	$x_{85,3}$ µm	$\Delta^{\#}$	$\dot{m}_{CO_2}$ g/min
1	original		3.3	15.2	37.8	1.1	-
2	323	20	0.5	0.7	0.9	0.3	13
3	343	20	0.6	0.7	0.9	0.2	11
4	363	20	0.4	0.7	1.3	0.6	9

$\Delta^{\#} = (x_{85,3} - x_{15,3})/2 \cdot x_{50,3}$

Figure 3 shows SEM images of unprocessed and processed Naproxen particles (T_E = 313 K, $p_0 = p_E$ = 20 MPa and T_0 = 343 K).

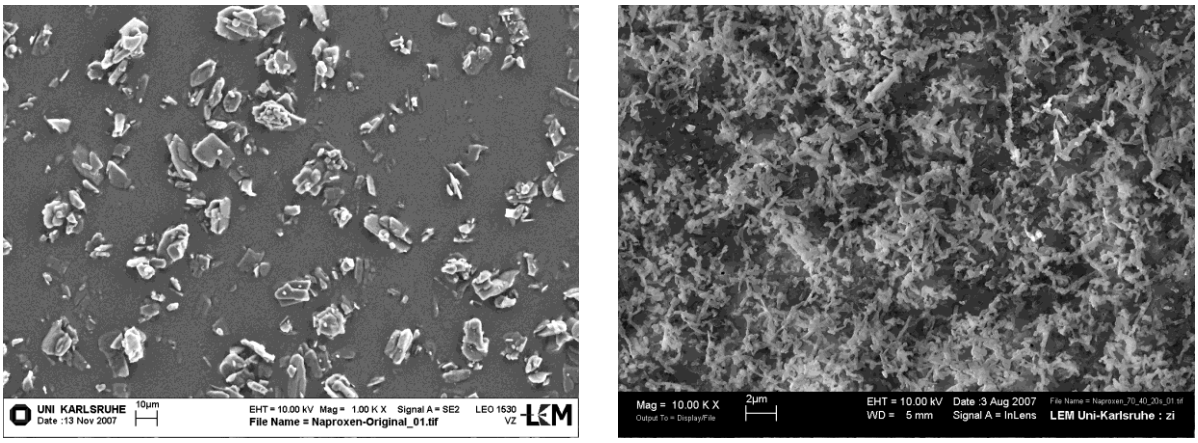

Fig. 3: SEM images of unprocessed Naproxen (left) and Naproxen processed with RESS.

These pictures are typical examples of the obtained product and show that the small primary particles are strongly agglomerated and coagulated. No significant change in particle morphology of RESS processed Naproxen was observed as the temperature T_0 was increased from 323 to 363 K at 20 MPa.

As is illustrated in Figure 4, similar DSC curves and XRD patterns were observed for the unprocessed material and the Naproxen particles produced by RESS. On the basis of the DSC measurements, and XRD investigations, it was confirmed that the processed Naproxen is still in a crystalline state. However, the average melting temperature and the average heat of fusion of the processed Naproxen (Δh_m = 125.7 J/g, T_m = 424.3 K) are lower compared to the unprocessed powder (Δh_m = 129.3 J/g, T_m = 427.6 K).

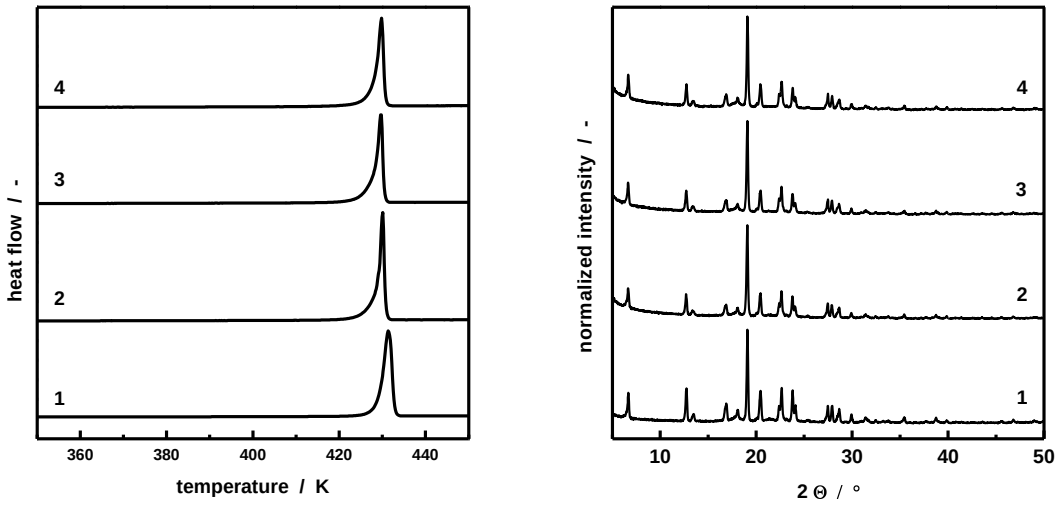

Fig. 4: DSC curves (left) and XRD pattern (right) of unprocessed (1) and processed Naproxen T_E = 313 K; $p_0 = p_E$ = 20 MPa and T_0 = 323 K (2); T_0 = 343 K (3); T_0 = 363 K (4).

3.4 Particle stabilization by RESSAS

RESSAS experiments were carried out to investigate the influence of surfactant concentration on particle size and drug loadings in the surfactant solutions. Based on the RESS results obtained for Naproxen, the RESSAS experiments were performed at an extraction temperature of T_E = 313 K, a pre-expansion pressure of $p_0 = p_E$ = 20 MPa, and a pre-expansion temperature of T_0 = 343 K. In these experiments the aqueous surfactant solution was held at a temperature of 298 K and the spray time was 60 min. The results of spraying a supercritical CO_2/Naproxen mixture into an aqueous PVP K25, Polydocanol or Tween®80 solution are summarized in Fig. 5. For comparison, the PSD for the unprocessed material and the RESS-product is also included. The figure shows the particle size distribution of Naproxen stabilized in aqueous solutions containing either 0.4 wt-% of PVP K25, Polydocanol or Tween®80. The characteristic data of RESS and RESSAS experiments are compared in Table 4.

Obviously Polydocanol and Tween®80 are not appropriate to stabilize sprayed Naproxen particles in aqueous solution although especially Tween®80 has been successfully used to stabilize RESSAS-produced Phytosterol, Ibuprofen and Salicylic acid [9]. The polymer PVP K25 in contrast is an effective stabilizing agent. PVP K25 stabilized solutions reach Naproxen concentrations up to 1.1 g/dm^3 which is $\geq$ 130 times higher than the

saturation concentration of unprocessed Naproxen in water and 55 times higher than the saturation concentration in a 0.5 wt-% PVP K25 solution, cf. Table 2. The mean particle size $x_{50,3}$ in the solution is 0.3 µm. Compared to the original powder the particles are ≥ 50 times smaller whereas they are just 2 times smaller than the comparable RESS product. Although the PSD of the RESSAS product intersects the PSD of the RESS product, more than 60% of the particles stabilized in aqueous solution are smaller than RESS particles. Compared to saturated Polydocanol or Tween®80 solutions the Naproxen concentration in the RESSAS product is low. On the other hand it is necessary to use ≥ 2 wt-% of surfactant to reach this concentration which is undesirable in pharmaceutical applications.

Table 4: Comparison between RESS and RESSAS experiments with Naproxen (T_E = 313 K, $T_N = T_0 + 5$ K, T_0 = 343 K, $p_E = p_0$ = 20 MPa, d_N = 50 µm).

No.		$x_{50,3}$ **µm**	$\Delta^{\#}$	$c_{Naproxen}$ **g/dm³**
1	original	15.2	1.1	-
3	RESS	0.7	0.3	-
a	H_2O / PVP K25	0.3	5.3	1.1
b	H_2O / Tween®80	7.8	1.0	0.5
c	H_2O / Polydocanol	13.7	4.1	-

$\Delta^{\#} = (x_{85,3} - x_{15,3})/2 \cdot x_{50,3}$

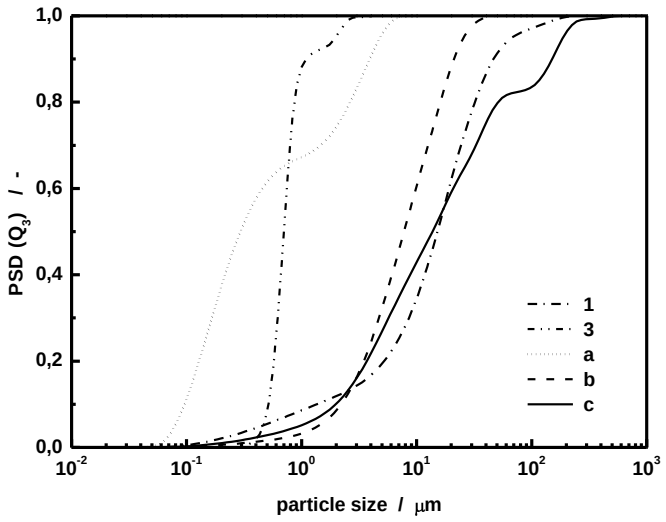

Fig. 5: PSD of Naproxen unprocessed (1), RESS processed (T_E = 313 K; $p_0 = p_E$ = 20 MPa and T_0 = 343 K) (3) and RESSAS-processed (T_E = 313 K; $p_0 = p_E$ = 20 MPa and T_0 = 343 K) with 0.4 wt-% PVP K25 (a), Tween®80 (b) and Polydocanol (c).

4. CONCLUSIONS

Submicron Naproxen particles were produced by rapid expansion of supercritical solution into air or aqueous surfactant solutions to minimize particle growth and to prevent particle agglomeration. RESS experiments resulted in rod shaped particles with a mean size ≤ 0.8 µm which was independent on the pre-expansion conditions temperature (323 – 363 K) and pressure (20 – 30 MPa). With RESSAS we were able to minimize the mean particle size to 0.3 µm and increase the Naproxen concentration in suspension up to 1.1 g/dm³. These results

could be reached applying the polymer PVP as stabilizing agent (0.4 wt-%). On the other hand the surfactants Polydocanol and Tween®80 were completely inefficient in stabilizing Naproxen although especially Tween®80 has been applied successfully in former works e.g. for Phytosterol [8,9]. Finally, our work indicates that RESSAS can be a promising process for producing stable suspensions of submicron water-insoluble substances.

5. ACKNOWLEDGMENTS

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