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THERMAL AND MORPHOLOGICAL CHARACTERISATION OF MICRONIZED ACETYLSALICYLIC ACID POWDERS PREPARED BY RAPID EXPANSION OF A SUPERCRITICAL SOLUTION

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Abstract

Aim of this work was to investigate the solid-state characteristics of micronized acetylsalicylic acid (ASA), produced by rapid expansion of a supercritical carbon dioxide solution (RESS) and to assess whether a correlation could be found between process parameters and solid-state characteristics. Drug solubility in supercritical CO_2 was first assessed under various pressure and temperature conditions. DSC, FT-IR, PXRD, SEM, laser light scattering and HPLC were used to characterise the solid phases produced by the RESS.

The obtained particles were crystalline, with spectroscopical and diffractometrical pattern overlapping those of the starting available product. However, a strong reduction of particle size was obtained, linearly correlated to pressure imposed during the RESS process, while temperature did not seem to have a major effect.

Similar influence of pressure was observed on the final melting temperature of the micronized ASA.

The application of a mathematical model allowed to conclude that the melting temperature depression of RESS-prepared ASA powders can be attributed to the decrease of particle dimension rather than to the formation of different solid phases or impurities.

Keywords: aspirin, micronisation, RESS, solid-state, supercritical CO_2

Introduction

Particle engineering is becoming a very important subject in the field of pharmaceutical solid dosage forms. Among various possible modifications of particles morphology, size reduction is one of the most frequently proposed changes in the material characteristics. Particle size reduction to nano- or micro-size is gaining increasing importance especially for injectable drug products and for the administration by the inhalatory route.

In the last decades atomisation processes such as spray-drying or spray-congealing have been successfully proposed as a valuable alternative to the classical jet milling process especially for thermally unstable drugs [1, 2]. Nevertheless, these processes do not eliminate completely the thermal stress and often require the use of organic solvents. For these reasons in recent years particle size reduction by means of techniques based on fluids in the supercritical state, generally carbon dioxide (T_c 31.1°C; P_c 73.8 bar), have at-

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tracted particular attention for those substances such as explosives or pharmaceutics that can be difficult to comminute. Supercritical CO_2 can be used either as a solvent or as an antisolvent for the drug to be micronized [3, 4]. The rapid expansion of a supercritical solution (RESS) of a drug in carbon dioxide, may be, perhaps, considered the simplest process for producing microparticles having a predefined particle size distribution [5]. Typically, the process consists on the formation of particles as a consequence of the rapid evaporation of the solvent from a supercritical drug/CO_2 solution sprayed with a nozzle to ambient pressure. Obviously, in order to assure adequate yield this technique can be applied only to drugs having 'high' solubility in SC CO_2, at least of the order of 10^{-4} mole of drug/mole of solution.

Particle size reduction, by any of the above-mentioned techniques, may result in profound modifications of the solid-state characteristics, such as amorphisation or formation of different polymorphs [5]; this can have a great impact on the biopharmaceutical (solubility, dissolution rate, bioavailability) and technological (flowability, packing, compactability) behaviour of the drug. In this regard the RESS process could be envisaged as very critical, since the fast particle nucleation stemming from then sudden loss of solvation power, as a consequence of pressure drop, very often gives rise to thermodynamically unstable phases [6].

Therefore, the accurate characterisation of the solid-state properties of the prepared particles is of outmost importance in order to be able to correctly address the preparation process toward a drug material showing the desired biopharmaceutical and technological behaviour.

Aim of this work was to investigate the solid-state characteristics of micronized acetylsalicylic acid, ASA, produced by RESS and to assess whether a correlation could be found between process parameters and said characteristics.

ASA was selected as a model drug because of its chemical similarity with salicylic acid whose high solubility in SC CO_2 is well known [7]. Moreover, it is a drug sensitive to heating, very easily undergoing deacetylation to salicylic acid. Furthermore, ASA can be considered as an interesting case of monomorphism. In fact, even though apparent evidence of polymorphism was provided in the late 60s and early 70s [8–13] on the other hand, on the basis of X-ray diffraction investigations, ASA polymorphism has been excluded [14]. Quite recently, the possible theoretical existence of different crystal forms for ASA has been reported [13].

In this work the solubility of ASA in supercritical CO_2 was first assessed under various pressure and temperature conditions. Differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), powder X-ray diffractometry (PXRD), scanning electron microscopy (SEM), laser light scattering and high performance liquid chromatography (HPLC) were used as analytical tools to characterise the solid phases produced by the RESS process.

Materials and methods

Materials

ASA (Lisapharma S.p.A., Italy, mean particle diameter 520 μm) was used as received from the supplier. All other chemicals were of analytical grade.

Measurement of ASA solubility in SC CO_2

Solubility measurements in supercritical CO_2 were performed by means of a laboratory scale apparatus (SPE-ED SFE, Applied Separation, USA), schematically reported in Fig. 1, operating under dynamic conditions at low flux according to the method described by Stassi *et al.* [16] and modified by Bettini *et al.* [17]. Data were collected at two temperatures (40 and 50°C) in the 100–250 bar pressure range.

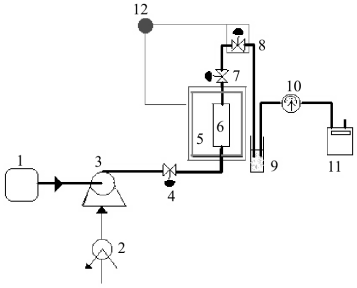

Fig. 1 Scheme of the apparatus for measurement of solubility in supercritical phase and for RESS process. 1 – CO_2 reservoir; 2 – chilling bath; 3 – pump; 4 – inlet valve; 5 – thermostatic chamber; 6 – saturation cell; 7 – outlet valve; 8 – micrometric valve; 9 – collecting flask; 10 – flow-meter; 11 – gas meter; 12 – temperature controller

Briefly, the saturation cell (internal volume 3 mL) was loaded with about 500 mg of ASA mixed with glass beads in order to allow a thorough diffusion of the fluid and placed in a thermostatic chamber. The pump operating at the constant-pressure mode imposed the pressure, while the micrometric (expansion) valve allowed a manual tune of the flow rate, normally set approximately at 100 μL of liquid CO_2 min^{-1} (~0.1 g min^{-1}). In a typical determination, the pressure of the saturation cell was raised up to the desired value, the outlet valve being closed. Then, the outlet valve was opened in order to allow the CO_2 to flow through the saturation cell. The run was stopped when 20–25 g of CO_2 had passed through the cell. The dissolved drug, conveyed by the SC–CO_2 stream, was collected in a 50 mL flask containing anhydrous methanol and positioned after the micrometric valve. To avoid the risk of missing portions of the solute, at the end of each measurement all valves and connection tubings were carefully washed with portions of methanol. The total amount of drug dissolved was determined spectrophotometrically (V 530, Jasco, Japan) at 276 nm. Possible presence of salicylic acid formed as a consequence of the process was checked by HPLC. Each measurement was performed at least in triplicate.

HPLC analysis

HPLC analysis was carried out on ASA samples according to a method similar to that described in the monography 'Aspirin' of the USP 25 (18): 20 μL of ASA solution in methanol, having a concentration of about 0.1 mg mL^{-1} were manually injected (Rheodyne, USA) in a liquid chromatographer (Pump LC-10AS ; detector UV set a

254 nm, SPD 10A, Integrator C-R6A Chromatopac, Shimadzu, Japan). The column was a μBondapak C_{18} 3.9×35 mm (Waters, USA), the mobile phase was constituted by a 150:850 acetonitrile water solution containing 2 g L^{-1} of sodium 1-heptanesulfonate, adjusted to pH 3.4 with glacial acetic acid. The flow rate was 1 mL min^{-1}.

ASA and salicylic acid were quantified by comparing the sample signal with that of a standard solution of both drugs with the same concentration. Retention times for ASA and salicylic acid were 15 and 10.5 min respectively.

Micronization by RESS

Micronized ASA powders were produced using the equipment above described for solubility measurements modified in order to collect solid particles. In particular, the outlet part of the micrometric valve was connected with a tube-shaped nozzle (length 10 cm, internal diameter 35 μm). Particles precipitated at the tip of the nozzle were collected in a 50 mL vial kept refrigerated with an ice/NaCl mixture. The flow rate of the expanded CO_2 was maintained around 150 mL min^{-1}.

Powder X-ray diffractometry, PXRD

X-ray diffraction patterns on powder were recorded on a Philips PW 1050 diffractometer using CuK_α radiation over the 5–35° 2θ interval (scan speed 0.5° min^{-1}). Analyses were performed on both untreated and micronized ASA powders.

Laser light scattering

The particle size distribution of the micronized ASA samples was determined by laser light scattering (2600 Series, Malvern Instruments, Malvern, UK). Before analysis, a few mg of sample were dispersed in 2 mL of cyclohexane containing 0.1% (v/v) of lecithin and placed in a sonicating bath for 6 min in order to disperse possible particles aggregates.

FT-IR spectroscopy

FT-IR spectra of untreated and micronized ASA samples were recorded by means of a Micro FT-IR microsampling (Jasco, Japan) in the 4000–400 wavenumber interval. Powder samples were placed on a KBr disc under the objective of the microscope and directly scanned.

Particle morphology

Morphological characterisation of powders was performed by scanning electron microscopy (SEM) (JEOL, 6400, Japan) at 10 kV on samples mounted on glass slides with double-sided adhesive tape and coated with gold to a thickness of 200–400 Å.

Thermal analysis

Differential scanning calorimetry (DSC) was performed on an indium calibrated Mettler DSC 821e (Mettler Toledo, USA) driven by STARe software (Mettler Toledo). DSC traces were recorded by placing precisely weighed quantities (1–5 mg) in a 40 μL aluminium pan sealed and pierced. Scans were performed with the so-called instant heating method according to EP 4th, 2002 [19] between 110 and 150°C at 10 K min^{-1} under a flux of dry nitrogen (100 mL min^{-1}), in order to minimize ASA degradation due to heating. Each power sample was analysed at least in triplicate.

Results and discussion

As stated above, the determination of drug solubility is a necessary prerequisite in order to select the appropriate precipitation method (CO_2 as solvent or antisolvent) and to set suitable process conditions. Therefore, the solubility of ASA in CO_2 was determined at different experimental conditions. Molar solubilities obtained at 40 and 50°C at 4 pressure values in the 100–250 bar range are reported in Table 1.

Table 1 ASA solubility (mol/mol) in SC CO_2 at 40 and 50°C and different pressures. Mean values, standard deviations in parenthesis, $n \geq 3$

Pressure/ bar	Mol ASA/mol SC solution (s.d.)·10^4	
	40°C	50°C
100	0.83 (0.05)	0.70 (0.19)
150	1.64 (0.002)	2.50 (0.75)
200	2.60 (0.28)	3.36 (0.02)
250	2.90 (0.38)	3.61 (0.6)

In general, the ASA solubility increased both with pressure and temperature. In particular, the solubility values recorded at 100 bar, at both temperatures, were below $1 \cdot 10^{-4}$ mol mol^{-1} which has been assumed as the minimal borderline value for the feasibility of RESS process. Therefore, only pressure values above 100 bar were considered for microparticles production.

A SEM photomicrograph of the particles produced by the RESS process is presented in Fig. 2 together with that of untreated ASA. While the latter was constituted by irregularly shaped particles (size in the range 0.5–1 mm), particles prepared by the RESS process were needle-shaped with average length well below 10 μm. Similar results were obtained by Domingo and co-workers [20].

FT-IR spectra of treated and untreated powders resulted completely superimposable indicating that the dissolution in SC CO_2 and subsequent particles precipitation did not modify the chemical nature of the material.

To confirm that neither the physical nature (crystal phase) had changed, PXRD experiments were performed on treated and untreated ASA. Figure 3 reports the dif-

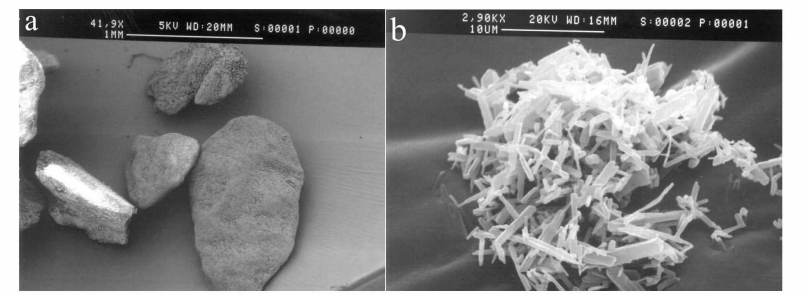

Fig. 2 SEM images of ASA untreated (panel a) and treated by RESS at 50°C and 200 bar (panel b)

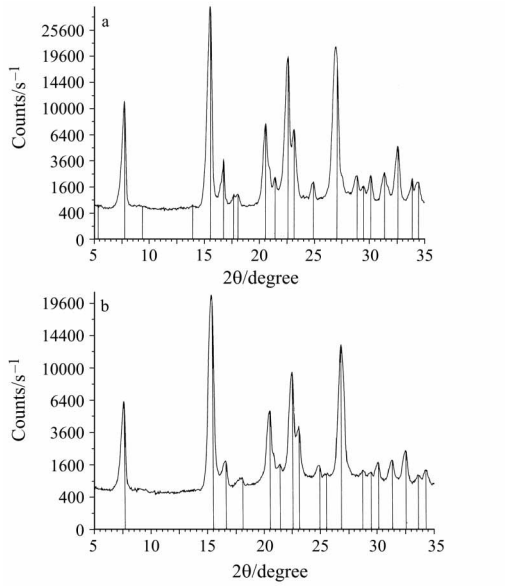

Fig. 3 X-ray powder diffraction patterns of untreated ASA (panel a) and treated by RESS at 50°C and 200 bar (panel b)

fraction pattern of untreated ASA and that of a micronized powder prepared at 50°C and 200 bar. As for IR spectra, also in this case SC treated and untreated drug powders afforded identical patterns, indicating that, upon precipitation from the supercritical solution, ASA microparticles recrystallized in the same solid phase as the starting drug. Therefore, in spite of the very rapid (instantaneous) precipitation of a saturated solution, no amorphous or new polymorphs of ASA were generated. Furthermore, it must be underlined that despite of the great difference in particle size (Fig. 2) no peak broadening could be evidenced as a consequence of RESS treatment.

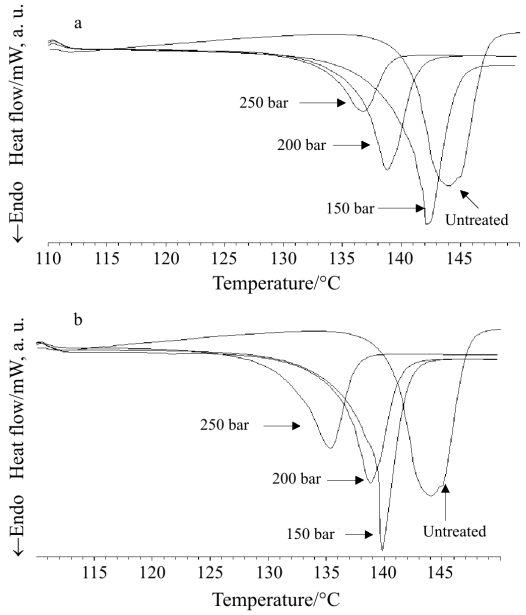

Fig. 4 DSC traces of ASA untreated and treated by RESS at different pressures and at 40°C (panel a) or 50°C (panel b)

Table 2 Melting temperature (T_f) and enthalpy of fusion (ΔH_f) for untreated ASA and for ASA powders obtained by RESS at different temperatures and pressures. Mean values, standard deviations in parenthesis, $n \geq 3$

Experimental conditions		T_f/ °C	ΔH_f/ J g^{-1}
T/°C	P/bar		
40	150	138.8 (3.5)	129.9 (48.63)
40	200	138.5 (2.42)	128.0 (28.38)
40	250	137.3 (1.3)	145.5 (10.73)
50	150	139.9 (1.2)	142.4 (6.77)
50	200	139.4 (3.7)	136.1 (28.31)
50	250	137.2 (2.5)	144.3 (14.09)
Untreated ASA		143.5 (0.7)	146.8 (18.07)

In Fig. 4 and Table 2, respectively, the DSC traces and the relevant parameters of thermal analyses performed on micronized samples and untreated ASA are reported.

No correlation could be found between temperature or pressure adopted during the RESS process and enthalpies of fusion, ΔH_f, these last data being poorly reproducible even for the untreated drug (relative standard deviation ranging from 5 and 37%). This can be justified by considering the ASA peculiar thermal instability [21].

For this reason, aiming at minimizing the ASA thermal decomposition during DSC scan, the thermal behaviour of treated and untreated drug was studied using the so-called instant heating method as suggested by the European Pharmacopeia. However, DSC scans were also performed in the 30–110°C range where no thermal events were recorded.

On the contrary, for powders prepared either at 40 or 50°C, the melting peaks shifted progressively to lower temperatures as the pressure of the RESS process was increased. The data variability was much less prominent than ΔH_f data, with a relative standard deviation ≤2.5%. All micronized ASA samples presented a melting peak temperature significantly different from that of untreated ASA (*t*-test, $P \leq 0.05$).

Using data taken from Table 2, melting temperatures were plotted *vs*. pressure, considering the untreated material as treated at zero pressure. Satisfactory linear correlation between the two variables was found (R^2=0.968 and 0.971 for 40 and 50°C, respectively). Both lines show practically the same slope (–0.0248 and –0.0237), thus indicating that, at both temperatures, pressure adopted for the RESS process had the same influence on the melting point depression of the product obtained.

Since the melting point depression could be due to salicylic acid formation during the RESS process, all micronized samples were tested for SA content by HPLC; in no case SA was ever detected.

Another source of SA from ASA could be represented by the DSC scan itself, as a consequence of the sample heating up to the final melting temperature. Thus, the possibility of thermal degradation was checked by heating micronized ASA up to 132°C, namely 3 K below the onset melting temperature, and recovering, after cooling, the pan content by dissolving it in methanol and analysing for SA content. HPLC revealed that samples were SA-free.

To further support these findings and considering that the SA detection limit by the HPLC method adopted is approximately 0.2 μg mL^{-1}, i.e. 0.2% of the concentration of the sample solution analysed, a solid mixture of 99.8 mg ASA and 0.2 mg SA was prepared by carefully mixing the components in an agate mortar by applying the 'doubling-up' technique to ensure an even distribution. 4 mg samples of this mixture were submitted to HPLC analysis and DSC scan. As expected, HPLC analyses confirmed the absence of SA while a not-significant 1 K (P=0.15) decrease of melting temperature was recorded by DSC with respect to pure ASA.

Therefore, the explanation for the melting point decrease observed had to be found elsewhere.

As already shown in Fig. 2, materials obtained by RESS were characterised by their extremely small particle size. Table 3 reports the particle size distribution (PSD) relevant to particles prepared by RESS process at the experimental conditions adopted. PSD is expressed as equivalent volume diameter values (in micrometers) below which the 10% *D* [0.1] 50% *D* [0.5] and the 90% *D* [0.9] of the measured particles can be found. The width of the distribution is summarized by the span value, which represents the geometric standard deviation.

Table 3 Volume diameters and span for ASA powders obtained by RESS at different temperatures and pressures. Mean values, standard deviations in parenthesis, $n \geq 3$

Experimental conditions		D [0.1]/ μm	D [0.5]/ μm	D [0.9]/ μm	Span
T/°C	P/bar				
40	150	2.14 (0.09)	5.39 (0.06)	12.36 (2.1)	1.89 (0.27)
40	200	2.07 (0.17)	4.75 (0.06)	10.62 (0.26)	1.79 (0.05)
40	250	2.12 (0.13)	4.02 (0.53)	13.41 (6.5)	2.94 (2.05)
50	150	2.10 (0.05)	4.70 (0.16)	8.60 (1.18)	1.37 (0.19)
50	200	2.20 (0.14)	4.60 (0.40)	18.6 (7.26)	3.40 (1.25)
50	250	2.06 (0.16)	4.04 (0.93)	7.22 (3.5)	1.28 (0.76)

Untreated ASA D [0.5]=520 μm

Taking into account that for pharmaceuticals particles having a diameter lower that 10 μm are considered as micronized, in general, RESS produced in all cases micronized powders of ASA.

The influence of the experimental parameters was quantified by looking at the relevant D [0.5], the value that splits in two equal parts the particle population, representing the median volume diameter. D [0.5] value differences were statistically significant only for powder samples prepared at 40°C at 150 and 200 bar ($P<0.01$) and 150 and 250 bar ($P<0.05$) while, comparing the results at the two experimental temperatures, a significant difference was found only at the lower pressure ($P<0.05$). Nevertheless, at both temperatures, the median volume diameter decreased linearly with pressure. The equations for the straight lines obtained by linear regression analysis in the D [0.5] *vs*. pressure plots were $y=-0.014x+7.5$, $R^2=1$ and $y=-0.01x+6.3$, $R^2=0.82$ for 50 and 40°C, respectively.

Carli and Colombo [22] proposed a model based on the Gibbs–Duhen–Laplace equation to explain the crystallization process of a drug into a silica gel carrier. This model enables to correlate the shifting of the melting temperature of the loaded drug with the size of the finely divided crystals. The simplified form of this model has the following expression:

$$T_e = -\frac{K}{R_p} + T_f$$

where T_e is the melting temperature observed for the micronized sample, R_p is the particle radius, K is a constant including various parameters such as entropy of fusion, surfacial tension of liquid and gas, molar volume of solid and liquid phases, T_f is the final melting temperature of the pure, non micronized compound. According to this model the plot of experimentally determined melting temperatures for micronized powders *vs*. the reciprocal of the particles radius should afford a straight line with a negative slope and an intercept equal to the melting temperature of the non micronized powder.

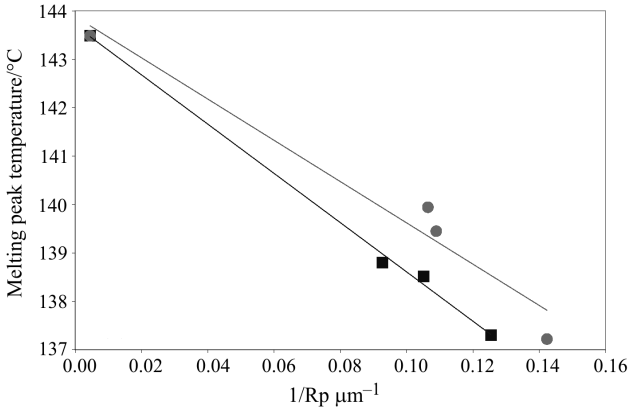

Fig. 5 Melting peak temperatures as a function of reciprocal of particle radius of ASA powders untreated and treated by RESS at 40°C (solid square) and 50°C (solid circle). The lines are linear regressions of the experimental points: relevant equations are y= –50.852x+143.69 R^2 0.997 and y= –42.583+143.88 R^2 0.9605 for 40 and 50°C, respectively. For sake of clarity, error bars are omitted; for y and x variability (Tables 2 and 3)

In Fig. 5 the ASA melting temperature (reported in Table 2) is plotted *vs*. the relevant reciprocal of particle radii calculated from the D [0.5] values of Table 3. Linear regression of experimental points gives straight lines with R^2 values of 0.9972 and 0.9605 for data at 40 and 50°C, respectively. This result indicates that the ASA melting point depression observed for RESS powders with respect to the commercial material can be attributed to the dramatic decrease of particle size as a consequence of micronisation.

Conclusions

Micronized ASA particles were produced by RESS process starting from a supercritical solution in CO_2. The obtained particles were crystalline, with spectroscopical and diffractometrical pattern overlapping those of the commercially available product.

The strong reduction of particle size can be linearly correlated to pressure imposed during the RESS process, while temperature does not seem to have a major effect.

Similar influence of pressure is observed on the final melting temperature of the micronized ASA.

The application of a mathematical model suggests that the melting temperature depression of RESS-prepared ASA powders can be attributed to the decrease of particle dimension and not to the formation of different solid phases or even impurities.

An additional conclusion of the present work is represented by the lack of isolation of a new ASA crystal phase as a consequence of the RESS process. On the other hand, this does not exclude, of course, the possibility of preparing ASA polymorphs, which has been envisaged merely from a speculative and theoretical point of view on the basis of computational molecular modelling [15].

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