Enhanced Dissolution Profile of Furosemide Treated by Supercritical Antisolvent Technology with Crospovidone

I. Kikic, N. De Zordi *, M. Moneghini*, D. Solinas

Department of Industrial Engineering and Information Technology University of Trieste, Via Valerio 10, 34127 Trieste, ITALY.
*Department of Chemical and Pharmaceutical Sciences University of Trieste, Via Giorgieri 1, 34127 Trieste, ITALY

ABSTRACT

An increasing number of poorly water-soluble drug candidates in pharmaceutical sciences provide challenges for the oral formulation since their water-solubility and rate of dissolution are a limiting step for their absorption and biological availability. Several strategies for improving the oral bioavailability of these drugs have been attempted; including particle size reduction, preparation of solid dispersions and drug loading in solid porous carriers. The purpose of the present investigation was to increase the solubility and dissolution rate of Furosemide by the preparation of its solid dispersion with crospovidone using supercritical antisolvent technology and two ratios 1:1 and 1:2 w/w drug polymer were prepared. Preliminary studies were conducted by means of Peng Robinson’s equation of state in order to predict the better operative conditions to precipitate Furosemide. Acetone, pressure of 100 and 200 bar and temperature of 313 K turn out to be the finest candidates. In order to understand the influence of the adopted technique on the drug solid state, Furosemide and Furosemide: crospovidone systems were compared with that obtained with the traditional method of rotavapor. Powder X-ray diffraction, particle size analysis and DRIFT studies were employed to investigate the physicochemical modifications of the drug. The 1:2 w/w drug polymer system processed with the antisolvent method at 100 bar lead to the amorphisation of Furosemide. This compound exhibits the better in vitro dissolution and solubility performances in the simulated gastric fluid (pH 1.2), where Furosemide (pKa~3.9) exists mainly in its unionized form that is poorly water soluble. These results, together with the presence of the selected carrier, determined a remarkable enhancement of solubility and in vitro dissolution rate of the drug, suggesting that the supercritical antisolvent technology can be considered as a promising way to prepare drug-polymer systems.
INTRODUCTION

The solubility of drug remains one of the most important issues in formulation development [1] and therefore it is necessary to improve it and the dissolution rate, reducing the particle size [2], preparing solid dispersions [3] or loading drug in solid porous carriers [4, 5]. Recently supercritical fluids (SCF) technology found a widely used in pharmaceutical area with two objectives: micronization and modification of solid state characteristics [2]. The use of carbon dioxide (CO₂) is one of the major advantages in the SCF process, as CO₂ is nontoxic and presents mild critical conditions, making it an ideal substitute for organic solvents. The low solubility of the majority of polar and ionic drug in CO₂ limits its use as pure solvent media. Alternative approaches to solve this problem are proposed combining CO₂ with an organic solvents characterized by solvating properties. The expansion degree of the organic solvent modulated by the CO₂ content in the mixture, offers different opportunities for the development of a suitable methodology for the processing of the materials, such as precipitation (DELOS, GAS and SAS) [6,7].

Supercritical fluids have found also application for the preparation of solid dispersions, i.e. cefuroxime axetil with HPMC 2910 and PVP K-30 [8] and felodipine with HPMC [9] were processed by means of supercritical antisolvent technology with the aim to increase their dissolution rate.

The variation of solid state characteristics such as crystal habit, crystallinity, and polymorphism has gained increasing attention in pharmaceutical research and has been successfully achieved through the precipitation of drug particles using various SAS processes [10-13]. In particular, SCF process operating parameters can be adjusted to vary supersaturation and conditions for nucleation and crystal growth across a wide range [14]. In this work, Furosemide treated with the supercritical antisolvent method was investigated alone and combining the drug with crospovidone. All the systems were also physicochemical characterized.

Furosemide (pKa 3.6) is a diuretic drug that explicates the action on the ascending loop of Henle in the kidney to allow the removal of unneeded water and salt from the body into the urine. This drug is practically insoluble in water (solubility 0.006 mg mL⁻¹).

Crospovidone is a water-insoluble disintegrant and dissolution agent. It rapidly exhibits high capillary activity and pronounced hydration capacity, with little tendency to form gels. It can also be used as a solubility enhancer [15].

After a preliminary phase equilibrium study to define the better operative conditions, acetone, temperature of 313 K and pressure of 100 and 200 bar were selected to precipitate the drug alone and with crospovidone in 1:1 w/w and 1:2 w/w drug polymer raitios. All the systems obtained by means of SAS were compared with the ones obtained with the traditional method of rotavapor (RV).

MATERIALS AND METHODS

Materials

Furosemide (FUR) and micronized Crospovidone (PVP CLM) were purchased from Farmalabor (Italy) and BASF (Germany), respectively. CO₂ (purity 99.9%) and organic solvents (acetone and methanol) were acquired from SIAD (Italy) and Baker (Germany), respectively. All other chemicals were reagent grade and used as received.
**Traditional evaporation method**

Furosemide was solubilized in acetone. The solvent was then removed under reduced pressure in a rotary evaporator (Buchi R-114, Flawill, Switzerland) at 313 ± 1 K for 30 min. Before the characterization, samples were kept for 3 days in desiccators under vacuum at room temperature. The same procedure was adopted for the binary systems composed by drug and the insoluble polymer crospovidone in the 1:1 and 1:2 w/w ratios (called FUR RV, FUR:PVP CLM 1:1 w/w RV and FUR:PVP CLM 1:2 w/w RV, respectively) to achieve a coprecipitation.

**Supercritical precipitation method**

A schematic diagram of equipment used in this study is reported in Figure 1. The precipitator (New ways of Analytics NWA, Lörrach, Germany, internal diameter and volume of 30 mm and 100 cm$^3$ respectively) was jacketed ensuring temperature to be kept within 313 ± 0.5 K. The sample solution, kept at the precipitator temperature was introduced into the precipitator. Liquid CO$_2$ was fed from the top of the precipitator by a high pressure pump (NWA, PM-101). The outlet flow was then filtered (0.22 µm) to prevent precipitate losses and regulated by a heated metering valve (Whitey SS-21RS4). Temperature and pressure values in the precipitator were measured by a Delta OHM thermometer (HD 9214, ± 0.1 K) and a DRUCK pressure transducer (DPI 260, ± 0.1 bar). The precipitator was filled with 10 mL of sample solution; then the CO$_2$ were pumped to the reactor at constant flow of 1 l/min$^{-1}$. The pressure and temperature selected for the precipitation of Furosemide and Furosemide :Crospovidone 1:1 and 1:2 w/w were 100 and 200 bar at 313K (called FUR 100 bar, FUR 200 bar, FUR:PVP CLM 1:1 w/w 100 bar and FUR:PVP CLM 1:1 w/w 200 bar, respectively). Saturated drug solutions were used for the precipitation.

![Figure 1: schematic diagram of equipment](image)

**Determination of drug organic solvent solubility**

Solubility of Furosemide in the considered organic solvents was measured by gravimetric analysis, dispersing a weighted amount of drug in 100 ml of solvent under stirring at room temperature (r.t.) as described [16].
Phase equilibria simulation

It is essential to determine the solubility of Furosemide as a function of the solvent and anti-solvent composition.

In the case of ternary systems Furosemide–carbon dioxide – organic solvents it is possible to find in literature experimental data for the different binary systems involved such as acetone or methanol – supercritical carbon dioxide binary systems [17, 18]. The behaviour of Furosemide – organic solvent binary mixtures can be study by determining solubility data of the drug in the different solvents.

The Peng-Robinson equation of state was used for the correlation of the different binary systems and for the prediction of the behaviour of the ternary following the procedure described in [16]. In the case of solid-fluid equilibrium, where it is assumed that the solid phase is pure solute, the fugacity of the pure solute is obtained as proposed by Poling et al. [19] on the basis of the knowledge of the heat ($\Delta H_f$) and temperature of fusion ($T_f$). Furosemide values of $\Delta H_f$ (58.53kJ/mole) and $T_f$ (479.15 K) were measured with the differential scanning calorimetric technique. Critical properties (critical temperature $T_c$, critical pressure $P_c$ and acentric factor $\omega$) of Furosemide were calculated using Simamora Yalkowsky and Lydersen group contribution method as reported in Kikic et al. [16].

Since the complete insolubility of crospovidone in both supercritical fluids and organic solvents, in this work the phase equilibria behaviors were conducted considering only the ternary system composed by drug-organic solvent and CO₂.

Table 1 reported the pure component properties of the different compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$T_c$ [K]</th>
<th>$P_c$ [bar]</th>
<th>$\omega$</th>
<th>$T_f$ [K]</th>
<th>$\Delta H_f$ [kJ/mole]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furosemide</td>
<td>828.6772</td>
<td>29.9164</td>
<td>1.1733</td>
<td>479.15</td>
<td>585320.1</td>
</tr>
<tr>
<td>CO₂</td>
<td>304.1</td>
<td>73.8</td>
<td>0.239</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>508.1</td>
<td>47</td>
<td>0.304</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>512.58</td>
<td>80.96</td>
<td>0.557</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: pure components properties

Particle size and shape analysis

Particle size and elongation factor characterizations of samples were determined using an optical microscope (Reichert Biovar, Wien, Austria) (magnification 1000 x). Small amounts of each sample were uniformly dispersed on a microscopy glass slide. For each powder batch, 10 microscopy glass slides were prepared, examining at least 5,000 particles per sample. Pictures were examined with the image analysis program ImageJ [20]. The size of each sample was determined as Feret diameter while the shape factor was expressed as roundness [20].

Powder X-ray diffraction studies (PXRD)

PXRD studies were done using a STOE D500 (Siemens, Monaco, Germany) diffractometer with Cu Kα radiation ($\lambda=1.5418$ Å), monochromatised by a secondary flat graphite crystal. The scanning angle ranged from 3° to 40° of 2θ, steps were of 0.05° of 2θ, and the counting time was of 5 s/step. The current used was 20 mA and the voltage 40 kV.
Determination of drug solubility in simulated gastric fluids

Solubility of commercial FUR and the treated ones alone and with crospovidone was measured in oversaturated conditions ($C>10\ C_s$), dispersing a weighted amount of drug in 10 mL of simulated gastric fluids at pH 1.2. The suspensions were stirred under constant speed at $3130 \pm 0.5 \ K$ for 24 h (appropriate time for equilibrium), filtered through a membrane (pore size 0.45 µm) and then assayed spectrophotometrically at 274 nm. The composition of the dissolution medium was 0.2 M NaCl/0.2 M HCl (pH 1.2).

Determination of drug dissolution

Profiles of Furosemide and Furosemide binary systems release were obtained according to the USP 33 paddle method: 100 rpm, 900 mL of simulated gastric fluids at pH 1.2, $T= 310 \pm 0.1 \ K$, sink conditions ($C<0.2 \ Cs$). The aqueous solution was filtered (0.45 µm porosity) and continuously pumped to a flow cell in a spectrophotometer and absorbance was recorded at 274 nm. Experimental points were the average of at least three replicates, and standard deviations did not exceed ± 5% of mean value.

RESULTS

Solubility of Furosemide in supercritical CO$_2$ at 313 K was $10^{-6}$ mole fraction from 100 to 200 bar.

Vapor liquid equilibrium data taken from literature for carbon dioxide – organic solvents were correlated. Solubility of Furosemide in the different organic solvents was determined using gravimetric method and is reported in Table 2.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>y ($10^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>9.32</td>
</tr>
<tr>
<td>Methanol</td>
<td>2.48</td>
</tr>
</tbody>
</table>

Table 2: mole fraction solubility of Furosemide in the organic solvents

These data were correlated with the Peng Robinson EOS in order to obtain the value of temperature independent $k_{ij}$ and $l_{ij}$ binary interaction parameter between organic solvent and Furosemide (Table 3).

<table>
<thead>
<tr>
<th>System</th>
<th>$k_{ij}$</th>
<th>$l_{ij}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furosemide – CO2</td>
<td>-0.0534</td>
<td>0</td>
</tr>
<tr>
<td>Furosemide – Acetone</td>
<td>-0.1272</td>
<td>0</td>
</tr>
<tr>
<td>Furosemide – Methanol</td>
<td>-0.0531</td>
<td>0</td>
</tr>
<tr>
<td>Acetone – CO2</td>
<td>0.0204</td>
<td>0.0292</td>
</tr>
<tr>
<td>Methanol – CO2</td>
<td>0.0732</td>
<td>0.0291</td>
</tr>
</tbody>
</table>

Table 3: binary interaction parameters obtained with PR EoS

Tri-phase phase equilibria were calculated for different liquid phase concentrations at 40°C and at the pressure of 100 and 200 bar. For brevity in figure 3 is reported the simulation diagram of furosemide processed with acetone at the pressure of 100 bar. The diagram reports the Furosemide concentration in the organic solvent as a function of the molar fraction of CO$_2$ in the mixture ($X_{CO2}$).
In the case of “Furosemide – methanol” solvent mixture the addition of carbon dioxide to the saturated solution does not yield the drug precipitation in all the CO₂ composition mixtures showing only the 30% of precipitate (data not show). Acetone was selected as optimal organic solvent because it carried a precipitation in the entire CO₂ ratio suggesting the highest yield of the assay. The above simulations were confirmed weighting the precipitate (Figure 2).

![Figure 2: ternary mixture precipitation behavior of Furosemide-Acetone-CO₂ at 100 bar and 313 K](image)

Figure 2: ternary mixture precipitation behavior of Furosemide-Acetone-CO₂ at 100 bar and 313 K

Powder X-ray analysis of treated and untreated Furosemide reveal the presence of two different crystalline forms of drug (Figure 3). By means of Retvield analysis, our samples were compared with the crystal structures reported in literature [21]. Raw Furosemide, Furosemide precipitated at 100 bar and in rotavapor in presence of acetone show the same

![Figure 3: powder x-ray diffractometer of: a) Furosemide CO₂ treated at 200 bar and 313 K, b) Furosemide CO₂ treated at 100 bar and 313 K, c) Furosemide RV, d) raw Furosemide](image)
diffractogram patterns that agree with triclinic crystal system (GOF 0.99, Rp 11.01 %, and Rwp 16.89 %) Instead, pattern of drug precipitated at 200 bar revealed the monoclinic crystal system (GOF 1.14, Rp 10.88 % and Rwp 15.35 %) even if the reflection at about 5 of 2 θ attests the concomitant presence of small percentage of triclinic form (about 20% estimated with Powder Cell 2.4 [22]).

Figure 4: DRIFT spectra of: a) Furosemide CO₂ treated at 200 bar and 313 K, b) Furosemide CO₂ treated at 100 bar and 313 K, c) Furosemide RV, d) raw Furosemide

Moreover, the above results were confirmed by DRIFT analysis (Figure 4) that are in agree with the literature spectra [21]. Raw Furosemide, Furosemide precipitated with CO₂ at 100 bar and in RV show the typical DRIFT peaks of Furosemide triclinic system with the asymmetric sulfonamide NH stretch, secondary amine NH and symmetric sulfonamide NH at 3400, 3351 and 3285 cm⁻¹, respectively; while Furosemide treated at 200 bar reports the typical spectra of monoclinic system showing two diagnostic peaks at 3347 and 3253 associated to the secondary ammine NH and symmetric sulfonamide NH, respectively.

The effect of pressure on the Furosemide’s solid state was investigated also for the drug polymer binary systems in both ratios of 1:1 and 1:2 w/w compared with the ones precipitated with RV method. From Powder X-ray diffraction (Figure 5) no difference were observed between the samples of Furosemide:crospovidone 1:1 w/w precipitated at 100 bar and with RV, while a reduction of drug crystallites dimensions appeared for samples at 200 bar and in 1:2 w/w ratio precipitated with traditional method. Amorphisation of Furosemide was observed only for Furosemide: crospovidone 1:2 w/w treated at 100 bar. For this reason a precipitation at 200 bar was not need. Moreover, 1:1 w/w system obtained at 200 bar revealed the presence of a mixture of both monoclinic and triclinic crystal forms (about 50 %), while the others samples show the monoclinic behavior.
Any evident interaction were observed by means of DRIFT analysis (data no show).
The particles size expressed like Feret’s diameter and shape factor of raw Furosemide and those precipitated at 100, 200 bar and with RV method were measured (Figure 6).

Considerable difference were observed for Furosemide precipitated at 200 bar that showed an
average diameter of about 3 µm while the others did not refer substantial changes and showed an average diameter of about 6 µm.

A decrease of particle size was also detected for FUR: PVP CLM 1:2 w/w 100 bar compared to the other considered binary systems. In figure 7, for brevity, are reported only the particle size of FUR: PVP CLM 1:2 w/w 100 bar and FUR: PVP CLM 1:2 w/w RV.

![Figure 7: particle size estimation of binary systems](image)

According to the USP solubility definition, Furosemide at pH 1.2 is practically insoluble. No appreciable variation in the solubility behaviors were observed between the two polymorphic forms. An increase in solubility was observed for all the binary mixtures due to the reducing of Furosemide cristallinity. In particular the FUR: PVP CLM 1:2 w/w 100 bar showed the best solubility enhancement ascribed to the drug amorphisation (table 4).

<table>
<thead>
<tr>
<th>System</th>
<th>Solubility pH 1.2 [µg ml⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Furosemide</td>
<td>21 ± 1.5</td>
</tr>
<tr>
<td>Furosemide 200 bar</td>
<td>21.5 ± 1.3</td>
</tr>
<tr>
<td>FUR: PVP-CLM 1:1 w/w RV</td>
<td>26.5 ± 2.6</td>
</tr>
<tr>
<td>FUR: PVP-CLM 1:1 p/p CO2</td>
<td>37.9 ± 3.6</td>
</tr>
<tr>
<td>FUR: PVP-CLM 1:2 w/w RV</td>
<td>37.9 ± 4.4</td>
</tr>
<tr>
<td>FUR: PVP-CLM 1:2 w/w CO2</td>
<td>50.6 ± 3.7</td>
</tr>
</tbody>
</table>

*Table 4: furosemide solubility at pH 1.2 and 310 K*

A remarkable increase of Furosemide dissolution rate (Figure 8) was observed when it was processed with supercritical CO₂ at the pressure of 200 bar as a comparison to the Raw and precipitated ones at 100 bar and in RV, which instead, do not show considerable differences. It is plausible that the particle sizes decreases, and the polymorphic form of the drug treated at 200 bar carry to an increase of surface area, suggesting the better enhancement of the dissolution rate.

The same phenomenon occurs for the binary systems that along the disintegrant PVP CLM
effect, they show a diminution in crystallite and particle size. These issues play an important role for the increase of surface area of the samples in contact with the dissolution medium. Clearly, the amorphisation of the drug obtained for FUR: PVP CLM 1:2 w/w at 100 bar refers the best dissolution performance. It must be point out that the amount of monoclinic form presents also in FUR:PVP CLM 1:1 w/w at 200 bar shows a similar dissolution profile of FUR:PVP CLM 1:2 w/w obtained with RV treatment, attesting, even in this case, the benefit of this crystal form. Any difference where observed between the RV system in 1:1 w/w ratio and the commercial Furosemide, while the system 1:1 w/w treated at 100 bar carry to an enhance of dissolution kinetic for the effect of particle size decrease as regards to the corresponding RV system.

Figure 8: in vitro dissolution profile of considered systems

CONCLUSION

Furosemide was processed alone and with crospovidone in 1:1 and 1:2 w/w drug-polymer ratios with acetone by means of supercritical antisolvent technology. Preliminary studies were conducted using the Peng Robinson’s equation of State with the aim to find the better operative condition to enable the Furosemide precipitation. A comparison with the ones obtained with the traditional method was also reported. The physicochemical characterizations highlight the evident amorphisation of the drug in the FUR: PVP CLM 1:2 w/w at 100 bar system. This amorphisation has carried to the better dissolution performance as well. On the contrary, the same system treated with the traditional method, shows again the presence of crystalline Furosemide.

In conclusion, it was proved that supercritical antisolvent technology is a viable and alternative mean to prepare solid dispersion. Moreover, using crospovidone it was possible to achieve a highest degree of bioavailability for poorly soluble drug such as Furosemide.
REFERENCES


[16] KIKIC, I., DE ZORDI, N., MONEGHINI, M., SOLINAS, D., Journal of Supercritical
Fluids, Vol. 55, **2010**, p. 616


[22] KRAUS W., NOLZE G., Powder Cell 2.4, Federal Institute for Materials Research and Testing (BAM), Berlin, Germany, **2000**