SOLUBILITY OF POORLY SOLUBLE DRUGS IN SUPERCRITICAL CARBON DIOXIDE: EXPERIMENTAL MEASUREMENT AND DENSITY-BASED CORRELATIONS

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Abstract. Supercritical fluid (SCF) technologies are being intensively used in the development and optimization of novel approaches to prepare pharmaceutical materials with improved properties. Among others, those technologies include the supercritical solvent impregnation/deposition (SSI) of drugs in different biocompatible support materials and the rapid expansion of supercritical solutions (RESS), which can be used to load bioactive substances and to modify solid particles shape, polymorphism, morphology and surface structure. These processes lead to an increase in the drugs efficiency since they improve their solubility (and consequently their bioavailability), without compromising their stability and pharmaceutical behavior. Moreover, these techniques can be integrated using a simple one-step process to simultaneously micronize and load the drug particles. These SCF-based processes always require an accurate knowledge of the experimental and of the modeled equilibrium solubility data between the solutes of interest and the SCF solvent (at different temperature and pressure conditions). In this work the equilibrium solubility of the drugs like norfloxacin, ofloxacin and dexamethasone in $scCO_2$ were studied at different temperature conditions (308.2 K, 318.2 K, and 323.2 K) in a pressure range between 10 and 35.7 MPa using a static analytic method. Solubility data was found to be low and between $0.4x10^{-6}$ and $24.4x10^{-6}$ (in terms of mole fraction). Experimental data were correlated using density semi-empirical based models namely the Mendez-Santiago-Teja, Bartle, Chrastil and Spark's models with global average absolute relative deviations (AARD) lower than 13%. For all the studied drugs, the models were able to correctly describe the temperature dependence but failed to describe its dependence with density. Experimental solubility data of norfloxacin and ofloxacin is only correctly correlated when temperature dependent parameters are considered.

Keywords: Norfloxacin, ofloxacin, dexamethasone, solid solubility, supercritical carbon dioxide, density-based correlations

1. Introduction

Different pharmaceutical processes are being developed to design a very wide range of clinically approved products that aim to enhance the bioavailability of poorly-soluble compounds though an efficient control of the drug release kinetic within the human body [1]. A key parameter that permits to enhance drugs bioavailability is their solubility in aqueous media which is essential to enhance the absorbability of oral administrations and consequently decrease the dose amount/frequency and the often associated undesirable side effects and costs. The synthesis of most drug delivery systems (DDS) relies on either melt processing or organic solvent based methods to incorporate the drug into a carrier matrix (usually polymeric). However, these processes usually require the use of harmful organic solvents and/or high processing temperatures which may compromise the bioactivity of the drug. Over the last years supercritical carbon dioxide ($scCO_2$) has attracted particular attention from the pharmaceutical industry since it can be considered as an environmentally friendly solvent that can replace conventional organic solvents and can be applied at moderate processing conditions [2]. Among several $scCO_2$ processing techniques, rapid expansion of

supercritical solutions (RESS) and supercritical solvent impregnation/deposition (SSI) of drugs into biocompatible polymeric matrices are being employed as tunable methods to micronize (or at least to reduce the size of) drug particles and/or to load different drugs (especially those that present an hydrophobic character) into different organic, inorganic or composite materials. In some cases both processes (micronization and impregnation/deposition) are achieved simultaneously with a significant improvement in the amount of drug impregnated and in the control of the release process which also depends on the drug affinity for the polymer matrix (or on specific drug-polymer interactions). Through the manipulation of process conditions (pressure, temperature, depressurization rate, processing time) the low viscosity, low surface tension and high diffusivity of $scCO_2$ permits to control the amount of drug that is solubilized in the solvent media and further into the matrix to be impregnated.

Norfloxacin, ofloxacin and dexamethasone (DXMT) are drugs commonly used in several DDS due to their high pharmaceutical potential [3-8]. Among fluoroquinolones (FQN), norfloxacin and ofloxacin are extensively used as antimicrobial agents due to their excellent pharmacokinetic profile in biological tissues [4]. The incorporation of these drugs into different polymers has been studied in order to develop compatible materials for drug delivery products for ophthalmic applications [3]. Dexamethasone is a synthetic glucocorticoid (GC) that mimics the action of cortisol, which have an important role in immunologic, cardiovascular, homeostatic and metabolic functions. Due to its powerful inflammatory actions and immunosuppressive properties, DXMT is used to regulate the production of several pro-inflammatory cytokines as well as modulator for the osteogenic differentiation of mesenchymal stem cells [9,10]. However these drugs present low solubility in aqueous systems which may compromise their bioavailability. In these cases SCFs technology may be an interesting alternative to improve their solubility and consequently their processability without compromising stability and pharmaceutical behavior. The design and scale up of any SCF based process requires the knowledge of phase equilibrium and drug solubility in the supercritical fluid to determine the most advantageous operating conditions. In this work the equilibrium solubility of norfloxacin, ofloxacin and dexamethasone in $scCO_2$ were studied at different temperature conditions (308.2 K, 318.2 K, and 323.2 K) in a pressure range between 10 and 35.7 MPa using a static analytic method. Experimental data were correlated using density semi-empirical based models namely the Mendez-Santiago-Teja, Bartle, Chrastil and Spark's models.

2. Experimental

2.1 Materials

The sources and purities of the materials used in this work are detailed in Table1.

Table 1. Purities and source of materials used in this work.

Chemical Name	Source	Purity (w/w)
Carbon dioxide	Praxair	0.9998
Ethanol	Panreac Quimica SA	0.995
Ethanol	Panreac Quimica SA	HPLC grade
Acetonitrile	Sigma-Aldrich	HPLC grade
Methanol	Sigma-Aldrich	HPLC grade
Norfloxacin	Sigma-Aldrich	0.98
Ofloxacin	Sigma-Aldrich	0.95
Dexamethasone	Fluka	0.98

2.2 Experimental Solubility Measurement Procedures

Experimental equilibrium solubility data were measured according to the static method previously reported in the literature [11,12]. The solubility apparatus comprises a high-pressure CO_2 syringe liquid pump and a sealed high pressure stainless steel vessel coupled to a known volume sampling loop which is connected through a six-port sampling valve to sampling lines and to a balloon (used to quantify the amount of CO_2 for each data point), both with previously calibrated volumes. The high pressure cell is loaded with an excess amount of solid (≈ 200 mg) and a magnetic stirrer (700 rpm). After pressure and temperature stabilization the mixture (CO_2+drug) is stirred for 3h followed by a stabilization period of 15 min. Temperature is maintained by means of thermostatic water baths to within ± 0.1 K and pressure is measured

by a high pressure transducer 0-34.4 ± 0.04 MPa in the cell and 0-0.175 ± 1.9×10^{-4} MPa in the calibrated balloon. After equilibrium, a portion of the saturated supercritical CO_2 is allowed to pass to the sampling loop. This volume was higher for DXMT than for the FQNs in order to increase the amount of solubilized sample and decrease experimental error due to the particularly low solubility of this drug. Then the loop was depressurized into the collection vial and ethanol is injected through the sample loop and the expansion lines to recover all the sampled solid substances. The final volume of the solution was 5 ml. The tubing lines are additionally cleaned/dried with fresh and slightly pressurized CO_2. The amount of norfloxacin and ofloxacin that was solubilized in $scCO_2$, at a given pressure and temperature conditions, was quantified by spectrophotometry (Jasco V-530, Japan) and a calibration curve previously determined at a fixed wavelength of 284 nm for norfloxacin and 300 nm for ofloxacin. DXMT quantification was done by HPLC. The amount of CO_2 in each sampling step was calculated using the Virial EOS (applied to pure CO_2). All the prepared solutions were carefully stored and protected from light in order to avoid degradation. Each reported data point is the average of at least three or six replicate measurements for FQNs and DXMT, respectively.

2.3 High-performance Liquid Chromatography (HPLC)

Analyses were carried out using a HPLC system (Prominence UFLC Shimadzu coupled to a photo diode array detector SPD-M20A) and using a Eurospher column 100-5C18RP (250 x 4 mm i.d., 5 mm, Germany) equipped with a precolumn. The chromatographic conditions were based on the ones previously reported in the literature [13]. A mobile phase, constituted by methanol/water in a proportion of 9:1 (v/v), was employed in an isocratic elution (15 min), at a flow rate of 1 $mL.min^{-1}$ and at 35ºC. A run with acetonitrile only was used between each sample to clean the column. The chromatographic profile of each injected sample (20 μL) was measured at 239 nm in duplicate, and DXMT was identified and quantified by comparison of the retention times of standard solutions previously prepared in the concentration range between (0 and 120) μg. mL^{-1}.

2.4 Correlation of experimental solubility data using density-based correlations

Density-based semi-empirical models were used to correlate the experimental equilibrium solubility data for drugs in terms of reduced variables and were expressed as:

$$S_{2,r}=\frac{S_2}{\rho_{c,1}};\quad \rho_{1,r}=\frac{\rho_1}{\rho_{c,1}};\quad T_r=\frac{T}{T_{c,1}};\quad P_r=\frac{P}{P_{c,1}} \tag{1}$$

where the subscripts 1 and 2 refer to the solvent ($scCO_2$) and to the solute (drugs), respectively. The applied equations are given in Table 2, where T_r and P_r are the reduced experimental temperatures (T) and pressures (P), respectively, $S_{2,r}$ is the reduced solubility of the solid in the supercritical phase, $\rho_{r,1}$ is the solvent reduced density, and y_2 is the solute mole fraction. The critical properties of carbon dioxide where obtained from the NIST Chemistry Webbook.

Table 2. Density-Based Models Used To Correlate the Experimental Solubility Data Measured for drugs in $scCO_2$.

Model	Equation	Adjust constants
Méndez-Santiago and Teja [15,16]	$T_r \ln(y_2 P_r) = A' + B'\rho_{r,1} + C'T_r$	y_2, A', B' and C'
Bartle [17]	$\ln\left(y_2 P / P_{ref}\right) = a_1 + a_2/T_r + C(\rho_{r,1}-1)$	a_1, a_2, A and C
Chrastil [18]	$S_{2,r} = \rho_{r,1}^{k}\exp\left(a+\beta/T_r\right)$	α, β
Sparks [19]	$S_{2,r} = \rho_{r,1}^{(e_0+e_1\rho_{r,1}+e_2\rho_{r,1}^2)}\exp\left(a+\beta/T_r+y/T_r^2\right)$	e_0, e_1, e_2, α, β, γ

The average absolute deviation relative (AADR) was adopted to evaluate the accuracy of prediction. AADR was estimated from the following equation between experimental and prediction values.

$$AARD(\%) = \frac{100}{N}\sum_{i=1}^{N}\frac{\left|y_{i,calc} - y_{i,\exp}\right|}{y_{i,\exp}} \quad (2)$$

where N is the number of experimental data points for each isotherm, y_{exp} is the experimental solubility of the solid for experimental point i, and y_{cal} is the calculated solubility for point i.

3. Results and discussion

The equilibrium solubility of norfloxacin, ofloxacin and dexamethosone in $scCO_2$ was determinated at different temperature conditions (308.2 K, 318.2 K, and 323.2 K), in a pressure range between 10 and 35.7 MPa and according to the static method previously detailed in the experimental section. In the present work, some modifications were introduced in the experimental methodology as well as in the drug quantification method, as previously described, in order to accurately measure the solubility of DXMT at mild experimental conditions. Figure 1 compares the experimental solubility data, expressed in terms of solid mole fraction, y_2, measured at 318.2K.

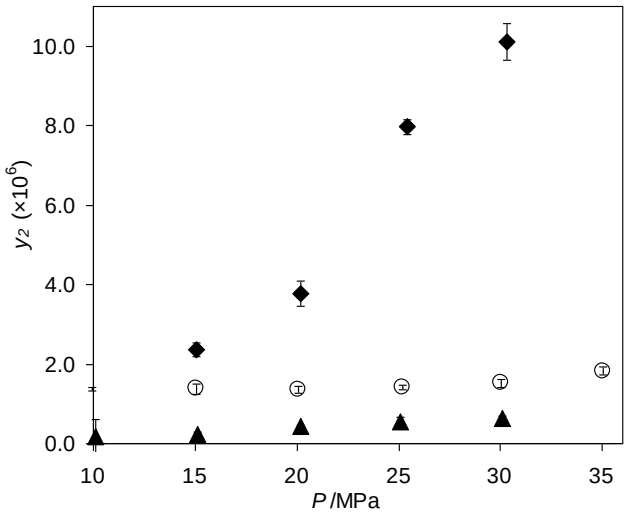

Figure 1. $scCO_2$ experimental solubility data expressed in terms of molar fraction of drug (y_2) at 318.2 K: (♦) Norfloxacin, (▲) Ofloxacin and (○) Dexamethasone

At the studied experimental conditions, the measured solubilities (in terms of mole fraction) varied between 1.4×10^{-6} (318.2 K; 10.1 MPa) and 24.4×10^{-6} (323.2 K; 30.1 MPa) for norfloxacin, 0.4×10^{-6} (313.2 K; 10.0 MPa) and 1.3×10^{-6} (323.2 K; 29.9 MPa) for ofloxacin, and 1.3×10^{-6} (308.2 K; 15.1 MPa) and 2.8×10^{-6} (328.2 K; 34.8 MPa) for DXMT. The observed deviations were within the ones expected for molecules with low solubility in $scCO_2$ with a global AARD lower than 11.4 % for FQNs and 5.8 % for DXMT. The isotherms presented in Figure 1 permit to observe that at constant temperature, the effect of the pressure on the solubility of norfloxacin is higher than for other compounds and follows an exponential increase with pressure which is an expected behavior for a solid/SCF system. This occurs due to an increase in the $scCO_2$ density and, consequently, to an enhancement in its dissolving power that result in the reduction of the intermolecular mean distance of the involved molecules that favor solute-solvent specific interactions. In the case of ofloxacin and DXMT, a smoother linear increase of the solubility with pressure was observed. In the case of DXMT, increasing the temperature from (308.2 to 328.2) K increases the solubility by 15 % at 15 MPa, by 19 % at 25 MPa, and by 46 % at 35 MPa due to predominant effect of the volatility of the solute. The crossover effect was not detected for these compounds, at the experimental conditions studied maybe due to their high molecular weight and polarity that does not compensate the effect of the solvent density on the solubility. According to Zhang and Wang [14] the solubility of ofloxacin in water is higher than that measured for norfloxacin, indicating that the former is highly polar and consequently less soluble in $scCO_2$ as observed in this work. After depressurization of the cell (at $10MPa.min^{-1}$) samples of processed and precipitated drugs were analyzed by SEM which revealed that the size of the particles was reduced after the

contact of the drugs with $scCO_2$ (3h), especially for norfloxacin due to the higher solubility [20]. This phenomenon is essential to improve the dissolution of these drugs in biological media and consequently its bioavailability. Experimental solubility data was correlated using the density-based correlations previously described (Méndez-Santiago-Teja, Bartle, Chrastil and Sparks' models). The correlation results and the corresponding AARD values obtained with the density-based models are shown in Table 3.

Table 3. Correlation results obtained with density-based models.

	Norfloxacin	Ofloxacin	Dexamethasone
Méndez-Santiago-Teja's Model			
A'	-82.04	-56.24	-21.86
B'	3.40	1.82	3.49
C'	61.53	37.51	2.68
AARD (%)	38.1	23.2	13.1
N	15	10	15
Bartle's Model			
a_1	74.05	55.20	0.67
a_2	-75.03	-54.03	-14.95
C	3.01	1.82	3.18
AARD (%)	37.3	23.1	13.4
N	15	10	15
Chrastil's Model			
k	3.71	2.61	2.96
α	-68.92	-32.58	-7.74
β	54.07	18.12	-4.94
AARD (%)	33.9	22.1	11.8
N	15	10	15
Sparks' Model			
e_0	1.43	-2.20	-1,01
e_1	0.93	2.25	1,07
α	-977.90	-978.26	-7,11
β	2073.80	2073.37	-4,31
γ	-1111.99	-1112.17	0
AARD (%)	26.9	8.6	7.7
N	15	10	15

In general terms the employed models were able to correlate the experimental solubility of the drugs in $scCO_2$ with acceptable AARD deviations considering the low solubility range measured in this work. In the case of FQNs these results can be justified by the different density dependences observed for each isotherm that do not permit that the same set of parameters is used to describe the entire set of data. Norfloxacin density and temperature dependency do not allow an improved correlation even when five adjustable parameters were used as in the case of the Sparks' model. Improvements can be obtained only if temperature dependent parameters are used [20]. In the case of DMTX the models were able to correctly describe the temperature dependence but failed to describe the sharp solubility increase that is verified for higher pressures (within the solubility range measured for this drug).

4. Conclusion

The solid solubilities of norfloxacin, ofloxacin and dexamethasone in $scCO_2$ were experimentally measured using a static analytical method at 308.2 K, 318.2 K, and 328.2 K, and for pressures between 10 and 35.7 MPa. The measured equilibrium solubility data showed to be low and varied between $1.4x10^{-6}$ and $24.4x10^{-6}$ (for norfloxacin), $0.4x10^{-6}$ and $1.3x10^{-6}$ (for ofloxacin) and 1.25×10^{-6} and 2.81×10^{-6} (for DXMT) in terms of mole fraction. Experimental solubility data were correlated with density-based models (Méndez-Santiago-Teja, Chrastil, Bartle and Sparks' models). For FQNs, the lowest AARD values were obtained with the Sparks' model being equal to 27 % for norfloxacin and 9 % for ofloxacin. For DMTX, the models were able to correctly describe the temperature dependence (AARD lower than 13.5 %) but failed to describe the sharp solubility increase that is verified for higher pressures. Moreover it was found that $scCO_2$ processing leads to particle size reduction of the commercial particles of norfloxacin and ofloxacin which largely

enhances the solubility of these drugs in aqueous media (and consequently their bioavailability) and broadens the possibilities for drug delivery systems applications.

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