

# Influence of Chemical Structure on the Equilibrium Solubility of Naphthoquinone Derivatives in Supercritical Carbon Dioxide: Experimental Determination and Correlation

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## Abstract

This work reports the experimental measurement and the correlation of the equilibrium solubility of three naphthoquinone derivatives, namely naphthoquinone (1,4-naphthoquinone), juglone (5-hydroxy-1,4-naphthoquinone) and plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) in supercritical carbon dioxide (scCO<sub>2</sub>). Results were obtained using a static analytical method, at 308, 318 and 328K, and in a pressure range from 9.0 up to 24.0MPa. Experimental data were correlated with three density-based models (Chrastil, Bartle and Méndez-Santiago-Teja) and with the PR cubic equation-of-state (PR-EOS) together with the conventional van der Waals mixing and combining rules. The best obtained average absolute relative deviations (AARD) were lower than 11 % for the semi-empirical models and lower than 12 % for the PR-EOS, after the proper choice of the employed critical and thermophysical properties estimation methods.

## INTRODUCTION

Plants produce a large number of distinct natural compounds, also known as secondary metabolites, that act as defence factors against disturbs caused by pathogens and/or by adverse environmental conditions [1,2]. Among these, phenolic compounds like naphthoquinones represent one of the most important groups.

Naphthoquinones, which in nature commonly occur in the reduced and glycosidic forms [3], represent an important subgroup from the quinones families and exist in large amounts in the roots, leaves, bark and wood of plants from the Bignoniaceae, Droseraceae, Ebenaceae, Juglandaceae and Plumbaginaceae families [4]. The main interest in these compounds arises from their well-known and broad-range biological activities which include phytotoxic, insecticidal, antibacterial, fungicidal and antiviral activities [4-9]. Among all naphthoquinones, juglone (5-hydroxy-1,4-naphthoquinone) and plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) are arousing great interest due to the potential treatment of neurodegenerative disorders [10,11] and effective anti-carcinogenic activities [12-14]. Therefore, their advantageous applications as natural pesticides as well as additives in cosmetic and pharmacological preparations may be easily foreseen. The potential benefits of these compounds for human health led to an increasing interest on the search of efficient and selective extraction procedures which usually largely depend on the characteristics of the raw vegetal matrix and on the specific targeted naphthoquinones. Moreover, the toxicological and pharmacological effects of naphthoquinones have proven to be strongly dependent on the chemical structure of these molecules namely on the presence and position of hydroxyl groups

which can significantly change the physical-chemical-biological properties of these compounds [15-17]. These findings strengthen the need for the use of selective extraction procedures to obtain the envisaged compounds.

Conventional extraction methods, such as maceration or hot extraction, are widely used extraction procedures. However, and considering the importance of these substances for pharmaceutical, food and/or personal care products, supercritical fluids (SCFs) and namely supercritical carbon dioxide (scCO<sub>2</sub>) represent a promising non-toxic alternative extraction solvent. The design of most SCF processes requires the knowledge of the experimental and of the modelled equilibrium solubility data between the solutes of interest and the SCF solvent, at different temperatures and pressures.

In this work, the equilibrium solubility of 1,4-naphthoquinone and two derivatives (namely juglone and plumbagin) in scCO<sub>2</sub> was experimentally measured at 308.2 K, 318.2 K and 328.2K, in the pressure range from 9.0 up to 24.5 MPa, using a static analytical method coupled to a spectrophotometric quantification method. Experimental solubility data were correlated with three semi-empirical density-based models and with the Peng-Robinson cubic equation of state (PR-EOS) with the conventional van der Waals mixing and combining rules.

## MATERIALS AND METHODS

**Materials.** Carbon Dioxide (>99.998%) and ethanol (>99.5%) were obtained from Praxair and Panreac Química SA, respectively. 1,4-naphthoquinone (≥97%), juglone (≥95%) and plumbagin (≥95%) were obtained from Sigma-Aldrich, Portugal and were used without further purification.

**Experimental procedure.** Experimental naphthoquinones-scCO<sub>2</sub> equilibrium solubility data were measured by a static analytical method and using a solubility apparatus already described and validated in previous works [18-24]. The apparatus consists in a high-pressure stainless-steel equilibrium cell coupled to a sampling loop which is connected, through a six-port sampling valve, to previously calibrated volumes which include tubing lines, a glass trap (immersed in ice) and a stainless steel balloon (immersed in a water bath at room temperature). The cell is loaded with an excess amount of each naphthoquinone and a magnetic stirrer and placed into a thermostatic water bath, equipped with a temperature controller that maintains the experimental temperature within ± 0.1 K. The cell is pressurized with liquefied CO<sub>2</sub> until the desired experimental pressure is achieved at the chosen operational temperature. Pressure is measured by a high-pressure transducer. After pressure and temperature stabilization, the mixture is stirred for one hour followed by a stabilization period of 15 min. A sample is then taken from the cell into the sampling loop using the six-port sampling valve. The dissolved naphthoquinone is forced to precipitate into the cold glass trap after expanding the compressed mixture into the trap + stainless steel balloon system which was previously brought to sub-atmospheric pressure. The resulting pressure increase is measured using a calibrated high precision low-pressure transducer. In order to recover all the sampled naphthoquinones, ethanol is injected through the sample loop and the expansion lines and recollected in the same cold glass trap. The tubing lines are additionally cleaned/dried with fresh and slightly pressurized CO<sub>2</sub>.

**Analytical method.** The amount of solid drug which was solubilized in scCO<sub>2</sub>, at the employed pressure and temperature conditions, was quantified by spectrophotometric analysis using a UV/Vis spectrophotometer (Jasco V-530, Japan) and a previously determined calibration curve (in the range 2.5×10<sup>-3</sup> and 55×10<sup>-3</sup> mg/ml). The solute that was collected in each sampling step was diluted to a convenient ethanol volume and the absorbance of the resulting solutions was measured at a fixed wavelength of 332,

423 and 420 nm for 1,4-naphthoquinone, juglone and plumbagin, respectively. The amount of CO<sub>2</sub> in each sampling step was calculated using the Virial EOS (applied to pure CO<sub>2</sub>) as previously reported and explained [18-24]. All the prepared solutions were carefully stored and protected from light to avoid naphthoquinones degradation.

**Correlation of experimental solubility data.** Naphthoquinones experimental solubility data were correlated using three semi-empirical density-based models, namely the Chrastil [25], Bartle [26] and Méndez-Santiago-Teja [27] models. Additionally, the well known Peng-Robinson cubic equation of state (PR-EOS) [28] was also employed (with the classic van der Waals mixing and combining rules). Experimental and estimated solute critical and thermophysical properties were used to PR-EOS correlations.

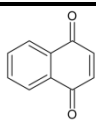
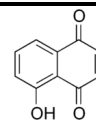
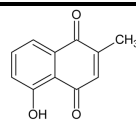
**Estimation of critical and thermophysical properties.** The critical pressure (P<sub>c</sub>) and critical temperature (T<sub>c</sub>) of juglone and plumbagin were calculated using the Wilson-Jasperson [29] method while the ones for 1,4-naphthoquinone were estimated by the Marrero and Gani [30] group contribution method. The Pitzer's acentric factor was estimated by the Ambrose-Walton corresponding states method [29] and the molar volume of naphthoquinones were estimated using the Fedors group contribution method [31]. Sublimation pressures for juglone and plumbagin were estimated by the Ambrose-Walton corresponding states method [29], assuming that the solids can be treated as sub-cooled liquids, while experimental data reported in the literature was used for 1,4-naphthoquinone [32]. Finally, the boiling temperatures for 1,4-naphthoquinone was estimated by the Marrero and Gani [30] group contribution method while the ones for juglone and plumbagin were estimated by the Stein and Brown method [33].

## RESULTS

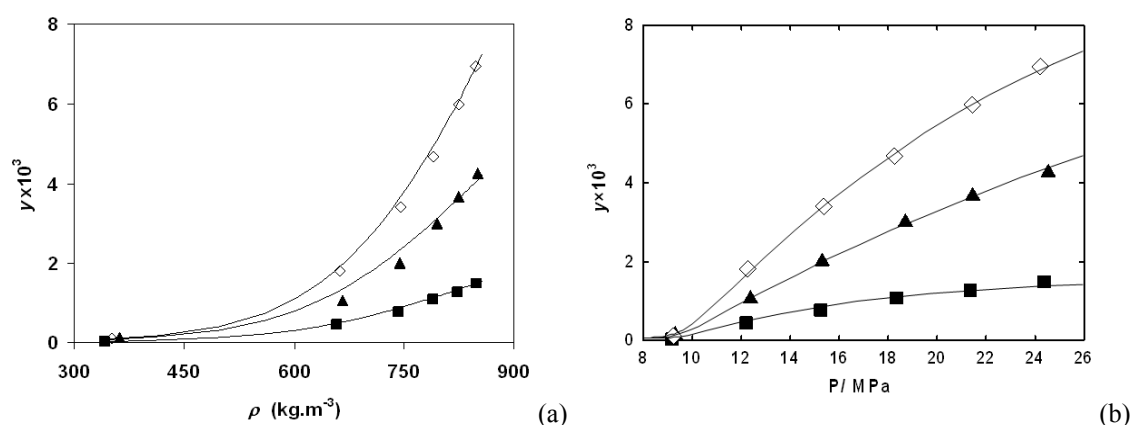
The equilibrium solubility of 1,4-naphthoquinone, juglone and plumbagin in scCO<sub>2</sub> was experimentally measured at 308.2 K, 318.2 K and 328.2 K, and in the pressure range from 9.0 up to 24.5 MPa. Each reported experimental data point is the average of, at least, three replicate measurements that lead to RSD values lower than 10 %. The solubility range observed for each solute as well as the relative standard deviations (RSD) for the mole fraction solubilities are presented in Table 1. The solubilities of all the solutes were found to increase with pressure for all isotherms. This is explained by the enhancement of the solute-solvent specific interactions that were favoured by the reduction of the intermolecular mean distance of the involved molecules. An example of the solute's solubility behaviour at 318.2 K is shown in Figure 1. According to the figure it can be seen that the experimental solubilities of these molecules follow the trend: plumbagin > 1,4-naphthoquinone > juglone. A comparison between the chemical structures of the three naphthoquinones shows that: i) when the hydroxyl group is "added" to the 1,4-naphthoquinone molecule (forming juglone), the solubility in scCO<sub>2</sub> decreases - due to the unfavourable hydroxyl/CO<sub>2</sub> interactions; and ii) when the methyl group is "added" to the juglone molecule (forming plumbagin), the solubility greatly increases and plumbagin becomes even more soluble in scCO<sub>2</sub> than 1,4-naphthoquinone - due to the favourable methyl/CO<sub>2</sub> interactions. This means that the low polarity methyl group contribution for the solubility overcomes the high polarity hydroxyl group unfavourable contribution.

Additionally, it was observed that the experimental solubilities of these substances follow the same trend as their sublimation pressures (for the three isotherms): as sublimation pressure increases, the corresponding solubilities will also increase [23,24]. This is a clear evidence that the solid sublimation pressure is a key factor in the solubilization of these solids into scCO<sub>2</sub>.

**Table 1:** Experimental and correlated data deviations for the solubility of 1,4-naphthoquinone, juglone and plumbagin in scCO<sub>2</sub>

Compound	1,4-Naphthoquinone	Juglone	Plumbagin
Chemical structure			
Solubility range	$5.0 \times 10^{-5}$ - $4.9 \times 10^{-3}$	$2.0 \times 10^{-5}$ - $1.6 \times 10^{-3}$	$5.0 \times 10^{-5}$ - $9.0 \times 10^{-3}$
Crossover region (MPa)	18 - 19	21 - 22	15 - 16
Mole fraction RSD (%)	0.6 - 8.5	0.8 - 7.4	0.2 - 10.9
Overall RSD (%)	2.6	3.4	2.6
	AARD obtained for different models (%)		
Chrastil	8.5	5.5	5.8
Bartle	10.1	6.7	10.5
Méndez-Santiago-Teja	10.8	6.4	10.3
PR-EOS-vdwl	8.7 - 12.9	4.2 - 13.1	5.9 - 20.8
PR-EOS-vdw2	6.1 - 9.5	4.1 - 10.1	5.1 - 12.2

The crossover effect, which is usually found for the solubility of organic solids in scCO<sub>2</sub>, was also observed for all the studied solids, with crossover regions located between 15 and 22 MPa as shown in Table 1. It was also observed that the pressure at which the crossover point occurs increases when solubility decreases, according to the following sequence: plumbagin ( $15 \pm 1$  MPa) < 1,4-naphthoquinone ( $18 \pm 1$  MPa) < juglone ( $21 \pm 1$  MPa) [23,24]. This retrograde solubility behaviour is known to be the result of the opposite effects of temperature on the SCF density and on the sublimation pressure of the solid solutes. At lower pressures, the decrease in the scCO<sub>2</sub> density with the increasing temperature is the dominant phenomenon, leading to a decrease of the solvent capacity and of solute solubility. Above the crossover region (the point at which the three isotherms intercept and cross over each other), the effect of temperature on the solute vapour pressure begins to prevail and thus the solute solubility will increase with increasing temperature.



**Figure 1:** (a) Naphthoquinones solubility ( $y$ ) as a function of pure scCO<sub>2</sub> density ( $\rho$ , kg m<sup>-3</sup>) at 318.2 K; (b) Solubility of naphthoquinones in scCO<sub>2</sub> at 318.2 K. Experimental: (■) Juglone; (▲) 1,4-Naphthoquinone; (◇) Plumbagin; Full lines represent the correlation obtained with the Bartle model (a) and with the PR-EOS model and the vdW2 mixing and combining rules (b).

The correlation results obtained using three density-based correlations (Chrastil, Bartle and Méndez-Santiago-Teja) and the PR-EOS with two adjusted parameters (vdW2) are given in Table 1 and represented in Figure 1a for the isotherm at 318.2 K. Good AARD correlation results were obtained with the density based models for all solid substances (between 5.5% and 10.8%), even at lower pressure/density which usually present the larger deviations for the fitted curves. The PR-EOS model was also able to accurately describe the pressure dependence of the solubility data for all isotherms as well as the already referred retrograde solubility behaviour (Figure 1b). As expected significantly better correlated results were obtained when using two instead of one adjustable parameter, mainly for plumbagin at 328.2 K, for which AARD values decreased from 20.8 to 12.2 %. Although not deeply discussed in the present work, it was previously reported that the choice of the proper critical and thermophysical solute's properties is crucial to obtain good PR-EOS correlation results [23,24]. Notice that the estimation methods that originated the best fit results were the ones previously presented on the estimation of critical and thermophysical properties section.

## CONCLUSIONS

The solid solubilities of 1,4-naphthoquinones derivatives in scCO<sub>2</sub> were experimentally measured using a static analytical method at 308.2, 318.2 and 328.2 K, and for pressures between 9.0 and 24.5 MPa. Experimental solubility data were correlated with three density-based models (Chrastil, Bartle and Méndez-Santiago-Teja models) and with the PR-EOS together with the conventional van der Waals mixing and combining rules. Employed semi-empirical density-based models led to AARD values lower than 11% (for all solid compounds). Good correlation results were also achieved when using the PR-vdW2 model (with AARD values between 4.0% and 12.2% for all substances and for different isotherms), despite the fact that the adequate choice of the employed critical and thermophysical properties estimation methods was critical for the obtained PR-EOS correlation results. The obtained experimental and correlated data is important for the design and optimization of supercritical fluid processes and technologies, including the extraction of naphthoquinones from different raw materials, their processability to increase bioavailability and their impregnation/deposition into solid matrices intended for controlled delivery applications.

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