# Solubility of 1,3-Dimethyl-7*H*-purine-2,6-dione (Theophylline) in Supercritical Carbon Dioxide

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This contribution provides complementary experimental data of solubility ($y_2$, molar fraction) of solid 1,3-dimethyl-7*H*-purine-2,6-dione (theophylline) in supercritical $CO_2$ as a function of temperature (313 K $\leq T \leq$ 333 K) and pressure (10 MPa $\leq P \leq$ 44 MPa). A static-analytic methodology was used with standard deviations from average solubility measurements of $\leq$ 38 % and with estimated inherit errors $\leq$ 25 %. The solubility of theophylline increased with the $CO_2$ density from $0.33 \cdot 10^{-5}$ mol·mol$^{-1}$ at 797.2 kg·m$^{-3}$ (313 K, 16 MPa) to $3.3 \cdot 10^{-5}$ mol·mol$^{-1}$ at 890.2 kg·m$^{-3}$ (333 K, 40 MPa). The solubility increased with pressure and temperature. Experimental solubilities were correlated with a density-based model with three adjustable parameters, which was valid for solvent densities in the range of (400 to 950) kg·m$^{-3}$.

## 1. Introduction

1,3-Dimethyl-7*H*-purine-2,6-dione (theophylline) with a molecular structure illustrated in Figure 1 is an alkaloid that belongs to the family of methylxanthines, 1,3,7-trimethylpurine-2,6-dione hydrate (caffeine) being the most known constituent. Theophylline is found naturally in foods such as chocolate and coffee, and due to its relevant physiological effect on human organism as a bronchodilator and muscle relaxant, theophylline has received great attention from food and pharmaceutical industries.[1]

The recovery or purification of high-value active principles (such as theophylline) from biological substrate using carbon dioxide ($CO_2$) at high pressure as solvent or altisolvent is an alternative to organic liquid solvents due to its inertness, nontoxicity, nonflammability, and low cost.[2−4] Extractions or purifications carried out with supercritical $CO_2$ (SC−$CO_2$) are environmentally friendly processes that can selectively isolate high-purity bioactive compounds and avoid deleterious chemical reactions. Solute solubility and extraction selectivity are thermodynamics constraints that have to be established to optimize the design and operational conditions for processes with SC−$CO_2$.

Johannsen and Brunner[5] measured the solubility of pure theophylline in SC−$CO_2$ at 313 K, 323 K, and 333 K, and pressures ranged from (20 to 35) MPa using a static-analytic method with direct coupling of the equilibrium cell to a high-pressure chromatographic system. Solubilities measured indicated that the content of theophylline in the $CO_2$-rich phase increased with temperature at constant pressure.

The objective of this work was to contribute with complementary experimental solubility data of theophylline in SC−$CO_2$ and with a correlation to represent the solubility values in terms of temperature and $CO_2$ density.

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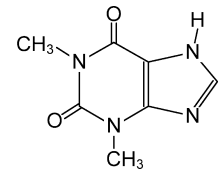

**Figure 1.** Chemical structure of 1,3-dimethyl-7*H*-purine-2,6-dione (theophylline).

## 2. Experimental Section

***2.1. Material.*** 1,3-Dimethyl-7*H*-purine-2,6-dione (theophylline, ≥ 99 %, anhydrous powder, CAS # 58-55-9) was from Sigma-Aldrich (St. Louis, MO), and carbon dioxide (99.99 % pure) was from AGA-Chile S.A. For the HPLC analysis, the reverse phase C18 column (LiCroCART), pro-analysis acetic acid, and HPLC-grade acetonitrile were from Merck (Darmstadt, Germany).

***2.2. Method.*** The solubility of solid theophylline in SC−$CO_2$ was measured at 313 K, 323 K, and 333 K and over a pressure range from (10 to 44) MPa using a static-analytic method. The experimental apparatus and procedure were described in detail by de la Fuente et al.[6] A high-pressure 50 cm$^3$ capacity equilibrium view cell (TharTech, Pittsburgh, PA) with two windows (2.22 cm diameter) was coupled to a high-performance liquid chromatograph (HPLC) system with a photodiode array detector (Hitachi LaChrom, Japan). A six-port high-pressure valve with a 20 $\mu$L loop (Rheodyne 7010, Rohnert Park, CA) was used to measure the composition of the $CO_2$-rich phase at equilibrium. The apparatus could be operated up to 363 K and 40 MPa, and the estimated uncertainties in the temperature and pressure were < 0.5 K and < 0.2 MPa, respectively.

To determine the solubility of theophylline (2) in SC−$CO_2$ (1), approximately 0.2 g of solid solute and the $CO_2$ were charged into the equilibrium cell. Temperature and pressure were adjusted according to the desired values. After 12 h of stirring, when the equilibrium was reached, the $CO_2$-rich phase was sampled using the six-port high-pressure valve, and 20 $\mu$L was injected into the HPLC system. The theophylline molar fraction in the $CO_2$-rich phase ($y_2$) was quantified according to eq 1.[6]

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**Table 1. Solubility Isotherms for 1,3-Dimethyl-7*H*-purine-2,6-dione (Theophylline) in SC−$CO_2$[a]**

| $T$/K = 313 | | $T$/K = 323 | | $T$/K = 333 | |
|---|---|---|---|---|---|
| $P$ | $y_2 \cdot 10^5$ | $P$ | $y_2 \cdot 10^5$ | $P$ | $y_2 \cdot 10^5$ |
| MPa | mol·mol$^{-1}$ | MPa | mol·mol$^{-1}$ | MPa | mol·mol$^{-1}$ |
| 15.7 | 0.53 ± 0.11 | 10.1 | 0.41 ± 0.04 | 15.2 | 2.5 ± 0.57 |
| 16.2 | 0.33 ± 0.03 | 14.7 | 0.97 ± 0.34 | 15.5 | 2.7 ± 0.04 |
| 16.5 | 0.35 ± 0.04 | 22.9 | 1.7 ± 0.53 | 15.8 | 2.7 ± 0.99 |
| 21.5 | 0.66 ± 0.13 | 24.3 | 1.6 ± 0.54 | 19.9 | 2.5 ± 0.95 |
| 24.3 | 0.48 ± 0.14 | 29.6 | 1.7 ± 0.14 | 35.3 | 3.2 ± 0.52 |
| 26.4 | 0.87 ± 0.21 | 34.7 | 2.5 ± 0.11 | 39.3 | 3.3 ± 1.0 |
| 29.7 | 0.95 ± 0.14 | 39.1 | 2.6 ± 0.82 | 40.0 | 3.3 ± 1.2 |
| 29.8 | 1.1 ± 0.30 | 41.2 | 2.6 ± 0.79 | | |
| 33.4 | 1.1 ± 0.13 | 40.2 | 3.0 ± 0.12 | | |
| 37.3 | 1.8 ± 0.13 | 44.1 | 2.8 ± 0.47 | | |

[a] Molar fraction of solute in a saturated $CO_2$ phase ($y_2$), calculated from an arithmetic average of three to five injections at each condition, as a function of system temperature ($T$) and pressure ($P$). Standard deviations for each point are also included.

$$y_2 = \left(\frac{A_{FP}}{A_S}\right) \cdot \left(\frac{V_{CL}}{V_{FP}}\right) \cdot \left(\frac{MW_1}{\rho_1}\right) \cdot C_S \qquad (1)$$

where $A_{FP}$ and $A_S$ are the chromatographic peak areas for the fluid $CO_2$-rich phase and standard injection, respectively; $V_{CL}$ is the volume of the HPLC sample loop injector (20 $\mu$L); $V_{FP}$ is the volume of sample of equilibrated fluid $CO_2$-rich phase (20 $\mu$L); $MW_1$ is the molecular weight of $CO_2$ (44 g·mol$^{-1}$); $\rho_1$ is the density of $CO_2$ at the test temperature and pressure conditions of the cell (calculated with NIST Standard Database v5.0 [13]); and $C_S$ is the concentration of solute in a standard solution employed for calibration of the HPLC. A sampling procedure was repeated for each value of $y_2$, and between three and five HPLC injections were analyzed. The values of $y_2$ informed in this work were calculated from an arithmetic average of these repetitions, at each condition.

HPLC analyses were performed according to the isocratic method of Huck et al.[7] The separation was carried out at 298 K using a reverse phase C18 column (LiCroCART, Merck, Darmstadt, Germany) and 0.8 cm$^3$·min$^{-1}$ of a 98:2 (v/v) mixture of acetic acid and acetonitrile as the mobile phase. Detection was done at 280 nm.

## 3. Results and Discussion

Table 1 reports molar fractions of theophylline in SC−$CO_2$ as a function of temperature and pressure. Values of solubility started from $y_2 = 0.33 \cdot 10^{-5}$ mol·mol$^{-1}$ at $T = 313$ K and $P = 16$ MPa and reached $y_2 = 3.3 \cdot 10^{-5}$ mol·mol$^{-1}$ at $T = 333$ K and $P = 40$ MPa. At isothermal condition, the solubility increased with pressure due to the increase in the density (solvent power) of $CO_2$. Similarly, the solubility increased with temperature at isobaric condition, indicating that the reduction in density was surpassed by the increase in the vapor pressure of theophylline. In addition, in Table 1 are included individual values of standard deviations for each point, calculated from the arithmetic average of between three and five solubility measurements. Deviations were $0.03 \cdot 10^{-5} \le \Delta y_2 \le 1.24 \cdot 10^{-5}$ mol·mol$^{-1}$ (equivalent to $1.5 \le 100 \cdot \Delta y_2/y_2 \le 38$ %), and the tendency observed was to increase the deviations as the temperature increased. The experimental inherit relative error in the solubility measurements was estimated according to the information available for the experimental apparatus. The uncertainties adopted for the parameters included in eq 1 were: $\Delta(A_{FP}, A_S) < 35000$ units; $\Delta C_S < 0.0005$ mol·cm$^{-3}$; $\Delta\rho_1 < 0.04$ kg·m$^{-3}$ (with $\partial\rho_1/\partial T < 0.02$ kg·m$^{-3}$/K and $\partial\rho_1/\partial P < 0.12$ kg·m$^{-3}$/MPa); $\Delta(V_{FP}, V_S) < 2$ $\mu$L. The relative experimental inherit errors for the average values of $y_2$ were estimated to be ≤ 25 %. The indicative contributions to the inherit error of the uncertainties of parameters included in eq 1 were: volumes of sample loops (HPLC injector and equilibrium cell), ∼80 %; chromatographic peak area, ∼15 %; density of $CO_2$, ∼4 %; concentration of solute in the standard solution, ∼1 %. The error estimated was approximately constant with temperature and pressure, taking into account that the uncertainty in the size loops was constant and the predominant contribution to the total value of the error.

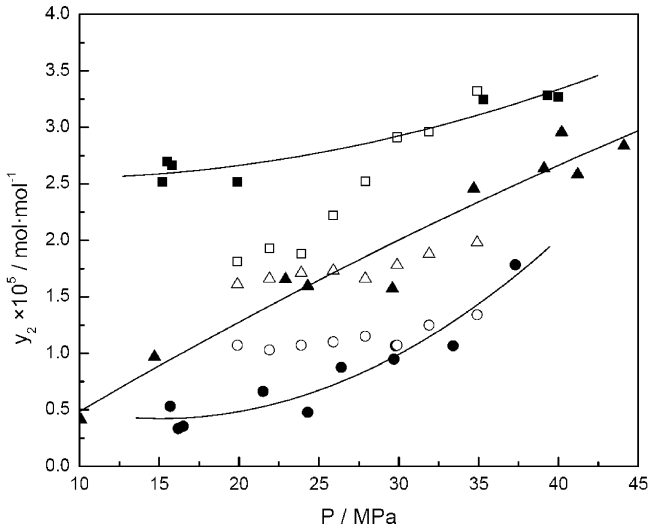


**Figure 2.** Solubility isotherms for theophylline (1,3-dimethylxanthine) in SC−$CO_2$ as a function of system pressure at ●, 313 K; ▲, 323 K; and ■, 333 K. Lines represent trends, and open symbols represent the results reported by Johannsen and Brunner[5] at the same temperatures.

Figure 2 shows the isothermal molar fraction of theophylline measured in this study as a function of equilibrium pressure, represented as closed symbols connected by trend lines. As a comparison, results reported by Johannsen and Brunner[5] were also included in this figure (open symbols). At 313 K, solubilities from ref 5 were higher than the values listed in Table 1 starting by a factor of 1.2 at 19.9 MPa and decreasing as the pressure increased up to 29.9 MPa where the deviations were not significant. For the isothermal of 323 K at (23.9 and 25.9) MPa, solubility values from this work and from ref 5 were similar. At pressures < 23.9 MPa, the solubilities from ref 5 were higher by a factor ≤ 1.2, and this behavior shifted at pressure > 25.9 MPa where the results from this work were higher by a factor ≤ 1.2. At 333 K, the molar fractions of theophylline reported in Table 1 were higher than the values from ref 5 for pressures in the range of (19.9 to 27.9) MPa by a factor in the range of 1.5 to 1.1, respectively. At pressures > 27.9 MPa, the solubilities from both data source were comparable. In summary, from 24

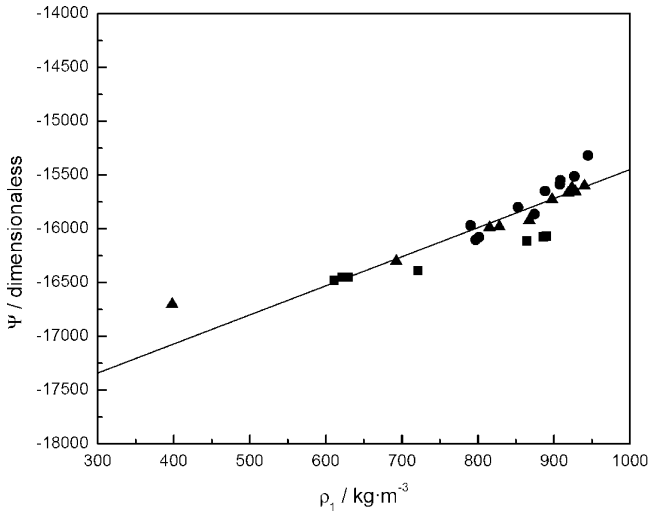


**Figure 3.** Solubility function $\Psi$ [$= \ln(y_2P) - C$] versus solvent density ($\rho_1$) showing the collapse of experimental isotherms for 1,3-dimethyl-7*H*-purine-2,6-dione (theophylline) in $CO_2$ to a single best-fit line. Symbols represents experimental data points at ●, 313 K; ▲, 323 K; and ■, 333 K, and the line corresponds to correlation of data points using eq 2.

experimental solubility points compared, 8 results were equivalent; in 9 points, solubilities measured in this work were higher than in ref 5; and in 7 points, results from ref 5 were higher than the values reported by this work.

Equation 2, the model proposed by Méndez-Santiago and Teja,[8] was utilized to correlate theophylline molar fractions listed in Table 1 as a function of solvent density.

$$\Psi(T, P, y_2) = T[\ln(y_2P) - C] = A + B\rho_1 \qquad (2)$$

where $\Psi$ represents the solubility function, and $A$, $B$, and $C$ are the model parameters. The optimal fitting parameters are presented in eq 3 that correlates the experimental solubility values measured in this work, with an average absolute deviation (AAD) value of 87.7 %.

$$y_2 = \frac{1}{P}\exp\left[\frac{-18153 + 2.7037\rho_1}{T} + 43.897\right] \qquad (3)$$

Figure 3 compares values of $\Psi$ calculated from experimental data ($= T\cdot[\ln(y_2\cdot P) - C]$) and from eq 2 ($= A + B\cdot\rho_1$) versus $\rho_1$, for a range of (400 to 950) kg·m$^{-3}$ in $CO_2$ density. Despite the scattering in Figure 3, solubility values measured for the three isotherms collapsed to a single line. This suggested reasonable autoconsistency for the experimental data, based on Méndez-Santiago and Teja,[8] who claimed that eq 2 can be employed as an autoconsistency test to identify questionable data. In addition, according to de la Fuente,[9] the relatively large value of AAD can be presented under conditions where values of solubility are extremely small as occur for theophylline [(0.33 to 3.3)·10$^{-5}$ mol·mol$^{-1}$]. Equation 3 could be utilized to estimate the molar fraction of theophylline in $CO_2$ for temperatures and pressures in the range of (313 to 333) K and (15.7 to 44.1) MPa, with differences between the predicted and the actual data by a factor in the range of 0.31 to 2.7 based on the scattering observed.

## 4. Conclusions

The molar fraction of 1,3-dimethyl-7*H*-purine-2,6-dione (theophylline) in SC−$CO_2$ has been measured for the isotherms 313 K, 323 K, and 333 K and pressures in the range of (15.7 to 44.1) MPa using a static-analytic method with standard deviations from average solubility measurements of ≤ 38 % and inherit errors ≤ 25 %. Solubility values obtained in this work, $0.33\cdot10^{-5} \le y_2 \le 3.3\cdot10^{-5}$ (mol·mol$^{-1}$), were compared with data available from the literature, and appropriate consistency was observed. A density-based model was utilized to correlate the solubility as a function of temperature and pressure for 400 ≤ $\rho_1$ ≤ 950 (kg·m$^{-3}$) with an average absolute deviation of 87.7 %.

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