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# Solubility of Corosolic Acid in Supercritical Carbon Dioxide and Its Representation Using Density-Based Correlations

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**ABSTRACT:** The solid solubilities of 2α,3β-dihydroxyurs-12-en-28-oic acid (corosolic acid) in supercritical carbon dioxide (SC-$CO_2$) have been measured using a dynamic method at (308.15, 313.15, 323.15, and 333.15) K over a pressure range of (8 to 30) MPa. Prior to solubility measurements, the accuracy of the experimental apparatus was examined by measuring solubilities of squalene in SC-$CO_2$ and comparing them with literature. The corosolic acid solubilities ranged from a corosolic acid mole fraction of $3.28 \cdot 10^{-11}$ at 333.15 K and 8 MPa to $7.43 \cdot 10^{-2}$ at 333.15 K and 30 MPa. The del Valle and Aguilera and Méndez-Santiago and Teja (density-based) models were used to correlate the experimental data. The calculated solubilities showed good agreement with the experimental data in the temperature and pressure ranges studied.

## INTRODUCTION

Diabetes mellitus is one of the world's major health problems. In the developed countries, diabetes mellitus patients may have proper treatments, such as controlled low sugar diet and administration of insulin and/or hypoglycaemic agents. Unfortunately, patients in the developing countries may not be able to afford these methods due to their socio-economic conditions.[1] This fact has prompted to the high prevalence of noncompliance observed in minority, disadvantaged communities in the developed countries, and rural folks in the developing countries.[2] In addition, medical centers are not always available within the reach of these population groups, and rural folks prefer to seek traditional medications rather than the modern ones. In Asia and South America, the development and use of inexpensive and easily accessible phytomedicines from plants of the genus *Syzygium* in the treatment of diabetes mellitus is encouraged to overcome these problems.[3]

Corosolic acid (2α,3β-dihydroxyurs-12-en-28-oic acid, IUPAC 1*S*,2*R*,4a*S*,6a*R*,6a*S*,6b*R*,10*R*,11*R*,12a*R*,14b*R*)-10,11-dihydroxy-1,2,6a,6b,9,9,12a-heptamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1*H*-picene-4a-carboxylic acid, CAS Registry No. 4547-24-4, Figure 1) is one of the secondary metabolites contained in the leaf of *Syzygium cumini*, which has antidiabetic activity.[4,5] Miura and coworkers[6] reported an acute hypoglycemic effect of corosolic acid, which was shown by an increase in GLUT4 translocation in mouse muscle. In addition, they also found that corosolic acid promotes glucose metabolism by reducing insulin resistance. The action of insulin is mediated by tyrosine phosphorylation and initiated by the binding of insulin to the insulin receptor. Corosolic acid may act as an insulin sensitizer, enhancing insulin receptor B phosphorylation indirectly by inhibiting certain nonreceptor protein tyrosine phosphatases.[7] Latter, Fukushima et al.[5] have discovered corosolic acid ability to reduce post challenge plasma glucose levels in humans. Another study reported that corosolic acid inhibited gluconeogenesis by increasing the production of the gluconeogenic intermediate fructose-2,6-bisphosphate in isolated hepatocytes.[8,9] Bhat et al.[10] have demonstrated various levels antidiabetic activity of corosolic acid obtained from aqueous and organic solvent extraction of *S. cumini* plant leaf. No antidiabetic activity was shown by aqueous and methanol extracts, while chloroform extract showed good antidiabetic activity. In addition, it has also been reported that in general, secondary metabolites are highly unstable depending on factors like temperature, light, oxygen, and solvent used in the extraction.[11−14]

Supercritical fluid extraction (SFE) has shown its superiority over conventional solvent extraction techniques. The absence of both light and air during the extraction process along with relatively mild operating temperatures can reduce the tendency of degradations, while the use of $CO_2$ as solvent allows working in an environmentally clean media. The possibility of adjusting the supercritical solvent power based on its density is the key factor in the extraction process. Then, information of solubility of corosolic acid in the supercritical condition is necessary for the analyses and optimization of corosolic acid extraction and purification units.

This article reports experimental measurements of corosolic acid solubilities in supercritical $CO_2$, which, to the best of the author knowledge, has not been reported in the literature until present. Four density-based correlations on the solubility of solute in supercritical $CO_2$ were used in this work to represent the solubility data.

## EXPERIMENTAL METHODS

**Materials.** Corosolic acid ($C_{30}H_{40}O_4$, purity > 0.96 mass fraction) was purchased from Dalian GreenPeak Biotech Co. Ltd. China and used as received without any further treatments. Deionized water, squalene (Fluka Chemie, high-performance liquid chromatography (HPLC) grade, purity ≥ 0.97 mass

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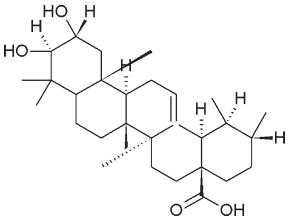


**Figure 1.** Structure of the corosolic acid molecule.

fraction) and anhydrous methanol (HPLC grade, purity ≥ 0.998 mass fraction) were purchased from Sigma-Aldrich Pte. Ltd. Singapore and stored at 291.15 K. Orthophosphoric acid (HPLC grade, purity ≥ 0.998 mass fraction) was purchased from Merck, Germany. Carbon dioxide (0.99 mass fraction) was provided in liquid form in cylinders equipped with a diptube, and it was further purified by passing it through a 2 $\mu$m filter (PT. Aneka Gas, Indonesia).

**Procedure.** The solubility of corosolic acid in supercritical carbon dioxide (SC-$CO_2$) was determined using a dynamic method. This method is based on the assumption that the solute−solvent system reaches equilibrium as the solvent flows over the solute.[15] Preliminary solubility measurements were carried out by delivering carbon dioxide (liquid based) into the equilibrium cell at flow rates ranging from ($6.67 \cdot 10^{-9}$ to $3.33 \cdot 10^{-8}$) $m^3 \cdot s^{-1}$ for about 4 h. Variation of the flow rate within this range was found to have no effect on the observed solubilities, thereby confirming that equilibrium between the solid phase and the fluid phase was achieved at these flow rates and that there were no mass-transfer limitations. Hence, the supercritical carbon dioxide solvent leaving the extraction vessel was saturated with the solute and equilibrium between the solid and the supercritical phase was achieved. The solubility of corosolic acid in SC-$CO_2$ was measured using the same apparatus that was described in the literature[16] and explained briefly here (see Figure 2). The main apparatus was a $1.6 \cdot 10^{-5}$ $m^3$ high-pressure stainless steel equilibrium cell (H) (10 mm inner diameter × 210 mm length) immersed in a water bath (A) controlled by an electrical heater (D) (model DC10, Thermo Haake) to within ± 0.1 K uncertainty. The system pressure was indicated by a pressure transducer (G) (model PDCR 961, Druck). Its uncertainty was ± 0.01 MPa in the pressure range of (8 to 30) MPa, while an HPLC pump (C) (model LC-6A, Shimadzu Co.) was used to compress the liquid $CO_2$ and to deliver it at a constant volumetric flow rate of $1.67 \cdot 10^{-8}$ $m^3 \cdot s^{-1}$. The $CO_2$ was depressurised to atmospheric by a back pressure regulator/BPR (J) (model BP 1580-81, JASCO) with uncertainty of ± 0.01 MPa and the amount of $CO_2$ consumed was quantified using a wet gas meter (L) (model DM 3A, Alaxander Wright & Co.) within the time of measurement to calculate the expanded gas flow rate (± $1.0 \cdot 10^{-10}$ $m^3 \cdot s^{-1}$). The solute collected was gravimetrically determined using an analytical balance (model AG 204, Mettler Toledo) with an accuracy of ± 0.0001 g. However, when the amount of corosolic acid was not possible to be measured using the analytical balance, then the HPLC analysis was applied to quantify the exact amount of corosolic acid in the vial. It was done by washing the vial using a solution, which was a mixture of methanol and aqueous orthophosphoric acid. The concentrations of methanol and orthophosphoric acid in the solution were 27.39 $mol \cdot kg^{1-}$ and $1.25 \cdot 10^{-2}$ $mol \cdot kg^{1-}$, respectively. The collected washing liquid was then subjected to corosolic acid analysis using the HPLC method.[16]

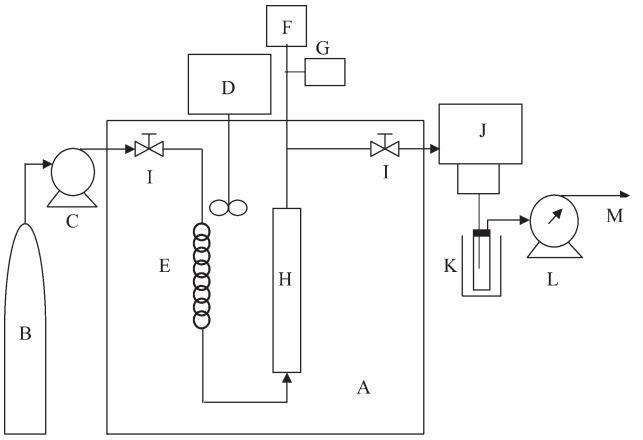


**Figure 2.** Schematic diagram of isothermal solubility measurement apparatus. A, constant temperature water bath; B, $CO_2$ tank; C, $CO_2$ pump; D, controlled electric heater with water circulation pump; E, heating coil; F, temperature indicator; G, pressure transducer; H, equilibrium cell; I, micrometer valve; J, backpressure regulator; K, cold trap; L, wet gas meter, and M, vent.

This method was valid for corosolic acid concentration ranged from (4.5 to 400) $g \cdot m^{-3}$. Prior to solubility experiments, the equilibrium cell was washed with ethanol, dried in an electric oven at 373.15 K for one hour, and cooled in a desiccator. On the other hand, the tube lines, fittings, and connections were cleaned up by flushing liquid $CO_2$ at $3.33 \cdot 10^{-8}$ $m^3 \cdot s^{-1}$ for about 900 s to totally remove dirt and solute.

A total of 10 g of corosolic acid was charged into a stainless-steel equilibrium cell by arranging it into alternative layers of about 500 mg and glass beads (30 to 60) mesh, with glass wool at either end. The function of the packing was to improve corosolic acid−SC-$CO_2$ contact, reduce channeling of the SC-$CO_2$, and prevent corosolic acid entrainment.[17] The equilibrium cell was heated to the prescribed temperature value by immersing it in a controlled temperature water bath. The liquid $CO_2$ was pressurized and delivered at a flow rate of $1.67 \cdot 10^{-8}$ $m^3 \cdot s^{-1}$ using a reciprocating HPLC pump to pass through a heating coil immersed in the water bath and enabled $CO_2$ to reach the desired temperature before entering the equilibrium cell at a constant pressure. The system pressure was maintained by a back-pressure regulator, and the value was indicated by a test gauge. The saturated SC-$CO_2$ was then depressurized by the BPR to atmospheric, and the dissolved compound was trapped and collected in a vial cooled in a table salt solution of 277.15 K after exiting from the BPR. To prevent precipitation of the solute between BPR and the trap, short tape heater tubing and the BPR-heater-jacket temperature of 333.15 K were used. The weight loss of the cell was also compared with the weight of the collected solute to ensure at least 96 % of trapping efficiency during solubility measurements. The solubility was defined as the mole fraction of the solutes in the expanded $CO_2$. The corosolic acid solubility experiments were performed at (308.15, 313.15, 323.15, and 333.15) K and at pressures ranging from (8 to 30) MPa. The reproducibility of the measurements was confirmed by making triplicates of each measurement to obtain reliable solubility values, and an average value was given. The estimated uncertainty of the solubility values based on error analysis and repeated observations was within 2 %. To verify the accuracy of the solubility measurements and the method employed, the solubility of squalene in SC-$CO_2$ had been measured and compared with the literature values.

## RESULTS AND DISCUSSION

**Apparatus Validation.** It was imperative for the author to ensure that the experimental technique was adequate. However, no literature values for corosolic acid solubility in SC-$CO_2$ could be found. Fortunately, the solubility of a close compound in the same homologous series (i.e., squalene) in SC-$CO_2$ is available in the literature, which could have served as an indirect way to validate the results. Experiments were performed at (323.15 and 333.15) K and at pressures of (10 to 27.5) MPa. The procedure was identical to that used for the corosolic acid experiments, including equilibrium determination and runs in triplicate. Table 1 presents and compares the experimental solubility of pure squalene in SC-$CO_2$ (grams of squalene per kilograms of $CO_2$) at pressures of (10 to 27.5) MPa obtained from this work and those reported by Catchpole and von Kamp.[18] The experimental results show good agreement with the literature, where the solubility values obtained in this work are almost equal with those reported in the literature. These results validate the experimental method used in this study.

**Solubility Data.** The solubility values of corosolic acid in pure SC-$CO_2$ obtained in this work are summarized in Table 2 and plotted in Figure 3. Most of the $CO_2$ density values were obtained from the National Institute of Standards and Technology (NIST) fluid property database.[19] When the $CO_2$ density value was not readily available, its value was then predicted using REFPROP program available in the NIST database. In general, as pressure increased at constant temperature, the solubility of corosolic acid in SC-$CO_2$ also increased. This was expected because an increase in SC-$CO_2$ pressure at a constant temperature results in increasing its density and thereby its solvent strength (ability of a solvent to dissolves solute).[20] The increases of $CO_2$ density with pressure causing a decrease of the intermolecular distances which increases the solute−solvent interaction. Therefore, at lower pressures, corosolic acid solubility in SC-$CO_2$ at 333.15 K is the lowest. In addition, temperature also affects the solute vapor pressure, the solvent density, and the intermolecular interactions in the fluid phase. At a constant pressure, an increase in temperature will reduce the ability of SC-$CO_2$ to dissolve corosolic acid due to the reduction of SC-$CO_2$ density.[20] On the other hand, an increase in temperature will increase the vapor pressure of corosolic acid, which leads to an increase in the dissolution of corosolic acid in SC-$CO_2$. Therefore, it is not surprising that a crossover pressure occurs between at about 20.0 MPa. Such retrograde behavior has also been reported before for different organic compounds.[21,22] The crossover phenomena could be attributed to the competitions between solute's vapor pressure and solvent's density, whose temperature dependences are in opposite directions. At the crossover point, the effects of solute vapor pressure and solvent density on solid solubility balance each other. From the crossover

**Table 1. Solubility of Squalene in $CO_2$**

| | 323.15 K/g·kg$^{-1}$ | | 333.15 K/g·kg$^{-1}$ | |
|---|---|---|---|---|
| $p$/MPa | Catchpole and von Kamp [17] | this work | Catchpole and von Kamp[17] | this work |
| 10.0 | 0.27 | 0.26 | | |
| 12.0 | | 4.42 | | |
| 15.0 | 10.63 | 10.65 | | 3.12 |
| 17.5 | 17.38 | | | |
| 18.0 | | 18.91 | | 12.60 |
| 20.0 | 23.84 | 23.79 | | 19.01 |
| 22.5 | 30.12 | | 27.05 | |
| 25.0 | | | 34.65 | 34.73 |
| 27.5 | | | 43.00 | |

**Table 2. Mole Fraction Solubility ($x_2$) of Corosolic Acid in Carbon Dioxide in the Temperature Range (308.15 to 333.15) K**

| $p$/MPa | $10^2 \cdot (x_2)$ |
|---|---|
| 308.15 K | |
| 8.00 | $6.36 \cdot 10^{-5}$ |
| 10.00 | $1.86 \cdot 10^{-1}$ |
| 12.00 | 2.06 |
| 15.00 | 3.42 |
| 16.00 | 3.71 |
| 18.00 | 4.17 |
| 20.00 | 4.54 |
| 25.00 | 4.85 |
| 28.00 | 4.92 |
| 30.00 | 4.95 |
| 313.15 K | |
| 8.00 | $1.79 \cdot 10^{-4}$ |
| 10.00 | $1.13 \cdot 10^{-1}$ |
| 12.00 | 1.08 |
| 15.00 | 3.00 |
| 16.00 | 3.47 |
| 18.00 | 4.07 |
| 20.00 | 4.56 |
| 25.00 | 5.24 |
| 28.00 | 5.41 |
| 30.00 | 5.48 |
| 323.15 K | |
| 8.00 | $4.18 \cdot 10^{-8}$ |
| 10.00 | $9.37 \cdot 10^{-5}$ |
| 12.00 | $1.91 \cdot 10^{-1}$ |
| 15.00 | 1.22 |
| 16.00 | 1.98 |
| 18.00 | 3.47 |
| 20.00 | 4.54 |
| 25.00 | 5.91 |
| 28.00 | 6.31 |
| 30.00 | 6.51 |
| 333.15 K | |
| 8.00 | $3.28 \cdot 10^{-9}$ |
| 10.00 | $7.58 \cdot 10^{-7}$ |
| 12.00 | $5.64 \cdot 10^{-4}$ |
| 15.00 | $1.69 \cdot 10^{-1}$ |
| 16.00 | $4.08 \cdot 10^{-1}$ |
| 18.00 | 1.54 |
| 20.00 | 4.55 |
| 25.00 | 6.30 |
| 28.00 | 7.10 |
| 30.00 | 7.43 |

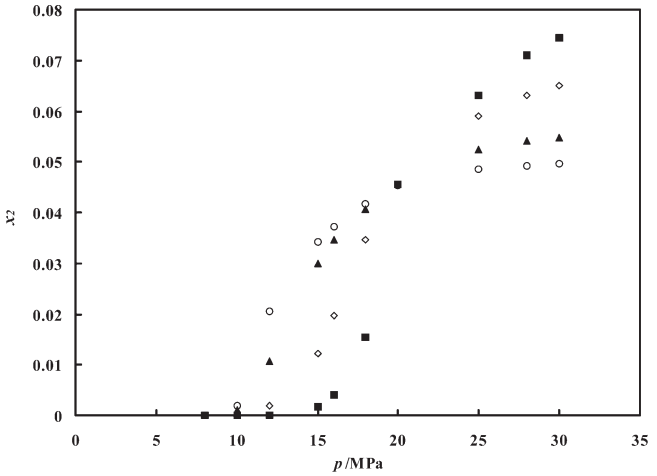


**Figure 3.** Mole fraction solubility ($x_2$) of corosolic acid in supercritical carbon dioxide at elevated pressure: ○, 308.15 K; ▲, 313.15 K; ◇, 323.15 K; ■, 333.15 K.

pressure point and upward, the solubilities of corosolic acid in SC-$CO_2$ at 333.15 K were higher than at any other temperatures in this study. This phenomenon indicated that the vapor pressure of corosolic acid began to have a dominant effect on solubility.[17,23]

**Modeling.** There are two most common approaches in the modeling of solubility of solute in supercritical fluid, namely, the so-called semiempirical methods and the equation-of-state (EOS) methods. Considering the calculation simplicity and further applications, some semiempirical correlations were tested in this work.

By assuming chemical association equilibrium between the solute and the compressed gas, Chrastil developed a theory based on the mass action law applied to the formation of a solvate complex between a solute molecule and a number of supercritical fluid (SCF) solvent molecules.[24] It should be noted that the associated solvate complex assumption is quite close to the cluster conception accepted by the community.[25] Based on the facts that $k$ is a function of density[26] and that the solubility is a function of temperature, del Valle and Aguilera proposed a model to help compensate for the change in the heat of vaporization with temperature in the original Chrastil model. They also recommended the equation to be used for a temperature range from (293 to 353) K and for pressure varying from (15 to 88) MPa.[27]

This idea leads to the following equation for the solubility of component 2 (solid or liquid) in component 1 (SCF):

$$\ln S = k \ln \rho_f + A + \frac{B}{T} + \frac{C}{T^2} \quad (1)$$

$$S = \frac{\rho_f MW_2 x_2}{MW_1(1 - x_2)} \quad (2)$$

In eq 1, $S$ is the concentration of solute in the supercritical fluid ($kg \cdot m^{-3}$); $\rho_f$ is the density of the supercritical fluid ($kg \cdot m^{-3}$); $k$ is an association number; $A$ is a function of the molecular weights of the solute and supercritical fluid; $B$ and $C$ are parameters related to the heat of solvation and heat of vaporization; and $T$ is temperature (K). In eq 2, $MW_1$, $MW_2$, and $x_2$ are the molecular weight of SCF and solute and mole fraction solubility of solute in SCF, respectively.

**Table 3. Optimized Parameters of the DVA and MST Equations for Corosolic Acid Solubility in Supercritical Carbon Dioxide**

| model | parameters | AARD, % |
|---|---|---|
| DVA | $A = -35.6134$ | 17.2 |
| | $B = -0.3234$ | |
| | $C = -0.8689$ | |
| | $k = 6.1584$ | |
| MST | $D = 11957.5581$ | 14.7 |
| | $F = 2.7811$ | |

The measured solubility values are usually much greater than predicted using ideal gas theory. The extent to which pressure enhances the solubility of the solid in the gas is shown by an examination of the enhancement factors $E$ of the solutes, where:

$$E = \frac{x_2 P}{P_2^{sat}} \quad (3)$$

and $P$ is the total system pressure, and $P_2^{sat}$ is the saturation (or, for solid solutes, sublimation) pressure of the pure solute. The vapor pressure of the solid, necessary to calculate the enhancement factor using eq 3, can be predicted according to the method proposed by Grain.[28] On the basis of the dilute solutions theory, Méndez-Santiago and Teja[29] proposed the other model to fit the corosolic solubility data, which started with Henry's constant of the dilute solution and demonstrated that the solubility plotted as $T \ln E$ against the density of the solvent would yield a single line. They also concluded that the fact that all isotherms collapse to a single line is thus a powerful tool to determine the self-consistency of experimental data. The model can be written as

$$T \ln E = D + F\rho_f \quad (4)$$

where $D$ and $F$ are the equation parameters. Both $D$ and $F$ are independent of temperature.

In the calculations, the objective function used is the average absolute relative deviation (AARD) between the calculated and experimental solubility:

$$AARD = \frac{1}{N}\sum_{1}^{N}\left(\frac{|x_{2calc} - x_{2exp}|}{x_{2exp}}\right) \quad (5)$$

where $N$ is the number of experimental data points. The $x_{2exp}$ and $x_{2calc}$ are experimental and calculated solubility values, respectively.

The parameters $A$, $B$, $C$, and $k$ to facilitate the incorporation of effect of temperature and solvent density of the del Valle and Aguilera (DVA) equation together with the AARD between experimental and calculated solubility are presented in Table 3. The plots of the experimental data and the solubility isotherms calculated with the DVA model are presented in Figure 4, where the quality of the correlations can be visually evaluated. It is obvious in Figure 4 and Table 3 that the model still produced reasonable solubility estimation compared with the experimental data, where an AARD of 17.2 % was obtained. The correlation improves largely when the correlation parameters were evaluated without taking into account the low solubility data since solubilities are often in the same order of magnitude, as this equation does not require the thermodynamic properties of the pure component which is not always available in the literature.

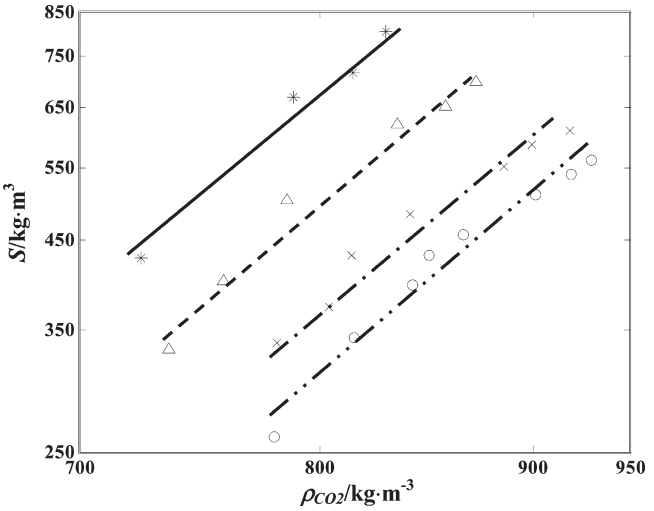


**Figure 4.** Solubility ($S$) of corosolic acid in carbon dioxide as a function of carbon dioxide density and temperature: ○, 308.15 K; ×, 313.15 K; △, 323.15 K; ∗, 333.15 K. The lines are the best fit of the experimental data calculated with DVA equation; — · ·, 308.15 K; — ·, 313.15 K; — —, 323.15 K; —, 333.15 K.

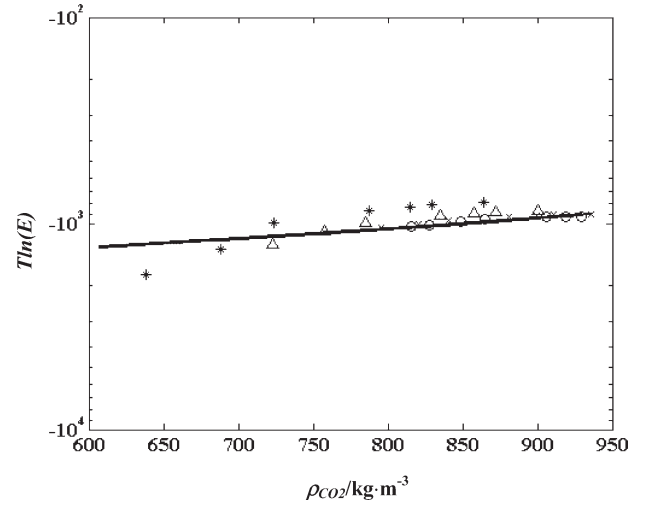


**Figure 5.** Enhancement factor ($E$) of corosolic acid solubility in supercritical carbon dioxide as a function of carbon dioxide density: ○, 308.15 K; ×, 313.15 K; △, 323.15 K; ∗, 333.15 K; — is the best fit line of the experimental data calculated with MST equation.

When vapor pressure data is available, the original version of the MST model is very useful for data correlation because of its simplicity and good fitting results. With the purpose of regression analysis of the MST equation, $P$, $\rho$, and $T$ were expressed in MPa, kg·m$^{-3}$, and K, respectively. The values for $D$ and $F$ obtained via multilinear regression are reported in Table 3. Although MST equation uses only two adjustable parameters, it results in better solubility calculations than DVA equation by generating an AARD as low as 14.7 %. Figure 5 shows that the data points for the four isotherms follow a single trend, indicating good consistency of the data over the range of temperatures tested. It may be observed that the model reproduces much better calculation when the parameters are obtained without including the solubilities obtained at pressures in the vicinity of the critical point of carbon dioxide.

## CONCLUSIONS

The solubility of corosolic acid in supercritical carbon dioxide has been measured experimentally. The solubility varied from a mole fraction of $3.28 \cdot 10^{-11}$ at 333.15 K and 8.0 MPa to $7.43 \cdot 10^{-2}$ at 333.15 K and 30.0 MPa. In general, the solubility of corosolic acid increased with increasing pressure at constant temperature. Overall, the solubility of corosolic acid in supercritical carbon dioxide increased with pressure at a constant temperature. Retrograde behavior of this system was indicated by a crossover pressure at 20.0 MPa. The solubility data were modeled with two density based semiempirical equations. The MST equation performed its superiority over the DVA models in term of accuracy with an AARD of 14.7 %, and its also successfully tested the consistency of the data. To the author's knowledge, this is the first reported data of corosolic acid solubility in SC-$CO_2$.

## AUTHOR INFORMATION

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