Measurement and correlation study of silymarin solubility in supercritical carbon dioxide with and without a cosolvent using semi-empirical models and back-propagation artificial neural networks

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ABSTRACT

The solubility data of compounds in supercritical fluids and the correlation between the experimental solubility data and predicted solubility data are crucial to the development of supercritical technologies. In the present work, the solubility data of silymarin (SM) in both pure supercritical carbon dioxide (SCCO2) and SCCO2 with added cosolvent was measured at temperatures ranging from 308 to 338 K and pressures from 8 to 22 MPa. The experimental data were fit with three semi-empirical density-based models (Chrastil, Bartle and Mendez-Santiago and Teja models) and a back-propagation artificial neural networks (BPANN) model. Interaction parameters for the models were obtained and the percentage of average absolute relative deviation (AARD%) in each calculation was determined. The correlation results were in good agreement with the experimental data. A comparison among the four models revealed that the experimental solubility data were more fit with the BPANN model with AARDs ranging from 1.14% to 2.15% for silymarin in pure SCCO2 and with added cosolvent. The results provide fundamental data for designing the extraction of SM or the preparation of its particle using SCCO2 techniques.

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1. Introduction

In recent years, supercritical fluids (SCF) have been widely applied in the fields of pharmaceuticals, food nutrition, and industrial materials [1–3] owing to their non-toxic, non-flammable, non-explosive and recyclable properties. On the other hand, working with SCF requires high-pressure processes which imply high investment costs for the machinery and the training of skilled staff [4]. For the application of SCF in extraction, reaction, waterless dyeing processes and particle preparation [5–8], the solubility of compounds in SCF is a key parameter in process design. Since experimentally acquiring solubility data is time consuming and laborious, understanding and ultimately predicting the solubility of related compounds are very helpful in supercritical process design or election parameter [9]. Supercritical carbon dioxide (SCCO2) is the most widely used SCF for its temperate critical conditions due to alcohol and toxins [10]. However, there have been only a few studies on the solubility of flavonoids [15,16], and no solubility data for silymarin has been analyzed thus far.

Silymarin (SM), a hepatoprotective agent obtained from the herb Silybum marianum (L.), is widely used in the treatment of liver diseases such as cirrhosis, hepatitis, and fatty infiltration due to alcohol and toxins [17]. A mixture of flavonolignan isomers, namely silybin, isosilybin, silydianin, and silychristin, collectively constitute SM. Among these isomers, silybin is the major component of SM, representing approximately 60–70%, and is responsible for its pharmacological activity [18–21]. Extraction of SM using SCCO2 has many advantages, such as lower extraction temperature, shorter extraction time, and no remains of toxic solvents [22,23]. Moreover, SCCO2 can be utilized to produce SM nanoparticles, solid dispersion and liposomes that may improve the bioavailability of SM, in which the supercritical anti-solvent (SAS) and solution-enhanced dispersion by supercritical fluids (SEDS) methods were often utilized [24–26]. Thus, the solubility of SM in SCCO2 is important for all of these processes. Among the factors responsible for the limited acceptance of SCF technologies, the insufficiency of supercritical solubility data has been frequently cited.

However, the measurement of equilibrium solubilities of solids in SCF at different temperatures and pressures is experimentally expensive and time consuming; hence, the modeling of the solubilities is essential. The models classically used to fit the solubility of solid solutes are semi-empirical equations such as Chrastil [27], Bartle [28], Mendez-Santiago and Teja (MST) [29] models. Semi-empirical models only need independent variables like pressure, temperature and density of SCF instead of solid properties [30,31]. They are based on simple error minimization. Nowadays, application of BPANN has been considered a promising tool because of their simplicity toward simulation, prediction and modeling. One of the characteristics of modeling based on BPANN is that it does not require the mathematical description of the phenomena involved in the process, and might therefore prove useful in simulating and up-scaling complex systems. So, it is preferable to use a nonparametric technique such as a neural network model to make reliable prediction of silymarin solubility in the SCCO2 and co-solvent system [32].

The purpose of this study is to provide fundamental data for the extraction and particle preparation process of SM using the SCCO2 technology, and to find a way of predicting the solubility of SM in SCCO2. During our research, the equilibrium solubility of SM was measured in SCCO2 with a static method in the pressure range of 8 to 22 MPa and at temperatures equal to 308, 318, 328 and 338 K. The influence of ethanol, acetone and dichloromethane as cosolvents on solubility was also investigated. Finally, three semi-empirical models (Chrastil, Bartle, and Mendez-Santiago and Teja models) and a back-propagation artificial neural networks (BPANN) model were applied to fit with the experimental solubility data and predict the solubility of SM in SCCO2 at different conditions.

2. Material and methods

2.1. Materials

Silymarin and oridonin were purchased from Dalian Meilun Biotech Co., Ltd. (Dalian, China). CO2 with a purity of 99.99% was obtained from SJTU chemical store (Shanghai, China). Ethanol, acetone and dichloromethane were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Apparatus and procedure

The solubility of SM in SCCO2 was measured by a semi-dynamic technique, as shown by the schematic (Fig. 1). Solubility measurements were taken in the pressure range of 8 to 22 MPa at temperatures of 308 to 338 K. A certain amount of SM powder was preloaded in the saturation cell and the procedure of solubility measurement was as follows: Before the experiment, CO2 was delivered by a high pressure piston pump into the saturation cell for air removal by adjusting the pressure relief valve V0. The high pressure CO2 then passed into the system, heated and kept at the desired temperature with a temperature sensor. After the desired conditions were achieved, the valves V2 and V4 were closed. The SCCO2 was made to circulate in the system by the circulating pump. Based on pre-experimental results, the circulating time was set for 90 min. When the system reached equilibrium, the valves V2 and V4 at the inlet and outlet of the 50 ml U-sample collector were closed, and the certain amount of SCCO2 with dissolved solute was sealed inside. The amount of SCCO2 can be calculated using the CO2 density corresponding to the operating conditions and the inner volume of the collector. Finally, the U-sample collector was then washed with ethanol more than three times, and the washing solvent was combined with the solution of the beaker. All experiments were performed in triplicate.

The procedure of measuring the SM solubility in SCCO2 with a cosolvent at a mole fraction of 0.02 was similar, except that at the beginning, a calculated amount of solvent was injected into the system in advance.
2.3. Analytical method

The obtained solution was analyzed by high-performance liquid chromatography (HPLC). The content of SM (based on silybin) was determined using a LC-2010A HT HPLC system (Shimadzu, Toyoto, Japan) with an Agilent Eclipse XDB-C18 column (5 μm, 4.6 × 250 mm) (Agilent, Shanghai, China). The mobile phase consisted of methanol and pure water (46:54, v/v) at a flow rate of 0.8 ml/min. The effluent was monitored at 288 nm. Each data point was measured at least three times and the average value was adopted. The SM solubility in SCCO\textsubscript{2} with or without a cosolvent was defined as the mole ratio of SM to CO\textsubscript{2} in the U-sample collector. The mole amount of CO\textsubscript{2} was calculated based on the inner volume of U-sample collector and the density of CO\textsubscript{2} corresponding to the operating conditions, which was obtained from the National Institute of Standards and Technology (NIST) fluid property database.

2.4. Correlation models

In this work, Chrastil, Bartle, Mendez-Santiago and Teja (MST), and BPANN models were used to fit with the experimental solubility data.

Chrastil is based on the association theory of formation of a solvate complex, which was assumed to be in equilibrium with the supercritical fluid. Mathematically, it is given by the expression.

\[
\ln y = A_1 + A_2 \ln \rho + \frac{A_3}{T}
\]

Where \(y\) is the solubility of solute, \(\rho\) is density of SCF, \(T\) is temperature, \(A_1\) and \(A_3\) are the regression coefficient and \(A_2\) is association number.

The next model proposed by Bartle and co-workers fit with the solubility using the following equation.

\[
\ln \left( \frac{yP}{P_c} \right) = A_1 + A_2 (\rho - \rho_c) + \frac{A_3}{T}
\]

Where \(y\) is the solubility of solute, \(\rho\) is density of SCF, \(P_c\) is 700 kg/m\textsuperscript{3}. \(P\) is the pressure, \(P_c\) is assumed as a standard pressure of 0.1 MPa. \(T\) is temperature, \(A_1\) and \(A_3\) are the regression coefficient and \(A_2\) is association number.

Another important semi-empirical model is MST model which derived a relation between the enhancement factor and the density using the theory of dilute solutions given by the following expression.

\[
T \ln (yP) = A_1 + A_3 \rho + A_2 T
\]

Where \(y\) is the solubility of solute, \(\rho\) is density of SCF, \(T\) is temperature, \(A_1\) and \(A_3\) are the regression coefficient and \(A_2\) is association number.

BPANN is a type of supervised network, and has been generally accepted for its excellent capability to train rapidly on sparse data sets. It has three layers. The number of neurons in the first layer (input layer) is equal to the number of inputs and the number of neurons in the third layer is equal to the number of outputs (output layer). The number of neurons in the second layer (hidden layer) is equal to or more than the number of training data sets. The data are scaled using a scaling function before it is used for training. During the training of BPANN, a neuron for each training pattern is assigned in the pattern layer. The weights between the pattern layer neurons and the input layer neurons are equal to the inputs of the training patterns. The outputs of the training patterns are assigned as the weights between the pattern layer neurons and the summation layer neurons. Fig. 2 presents the BPANN block diagram.
3. Results and discussion

3.1. Experimental solubility equilibrium data

To start with, the reliability and efficiency of the solubility measurement apparatus and the procedures were verified by measuring the solubility of oridonin in 
SCCO\textsubscript{2} at 308 K and 328 K and comparing with literature data \cite{33}. The results are shown in Table 1 and Fig. 3, where satisfactory agreement between various measurements can be observed.

Isothermal solubility for the system in pure 
SCCO\textsubscript{2} over the pressure range of 8 to 22 MPa and temperature range of 308 to 338 K are tabulated in Table 2 and solubility of SM in 
SCCO\textsubscript{2} with a cosolvent (ethanol, acetone or dichloromethane) at a mole fraction of 0.02 under the same conditions are shown in Table 3. Each reported data point is the average of at least three replicate measurements with relative standard deviations of less than 2.6 \%.

The solubility increases with the increase of pressure at a constant temperature. This can be explained by the increase of solvent density attributed to pressure growth along an isotherm as well as specific interaction between dissolved substances and solvent molecules in elevated pressure. Increase of carbon dioxide density will also increase the polarity of its molecules \cite{34}.

Crossover points have been observed for all groups as shown in Fig. 4. The crossover pressure regions of SM without a solvent and with ethanol, acetone and dichloromethane are from 12.5

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{T} \textsuperscript{a} (K) & \textbf{P} (MPa) & \textbf{P} \textsuperscript{b} (g/L) & \textbf{Oridonin} \textsuperscript{y.10\textsuperscript{6}} \\
\hline
308 & 9 & 662.13 & 0.57 \\
 & 11 & 743.95 & 1.62 \\
 & 13 & 785.70 & 3.29 \\
 & 15 & 815.06 & 5.41 \\
 & 17 & 838.09 & 7.60 \\
328 & 9 & 255.54 & 0.36 \\
 & 11 & 414.90 & 0.60 \\
 & 13 & 571.33 & 0.77 \\
 & 15 & 653.50 & 1.32 \\
 & 17 & 703.82 & 1.98 \\
\hline
\end{tabular}
\caption{Solubility of oridonin in pure 
SCCO\textsubscript{2} at temperatures of (308 and 328) K and pressures from (9 to
17) MPa.}
\\textsuperscript{a} Standard uncertainties \textit{u} are the following: \textit{u} (T) = 0.1 K,\textit{u} (P) = 0.2 MPa, \textit{u} (y) = 2.3 \%.
\textsuperscript{b} \textit{p} is the density of pure \textit{CO}\textsubscript{2} obtained from the National Institute of Standards and Technology (NIST) fluid property database.
\end{table}
to 12.8 MPa, 11.4 to 11.7 MPa, 11.8 to 12.3 MPa and 13.9 to 14.2 MPa, respectively. The crossover phenomena may be attributed to competition between the solute’s vapor pressure and the solvent’s density, which have opposing temperature dependences.

Below the crossover pressure region, the effect of density, which is sensitive to the solute’s vapor pressure, is dominant so that the solute is more soluble at a low temperature. However, above the crossover pressure region, the solute’s vapor pressure becomes

| Table 3 – Solubility of SM in SCCO$_2$ with a cosolvent (ethanol, acetone or dichloromethane) at a mole fraction of 0.02 at temperatures of (308, 318, 328 and 338) K and pressures from (8 to 22) MPa. |
|---|---|---|---|---|
| $T$ (K) | $P$ (MPa) | $y \cdot 10^5$ SM + ethanol + CO$_2$ | $y \cdot 10^5$ SM + acetone + CO$_2$ | $y \cdot 10^5$ SM + dichloromethane + CO$_2$ |
| 308 | 8 | 7.12 | 10.75 | 5.78 |
| | 10 | 7.21 | 12.33 | 6.12 |
| | 12 | 7.76 | 15.16 | 6.78 |
| | 14 | 8.45 | 19.32 | 7.61 |
| | 16 | 9.33 | 25.88 | 8.59 |
| | 18 | 10.87 | 33.11 | 10.01 |
| | 20 | 13.07 | 44.08 | 12.55 |
| | 22 | 18.88 | 54.81 | 14.08 |
| 318 | 8 | 6.71 | 8.97 | 4.37 |
| | 10 | 7.23 | 12.02 | 5.08 |
| | 12 | 8.55 | 16.11 | 6.11 |
| | 14 | 10.92 | 21.74 | 7.92 |
| | 16 | 14.65 | 31.65 | 9.29 |
| | 18 | 17.71 | 42.17 | 12.9 |
| | 20 | 22.11 | 56.14 | 16.33 |
| | 22 | 30.54 | 68.90 | 18.76 |
| 328 | 8 | 4.45 | 7.66 | 3.12 |
| | 10 | 6.78 | 10.13 | 4.37 |
| | 12 | 9.66 | 17.65 | 5.86 |
| | 14 | 13.12 | 26.72 | 7.42 |
| | 16 | 18.46 | 37.19 | 10.16 |
| | 18 | 24.77 | 50.08 | 15.67 |
| | 20 | 33.14 | 65.72 | 21.71 |
| | 22 | 41.99 | 83.24 | 27.18 |
| 338 | 8 | 3.89 | 5.17 | 2.99 |
| | 10 | 5.99 | 8.22 | 4.87 |
| | 12 | 10.12 | 15.51 | 6.81 |
| | 14 | 16.98 | 26.40 | 11.14 |
| | 16 | 24.11 | 41.73 | 16.32 |
| | 18 | 34.24 | 60.06 | 23.82 |
| | 20 | 47.76 | 80.06 | 31.19 |
| | 22 | 60.12 | 101.37 | 40.33 |

* Standard uncertainties $u$ are the following: $u(T) = 0.1$ K, $u(P) = 0.2$ MPa, $u(y) = 1.4$ %.
dominant at higher temperature and the density of the solvent turns is less sensitive to the solute’s vapor pressure. At the crossover point, these two competitive factors effect rather intense.

Moreover, systems with a cosolvent have greatly increased SM solubility due to the solubility of SM in these organic solvents is much bigger than that in SCCO2. The solubility of SM in different solvents are as follows: acetone > ethanol > dichloromethane. This may be due to the fact SM can form a strong intermolecular hydrogen bonds with acetone and ethanol [35]. Moreover, the polarity of acetone is smaller than that of ethanol, which makes it easier to dissolve SM. All of these make the solubility discrepancy of SM in SCCO2 with different cosolvents

3.2. Correlation results of solubility data

Experimental data were fit with several semi-empirical models as well as BPANN, which enable the prediction of compound solubility in pure SCCO2 and cosolvent-added SCCO2. The correlation results and detailed descriptions of equations are listed in Table 4 and Table 5. The percentage of average absolute relative deviations (AARD%) was applied between the calculated and experimental solubility to study the correlation of the analytes in the models as is mentioned in the following equation:

\[
\text{AARD}(\%) = \frac{100}{N} \sum_{i=1}^{N} \frac{|y_{\text{cal}} - y_{\text{exp}}|}{y_{\text{exp}}}
\]

where \(y_{\text{cal}}\) is the calculated value of the mole fraction solubility of solute, \(y_{\text{exp}}\) is the experimental value of the mole fraction solubility of solutes, and \(N\) is the number of experimental points.

As shown in the Table 4 and 5, the AARD% values of the calculated equilibrium solubility for all models are below 11.23%, showing satisfactory accuracy of the experimental results.

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Among the semi-empirical models, the MST models provide the minimum AARDs (%) at 4.86, 4.58, 3.90 and 4.85 for SM in pure SCCO2 and in cosolvent-added SCCO2 with ethanol, acetone, and dichloromethane, respectively, indicating a better predictive ability. Fig. 5 compares the calculated isotherms from Chrastil, Bartle, and MST models with the experimental data. As seen, the agreements between the experimental and calculated values are satisfactory and each model agrees well with the experimental data. Moreover, the MST model has a better linear relation, with the plots of \(T \ln (y \cdot 10^5) - A_1 \cdot A_2 \cdot T\) versus \(\rho\) resulting in a nearly straight line.

In this study of BPANN model, it was able to predict the testing subset of solubility by the AARDs (%) of 1.14, 1.70, 1.94, and 2.15 for SM in pure SCCO2 and in cosolvent-added SCCO2 with ethanol, acetone, and dichloromethane, respectively, which are much less than the AARDs of semi-empirical models. After the training, the fitted responses surface was very close to the desired values. For instance, Fig. 6 compares the BPANN predictions to the experimental data via scatter plots for training set of SM in SCCO2 without cosolvent. The overall performance of training set was evaluated by the determination coefficient (\(R^2\)) values (\(R^2 = 0.9998\)). The proposed model had impressively learned the non-linear relationship between the input and the output variables, exhibiting a successful performance of the BPANN model for prediction of SM solubility in SCCO2 analog in the training stage.

4. Conclusion

In this work, the solubility of silymarin in pure SCCO2 and cosolvent-added SCCO2 was determined at 308, 318, 328
and 338 K, over a pressure range of 8.0 to 22.0 MPa. The solubility of SM ranges from $0.27 \times 10^{-5}$ to $8.01 \times 10^{-5}$ in pure SCCO$_2$, $3.89 \times 10^{-5}$ to $60.12 \times 10^{-5}$ in ethanol-added SCCO$_2$, $5.17 \times 10^{-5}$ to $101.37 \times 10^{-5}$ in acetone-added SCCO$_2$ and $2.99 \times 10^{-5}$ to $40.33 \times 10^{-5}$ in dichloromethane-added SCCO$_2$ (at solvent mole fractions of 0.02). Then the experimental solubility data were fit with three semi-empirical models (Chrastil, Bartle and MST models). All fitted models were shown to successfully correlate experimental solubility data with AARDs of 3.90 to 11.23%. Meanwhile, BPANN model was established to fit the solubility data. The BPANN model obtained a more satisfactory accuracy with the AARDs from 1.14 to 2.25% which was lower than those of the other models, suggesting that the BPANN model had a better predictive ability in our study.

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### Conflicts of interest

The authors declare that there is no conflicts of interest.
**REFERENCES**

[1] Girotra P, Singh SK, Nagpal K. Supercritical fluid technology: a promising approach in pharmaceutical research. Pharm Dev Technol 2013;18:22–38.

[2] Brunner G. Supercritical fluids: technology and application to food processing. J Food Eng 2005;67:21–33.

[3] Reverchon E, Adami R. Nanomaterials and supercritical fluids. J Supercrit Fluids 2006;37:1–22.

[4] Khazaiepour A, Soleimani M, Salahi S. Solubility prediction of dispersed dyes in supercritical carbon dioxide and ethanol as co-solvent using neural network. Chin J Chem Eng 2016;24:491–498.

[5] Zuknik MH, Norulaini NAN, Omar AKM. Supercritical carbon dioxide extraction of lycopene: a review. J Food Eng 2012;112:253–262.

[6] Sahena F, Zaidul ISM, Jinap S. Application of supercritical CO2 in lipid extraction – A review. J Food Eng 2009;95:240–253.

[7] Park MW, Bae HK. Dye distribution in supercritical dyeing with carbon dioxide. J Supercritical Fluid. 2002;22:65–73.

[8] Byarappa K, Ohara S, Adschiri T. Nanoparticles synthesis using supercritical fluid technology towards biomedical applications. Adv Drug Deliv Rev 2008;60:297–307.

[9] Shen BQ, Feng LF, Li SF, et al. Correlating and predicting the solubilities of polycyclic aromatic hydrocarbons in supercritical fluids using the compressed gas model and the reference solubilities. Fluid Phase Equilib 2013;337:100–108.

[10] Wu H, Zhu J, Wang YW, et al. Measurement and modeling for solubility of 3-hydroxybenzaldehyde and its mixture with 4-hydroxybenzaldehyde in supercritical carbon dioxide. Fluid Phase Equilib 2016;409:271–279.

[11] Peng J, Yang HJ, Yang HW. Solubilities and partial molar volumes of three bis (2-ethoxyethyl) ethanediol derivatives in supercritical carbon dioxide. J Chem Thermodyn 2015;83:33–42.

[12] Li HR, Jia DD, Liu RH, et al. Correlating and estimating the solubilities of solid organic compounds in supercritical CO2 based on the modified expanded liquid model and the group contribution method. Fluid Phase Equilib 2014;385:10–24.

[13] Lamba N, Narayan RC, Modak J, et al. Solubilities of 10-undecenoic acid and geraniol in supercritical carbon dioxide. J Supercrit Fluids 2015;107:384–391.

[14] Asahi H, Yamini Y, Latifah F, et al. Solubilities of four macrolide antibiotics in supercritical carbon dioxide and their correlations using semi-empirical models. J Supercrit Fluids 2015;104:62–69.

[15] Sato M, Takeda K, Ota M, et al. Solubility estimation of high-value natural products in supercritical CO2 based on solubility parameter. Kagaku Kogaku Ronbunshu 2010;36:466–471.

[16] Kiyoshi M, Kenji M, Ryugen O, et al. Solubilities of 7, 8-dihydroxystrophanthidine and 3,3’,4’,5,7-pentahydroxystrophanthidine in supercritical carbon dioxide. J Chem Eng Data 2003;48:1040–1043.

[17] Dixit N, Baboota S, Kohli K, et al. Silymarin: a review of pharmacological aspects and bioavailability enhancement approaches. Indian J Pharmacol 2007;39:172–179.

[18] Campodónico A, Collado E, Ricci R, et al. Dissolution test for silymarin tablets and capsules. Drug Dev Ind Pharm 2001;27:261–265.

[19] Jia JF, Zabihi F, Gao YH, et al. Solubility of glycyrrhizin in supercritical carbon dioxide with and without cosolvent. J Chem Eng Data 2015;60:1744–1749.