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Solubility of Puerarin in Ethanol + Supercritical Carbon Dioxide

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Equilibrium solubility of puerarin, a naturally occurring isoflavone *C*-glycoside, in the ethanol + supercritical carbon dioxide was measured at (308, 318, and 328) K and pressures up to 20 MPa. The results obtained are useful for the design of supercritical processes with this drug.

Introduction

Recently, researchers are very interested in gas antisolvent (GAS) crystallization or supercritical antisolvent (SAS) precipitation to produce micron or submicron particles with controlled particle size and particle size distribution.[1,2] One of the significant reasons is that in products for medical and pharmaceutical applications, the presence of residual organic solvents is rigorously controlled by international safety regulations. Thus, it is necessary to warrant the complete removal and absence of these substances without exposing the polymers and drugs contained in a typical polymeric controlled drug release system to high temperatures that could degrade them. In this sense, supercritical fluids (SCFs) can be very attractive solvents. Until now, carbon dioxide (CO_2) has been the most common supercritical fluid for drug process due to its relatively low critical temperature. It has other import advantages, including its non-toxicity, non-flammablity, and inexpensiveness.

The design of chemical and pharmaceutical processes based on SCFs and the determination of their best operating conditions require knowledge of phase equilibria and drug solubility in a SCF or in a solution with a SCF. In the last two decades, the solubility of a large number of compounds in SCFs has been measured, reported, and reviewed.[3−5] However, the development of new supercritical processes and new applications for existing substances has maintained the need for new experimental solubility determinations.

In this work, equilibrium solubility of puerarin in ethanol + CO_2 was measured at (308, 318, and 328) K and pressures up to 20 MPa. Puerarin, shown in Figure 1, is a naturally occurring isoflavone *C*-glycoside from the natural product named Pueraria, one of the most popular Chinese herbal medicines, traditionally used to reduce febrile symptoms and also used as an anti-inebriation agent.[6] Because the solubility of puerarin in water solutions is poor, recrystallization of this compound by GAS process to produce micron particles may increase its bioavailability and also reduce side effects.

Experimental Section

Meterials. The puerarin of pharmaceutical purity grade was kindly provided by Zhejiang Chemical Reagent Plant (China). All samples of puerarin used, without further purification, were dried in a vacuum at (105 to 110) °C for 2 h. Other reagents used such as ethanol were of analytical purity grade. CO_2 (more than 99.5 % pure) was supplied by Kaitian Gas Company (China).

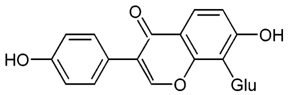

Figure 1. Chemical structure of puerarin, CAS Registry Number 3681-99-0, ($C_{21}H_{20}O_9$), 8-β-D-glucopyransyl-7-hydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one.

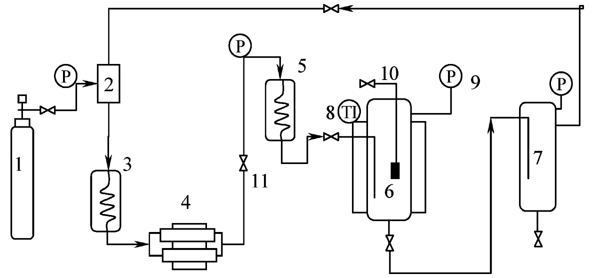

Figure 2. High-pressure apparatus for solubility measurements: 1, CO_2 cylinder; 2, drier; 3, cooler; 4, high-pressure pump; 5, heater; 6, equilibrium cell; 7, depressurizing vessel; 8, temperature controller; 9, pressure gauge; 10, liquid example exit; 11, valves.

Apparatus and Procedures. The solubility of puerarin in ethanol (1) + CO_2 (2) solutions was measured using a high-pressure apparatus, schematically presented in Figure 2. The determination of the solubility was performed in a manner similar to that described by Guan et al.[7] A stainless steel equilibrium cell equipped with a magnetic stirrer, with an internal volume of approximately 1000 mL, was heated by means of a circulating water controller that maintained the temperature within ± 0.2 K. A high-pressure pump (model JW-15-30) was coupled to the system for pumping CO_2 into the heater, where the SCF was heated and kept at constant temperature with circulating water coming from a thermostatic bath. The uncertainty of the temperature measurement is ± 0.1 K, and that for pressure is ± 0.05 MPa.

In the experiment, 200 mL of puerarin-saturated ethanol solution with some solute crystals in it was loaded into the cell. CO_2 was charged carefully into the cell from the top of the cell until a test pressure was reached at the controlled temperature. After the pressure and temperature had remained unchanged for 30 min, a sample of

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Table 1. Mole Fraction of CO_2 (2), x_2, and Puerarin (3), x_3, in Solution Phase

T/K								
308			318			328		
P/MPa	x_2	$10^3\,x_3$	*P*/MPa	x_2	$10^3\,x_3$	*P*/MPa	x_2	$10^3\,x_3$
0.0	0.000	7.687	0.0	0.000	7.021	0.0	0.000	6.171
2.0	0.153	7.921	2.1	0.096	7.281	2.4	0.061	6.298
3.3	0.241	7.998	3.3	0.156	7.351	3.5	0.101	6.273
4.0	0.290	7.578	4.2	0.191	7.108	4.0	0.125	6.038
4.5	0.350	6.054	4.6	0.232	6.202	4.4	0.154	5.197
5.0	0.391	4.515	5.1	0.262	4.680	5.2	0.176	3.882
5.5	0.450	2.894	5.5	0.305	3.170	5.5	0.207	3.327
6.0	0.528	1.920	6.0	0.362	2.313	6.0	0.248	2.745
7.0	0.770	1.212	7.0	0.540	1.714	7.0	0.379	1.880
8.0	0.810	0.695	8.0	0.581	1.293	8.0	0.417	1.539
9.0	0.845	0.584	9.0	0.620	0.907	9.0	0.456	1.083
10	0.876	0.394	10	0.659	0.542	10	0.495	0.858
12	0.892	0.242	12	0.705	0.287	12	0.557	0.526
14	0.904	0.178	14	0.752	0.225	14	0.666	0.355
17	0.928	0.112	17	0.841	0.188	17	0.810	0.295
20	0.945	0.108	20	0.936	0.147	20	0.926	0.234

liquid phase, about 0.50 mL, was taken from the liquid example exit for composition analysis. Then CO_2 was charged once again into the cell and when the pressure of the system reached the next test point and kept on for 30 min for approaching to equilibrium another sample was taken and so on.

Analytical Method. The collected samples were diluted in ethanol to a convenient volume. To determine the puerarin concentration, the resulting solutions were analyzed by UV spectrophotometry (Spectrnblab 52, Lengguang Instrument Factory, Shanghai, China). Puerarin absorbs in the UV region, with a maximum absorbance at 308 nm. Calibration was obtained by preparing standard samples with concentrations between (5.0 and 30.0) mg/L. Each data point is the average of at least three measurements. The uncertainty of the concentration measurement is ± 2.0 % relatively.

Results and Discussion

Measured solubilities of puerarin in ethanol + CO_2 solution at (308, 318, and 328) K are listed in Table 1, where x_3 is the mole fraction experimental solubility of puerarin. Meanwhile, the balance mole fraction of ethanol, x_2, was calculated or extracted from the experimental data of Day et al.[8] Day and co-workers have studied phase equilibrium of the ethanol + CO_2 system from (291 to 313) K.[8] The values of x_2 at (318 and 328) K in Table 1 were calculated by using the modified Apelblat equation[9]:

$$\ln x_2 = A + \frac{B}{T/\mathrm{K}} + C \ln (T/\mathrm{K}) \tag{1}$$

where parameters A, B, and C were calculated from their results. From Table 1, we can find that supercritical or near-critical CO_2 has versatile effects on solubility behavior of this drug compound in ethanol solutions and that supercritical CO_2 can be used as an antisolvent for crystallization.

Influence of Pressure. Solubility curves of puerarin in the ethanol + CO_2 solution at (318 and 328) K are shown in Figure 3. At all temperatures, as the pressure of the system increases and the density decreases by adding CO_2, the solubility of the solute first retains constant or slightly increases with the addition of CO_2, and then decreases with crease in pressure.

As a consequence of this information, recrystallization of puerarin from ethanol solutions would be possible by the GAS process. In such a process, CO_2 would be added to the organic solution at the working temperature until a final pressure of 15 MPa, where most of the solute would precipitate.

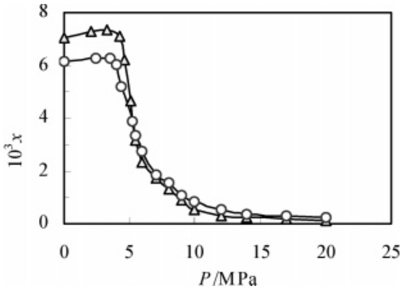

Figure 3. Solubility of puerarin in CO_2 + ethanol solution: △, at 318 K; ○, at 328 K.

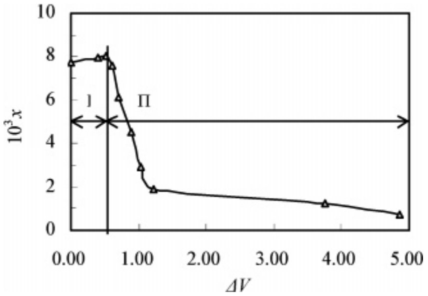

Figure 4. Cosolvent and antisolvent effects of CO_2 on puerarin solution at 308 K (I, cosolvent; II, antisolvent).

Cosolvent and Antisolvent Effects. Figure 4 shows the change of puerarin solubility via the volume expansion ΔV of ethanol solution at 308 K. The data of ΔV are calculated according to following equation:

$$\Delta V = \frac{44 \times x_2 \times (\rho/\mathrm{g{\cdot}dm^{-3}}) + 46 \times (1 - x_2)(\rho/\mathrm{g{\cdot}dm^{-3}} - \rho^{\mathrm{L}}/\mathrm{g{\cdot}dm^{-3}})}{46 \times (1 - x_2)(\rho^{\mathrm{L}}/\mathrm{g{\cdot}dm^{-3}})} \tag{2}$$

where 44 and 46 are the molar mass of CO_2 and ethanol, respectively; x_2 is the mole fraction of CO_2 in the solution; ρ is the density of ethanol at 308 K; ρ^{L} is that of the ethanol + CO_2 solution, which are also from the experimental results of Day et al.[8]

In general, CO_2 acts as an antisolvent when mixed with an ethanol solution of puerarin. However, it is much interesting to notice that in the system we investigated, CO_2 acts not only as an antisolvent that decreases the solubility of the solute but also as a cosolvent, increasing its solubility in the CO_2 + ethanol mixture as compared to pure ethanol. These functions of CO_2 are completely opposed. Such behavior has been reported by De Gioannis et al. for griseofulvine + ethanol + CO_2.[10] The system presented here is another example where CO_2 acts as a cosolvent before acting as an antisolvent.

During the process of adding CO_2 into the ethanol solution, as shown in Figure 4, there may be a changing point, before that addition of CO_2 can increase the solubility of puerarin in ethanol solution and after that CO_2 then acts as an antisolvent. This phenomenon implies that the effect of supercritical fluids on solubility of solution is much more complicated than what has been known. The reason for this phenomenon needs to be studied further.

Influence of Temperature. The solubility of puerarin in the liquid phase at (308, 318, and 328) K is shown graphically in Figure 5. Over the range studied, the

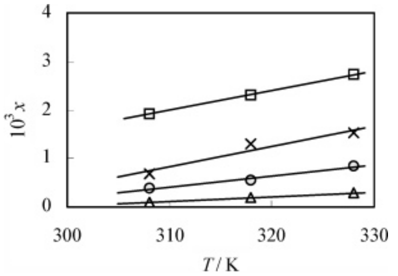

Figure 5. Effect of temperature on puerarin solubility in solution (□, 6MPa; ×, 8MPa; ○, 10MPa; △, 17MPa).

solubility of puerarin increases with increase in temperature at the same pressure. Since the solubility of puerarin in pure ethanol decreases with increase in temperature,[11] it would be better to recrystallizate puerarin using the GAS process at a lower temperature.

Conclusions

Equilibrium solubility of puerarin in ethanol + CO_2 solution was measured at (308, 318, and 328) K and pressure up to 20 MPa. The experimental data indicated that, because of its nonpolar chemical structure, CO_2 has a strong effect of antisolvent on puerarin in ethanol solution; therefore, it can be used in the GAS process for recrystallization of puerarin. The solubility of puerarin in ethanol + CO_2 increases with an increase in temperature, which is opposite to that in the pure alcohol solvent. For the system we studied, CO_2 can behave as a cosolvent before acting as an antisolvent.

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