Solubility of Pyoluteorin in Water, Dichloromethane, Chloroform, and Carbon Tetrachloride from (278.2 to 333.2) K

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The solubility of pyoluteorin (Plt) in water, dichloromethane, chloroform, and carbon tetrachloride was determined using an analytical method over the temperature range of (278.2 to 333.2) K. The solubility of Plt in chloroform is much higher than that in water, dichloromethane, and carbon tetrachloride. The solubility of Plt increases with increasing temperature in the four solvents, and all measurements were correlated with the Apelblat equation.

Introduction

Pyoluteorin (Plt) is a yellow aromatic polyketide antibiotic, which is composed of a bichlorinated pyrrole linked to a resorcinol moiety (see Figure 1).[1] It is a promising biopesticide produced by certain strains of *Pseudomonas* species,[2−4] which can inhibit phytopathogen fungi, including the plant pathogen *Pythium ultimum* effectively and suppress plant disease caused by this fungus and in some instances contributes to the ecological competence of the producing strain within the Rhizosphere.[4−6]

No experimental solubility data have been reported from a review of the literature on Plt. The scarcity of basic solubility data hinders progress in the design and scale-up of the bioproduction process of Plt.

Methods of measuring the solubility of a solid-in-liquid system can be classified as analytical[7−9] and synthesis.[10−12] Despite its disadvantages such as being time-consuming, the analytical method is widely used for the possibility of measuring a large number of samples simultaneously.[7] In this paper, the solubility of Plt in water, dichloromethane, chloroform, and carbon tetrachloride over the temperature range of (278.2 to 333.2) K was measured by the analytical method. The concentrations of Plt were determined by high-pressure liquid chromatography (HPLC).

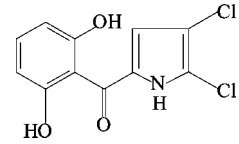

Figure 1. Chemical structure of Plt.

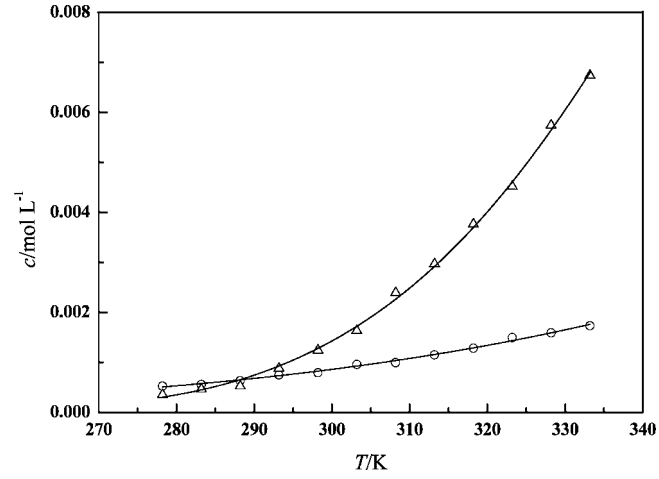

Figure 2. Solubility of Plt in ○, water; Δ, carbon tetrachloride. Solid lines represent the correlated results.

Experimental Section

Materials. Yellow crystal powder of Plt was prepared by our laboratory and had a purity of 99 %, determined by HPLC. It was dried in a vacuum at 40 °C for 48 h and stored in a desiccator. Analytical purity grade dichloromethane, chloroform, carbon tetrachloride, and ethyl acetate and HPLC grade methanol and redistilled deionized water were also used in this study.

Sample Preparation. Plt solubilities were measured in an experimental system as described by Mohsen-Nia et al. and Yang et al.[7,9] An excess amount of Plt was added to the solvents in a specially designed sealed dual-wall flask maintained at the desired temperature through circulating water. The water temperature was controlled by a thermostat within ± 0.1 K. Continuous stirring was achieved by a magnetic stirrer. Attainment of equilibrium was verified by repetitive measurements after several additional days and by approaching equilibrium from supersaturation by preequilibrating the solutions at a higher temperature. After attaining equilibrium, the stirrer was turned off to let the solution settle for 2 h. Then, the upper portion was taken to prepare the solutions for HPLC analysis.

Sample Analysis. To determine the Plt concentration in water, a volume of 200 μL of cultures was mixed well with 600 μL of ethyl acetate, and the ethyl acetate phase was taken and evaporated to dryness. To determine the Plt concentration in the other three organic solvents, a volume of 200 μL of cultures was sampled and evaporated to dryness. All dried samples were added with an appropriate amount of HPLC grade methanol to adjust the Plt concentration to about (0.1 to 2) $mg \cdot mL^{-1}$ according to its standard curve. The Plt concentrations were

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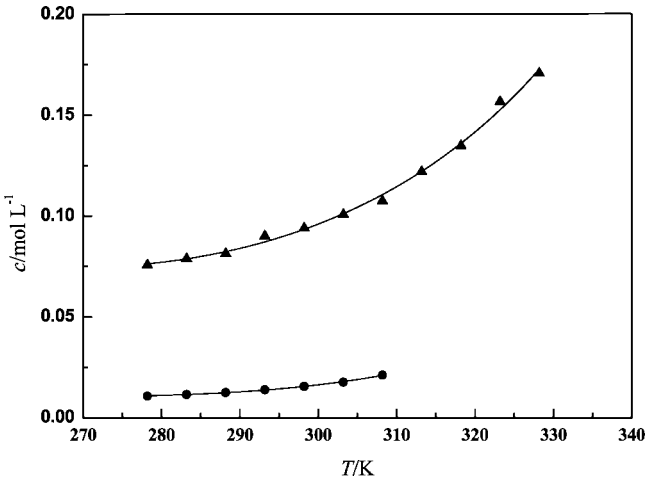

Figure 3. Solubility of Plt in ●, dichloromethane; ▲, chloroform. Solid lines represent the correlated results.

determined by reversed-phase HPLC using a Shimadzu LC-8A HPLC apparatus equipped with a variable-wavelength UV detector (Shimadzu, SPD-8A) as described by Zhang et al.[13] A Zorbax SB-C18 column (250 mm × 4.6 mm; 5 μm) was used at 25 °C with 70 % methanol as the mobile phase. The flow rate was kept constant at 1 mL·min^{-1}, and Plt was monitored at λ = 308 nm, identified by comparison with its authentic sample. Multiple flasks were run under each temperature, and the experimental data represent the means with deviations calculated from three independent samples.

Results and Discussion

The experimental solubility data of Plt in pure water, dichloromethane, chloroform, and carbon tetrachloride at different temperatures are shown in Table 1 and plotted in Figures 2 and 3. The experimental data show that the solubility of Plt in these solvents increases with increasing temperature. The solubility of Plt in chloroform was the highest, and its solubility in dichloromethane was higher than those in water and carbon tetrachloride.

Table 2. Parameters of Equation 1 for Plt Solubility in the Studied Solvents

solvent	*A*	*B*	*C*	10^3 rsmd
water	−30.745	−710.30	4.5679	0.03
dichloromethane	−558.87	22700	83.996	0.12
chloroform	−313.63	12734	47.133	1.94
carbon tetrachloride	299.51	−18287	−42.973	0.08

The temperature dependence of Plt solubility in pure water, dichloromethane, chloroform, and carbon tetrachloride can be described by the modified Apelblat equation[8−12]

$$\ln(c/\text{mol}\cdot\text{L}^{-1}) = A + \frac{B}{T/\text{K}} + C\ln(T/\text{K}) \qquad (1)$$

where c is the solubility of Plt (mol·L^{-1}); T is the absolute temperature (K); and A, B, and C are the fitting parameters. The difference between the experimental and the calculated solubilities of Plt are also given in Table 1. The values of the parameters A, B, and C and the root-mean-square deviations (rmsd's) are listed in Table 2. The rmsd is defined as

$$\text{rmsd} = \sqrt{\frac{\sum_{i=1}^{n}(c_i^{\text{calcd}} - c_i)^2}{n}} \qquad (2)$$

where n is the number of experimental data points and c_i^{calcd} is the calculated solubility.

From the data listed in Table 2 and the curves shown in Figures 2 and 3, it can be seen that the calculated solubilities are in good agreement with the experimental values, which indicate that the modified Apelblat equation is suitable to correlate the solubility data of Plt in water, dichloromethane, chloroform, and carbon tetrachloride as a function of temperature.

Table 1. Solubility of Plt in Water, Dichloromethane, Chloroform, and Carbon Tetrachloride

T/(K)	$10^3\ c$/(mol·L^{-1})	$10^3\ (c - c^{\text{calcd}})$/(mol·L^{-1})	T/(K)	$10^3\ c$/(mol·L^{-1})	$10^3\ (c - c^{\text{calcd}})$/(mol·L^{-1})
		Water			
278.2	0.53 ± 0.02	0.02	308.2	0.99 ± 0.03	−0.04
283.2	0.56 ± 0.01	−0.02	313.2	1.15 ± 0.04	−0.01
288.2	0.63 ± 0.03	−0.02	318.2	1.29 ± 0.02	0.00
293.2	0.75 ± 0.01	0.01	323.2	1.50 ± 0.05	0.06
298.2	0.79 ± 0.02	−0.03	328.2	1.59 ± 0.01	0.00
303.2	0.96 ± 0.03	0.03	333.2	1.73 ± 0.04	−0.03
		Dichloromethane			
278.2	10.76 ± 0.17	−0.10	298.2	15.53 ± 0.24	−0.01
283.2	11.52 ± 0.39	0.04	303.2	17.65 ± 0.12	−0.26
288.2	12.49 ± 0.28	0.06	308.2	21.10 ± 0.37	0.11
293.2	13.82 ± 0.11	0.06			
		Chloroform			
278.2	75.7 ± 3.1	−0.59	308.2	107.5 ± 4.6	−3.16
283.2	78.8 ± 4.4	0.03	313.2	122.0 ± 2.4	−0.20
288.2	81.3 ± 2.2	−1.10	318.2	134.8 ± 4.3	−1.21
293.2	90.1 ± 3.1	2.83	323.2	156.7 ± 2.9	3.97
298.2	94.0 ± 1.9	0.56	328.2	170.8 ± 8.3	−1.97
303.2	100.7 ± 7.0	−0.46			
		Carbon Tetrachloride			
278.2	0.35 ± 0.02	0.04	308.2	2.39 ± 0.02	0.13
283.2	0.46 ± 0.02	0.01	313.2	2.97 ± 0.03	0.05
288.2	0.52 ± 0.02	−0.14	318.2	3.76 ± 0.03	0.06
293.2	0.88 ± 0.03	−0.05	323.2	4.52 ± 0.03	−0.09
298.2	1.25 ± 0.01	−0.03	328.2	5.74 ± 0.04	0.10
303.2	1.63 ± 0.03	−0.08	333.2	6.74 ± 0.01	−0.06

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