Solubility of Phenazine-1-carboxylic Acid in Water, Methanol, and Ethanol from (278.2 to 328.2) K

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The solubility of phenazine-1-carboxylic acid (PCA) in water, methanol, and ethanol solutions was measured over the temperature range of (278.2 to 328.2) K. The solubility of PCA in methanol and ethanol is higher than that in water and increases with increasing temperature in the three solvents. All measurements were correlated with the Apelblat equation. The equation was found to provide an accurate representation of the experimental data.

Introduction

Phenazine-1-carboxylic acid (PCA) is an antibiotic that could be used to protect crops against a broad spectrum of soil-borne fungal phytopathogens.[1] It is a yellow or almost yellow powder. Figure 1 shows the chemical structure of PCA. The PCA, isolated from the fermentation broth of *Pseudomonas* M-18,[2] was investigated as a promising biopesticide,[1,3] while PCA generally is extracted and analyzed with methanol and ethanol.

From a review of the literature on PCA, it was found that no experimental solubility data in aqueous or organic solvents have been reported. The scarcity of basic solubility data hinders progress in the design and scale-up of the production process of PCA.

Methods of measuring the solubility of a solid-in-liquid mixture can be classified as analytical[4,5] and synthetic.[6,7] The advantage of the analytical method lies in the possibility of measuring a large number of samples simultaneously with a reliable method.[7] In the present study, the solubility of PCA in water, methanol, and ethanol over the temperature range of (278.2 to 328.2) K was measured by the analytical method. The concentrations were determined by high-pressure liquid chromatography (HPLC).

Experimental Section

Reagents and Apparatus. PCA was prepared by our laboratory and had a purity of 99 %, determined by HPLC. It was dried in vacuum at 50 °C for 48 h and stored in a desiccator. Other reagents used such as methanol and ethanol were of analytical purity grade, and re-distilled deionized water was used. The concentration measurements were carried out on HPLC (Shimadzu LC8A, Kyoto, Japan).

Sample Preparation. An excess amount of PCA 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. After attaining equilibrium, the stirrer was turned off to let the solution settle for 2 h.[4] Then the upper portion was taken to prepare the solutions for HPLC analysis. All the measurements were repeated three times.

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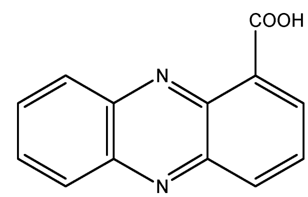

PCA

Figure 1. Molecular structure of PCA.

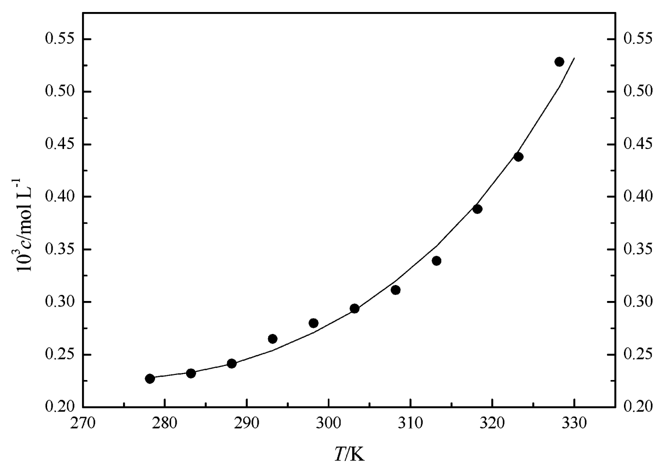

Figure 2. Solubility c of PCA in water. Line represents the correlation.

Sample Analysis. To determine the PCA concentration in the water, a volume of 180 μL cultures were adjusted to pH 2.0 with HCl, mixed well with 540 μL of chloroform. The chloroform phase was taken for HPLC analysis after discarding the upper layer. To determine the PCA concentration in the other two organic solvents, a volume of 180 μL cultures was added to 540 μL of chloroform after evaporation. PCA extracted from the samples was then subjected to HPLC for quantity analysis using C18 reverse-phase column eluted with methanol + water (where the volume fraction of methanol was 70 %) at a flow rate of 1 mL·min^{-1}, detected by UV spectroscopy at $\lambda = 248$ nm and retention time of 1.78 min.[8] The calibration curve for PCA was prepared by using the standard solutions in the concentration range of (0 to 30) 10^{-3} mol·L^{-1} at 25 °C.

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Table 1. Solubility *c* of PCA in Water (1), Methanol (2), and Ethanol (3)

T/K	c_1/10^{-3} mol·L^{-1}	$c_1 - c_1^{calcd}$/10^{-3} mol·L^{-1}	c_2/10^{-3} mol·L^{-1}	$c_2 - c_2^{calcd}$/10^{-3} mol·L^{-1}	c_3/10^{-3} mol·L^{-1}	$c_3 - c_3^{calcd}$/10^{-3} mol·L^{-1}
278.2	0.23 ± 0.01	0.00	3.40 ± 0.12	0.20	3.70 ± 0.01	0.90
283.2	0.23 ± 0.00	0.00	3.70 ± 0.03	0.20	4.30 ± 0.13	0.00
288.2	0.24 ± 0.01	0.00	4.10 ± 0.06	0.10	4.90 ± 0.07	−0.70
293.2	0.25 ± 0.02	−0.01	4.60 ± 0.00	0.70	5.60 ± 0.22	−0.90
298.2	0.27 ± 0.02	−0.01	5.40 ± 0.08	−0.40	6.40 ± 0.19	−0.80
303.2	0.29 ± 0.05	0.00	6.30 ± 0.15	0.10	7.40 ± 0.05	−0.40
308.2	0.32 ± 0.06	0.01	7.60 ± 0.01	−0.40	8.40 ± 0.28	0.30
313.2	0.35 ± 0.04	0.01	9.30 ± 0.59	−0.40	9.60 ± 0.07	0.20
318.2	0.39 ± 0.04	0.00	11.60 ± 0.37	0.20	10.90 ± 0.12	0.20
323.2	0.44 ± 0.05	0.00	14.60 ± 0.50	0.50	12.30 ± 0.57	0.40
328.2	0.51 ± 0.06	−0.02	18.80 ± 0.91	1.20	13.90 ± 0.18	0.90

Results and Discussion

The solubility data of PCA in pure water, methanol, and ethanol at different temperatures are listed in Table 1 and visually shown by Figures 2 and 3, where c is the experimental values with the measurement deviations. The solubility of PCA in water, methanol, and ethanol increases with increasing temperature. Moreover, the solubility of PCA in water is lower than in methanol and ethanol.

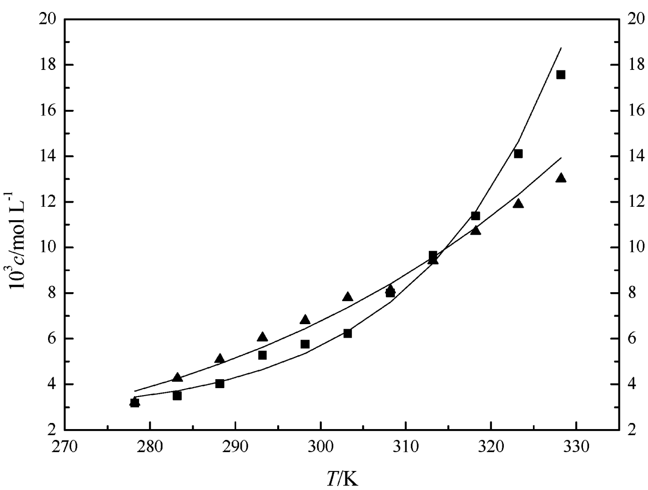

Figure 3. Solubility c of PCA in ■, methanol; ▲, ethanol. Lines represent the correlation.

The temperature dependence of PCA solubility in pure solvents can be described by the modified Apelblat equation:[7,9,10]

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

where c is the solubility of PCA; T is the absolute temperature; and A, B, and C are the parameters. The different values between the experimental solubility and the calculated solubility of PCA are also given in Table 1. The values of parameters A, B, and C and the root-mean-square deviations (rmsd) are listed in Table 2. The rmsd is defined as

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

Table 2. Parameters of Equation 1 for PCA in the Solvents

solvent	A	B	C	100 rmsd
water	−362.79	14716	53.569	0.10
methanol	−604.09	24311	90.796	5.2
ethanol	−52.359	68.981	8.2637	6.4

where n is the number of experimental values, c_i^{calcd} represents the solubility calculated, and c_i represents the experimental solubility values. From the data listed in Table 2, it can be seen that the calculated solubilities show good agreement with the experimental values, which indicates that the modified Apelblat equation is suitable to correlate the solubility data of PCA in the three solvents.

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