Solubility of Delphinidin in Water and Various Organic Solvents between (298.15 and 343.15) K

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The solubilities of 2-(3,4,5-trihydroxyphenyl)chromenylium-3,5,7-triol (delphinidin) in water, methanol, ethanol, and acetone have been measured spectrophotometrically at various temperatures ranging from (298 to 343) K under atmospheric pressure. Delphinidin is most soluble in methanol, followed by water, ethanol, and acetone at all measured temperatures. The experimental data were correlated using the modified Apelblat equation. The calculated solubilities for all solvents showed good agreement with the experimental data in the temperature range studied.

Introduction

Delphinidin [2-(3,4,5-trihydroxyphenyl)chromenylium-3,5,7-triol, CAS Registry No. 13270-61-6, Figure 1] is one of the major anthocyanidin molecules contained in the vacuolar sap of the epidermal tissues of flowers and fruit, to which they impart a pink, red, blue, or purple color.[1] It is the principal and basic skeleton of flower color pigments, so it is the most widespread in nature. It belongs to the group of polyphenolic antioxidants since it contains at least one hydroxyl group attached to a benzene ring and has been reported to possess antioxidant, antiinflammatory, and antiangiogenic properties in vitro.[2] It is commonly administered orally.[1,2] To ensure that the release of this drug material fits the patient needs, researchers try to formulate a dosage form of this drug into controllable release granules. Conventional micronization of drug through recrystallization and comminution have several drawbacks, such as wide size distribution, high thermal and mechanical stress, environmental pollution, large quantities of residual organic solvent, and multistage processes.[3] One of the better methods of preparing controllable release granules is supercritical fluid granulation, which utilizes carbon dioxide as an antisolvent.[4,5] Supercritical fluids offer considerable advantages as solvents or antisolvents in crystallization and precipitation processes. This is why their role has been upscaled and their use as solvents and antisolvents has been nowadays in the center of attention. In the sensitive area of pharmaceuticals processing, various requirements need to be fulfilled: use of the smallest possible amounts of organic solvents, molecular control of the process, one-stage technique that leads to pure product with no residual solvent, control of the properties of the formed microparticles, and application on a large field of pharmaceutical compounds.[6] Since delphinidin is usually obtained from its natural sources through solvent extraction, the knowledge of delphinidin solubility in water and certain organic solvents is important. Furthermore, this information is also necessary in the selection of the most appropriate supercritical antisolvent methods that could be applied. However, from a thorough study on delphinidin literature, it was found that no experimental solubility data of delphinidin in aqueous or organic solvents have been reported.

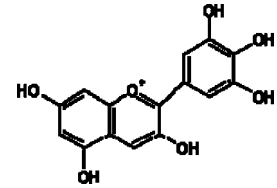

Figure 1. Structure of the delphinidin molecule.

The solubility of delphinidin in water and various organic solvents and at temperatures near the critical point of carbon dioxide was investigated. Solubility measurements were conducted by the pH differential spectrophotometric method.

Experimental Section

Materials. Delphinidin (CAS Registry No. 13270-61-6, > 0.99 mole purity) was purchased from Sigma-Aldrich and used as received without any further treatments. Deionized water, absolute ethanol (CAS Registry No. 64-17-5, > 0.998 mole purity), anhydrous methanol (CAS Registry No. 67-56-1, > 0.998 mole purity), and acetone (CAS Registry No. 67-64-1, > 0.998 mole purity) were purchased from Scharlab S.L., Mallinckrodt, and Merck, respectively, and were also used as solvents without further purification.

Apparatus and Procedure. The solubility of delphinidin was determined using the same apparatus that was described in the literature[7] and explained briefly here (see Figure 2). The experiment was conducted in a glass tube immersed into a constant temperature water bath, which was controlled at the desired temperature by continuous forced water circulation from a thermostat. A mercury-in-glass thermometer (uncertainty of ± 0.1 K) was used for the measurement of the actual temperature inside the glass tube. A predetermined excess amount of delphinidin was charged into 100 mL of solvent contained in a sealed glass tube. The mixture was then agitated using a magnetic stirrer for 1.5 h. The solution was then left for 2 h, to allow the undissolved solids to settle. Prior to the solubility study, some different agitation and settling times were tested to determine a suitable equilibrium time. It was found

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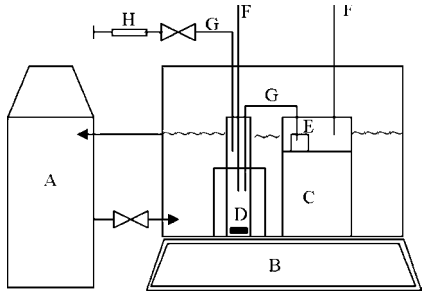

Figure 2. Schematic diagram of isothermal solubility measurement apparatus. A, thermoregulator and refrigerated bath; B, magnetic stirrer; C, sampling compartment; D, test tube (dissolution system); E, weighing bottle; F, thermometer; G, sampling line tubing; and H, syringe.

that 1.5 h of agitation and 2 h of settling time were enough for delphinidin to reach equilibrium in all solvents studied. Then, an approximately 5 mL of the upper clear aliquot was withdrawn for delphinidin analysis using the pH differential spectrophotometric method using AOAC Official Method 2005.02.[8] The samples were conditioned to pH 1.0 and 4.5 by addition of buffer solution of potassium chloride, 0.025 mol·L^{-1}, and sodium acetate, 0.4 mol·L^{-1}, respectively. This is because a monomeric anthocyanin pigments reversibly change color with a change in pH; the colored oxonium form exists at pH 1.0, and the colorless hemiketal form predominates at pH 4.5. The difference in the absorbance (A) of the pigments at 520 nm is proportional to the pigment concentration. To correct the haze, the absorbance of the samples was also measured at 700 nm. Degraded anthocyanins in the polymeric form are resistant to color change regardless of pH and are not included in the measurements because they absorb at pH 4.5 as well as pH 1.0. The delphinidin concentration (DC) was then calculated as cyanidin-3-glucoside equivalent (mg·L^{-1}) as follows:

$$\mathrm{DC} = \frac{A \times \mathrm{MW} \times \mathrm{DF} \times 1000}{\varepsilon \times l} \quad (1)$$

where $A = (A_{520\mathrm{nm}} - A_{700\mathrm{nm}})$ at pH 1.0 and $-(A_{520\mathrm{nm}} - A_{700\mathrm{nm}})$ at pH 4.5, MW = molecular weight (g·mol^{-1}), DF = dilution factor, ε = 26 900 molar extinction coefficient (L·mol^{-1}·cm^{-1}), and l = path length (cm).

The delphinidin solubility measurements were carried out at temperatures of (298.15, 303.15, 308.15, 313.15, 318.15, 323.15, 328.15, 333.15, 338.15, and 343.15) K. The reproducibility of the measurements was checked 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 %.

Results and Discussion

The solubilities of delphinidin in water, methanol, ethanol, and acetone with respective standard deviations (SDs) are listed in Table 1 and presented in Figure 3. To verify the reproducibility of the pH differential spectrophotometric determination, the mole fraction solubility value of delphinidin in water was measured using the gravimetric method at 333.15 K. The value obtained (126.54·10^{-8}) agreed with that measured by the spectrophotometric technique (123.55·10^{-8}), showing the adequacy of the spectrophotometric analysis.

It was obviously found that the solubility values are dependent on the system temperature. Increasing the system temperature increased the delphinidin solubility in all solvents. To some degree, the solubility of delphinidin in three organic solvents

Table 1. Mole Fraction Solubility x_1, Activity Coefficient γ, and Relative Deviation $(x_1 - x_{1\mathrm{calc}})/x_1$ of Delphinidin in Pure Solvents from T = (298.15 to 343.15) K

T/K	10^8 (x_1 ± SD)	γ	$(x_1 - x_{1\mathrm{calc}})/x_1$
	Water		
298.15	53.53 ± 0.06	415.64	−0.09
303.15	63.87 ± 0.03	451.54	0.07
308.15	69.77 ± 0.05	531.21	−0.08
313.15	78.66 ± 0.04	600.78	−0.05
318.15	86.85 ± 0.01	688.42	−0.04
323.15	102.05 ± 0.03	735.96	0.05
328.15	111.35 ± 0.01	841.31	−0.02
333.15	123.55 ± 0.02	939.51	−0.03
338.15	147.54 ± 0.03	968.94	0.04
343.15	163.71 ± 0.02	1075.26	0.04
	Methanol		
298.15	58.61 ± 0.01	378.16	−0.01
303.15	66.24 ± 0.04	430.22	−0.06
308.15	77.12 ± 0.01	486.61	0.03
313.15	87.69 ± 0.01	547.37	0.02
318.15	97.08 ± 0.01	612.51	0.03
323.15	105.43 ± 0.02	682.01	−0.04
328.15	125.89 ± 0.04	755.83	0.07
333.15	140.33 ± 0.02	833.91	0.04
338.15	152.62 ± 0.01	916.15	−0.02
343.15	168.64 ± 0.02	1002.43	−0.04
	Ethanol		
298.15	5.73 ± 0.02	3708.06	−0.04
303.15	6.13 ± 0.04	4358.87	0.07
308.15	7.04 ± 0.02	5060.02	0.03
313.15	7.70 ± 0.02	5804.89	0.05
318.15	8.59 ± 0.02	6585.49	−0.05
323.15	9.80 ± 0.01	7392.77	0.03
328.15	10.58 ± 0.04	8216.81	−0.07
333.15	12.31 ± 0.01	9047.20	0.04
338.15	13.69 ± 0.01	9873.26	−0.05
343.15	15.59 ± 0.02	10684.39	0.04
	Acetone		
298.15	0.0055 ± 0.0012	3546457.82	−0.12
303.15	0.0066 ± 0.0019	4149283.74	0.02
308.15	0.0071 ± 0.0020	4802034.46	0.05
313.15	0.0077 ± 0.0011	5500698.58	0.08
318.15	0.0087 ± 0.0022	6240213.76	0.07
323.15	0.0098 ± 0.0014	7014587.80	0.06
328.15	0.0106 ± 0.0014	7817046.22	−0.10
333.15	0.0124 ± 0.0021	8640198.94	0.05
338.15	0.0135 ± 0.0024	9476218.53	−0.09
343.15	0.0157 ± 0.0013	10317022.81	0.05

increased with the increase of polarity of the solvent, from acetone to ethanol to methanol. Therefore, delphinidin as a polar compound will be more soluble in methanol and less soluble in acetone. However, the polarity of the solvent is not an absolute measure to determine the solubility.[8] The solubility behavior may also be influenced by molecular structure of the solute which determines the polarizability and interaction between the solute and the solvent molecules in the solution[9] and hydrogen bond acidity and hydrogen bond basicity of the solvent.[10,11] Although acetone is also a polar solvent, no intermolecular hydrogen-bonding exists in the solvent.[11] For aliphatic alcohols, there exists strong solvent−solvent interaction due to hydrogen bonding, and this explains the difference in behavior for this class of solvents. In the normal alcohol series the solubility decreases as the number of CH_2 units increases,[12] as proven in this work by a higher solubility of delphidin in methanol as compared to that in ethanol. Further discussion of dissolution phenomena of organic solute in an organic solvent is complicated and beyond the scope of this article.

Water has a higher polarity than methanol, but the solubility of delphinidin in water is slightly lower than that in methanol. It is presumably due to the higher tendency for water

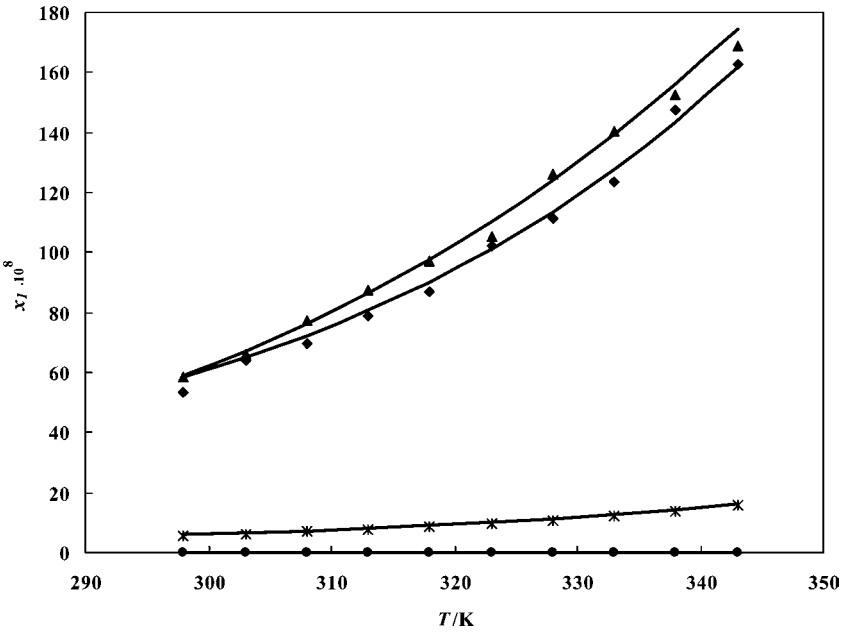

Figure 3. Mole fraction solubility (x_1) of delphinidin in various solvents: ◆, water; ▲, methanol; ∗, ethanol; ●, acetone. The line is the best fit of the experimental data calculated with the modified Apelblat equation.

molecules to remain self-associated through hydrogen bonding than that of methanol molecules. In the case of aromatic solutes, an increase in the solvent−solvent interaction inducing self-cohesiveness has an unfavorable influence on the solubility of the solute.[13] However, it should be remembered that the aforementioned explanation is only an estimation of many factors influencing the dissolution characteristics.

The dependence of delphinidin solubility in pure solvent on the temperature can be described by many thermodynamics approximation methods. A model commonly used in solubility correlation based on the nonideal solution is the modified Apelblat equation (eq 2).[14]

$$\ln x_1 = a + \frac{b}{T} + c \ln(T) \tag{2}$$

where x_1 and T are the mole fraction of the solute and absolute temperature (K), respectively, and a, b, and c are the empirical constants. The c value represents the effect of temperature on the fusion enthalpy, as a deviation of heat capacity (ΔC_p). The values of constants a and b reflect the variation in the solute activity coefficient and provide an indication of the effect of solution nonidealities on the solubility of the solute. The values of parameters a, b, and c were evaluated by multidimensional unconstrained nonlinear minimization using MATLAB software.

Delphinidin is a rigid molecule. However, no solid phase analysis was performed. It is therefore assumed that a solid−solid transition does not occur and the solute activity coefficient (γ) can be evaluated using the equation below:[15,16]

$$\ln(\gamma x_1) = \frac{-\Delta_{\text{fus}}H}{RT_{\text{m}}}\left(\frac{T_{\text{m}}}{T} - 1\right) \tag{3}$$

where $\Delta_{\text{fus}}H$ and T_{m} are the heat of fusion ($\text{J}\cdot\text{mol}^{-1}$) and melting point (K) of the solute, respectively. The $\Delta_{\text{fus}}H$ and T_{m} values were estimated using group contribution method suggested by Jain and Yalkowsky.[17]

The correlated solubility values of delphinidin and the corresponding activity coefficients are shown in Table 1,

Table 2. Optimized Adjustable Parameters of the Modified Apelblat Equation (eq 2) for Delphinidin Solubility in Water and Various Organic Solvents

solvent	a	b	c	rmsd
water	−153.8208	4626.9892	21.7474	0.0120
methanol	−47.306	−531.6297	6.0909	0.0047
ethanol	−194.5069	6467.139	27.4032	0.0041
acetone	−163.0732	4676.2559	21.7285	0.072

whereas the values of parameters a, b, and c and the root-mean-square deviation (rmsd) are given in Table 2. The rmsd is defined as

$$\text{rmsd} = \frac{1}{N}[\sum_{i=1}^{N}(x_{1,i} - x_{1,i}^{\text{calcd}})^2]^{1/2} \tag{4}$$

where N is the number of experimental points; $x_{1,i}^{\text{calcd}}$ is the solubility calculated from the modified Apelblat model; and $x_{1,i}$ is the experimental value of solubility. From Tables 1 and 2, it can be observed that the correlated solubility agreed well with the experimental values. This indicated that the modified Apelblat equation is suitable to correlate the measured value of delphinidin solubility in the four solvents and in the tested temperature range. Unfortunately, there is no such trend that can be obtained from a and b values presented in Table 2 which represent the variation of solute activity coefficient. Clearer consideration can be drawn from the activity coefficient values obtained in this work (Table 1). All of them were far higher than unity, showing the high nonideality of the system. The activity coefficients for the system delphinidin−water and delphinidin−methanol were very close at respective temperatures. The activity coefficients for system delphinidin−ethanol were about 10 times of those of delphinidin−water and delphinidin−methanol systems. With the fact that acetone is the less polar solvent in this study, the system of acetone−delphinidin was found to be the most nonideal system as indicated by highest activity coefficient values.

Conclusions

In this work, new data were measured for the solubility of delphinidin in water, methanol, ethanol, and acetone at tem-

peratures between (298 and 343) K using pH differential spectrophotometric observation technique. On the basis of the results of the experiment, the following conclusions can be drawn: (i) The solubility of delphinidin in all solvents studied increases as the temperature increases. (ii) The solubility of delphinidin also depends on the polarity of the solvent to some degree. The title compound is soluble in polar solvents (water, methanol, and ethanol), except for acetone. (iii) The modified Apelblat equation is appropriate to describe the temperature dependence of delphinidin in pure solvents, and its parameters are obtained by regression. Therefore, the experimental solubility and correlated equation in this work can be used as essential data and models for the production of controllable release delphinidin granules.

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