Solubility of Gallic Acid, Catechin, and Protocatechuic Acid in Subcritical Water from (298.75 to 415.85) K

Keerthi Srinivas,[†] Jerry W. King,*[,†] Luke R. Howard,[‡] and Jeana K. Monrad[‡]

Ralph E. Martin Department of Chemical Engineering, University of Arkansas, 3202 Bell Engineering Center, Fayetteville, Arkansas 72701, and Department of Food Science, University of Arkansas, 2650 North Young Avenue, Fayetteville, Arkansas 72704

The solubility of gallic acid hydrate, protocatechuic acid, and (+)-catechin hydrate was measured between 298.75 K and 415.85 K using a dynamic flow apparatus. The aqueous solubility of gallic acid hydrate was found to vary between 12.6 $g \cdot L^{-1}$ at 298.75 K and 2870 $g \cdot L^{-1}$ at 415.85 K. The aqueous solubility of protocatechuic acid at the same temperatures varied between 29.4 $g \cdot L^{-1}$ and 1180 $g \cdot L^{-1}$, respectively, while that of (+)-catechin hydrate varied between 2.26 $g \cdot L^{-1}$ and 576 $g \cdot L^{-1}$, respectively. The aqueous solubility of the phenolic compounds was found to increase exponentially with temperature. The temperature dependence of the aqueous solubility of the phenolic compounds was estimated using empirical correlations based on the data presented in this work. The thermodynamic properties such as standard molar enthalpy, standard molar entropy, and standard molar Gibbs energy of solution were also calculated from the solubility data.

Introduction

Flavonoids are a diverse group of polyphenolic compounds present in plants and natural products. They are used in food as colorants[1] and in nutraceutical industries for their antioxidant, antibacterial, antiviral, and anti-inflammatory properties.[2] Studies have indicated that the flavonoids exhibit antiproliferative activity toward coronary heart disease,[3] lung cancers,[4] and other types of cancers.[5] Flavonoids have also been used in skin rejuvenating creams to heal and moisturize aged and sun burnt skin.[6] The extraction of such flavonoid compounds from plants and natural products has been performed using solvent extraction,[7] microwave-assisted extraction,[8] ultrasound extraction,[9] solid phase extraction,[10] and supercritical fluid extraction.[11] For example, studies have indicated that supercritical carbon dioxide can be used to extract about 79 % of flavonoids from grape seeds,[12] and the use of methanol[13,14] and ethanol[15] as cosolvents was found to increase the flavonoid yields from grape seeds. The studies indicated that the alcoholic cosolvents increased the polarity of supercritical carbon dioxide to aid in the extraction of the flavonoids.

Water heated to higher temperatures above its boiling point and under pressure, also known as "subcritical" water, has been used in the extraction of antioxidants from aspen knotwood,[16] rosemary plants,[17] oregano,[18] Brazilian propolis,[19] and berry substrates.[20] The increased interest in the subcritical water extraction of flavonoid compounds from natural products was previously related to the decrease in the dielectric constant of water.[21] However, recently, the authors have shown that the effect can be related to a significant decrease in the hydrogen bonding propensity of the solubility parameter for water with an increase in temperature.[22] Apart from being a "green", environmentally friendly, and cheap solvent, subcritical water also has a greater affinity to extract polar compounds such as the flavonoids from plants and natural products. To better apply the subcritical water for the extraction of flavonoids, knowledge of the physicochemical properties of the flavonoids in water such as solubility is essential. However, the availability of such data is limited in the literature and is virtually nonexistent at subcritical water temperatures. This study reports on the aqueous solubility of certain flavonoids (phenolic compounds), contained in grape pomace[23] as a function of temperature ranging between 298.75 K and 415.85 K. The chemical structures of the phenolic compounds used in this study are shown in Figure 1.

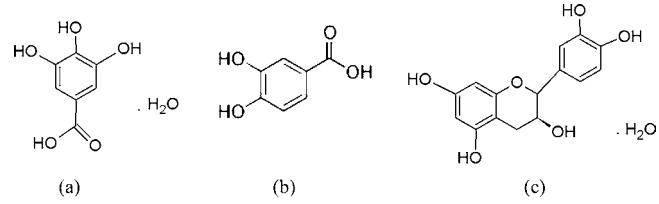

Figure 1. Structure of phenolic compounds: (a) gallic acid hydrate, (b) protocatechuic acid, and (c) (+)-catechin hydrate.

As indicated previously, there exists some literature data on the aqueous solubility of gallic acid and protocatechuic acid in water. Lu and Lu[24] measured the aqueous solubility of gallic acid and its esters between 273.15 K and 363.15 K using a traditional shake flask method. However, this method can prove disadvantageous in accurately measuring the effective aqueous solubility of gallic acid, especially when the temperatures approach the boiling point of water. Studies conducted by Noubigh et al.,[25] Mota et al.,[26] and Daneshfar et. al[27] measured the aqueous solubility of gallic acid until 318.20 K, 323.15 K, and 333.20 K, respectively. In these studies, the aqueous solubility measurements were performed using a constant-temperature stirred-type reactor with analysis performed using a HPLC and/or a spectrophotometer. Similar studies were performed by Noubigh et al.[25] and Queimada et al.[28] to measure the aqueous solubility of protocatechuic acid up to 318.15 K and 323.15 K, respectively. Additional studies performed by

* Corresponding author. Tel.: 01-(479)-575-5979. Fax: 01-(479)-575-7926. E-mail: jwking1@uark.edu.
[†] Ralph E. Martin Department of Chemical Engineering.
[‡] Department of Food Science.

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Noubigh et al.[29–31] reported the solubility of gallic acid and protocatechuic acid in pure water at room temperature and investigated the effect of salts and nitrates on their aqueous solubilities. There exist no data on the aqueous solubility of catechin in the literature.

Other measurements on the aqueous solubility of various compounds such as polycyclic aromatic hydrocarbons,[21] alkylcyclohexanes,[32] and pesticides[33] reported that pressure had a minimal effect on the aqueous solubility in subcritical water temperatures. These studies used pressures that are sufficient to maintain water in its liquid state above the boiling point of water.

In our studies, a dynamic flow apparatus was used to measure the aqueous solubility of gallic acid hydrate, protocatechuic acid, and (+)-catechin hydrate in subcritical water. This dynamic flow apparatus allowed a continuous flow of water through a saturation cell containing the dispersed phenolic compound, and the outlet concentration of the solute (after equilibration at a particular temperature) was measured to determine the aqueous solubility of the compound at that particular temperature. A decrease in the concentration of the compound collected in the outlet vial with time at a particular temperature can be attributed to either a decreasing amount of feed mixture into the saturation cell or thermal degradation of the phenolic compounds.

In this study, a new and novel approach has been developed for measuring solute solubilities in water at temperatures approaching the boiling point of water, including at temperatures of 40 °C above the boiling point of water. Measurements in this temperature region using more conventional solubility measurement techniques are difficult to execute due to the vaporization of the solvent (water) and thermal degradation of the solutes as a function of time. The continuous flow method that we have developed avoids such pitfalls by allowing precise and continuous flow of solvent through a packed solute bed over a short period of time with sampling done at regular time intervals as briefly described above.

Solution thermodynamic properties such as the molar enthalpy, molar entropy, and molar Gibbs free energy of solution of the phenolic compounds as a function of temperature were calculated from the solute's aqueous solubility values. These thermodynamic properties were used to understand the dissolution of these phenolic compounds in water. The aqueous solubility of the phenolic compounds was also correlated using empirical equations such as the modified Apelblat equation.[34] Such empirical equations can provide a method for predicting the aqueous solubility of the phenolic compounds as a function of temperature.

Using the above technique, we have determined solubility data on flavonoids where none has existed previously. This has allowed us to establish the validity of models for predicting flavonoid solubilities over this extended temperature range, inclusive of the subcritical water region. This solubility and corresponding thermodynamic properties data are critical to optimizing the design of extraction processes using hot pressurized water in place of organic solvents or hydroethanolic solvent media.

Experimental Section

Chemicals. (2*R*,3*S*)-2-(3,4-Dihydroxyphenyl)-3,4-dihydro-1*H*-chromene-3,5,7-triol ((+)-catechin hydrate; CAS# 225937-10-0; Lot# 1386954; Stock# 31508270; mass fraction purity ≥ 0.97 purum) and 3,4-dihydroxybenzoic acid (protocatechuic acid; CAS# 99-50-3; Lot# 0001400812; mass fraction purity ≥ 0.98 powder) were obtained from Sigma Aldrich (St. Louis, MO, USA), and 3,4,5-trihydroxybenzoic acid (gallic acid hydrate; CAS# 149-91-7; Lot# CBEJB; mass fraction purity ≥ 0.97 powder) was obtained from VWR (Batavia, IL, USA). Sea sand (washed) was acquired from EMD (Gibbstown, NJ, USA). Water (1−5 ppb TOC, 18.2 MΩ c and < 0.001 EU·mL^{-1} pyrogen levels) purified using a Milli-Q Synthesis A10 system (Millipore, Bellerica, MA, USA) was used as the solvent. The water was degassed using a nitrogen purge. HPLC-grade methanol (CAS# 67-56-1; Lot# 49204) that was used as a dilution solvent in some experiments and the other HPLC-grade reagents were purchased from VWR (Batavia, IL, USA).

Dynamic Flow Apparatus. The flow apparatus for measuring the aqueous solubility of the phenolic compounds is shown in Figure 2. The apparatus is based on a modification of the system used by Miller and Hawthorne.[35] In this study, a high-pressure saturation cell was placed in a Hewlett-Packard (HP) model 5890 oven. The temperature in the oven varied with an error of not more than ± 0.1 K. The temperature inside the oven was measured using a J-type thermocouple coupled with an Omega DP703 thermocouple microcomputer (Stamford, CT, USA). The saturation cell consisted of a hollow column made up of SS-316 tubing fitted with proper end fittings (Parker Hannifin Corp., Columbus, OH, USA). The size of the saturation cell was scaled in proportion to the increase in the aqueous solubility of the phenolic compounds at higher temperature. Aqueous solubility measurements until around 355.25 K were performed using a hollow TSK column (TOSOH Bioscientific, PA, USA, 0.2755 in. i.d. × 2.98 in. length), while the measurements at 375.35 K and 395.85 K were performed using a self-designed column (0.2755 in. i.d. × 6.01 in. length) and at 415.85 K using a similar column (1 in. i.d. × 6.01 in. length). The system pressure varied between 1 atm at temperatures up to 353.75 K and as high as 3.5 atm at higher temperatures, sufficient pressures to sustain water above its boiling point in the subcritical state.

The phenolic compound was mixed with sand in 1:2 ratio (by weight) and placed in the saturation cell. Water was allowed to flow through the saturation cell at a flow rate of (0.1 to 0.5) mL·min^{-1} using a high-pressure ISCO 260D pump (Lincoln, NE, USA) through a 3 m preheating coil placed in the oven. Another ISCO 260D pump was used to supply excess water which contacts the saturated solution exiting from the cell at a mixing tee (High Pressure Equipment Inc. (HIP), Eric, PA, P/N# HIP15-23AF1) placed in the oven. The excess solvent, flowing at (0.4 to 2.0) mL·min^{-1}, was intended to prevent the precipitation of the phenolic compound solution due to a decrease in temperature when it exits the oven into a collection vial through a 1.5 m cooling coil. The rapid decrease in the temperature of the outlet line would result in clogging of the system, thereby affecting effective solubility measurements. The flow rates in both the ISCO pumps were controlled using an ISCO SFX 200 controller (Lincoln, NE, USA), and a consistent dilution factor of 4 was maintained in all the experiments. An on/off switching valve (High Pressure Equipment Inc. (HIP), Eric, PA; P/N # HIP15-11AF1) was placed at the outlet from the oven to be used as a back-pressure regulator to throttle the water flow rate and prevent conversion to steam.

The solvent was allowed to flow through the saturation cell for (10 to 20) min until steady state was achieved. After equilibration, 10 fractions were collected every (1 to 3) min in the collection vial and analyzed using HPLC. For solubility measurements above the boiling point of water (i.e., 373.15 K), methanol was used as the dilution solvent, primarily due to a greater increase in the aqueous solubility of the phenolic compound relative to its solubility at room temperature. The

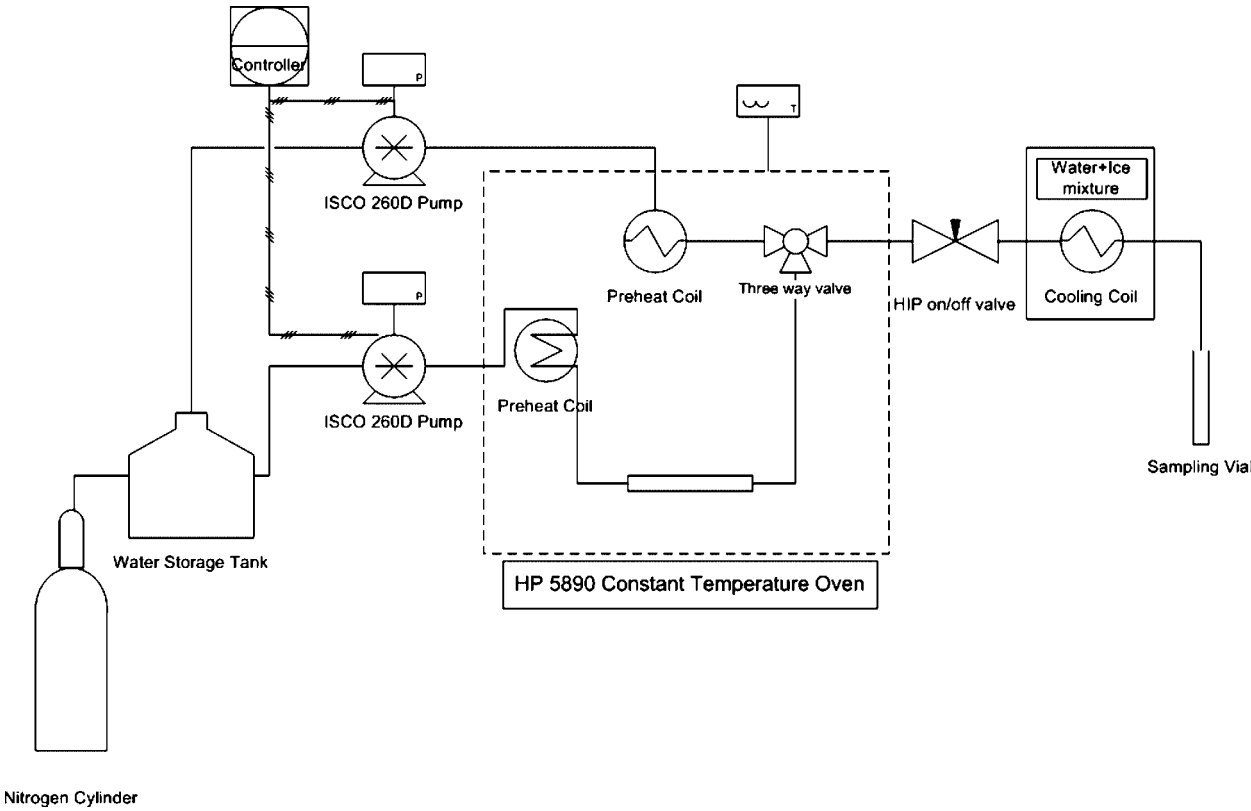

Figure 2. Dynamic flow apparatus for measuring the aqueous solubility of phenolic compounds.

aqueous solubility measurements were performed in triplicate for each solute and each temperature.

The aqueous solubility of the phenolic compounds measured can be expressed in terms of mole fraction (x_s) using eq 1.

$$x_s = \frac{1}{1 + \left[\frac{M_s}{M_w}\left(\frac{1}{S} - 1\right)\right]} \quad (1)$$

where M_s and M_w are the molecular weights of the phenolic compounds and water, respectively, and S is the aqueous solubility of the phenolic compound in grams per liter of solvent.

HPLC Analysis. The quantitative analysis of the phenolic compounds dissolved in the solution collected in the vial at different time intervals was performed using the method described by Scheiber et al.[36] The mobile phase consisted of 2 % (v/v) acetic acid in water (eluent A) and (50:50, v/v) 0.5 % acetic acid in water and acetonitrile (eluent B). An amount of 0.5 mL of the phenolic compound solution was mixed with 0.5 mL of methanol and placed in the injection vial. An amount of 100 μL of the mixture was analyzed using a Phenomenex Aqua C18 column (250 mm × 4.6 mm, 0.5 μm particle size) (Torrance, CA, USA). The following HPLC gradient program was used: 10 % B to 55 % B (30 min); 55 % B to 10 % B (35 min); 1 mL·min^{-1} flow rate. The samples containing the phenolic compounds were monitored at 280 nm using a Waters Photodiode Array Detector model 2998 (Milford, MA, USA). The concentration of the phenolic compounds in the samples analyzed using HPLC was calculated and recorded using Waters Empower chromatography data software (ver. 2; Milford, MA, USA).

Results and Discussion

The aqueous solubilities of the selected phenolic compounds as a function of temperature are given in Tables 1 to 3 and Figures 3 to 5. It can be seen from the figures that the aqueous solubility of the phenolic compounds increased exponentially with temperature. The reported solubility values in Tables 1 to 3 were an average of three experiments with the corresponding standard deviations calculated using the following equation

$$\text{rmsd} = \sqrt{\frac{\sum_{i=1}^{n}(X_{g,i} - \bar{X}_g)^2}{(n-1)}} \quad (2)$$

where $X_{g,i}$ = solubility for the ith sample at a particular temperature for a selected solute; $\bar{X}_g$ = average solubility of the selected solute at a particular temperature; and n = number of samples. All the data were reported to three significant digits after statistical analysis accompanied by the analysis of variances performed at the $P < 0.005$ level.

Table 1. Solubility of Gallic Acid Hydrate in Water

T	S	$10^3 x_s$				
K	g·L^{-1a}	exptl	lit.	eq 3	eq 5	eq 7
298.75	13.0 (± 0.65)	1.24	0.99,[24] 1.17,[25] 1.47,[26] 1.52[27]	1.25	1.24	1.24
314.65	25.0 (± 0.30)	2.40	2.35,[24] 1.38,[25] 2.65,[26] 2.53[27]	2.41	1.71	2.97
334.95	83.7 (± 2.1)	8.08	6.81,[24] 7.33[27]	6.11	2.57	8.23
355.25	211 (± 15)	20.3	18.9[24]	16.3	3.91	20.3
375.35	532 (± 15)	50.4		47.5	6.15	45.1
395.65	1000 (± 5.0)	92.7		142	10.5	92.9
415.85	2870 (± 110)	233		430	19.8	178

[a] The standard deviations were calculated from three replicate measurements using eq 2.

Table 2. Solubility of Protocatechuic Acid in Water

T	S	$10^3 x_s$				
K	g·L^{-1}*	exptl	lit.	eq 3	eq 5	eq 7
298.75	29.4 (± 0.98)	3.55	2.23,[25] 2.20[28]	3.53	3.55	3.55
314.65	44.3 (± 0.25)	5.25	2.44,[25] 2.46[28]	5.22	4.73	8.23
334.95	95.3 (± 1.6)	11.3		9.21	6.71	21.5
355.25	175 (± 9.3)	20.9		17.2	9.64	50.1
375.35	305 (± 10)	35.9		33.4	14.5	106
395.65	773 (± 35)	88.6		67.2	23.6	209
415.85	1180 (± 84)	126		137	42.9	385

* The standard deviations were calculated from three replicate measurements using eq 2.

Table 3. Solubility of (+)-Catechin Hydrate in Water

T	S	$10^3 x_s$			
K	g·L^{-1a}	exptl	eq 3	eq 5	eq 7
298.75	2.26 (± 0.50)	0.132	0.149	0.132	0.132
314.65	5.03 (± 0.57)	0.294	0.294	0.208	0.362
334.95	44.4 (± 2.7)	2.63	1.05	0.357	1.14
355.25	128 (± 3.5)	7.65	5.24	0.606	3.15
375.35	263 (± 23)	15.6	33.56	1.05	7.72
395.65	423 (± 23)	25.6	267	1.97	17.4
415.85	576 (± 9.5)	35.2	2450	4.04	36.2

[a] The standard deviations were calculated from three replicate measurements using eq 2.

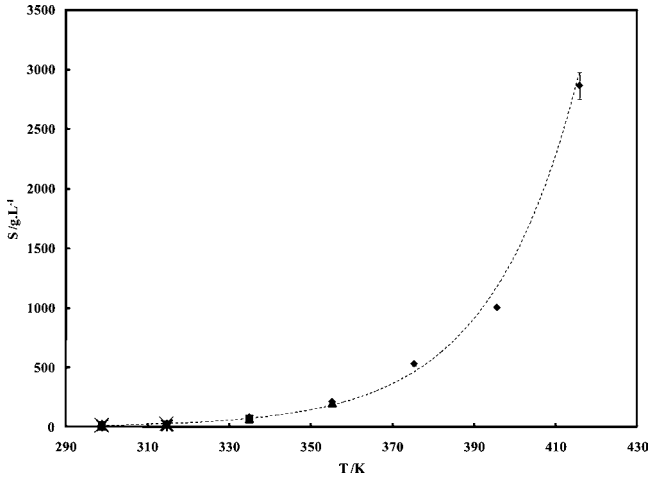

Figure 3. Variation of the aqueous solubility (g·L^{-1}) of gallic acid hydrate as a function of temperature and comparison with available literature data: ♦, experimental data; ▲, literature;[24] *, literature;[25] ×, literature;[26] ■, literature;[27] - - -, correlation of experimental data.

The experimentally measured aqueous solubility of the selected phenolic compounds was compared with those available in the literature. It can be seen from Figure 3 that there is a good agreement between the aqueous solubility of the gallic acid hydrate measured in this work and that available in the literature. A similar trend was also seen for protocatechuic acid as shown in Figure 4. However, as can be seen in Figure 5, there is no literature data on the aqueous solubility of (+)-catechin hydrate as a function of temperature. It can also be seen from Tables 1 and 2 that the aqueous solubility of gallic acid hydrate is lower than that of protocatechuic acid until approximately 334.95 K, above which the solubility of gallic acid hydrate became greater than protocatechuic acid and rapidly increased as a function of temperature. This is in agreement with the trends observed in Noubigh et al.[25] for gallic acid's solubility in water as a function of temperature. However, this trend was opposite to the gallic acid and protocatechuic acid

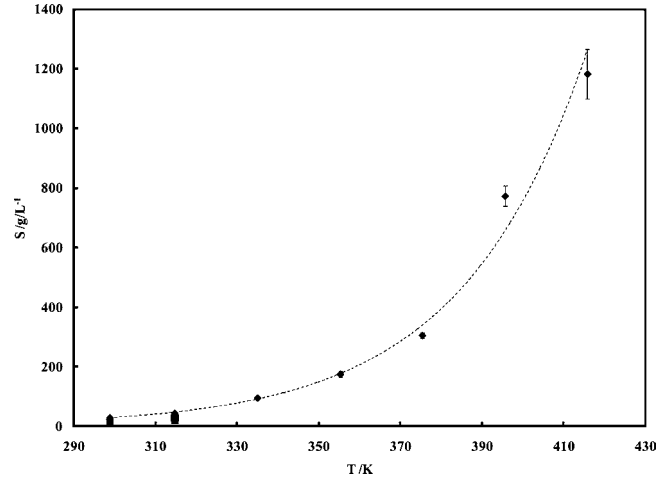

Figure 4. Variation of the aqueous solubility (g·L^{-1}) of protocatechuic acid as a function of temperature and comparison with available literature data: ♦, experimental data; ▲, literature;[25] ■, literature;[28] - - -, correlation of experimental data.

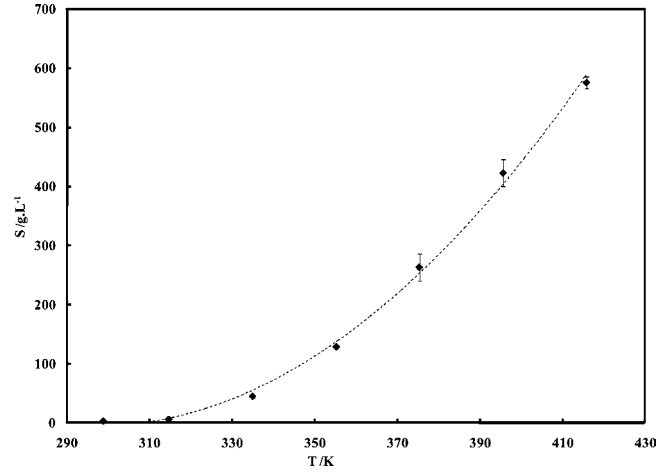

Figure 5. Variation of the aqueous solubility (g·L^{-1}) of (+)-catechin hydrate as a function of temperature: ♦, experimental data; - - -, correlation of experimental data.

solubility trends observed by Queimada et al.[28] as a function of temperature. It can also be observed from the examination of Figures 3 to 5 that the aqueous solubility of the selected phenolic compounds at room temperature decreased in the following order: protocatechuic acid > gallic acid hydrate > (+)-catechin hydrate.

The mole fraction solubility of the selected phenolic compounds was calculated from the measured solubility (in grams per liter) using eq 1. The natural logarithm of the mole fraction solubility of the selected phenolic compounds is plotted as a function of inverse of temperature as shown in Figure 6. It can be seen from Figure 6 that the solubility trend expressed as the natural logarithm of the mole fraction units decreased with an increase in the molecular weight (MW) of the phenolic compounds (MW protocatechuic acid = 154.12 g·mol^{-1}; MW gallic acid hydrate = 180.14 g·mol^{-1}; MW (+)-catechin hydrate = 308.28 g·mol^{-1}). Even though there is a slight difference between the molecular weights of the solutes, the mole fraction convention is used in comparing the solubility trends of the phenolic compounds as have been used in previous literature.[24,25,27,32,37]

As discussed before, the aqueous solubility of protocatechuic acid becomes lower than that of gallic acid hydrate above 334.95

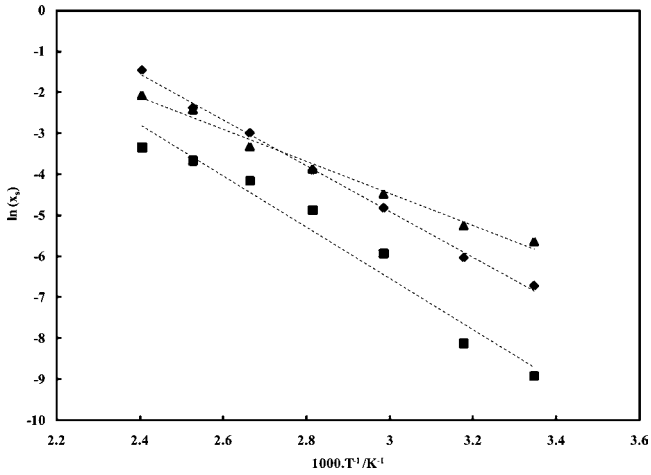

Figure 6. Variation of the natural logarithm of aqueous solubility (mole fraction) of the selected phenolic compounds as a function of inverse of temperature ($10^{-3}K^{-1}$): ♦, gallic acid hydrate; ▲, protocatechuic acid; ■, (+)-catechin hydrate; - - -, correlation of experimental data.

Table 4. Modified Apelblat Equation Parameters

solute	*A*	*B*	*C*	rms[a]
gallic acid hydrate	−343.53	12054.7	52.02	0.0767
protocatechuic acid	−236.40	8556.58	35.46	0.0102
(+)-catechin hydrate	−947.52	39374.53	141.57	0.9169

[a] The root-mean-square deviation was calculated from three replicate measurements using eq 4.

K. It was observed that the aqueous solubility trends of these two compounds as a function of temperature cross at around 343.15 K. It can also be seen from Figure 6 that the natural logarithm of the mole fraction solubility of (+)-catechin hydrate in water does not exhibit a perfectly linear trend as a function of inverse temperature ($R^2 = 0.925$). This variation in the trend of the aqueous solubility of (+)-catechin hydrate is a result of the high molecular weight of the compound relative to the other phenolic compounds when the mole fraction solubility is calculated using eq 1. The deviation from a perfectly linear trend obtained when the natural logarithm of the aqueous solubility of (+)-catechin hydrate was plotted as a function of inverse temperature can also be related to a secondary transition between the crystalline forms of the phenolic compound in water occurring especially at high temperatures.

The measured aqueous solubilities of the selected phenolic compounds as a function of temperature can be correlated using the modified Apelblat equation provided by Heidman et al.[38] given below

$$\ln(x_s) = A + \frac{B}{T} + C\ln(T) \tag{3}$$

where, x_s is the mole fraction solubility of the compound in water at temperature T (K) and A, B, and C are empirically derived constants. The values of the empirical constants for the selected phenolic compounds are given in Table 4 with the corresponding root-mean-square deviations (rms) between the predicted and the measured solubility values, calculated as follows

$$\text{rms} = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{x_{s,i}^{\text{calcd}} - x_{s,i}^{\text{exptl}}}{x_{s,i}^{\text{exptl}}}\right)^2 \tag{4}$$

where, $x_{s,i}^{\text{calcd}}$ and $x_{s,i}^{\text{exptl}}$ are the mole fraction solubilities of the selected phenolic compounds in water at a particular temperature calculated using eq 3 and the determined experimental values, respectively. The empirical constants are provided with more than three significant digits to improve the accuracy of the prediction using the modified Apelblat equation.

The mole fraction solubilities of the selected phenolic compounds as a function of temperature calculated using eq 3 are provided in Tables 1 to 3. It can be seen from the root-mean-square deviation (rms) reported in Table 4 that the solubilities predicted by the modified Apelblat equation are in good agreement with the experimentally determined values. The only phenolic compound that showed poor agreement with the aqueous solubilities measured in this work and that calculated by eq 3 is (+)-catechin hydrate. This is due to a slight scatter in the solubility observed when the natural logarithm of the mole fraction solubility of (+)-catechin hydrate is plotted as a function of temperature.

Another possible method to allow the prediction of the aqueous solubility of the phenolic compounds as a function of temperature, if its solubility in water at room temperature is known, is due to Miller et al.,[21] as given by eq 5 below

$$\ln x_s(T) = \frac{T_o}{T}\ln x_s(T_o) + 15\left(\frac{T}{T_o} - 1\right)^3 \tag{5}$$

where $x_s(T)$ and $x_s(T_o)$ are the mole fraction solubilities of the phenolic compounds at temperature T and reference temperature T_o, respectively. The aqueous solubilities of the selected phenolic compounds calculated as a function of temperature using eq 5 are also given in Tables 1 to 3. The root-mean-square deviation (rms) between the experimental data and the predicted values can be calculated using eq 4 and is found to be 0.0882, 0.0408, and 0.0160 for gallic acid hydrate, protocatechuic acid, and (+)-catechin hydrate, respectively. It can be seen from Table 3 that the aqueous solubility of (+)-catechin hydrate predicted using eq 5 is more accurate when compared with that predicted using the modified Apelblat equation. However, similar conclusions cannot be made for the aqueous solubility prediction for gallic acid hydrate and protocatechuic acid.

Assuming the zeroth approximation,[21] i.e., the molar Gibbs free energy of solution for the phenolic compounds in water remains constant as a function of temperature, the aqueous solubility of any solute can be predicted using an equation of the following form

$$\ln x_s(T) \approx \left(\frac{T_o}{T}\right)\ln x_s(T_o) \tag{6}$$

The approximation given for solute solubility in water (eq 6) is the basis for the formulation of the solubility prediction model given in eq 5. A plot of $\ln[x_s(T)] - (T_o/T)\ln[x_s(T_o)]$ as a function of inverse of temperature (Figure 7) can be used to check the validity of eq 6. It can be seen from Figure 7 that the deviation from linearity (or zeroth approximation) increases with an increase in temperature. This particular trend was also noticed by Miller et al.[21] and indicated that the zeroth approximation which is related to a constant molar Gibbs free energy of the solution as a function of temperature is not valid. From the limited solubility data presented in this work, a good linear fit can be seen when the term $11(1 - T_o/T)$ is added to the right-hand side of eq 6. The aqueous solubility of the selected phenolic compounds as a function of temperature can then be predicted using the following equation to a first approximation

$$\ln x_s(T) = \left(\frac{T_o}{T}\right)\ln x_s(T_o) + 11\left(1 - \frac{T_o}{T}\right) \tag{7}$$

The aqueous solubilities of the selected phenolic compounds calculated as a function of temperature using eq 7 are also given

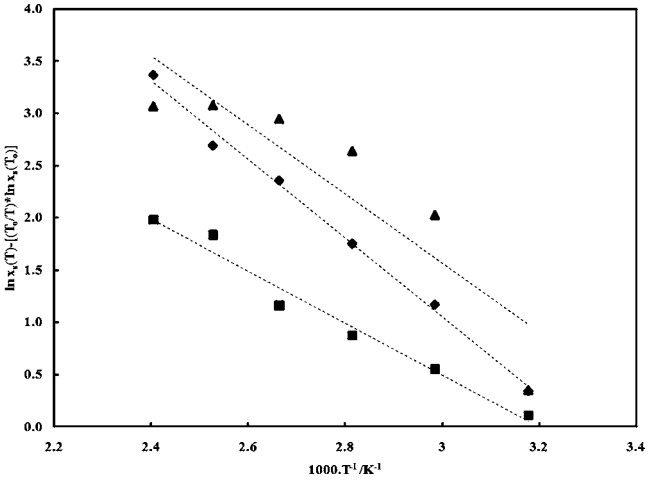

Figure 7. Plot of ln $x(T) - [(T_o/T)\ln x(T_o)]$ as a function of inverse of temperature to verify the zeroth approximation indicating a linear relationship between solubilities as a function of temperature: ♦, gallic acid hydrate; ▲, protocatechuic acid; ■, (+)-catechin hydrate; - - -, correlation of experimental data.

in Tables 1 to 3. The rms values calculated using eq 4 are found to be 0.0209, 0.112, and 0.00465 for gallic acid hydrate, protocatechuic acid, and (+)-catechin hydrate, respectively. On comparison between the aqueous solubilties of (+)-catechin hydrate predicted as a function of temperature using eqs 5 and 7, the latter prediction showed a good agreement with the experimentally determined values. It can be seen from Table 2 that the solubility prediction using eq 7 did not provide good agreement for the aqueous solubility of protocatechuic acid with the experimental data in comparison with that predicted using eqs 3 and 5. However, by altering the constant in the second term on the right-hand side of eq 7, the agreement between predicted and experimental values can be improved. Overall, it was found that solubility predictions using the modified Apelblat equation provided the best agreement with experimental data, although eq 7 does have some utility for the subcritical water extraction of phenolic compounds.

The thermodynamic properties of solution such as standard enthalpy, standard entropy, and standard Gibbs free energy can be used to elucidate and better understand the relative differences in the solubility trends observed in this work. The standard enthalpy ($\Delta H_{sol}{}^0$) of solution of the phenolic compounds can be calculated from the Gibbs−Helmholtz equation as given by Queimada et al.[28]

$$\Delta H_{sol}{}^0 = RT^2\left(\frac{d \ln x_s}{dT}\right)_p \quad (8)$$

where R is the universal gas constant; T is the temperature (K); and x_s is the experimentally measured mole fraction solubility of the phenolic compounds in water. The differential term in eq 8 is calculated by measuring the slope of the straight line obtained by plotting the natural logarithm of aqueous solubility of the compound, expressed in mole fraction units, as a function of temperature.

The standard Gibbs free energy ($\Delta G_{sol}{}^0$) and standard entropy ($\Delta S_{sol}{}^0$) of solution can then be calculated using eqs 9 and 10, respectively, as given below

$$\Delta G_{sol}{}^0 = -RT \ln(x_s)_p \quad (9)$$

$$\Delta S_{sol}{}^0 = \frac{\Delta H_{sol} - \Delta G_{sol}}{T} \quad (10)$$

The thermodynamic properties of solution calculated from the experimentally measured aqueous solubility data using eqs 8, 9, and 10 are given in Table 5. It can be seen from the values reported in Table 5 that the standard Gibbs free energy of solution of the phenolic compounds is not constant but decreases with increasing temperature. This is in agreement with the assumption made while formulating eq 7 to the first approximation. A positive standard Gibbs free energy of solution decreases with temperature ($\Delta G_{sol}{}^0 > 0$) indicating that the dissolution of phenolic compounds in water increases with temperature.[25,28] In contrast to the solubility trends, the thermodynamic properties of dissolution of phenolic compounds in water as a function of increasing temperature occur in the following order: (+)-catechin hydrate > gallic acid hydrate > protocatechuic acid.

Table 5. Thermodynamic Properties of the Dissolution of the Phenolic Compounds in Water as a Function of Temperature

T / K	$\Delta H_{sol}{}^0$ / kJ·mol^{-1}[a]	$\Delta G_{sol}{}^0$ / kJ·mol^{-1}[a]	$\Delta S_{sol}{}^0$ / J·mol^{-1}·K^{-1}[a]
	Gallic Acid Hydrate		
298.75	33.6 (± 0.2)	16.7 (± 0.1)	56.7 (± 0.2)
314.65	37.3 (± 0.2)	15.8 (± 0.0)	68.4 (± 0.6)
334.95	42.3 (± 0.2)	13.4 (± 0.1)	86.1 (± 0.6)
355.25	47.5 (± 0.2)	11.5 (± 0.0)	101 (± 0.5)
375.35	53.1 (± 0.2)	9.33 (± 0.1)	117 (± 0.6)
395.65	59.0 (± 0.3)	7.82 (± 0.0)	129 (± 0.7)
415.85	65.1 (± 0.3)	5.04 (± 0.0)	145 (± 0.8)
	Protocatechuic Acid		
298.75	24.6 (± 0.2)	14.0 (± 0.0)	35.6 (± 0.7)
314.65	27.3 (± 0.2)	13.7 (± 0.0)	43.2 (± 0.7)
334.95	31.0 (± 0.2)	12.5 (± 0.0)	55.2 (± 0.6)
355.25	34.8 (± 0.3)	11.4 (± 0.1)	65.9 (± 0.4)
375.35	38.9 (± 0.3)	10.4 (± 0.1)	75.9 (± 1.0)
395.65	43.2 (± 0.3)	7.97 (± 0.1)	89.1 (± 1.1)
415.85	47.7 (± 0.4)	7.17 (± 0.1)	97.5 (± 0.8)
	(+)-Catechin Hydrate		
298.75	47.9 (± 0.4)	22.2 (± 0.1)	86.2 (± 1.1)
314.65	53.2 (± 0.4)	21.3 (± 0.2)	101 (± 0.5)
334.95	60.3 (± 0.5)	16.5 (± 0.1)	131 (± 1.0)
355.25	67.8 (± 0.5)	14.4 (± 0.1)	150 (± 1.5)
375.35	75.7 (± 0.6)	13.0 (± 0.1)	167 (± 1.8)
395.65	84.1 (± 0.6)	12.1 (± 0.1)	182 (± 1.3)
415.85	92.9 (± 0.7)	11.6 (± 0.0)	196 (± 1.8)

[a] Standard deviations were calculated from three replicate measurements using eq 2.

The standard enthalpy of solution of the phenolic compounds ($\Delta H_{sol}{}^0$) is plotted as a function of temperature as given in Figure 8. It can be seen from Figure 8 that the phenolic compounds showed a linear increase in its $\Delta H_{sol}{}^0$ with increasing temperature. The specific heat capacity at constant pressure (C_p) calculated from the slope of the linear trends observed in Figure 8 yielded a value of 0.269 kJ·mol^{-1}·K^{-1} for gallic acid hydrate, 0.197 kJ·mol^{-1}·K^{-1} for protocatechuic acid, and 0.291 kJ·mol^{-1}·K^{-1} for (+)-catechin hydrate. The higher heat capacity observed for gallic acid hydrate and (+)-catechin hydrate when compared to that of protocatechuic acid can be attributed to the greater number of hydroxyl groups associated with the first two compounds.[39] The thermodynamic properties of dissolution of phenolic compounds in water as a function of temperature given in Table 5 were not in as good agreement with those reported in Noubigh et al.[25] and Queimada et al.[28] However, all the thermodynamic properties calculated in this work were closer to that reported in Queimada et al.[28] than to the ones reported in Noubigh et al.[25]

It can also be seen from the data reported in Table 5 that the dissolution of phenolic compounds in water consisted of positive enthalpic and entropic contributions. While a positive standard enthalpy of solution can be related to an exothermic process, positive standard entropy of solution also contributes toward

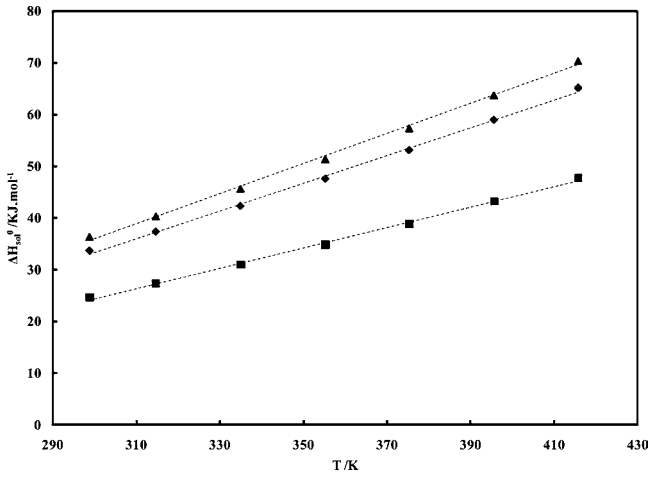

Figure 8. Variation of the molar enthalpy of solution (ΔH_{sol}^0) of the selected phenolic compounds as a function of temperature: ♦, gallic acid hydrate; ▲, protocatechuic acid; ■, (+)-catechin hydrate; - - -, correlation of experimental data.

the dissolution of the phenolic compounds.[40] This is contrary to the conclusions made by Noubigh et al.[25] where the authors indicated that the dissolution of the phenolic compounds in water was completely enthalpy driven.

Conclusions

The aqueous solubilities of selected phenolic compounds were measured as a function of temperature using a dynamic flow apparatus. The aqueous solubilities of the phenolic compounds were found to increase exponentially with temperature. The measured solubility data and the calculated thermodynamic properties were in good agreement with the limited data available in the literature. The solubility data were fitted with empirical equations such as the modified Apelblat equation[38] as well as other semiempirical equations to predict the aqueous solubility of the phenolic compounds where a solubility value at room temperature conditions is known. These predictive equations, especially the modified Apelblat equation, provided a better agreement with the solubility data reported in our study and could be optimized further as more solubility data become available. The solution thermodynamic properties of the phenolic compounds such as standard molar enthalpy, standard molar entropy, and standard molar Gibbs free energy were calculated from the solubility data as a function of temperature. The study of the solution thermodynamic properties indicated that the dissolution process of the phenolic compounds in water is endergonic, exothermic, and entropy-driven.

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