Dissolution thermodynamics and solubility of silymarin in PEG 400-water mixtures at different temperatures

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Abstract
An isothermal method was used to measure the solubility of silymarin in binary polyethylene glycol 400 (PEG 400) + water co-solvent mixtures at temperatures T = 298.15–333.15 K and pressure p = 0.1 MPa. Apelblat and Yalkowsky models were used to correlate experimental solubility data. The mole fraction solubility of silymarin was found to increase with increasing the temperature and mass fraction of PEG 400 in co-solvent mixtures. The root mean square deviations were observed in the range of 0.48–5.32% and 1.50–9.65% for the Apelblat equation and Yalkowsky model, respectively. The highest and lowest mole fraction solubility of silymarin was observed in pure PEG 400 (0.243 at 298.15 K) and water (1.46 × 10⁻⁵ at 298.15 K). Finally, thermodynamic parameters were determined by Van’t Hoff and Krug analysis, which indicated an endothermic and spontaneous dissolution of silymarin in all co-solvent mixtures.

Introduction
Bioflavonoids are biologically active polyphenolic compounds present in the plants, which provide a wide range of biological activities such as anti-inflammatory, antiviral, antibacterial and antioxidative activities¹,². Silymarin, 2-(2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-1,4-benzodioxin-6-yl)-2,3-dihydro-3,5,7-trihydroxy-4H-1-benzopyran-4-one (Figure 1) belongs to the bioflavonoid class of phytopharmaceuticals³. The molecular formula and molar mass of silymarin are C₂₅H₂₂O₁₀ and 482.44 g mol⁻¹, respectively⁴. It is obtained from a purified extract of seeds and fruits of Carduus marianus which shows good hepatoprotective activity⁵. Most of the bioflavonoids including silymarin have been reported as poorly water-soluble bioactive compounds; these biologically active compounds show poor in vivo oral bioavailability⁶,⁷. The physicochemical data of such bioactive compounds such as solubility, diffusion coefficients and transport parameters are important to optimize food engineering design process⁸. Such physicochemical data of bioactive compounds could also have applications in other fields such as purification, recrystallization, formulation design and extraction of these compounds⁹,¹⁰. With respect to bioactive compounds, the most important application of physicochemical data could be in their extraction from plant materials and food engineering process⁵,⁷. These bioactive compounds are usually extracted from plant materials using toxic solvents which could provide significant problems in development of their formulation⁸,⁹. Several physiologically compatible and non-toxic solvents such as ethanol, propylene glycol, polyethylene glycol 400 (PEG 400) and Transcutol have been used to enhance the solubility of various poorly soluble compounds¹⁰–¹⁴. These non-toxic solvents could help in formulation development and food engineering design of poorly water soluble bioactive compounds including silymarin. Various formulation approaches have been used by pharmaceutical scientists to enhance solubility and bioavailability of silymarin or its components³,⁶,¹⁵–²³. The Apelblat and Yalkowsky models are commonly used mathematical models for the correlation of experimental solubilities with calculated ones on mole fraction solubility of solute, respectively²⁴–²⁷. Recently, the temperature dependent solubility data of silymarin in various physiologically compatible solvents such as water, ethanol, isopropyl alcohol, PEG 400 and β-cyclodextrin aqueous solution have been reported⁷. However, the temperature dependent solubility data of silymarin in various PEG 400 + water co-solvent mixtures has not been reported in the literature so far. Therefore, the aim of this study was to measure and correlate the solubilities of bioactive compound silymarin in various PEG 400 + water co-solvent mixtures at temperatures T = 298.15–333.15 K and pressure p = 0.1 MPa.

Materials and methods

Materials
Silymarin and PEG 400 were purchased from Sigma Aldrich (St. Louis, MO). Distilled water was collected from the
distillation unit in the laboratory. All other chemicals used were of analytical/pharmaceutical grades.

Measurement of silymarin solubility

The solubility of silymarin was measured against mass fraction of PEG 400 (from m = 0.0 to 1.0) in PEG 400 + water co-solvent mixtures at temperatures T = 298.15–333.15 K and pressure p = 0.1 MPa using an isothermal method\(^7\). These experiments were carried out by using an excess amount of solute in known amount of each co-solvent mixture in 10 mL capacity flasks each evaluated in triplicate. These samples were equilibrated by continuous shaking in a biological shaker (Julabo, PA) at 100 rpm for 72 h. After 72 h, all the samples were removed from the biological shaker and solute (silymarin) particles were allowed to settle for 2 h at the bottom of each flask\(^7,12\). The supernatants from each sample were taken, diluted suitably with water and subjected for analysis of silymarin using a UV–Visible spectrophotometer at the wavelength of 286 nm as reported previously\(^7\). The standard uncertainty for the temperatures u(T) was found to be ± 0.12 K. However, the relative standard uncertainty in solubility u\(x_e\) was found to be ± 0.12 K. However, the relative standard uncertainty in solubility u\(x_e\) of crystalline silymarin was observed as 1.62%. The experimental mole fraction solubilities (\(x_e\)) of silymarin were calculated as reported previously\(^7,14\).

Results and discussion

Solubility data of silymarin

The mole fraction solubilities of bioactive compound silymarin against mass fraction of PEG 400 (m) in PEG 400 + water co-solvent mixtures at temperature T = 298.15–333.15 K and pressure p = 0.1 MPa are presented in Table 1. The mole fraction solubility of silymarin in pure water has been reported as 1.46 × 10\(^{-5}\) at 298.15 K\(^3,7\). However, its mole fraction solubility in pure PEG 400 has been reported as 0.24 at 298.15 K\(^3,7\). In this study, the mole fraction solubility of silymarin in water and PEG 400 was observed as 1.46 × 10\(^{-5}\) at 298.15 K and 0.24 at 298.15 K, respectively. These results indicated that solubility data of present investigation were in good agreement with the literature values of silymarin. On the other hand, the temperature-dependent solubilities of silymarin in PEG 400 + water co-solvent mixtures have not been reported in the literature so far. In general, the solubilities of silymarin were found to be increase exponentially with an increase in temperature in all co-solvent mixtures. The mole fraction solubilities of silymarin were observed highest in pure PEG 400 (m = 1.0; 0.24 at 298.15 K) as compared to pure water (m = 0.0; 1.46 × 10\(^{-5}\) at 298.15 K) at each temperature investigated (Table 1). The mole fraction solubility of silymarin in pure PEG 400 was significantly higher with respect to its aqueous solubility at each temperature investigated. The most probable reason for this observation could be due to the polarity difference between PEG 400 and water\(^13\). Based on these results, the bioactive compound silymarin has been considered as freely soluble in PEG 400 and practically insoluble in water according to USP and BP classification. The solubility data of this study could be extremely useful in the purification, recrystallization, formulation design and extraction of silymarin.

Correlation of experimental solubilities of silymarin with the Apelblat equation

The Apelblat equation was applied to correlate the experimental solubilities of silymarin with calculated ones and to evaluate the influence of temperature on the mole fraction solubility of silymarin\(^24-26\). According to this equation, the temperature-dependent solubility of silymarin can be calculated using Equation (1)\(^24,25\):

\[
\ln x = A + \frac{B}{T} + C \ln(T)
\]

in which, x is the calculated mole fraction solubility of silymarin and T is the absolute temperature (K). The empirical constants A, B and C are Apelblat coefficients which were calculated by nonlinear multivariate regression analysis of experimental solubilities as reported in the literature\(^6,26\). Apelblat/calculation solubilities (\(x_e\)) were calculated using these coefficients. The root mean square deviations (RMSD) between \(x_e\) and x of silymarin for Apelblat equation were calculated with the help of Equation (2)\(^7\):

\[
\text{RMSD} = \left[\frac{1}{N} \sum_{i=1}^{N} \left(\frac{x_i - x_e}{x_e}\right)^2\right]^{1/2}
\]

![Figure 1. Molecular structure of bioactive compound silymarin.](image)

Table 1. Experimental mole fraction solubilities (\(x_e\)) of bioactive compound silymarin against mass fraction of PEG 400 (m) in various PEG 400 + water co-solvent mixtures at temperatures T = 298.15–333.15 K and pressure p = 0.1 MPa.

| \(m\) | \(T = 298.15 \text{ K}\) | \(T = 303.15 \text{ K}\) | \(T = 313.15 \text{ K}\) | \(T = 323.15 \text{ K}\) | \(T = 333.15 \text{ K}\) |
|---|---|---|---|---|---|
| 0.0 | 1.46 × 10\(^{-5}\) | 1.68 × 10\(^{-5}\) | 2.09 × 10\(^{-5}\) | 2.54 × 10\(^{-5}\) | 3.06 × 10\(^{-5}\) |
| 0.1 | 4.46 × 10\(^{-5}\) | 4.95 × 10\(^{-5}\) | 5.99 × 10\(^{-5}\) | 7.02 × 10\(^{-5}\) | 8.05 × 10\(^{-5}\) |
| 0.2 | 1.06 × 10\(^{-4}\) | 1.19 × 10\(^{-4}\) | 1.48 × 10\(^{-4}\) | 1.75 × 10\(^{-4}\) | 2.08 × 10\(^{-4}\) |
| 0.3 | 2.77 × 10\(^{-4}\) | 3.14 × 10\(^{-4}\) | 3.87 × 10\(^{-4}\) | 4.60 × 10\(^{-4}\) | 5.65 × 10\(^{-4}\) |
| 0.4 | 7.37 × 10\(^{-4}\) | 8.45 × 10\(^{-4}\) | 9.78 × 10\(^{-4}\) | 1.46 × 10\(^{-3}\) | 1.35 × 10\(^{-3}\) |
| 0.5 | 1.99 × 10\(^{-3}\) | 2.13 × 10\(^{-3}\) | 2.56 × 10\(^{-3}\) | 3.06 × 10\(^{-3}\) | 3.56 × 10\(^{-3}\) |
| 0.6 | 5.05 × 10\(^{-3}\) | 5.65 × 10\(^{-3}\) | 6.52 × 10\(^{-3}\) | 7.55 × 10\(^{-3}\) | 9.01 × 10\(^{-3}\) |
| 0.7 | 1.38 × 10\(^{-2}\) | 1.55 × 10\(^{-2}\) | 1.77 × 10\(^{-2}\) | 2.10 × 10\(^{-2}\) | 2.42 × 10\(^{-2}\) |
| 0.8 | 3.51 × 10\(^{-2}\) | 3.95 × 10\(^{-2}\) | 4.53 × 10\(^{-2}\) | 5.18 × 10\(^{-2}\) | 5.92 × 10\(^{-2}\) |
| 0.9 | 9.69 × 10\(^{-2}\) | 1.07 × 10\(^{-1}\) | 1.21 × 10\(^{-1}\) | 1.35 × 10\(^{-1}\) | 1.53 × 10\(^{-1}\) |
| 1.0 | 2.24 | 0.26 | 0.29 | 0.34 | 0.38 |

The standard uncertainty for the temperatures u(T) is ± 0.12 K, the relative standard uncertainty in solubility u\(x_e\) for silymarin is 1.62%.
represents the experimental temperature points. The correlations and curve fitting data between \( x_e \) and \( x \) in various PEG 400 + water co-solvent mixtures from 298.15 to 333.15 K are presented in Supplementary Figure S1.

The values of Apelblat coefficients \( A, B \) and \( C \), correlation coefficients (\( R^2 \)), standard errors (SE) and RMSD for bioactive compound silymarin in various PEG 400 + water co-solvent mixtures are presented in Supplementary Table S1. The RMSD value for silymarin in pure water and pure PEG 400 were found to be 3.70 and 0.48%, respectively as shown in Supplementary Table S1. However, the lowest one was observed in pure PEG 400 (0.48%). The \( R^2 \) values for silymarin in pure water and PEG 400 were found to be 0.997 and 0.999, respectively. However, the lowest one was observed in pure PEG 400 (0.48%). The \( R^2 \) values in other co-solvent mixtures were found to be 0.994–0.998. The values of SE were observed in the range of 0.001–0.024 (Supplementary Table S1). The data of RMSD, \( R^2 \) and SE indicated good fitting of experimental data of silymarin with the Apelblat equation.

Correlation of experimental solubilities of silymarin with the Yalkowsky model

The model of Yalkowsky was also sued to correlate experimental solubilities with calculated ones and to investigate the impact of co-solvent mixtures on mole fraction solubility. According to Yalkowsky model, the mole fraction solubility of silymarin in co-solvent mixtures can be calculated using Equation (3):

\[
\log x_{\text{Yal}} = m_1 \log x_1 + m_2 \log x_2
\]

where, \( x_{\text{Yal}} \) is calculated mole fraction solubility of silymarin in co-solvent mixtures; \( x_1 \) and \( x_2 \) are mole fraction solubility of silymarin in pure solvents 1 (PEG 400) and 2 (water), respectively; and \( m_1 \) and \( m_2 \) are the mass fractions of solvent 1 and solvent 2 in the absence of silymarin. The experimental solubilities of silymarin were correlated with \( x_{\text{Yal}} \) (Yalkowsky solubilities) and RMSD values were calculated again using Equation (2).

The log \( x_{\text{Yal}} \) values of silymarin along with RMSD values in various PEG 400 + water co-solvent mixtures from 298.15 to 333.15 K are presented in Supplementary Table S2. The RMSD values in various co-solvent mixtures were found to vary from 1.50% to 9.65% from 298.15 to 333.15 K as shown in Supplementary Table S2. These results also indicated a good correlation between experimental and calculated solubilities of silymarin using the Yalkowsky model. The impact of mass fraction of PEG 400 on the natural logarithm of mole fraction solubility of silymarin (ln \( x_e \)) from 298.15 to 333.15 K were also investigated and results are presented in Figure 2. The solubility of silymarin was found to be increase significantly with increase in mass fraction of PEG 400 in all PEG 400 + water co-solvent mixtures from 298.15 to 333.15 K. For the correlation of experimental solubilities with calculated ones, Apelblat model was found to be more reliable and accurate than Yalkowsky model based on RMSD values.

Thermodynamic parameters for dissolution thermodynamics of silymarin

Various thermodynamics parameters such as molar enthalpy (\( \Delta H^0 \)), Gibbs energy (\( \Delta G^0 \)) and molar entropy (\( \Delta S^0 \)) of silymarin were measured by Van’t Hoff and Krug analysis. The \( \Delta H^0 \) values for the dissolution thermodynamics of silymarin in various...
The dissolution of silymarin in various PEG 400 + water co-solvent (m) mixtures were derived by Van’t Hoff analysis\textsuperscript{28,29}. The $\Delta H^0$ values were measured at mean harmonic temperature ($T_{hm}$) using Equation (4):

$$\left( \frac{\partial \ln x}{\partial \left( \frac{1}{T} - \frac{1}{T_{hm}} \right)} \right)_{p} = - \frac{\Delta H^0}{R}$$ (4)

in which, $R$ is the universal gas constant (8.314 J mol\(^{-1}\) K\(^{-1}\)). The $T_{hm}$ value in present study was 313.61 K. The Van’t Hoff plots were constructed between ln $x$ and $\frac{1}{T} - \frac{1}{T_{hm}}$ for various co-solvent mixtures using Equation (4). These plots were observed linear with $R^2$ values in the range of 0.995–0.999 (Table 2).

According to Equation (4), the slopes of Van’t Hoff plots were equal to $-\Delta H^0/R$ and hence the $\Delta H^0$ values were determined from the slope of each plot. The $\Delta G^0$ values for the dissolution of silymarin were determined at $T_{hm}$ by Krug analysis using Equation (5)\textsuperscript{30}:

$$\Delta G^0 = -RT_{hm} \times \text{intercept}$$ (5)

in which, the intercept was determined from the Van’t Hoff plots of silymarin using Supplementary Figure S2.

Finally, the $\Delta S^0$ values for the dissolution of silymarin were calculated using Equation (6).

$$\Delta S^0 = \frac{\Delta H^0 - \Delta G^0}{T_{hm}}$$ (6)

The resulting data of Van’t Hoff and Krug analysis are listed in Table 2.

The $\Delta H^0$ values for the dissolution of silymarin were observed as positive values in all co-solvent mixtures, indicating endothermic dissolution of silymarin. The $\Delta H^0$ value for the dissolution of silymarin in pure water and pure PEG 400 was found to be 17.31 and 10.65 kJ mol\(^{-1}\), respectively. However, the $\Delta H^0$ values for the dissolution of silymarin in other co-solvent mixtures were found vary from 10.43 to 16.49 kJ mol\(^{-1}\) (Table 2). The $\Delta G^0$ values for the dissolution of silymarin were also observed as positive values in all co-solvent mixtures, indicating both the Apelblat and Yalkowsky models from 298.15 to 333.15 K. The solubilities were measured using an isothermal method, which were found to be increase exponentially with increase in temperature. The mole fraction solubility of silymarin was significantly higher in PEG 400 as compared to its mole fraction solubility in water. The experimental solubilities of silymarin were correlated well with both the Apelblat and Yalkowsky models from 298.15 to 333.15 K. However, Apelblat model was found to be more reliable and accurate than Yalkowsky model based on RMSD values. The dissolution thermodynamics of silymarin was observed as endothermic and spontaneous in all co-solvent mixtures. Based on these results, silymarin has been considered as practically insoluble in water and freely soluble in PEG 400. The solubility data of this study could be useful in purification, recrystallization, formulation design and extraction of silymarin.

### Conclusion

The solubilities and dissolution thermodynamics of bioactive compound silymarin in various PEG 400 + water co-solvent mixtures were measured from 298.15 to 333.15 K. The solubilities were measured using an isothermal method, which were found to be increase exponentially with increase in temperature. The mole fraction solubility of silymarin was significantly higher in PEG 400 as compared to its mole fraction solubility in water. The experimental solubilities of silymarin were correlated well with both the Apelblat and Yalkowsky models from 298.15 to 333.15 K. However, Apelblat model was found to be more reliable and accurate than Yalkowsky model based on RMSD values. The dissolution thermodynamics of silymarin was observed as endothermic and spontaneous in all co-solvent mixtures. Based on these results, silymarin has been considered as practically insoluble in water and freely soluble in PEG 400. The solubility data of this study could be useful in purification, recrystallization, formulation design and extraction of silymarin.

### Declaration of interest

The authors report no conflict of interest. The authors would like to extend their sincere appreciation to the Kayali Chair for Pharmaceutical Industry at King Saud University for supporting this work (FN-2015, Research Chair, Deanship of Scientific Research).

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Supplementary Material available online.
Supplemental Tables S1 and S2, Figures S1 and S2.