Measurement and correlation of the solubility of gossypol acetic acid and gossypol acetic acid of optical activity in different solvents

B Zhang, H Tang¹, X Y Liu, X Zhai and X C Yao

School of Pharmaceutical Science, Shihezi University, Shihezi 832002, China

¹E-mail: Th_pha@shzu.edu.cn

Abstract. The equilibrium method was used to measure the solubility of gossypol acetic acid and gossypol acetic acid of optical activity in isopropyl alcohol, ethanol, acetic acid and ethyl acetate at temperature from 288.15 to 315.15 K. The Empirical equation and the Apelblat equation model were adopted to correlate the experimental data. For gossypol acetic acid, the root-mean-square deviations (RMSD) were observed in the range of 0.023-4.979 and 0.0112-0.614 for the Empirical equation and the Apelblat equation, respectively. For gossypol acetic acid of optical activity, the RMSD were observed in the range of 0.021-2.211 and 0.021-2.243 for the Empirical equation and the Apelblat equation, individually. And the maximum relative average deviation was 7.5%. Both equations offered an accurate mathematical expression of the experimental results. The calculated solubility showed a good relationship with the experimental solubility for most of solvents. This study provided valuable datas not only for optimizing the process of purification of gossypol acetic acid of optical activity in industry but also for further theoretical studies.

1. Introduction

The Gossypol ((±)-GP, figure 1) is a natural polyphenol derived from cotton plant. Since the chemical bond connecting two naphthalenes has steric effect in the gossypol structure, there are two optical isomers of it [1]. Racemic gossypol ((±)-GP) is a mixture composed of (-)-GP and (+)-GP with different physiological activity [2]. (+)-GP is an effective natural antitumor and anticancer compound. Researchers have evaluated the antitumor property of gossypol against many types of cancer cell lines [3, 4]. The effect on cancer cells of (-)-GP is 2-5 times higher than racemic gossypol [5]. Furthermore, pharmacological researches showed that the side effects of (-)-GP is obviously lower than (+)-GP. In the process of deriving racemic gossypol into the gossypol acetic acid of optical activity, the solubility of (-)-GP in solvents is an important factor in the process of optimizing the conditions of derivatization. The solubility of gossypol acetic acid has been reported in some literatures. However, no experimental solubility data of gossypol acetic acid of optical activity is reported in the open literatures so far. What’s more, it is necessary to know the solubility of the (-)-GP in different solvent systems to choose proper solvents.

In this work, the solubility datas of gossypol acetic acid of optical activity in pure isopropyl alcohol, ethanol, acetic acid and ethyl acetate were measured by the equilibrium method at temperature from 288.15 to 315.15 K under atmospheric pressure. The experimental datas were correlated by the Empirical equation and the Apelblat equation.
2. Experimental

2.1. Materials and Apparatus
Gossypol acetic acid (±)-GA and gossypol acetic acid of optical activity (+)-GA with purity ≥ 99.0 % (determined by HPLC Chiral column) prepared in Key Laboratory of Xinjiang Phytomedicine Resource. It was dried in vacuo at 323 K for 24 h and then stored in desiccator. All the organic solvents (HPLC grade) used for the experiment were purchased from Tianjin Fuyu Chemical Company, Ltd. The smart thermostatic bath (model: DC-2006) was provided by Ningbo Scientz Biotechnology Company, Ltd. Digital thermostat magnetic stirrer (model: 85-2), Japan Shimazu ultraviolet spectrophotometer (model: UV-2550), and distilled water was prepared in our laboratory.

2.2. Methods
The method of measuring the solubility of solid in solvents is analysis and synthesis [6-8]. In this work, we adopted an analytical method to determine the solubility of (+)-GA and (±)-GA in six selected solvents, with the temperature ranging from 288.15 to 315.15 K. Our analytical method is simple and reliable, and it allows the simultaneous measurement of a large number of samples.

The method of solubility measurement is similar to the method described in the literatures [9-11]. In the experiment, the equilibrium method was used to study the solubility of (+)-GA and (±)-GA in different organic solvents. The temperature was controlled by an intelligent thermostatic bath. The experiment was conducted in a closed small room with constant temperature and humidity and static air. (+)-GA and (±)-GA dissolved in 10 mL organic solvent were placed into a 50 mL three-necked flask with a stopper, and stirred continuously by a magnetic stirrer at the temperature in the thermostatic bath. The precision of the temperature should be controlled within ±0.01 K. 12 h was conducted to ensure the solid–liquid equilibrium was established in the tube. At least 6 h was needed to settle the solution after turning off the stirring. Filtrated the supernatant by 0.45 µm microporous membrane and diluted it to appropriate concentration, and then determined it by UV. Each experiment was repeated three times to obtain the mean value. The saturated solution mole fraction solubility of solute(X) in each solvent can be calculated using the following equation:

\[
X = \frac{m_A}{m_A + M_S} \frac{M_A}{M_A + M_S}
\]  

Where \(m_A\) and \(m_s\) represent the mass of the solute and the solvent, and \(M_A\) and \(M_S\) are the molar mass of the solute and the solvent, respectively.

2.3. Test of the Apparatus
To prove the feasibility and demonstrate the uncertainties of the measurements, the solubility of NaCl in water was measured and compared with values reported in the literature. The results agreed with...
the reported values with a mean relative deviation (RD) of 0.30%. The measured values were listed in table 1.

**Table 1.** Mole fraction of NaCl in water.

| T (K) | X    | X (NaCl) [12-13] | $10^2$RD |
|-------|------|------------------|----------|
| 293.15| 0.0996    | 0.0993±0.0004 | 0.30     |
| 303.15| 0.1001    | 0.0999±0.0005 | 0.19     |
| 313.15| 0.1009    | 0.1007±0.0006 | 0.20     |
| 323.15| 0.1019    | 0.1017±0.0005 | 0.20     |

3. Results and Discussion

In order to use the UV method to calculate the concentrations, calibration curves were needed. The measured values were listed in table 2 and table 3.

**Table 2.** Standard curve equation of (-)-GA in four organic solvents.

| Solvent          | Calibration curves equation | $R^2$   | The linear range          |
|------------------|-----------------------------|---------|---------------------------|
| Isopropyl alcohol| $A=0.0273*C+0.0051$         | 0.9990  | $10.21\mu g.mL^{-1}~35.74\mu g.mL^{-1}$ |
| Ethanol          | $A=0.0285*C-0.0007$         | 0.9998  | $9.99\mu g.mL^{-1}~34.97\mu g.mL^{-1}$ |
| Acetic acid      | $A=0.0301*C-0.0139$         | 0.9992  | $10.05\mu g.mL^{-1}~35.18\mu g.mL^{-1}$ |
| Ethyl acetate    | $A=0.0305*C-0.0007$         | 0.9999  | $10.08\mu g.mL^{-1}~35.28\mu g.mL^{-1}$ |

**Table 3.** Standard curve equation of (+)-GA in four kinds of solvents.

| Solvent          | Calibration curves equation | $R^2$   | The linear range          |
|------------------|-----------------------------|---------|---------------------------|
| Isopropyl alcohol| $A=0.0496*C-0.0677$         | 0.9992  | $3.36\mu g.mL^{-1}~20.16\mu g.mL^{-1}$ |
| Ethanol          | $A=0.0550*C-0.0449$         | 0.9995  | $5.15\mu g.mL^{-1}~25.75\mu g.mL^{-1}$ |
| Acetic acid      | $A=0.0513*C+0.015$          | 0.9996  | $5.47\mu g.mL^{-1}~19.15\mu g.mL^{-1}$ |
| Ethyl acetate    | $A=0.0636*C+0.3452$         | 0.9995  | $5.26\mu g.mL^{-1}~15.78\mu g.mL^{-1}$ |

The measured mole fraction of (-)-GA in pure isopropyl alcohol, ethanol, acetic acid and ethyl acetate at the temperature from 288.15 to 315.15 K were presented in table 4 and the measured mole fraction solubility of (+)-GA in table 5. The mole fraction $X$ values in table 4 and table 5 was the average value of three values taken from the same solvent. The RDs between the experimental values and the calculated values were also listed in table 4 and table 5.
Table 4. Mole fraction solubility data and correlation results of (-)-GA in four organic solvents.

| Solvents         | T/K   | $10^{-4}x_{exp}$ | Empirical equation | Apelblat equation |
|------------------|-------|------------------|-------------------|------------------|
|                  |       |                  | $10^{-4}x_{cal}$  | $10^{-4}x_{exp}$ |
|                  |       |                  | 10$^{2}RD$        | 10$^{2}RD$       |
| Isopropyl alcohol| 288.15| 11.223           | 11.489            | 2.37             | 11.533          | 2.76          |
|                  | 289.15| 11.415           | 11.370            | 0.39             | 11.425          | 0.09          |
|                  | 293.15| 11.607           | 11.113            | 4.26             | 11.208          | 3.44          |
|                  | 298.15| 11.703           | 11.285            | 3.57             | 11.388          | 2.69          |
|                  | 303.15| 11.990           | 12.006            | 0.13             | 12.063          | 0.61          |
|                  | 308.15| 12.757           | 13.277            | 4.08             | 13.286          | 4.15          |
|                  | 313.15| 15.153           | 15.096            | 0.38             | 15.176          | 0.15          |
|                  | 315.15| 16.399           | 15.978            | 2.57             | 16.161          | 1.45          |
| Ethanol          | 288.15| 12.143           | 12.236            | 0.77             | 12.298          | 1.28          |
|                  | 289.15| 12.252           | 12.204            | 0.39             | 12.277          | 0.20          |
|                  | 293.15| 12.719           | 12.229            | 3.86             | 12.330          | 3.06          |
|                  | 298.15| 12.737           | 12.602            | 1.06             | 12.705          | 0.25          |
|                  | 303.15| 13.372           | 13.357            | 0.12             | 13.430          | 0.43          |
|                  | 308.15| 14.502           | 14.492            | 0.07             | 14.539          | 0.26          |
|                  | 313.15| 15.860           | 16.009            | 0.94             | 16.094          | 1.47          |
|                  | 315.15| 17.149           | 16.722            | 2.49             | 16.862          | 1.68          |
| Acetic acid      | 288.15| 0.550            | 0.578             | 5.04             | 0.573           | 2.80          |
|                  | 289.15| 0.570            | 0.574             | 0.72             | 0.572           | 0.85          |
|                  | 293.15| 0.604            | 0.575             | 4.78             | 0.581           | 4.85          |
|                  | 298.15| 0.604            | 0.612             | 1.37             | 0.619           | 1.28          |
|                  | 303.15| 0.649            | 0.689             | 6.22             | 0.689           | 4.79          |
|                  | 308.15| 0.807            | 0.807             | 0.04             | 0.798           | 2.29          |
|                  | 313.15| 0.964            | 0.966             | 0.17             | 0.962           | 1.43          |
|                  | 315.15| 1.023            | 1.040             | 1.76             | 1.047           | 1.19          |
| Ethyl acetate    | 288.15| 131.730          | 131.726           | 0.01             | 131.957         | 0.17          |
|                  | 289.15| 132.046          | 132.878           | 0.63             | 133.188         | 0.86          |
|                  | 293.15| 141.443          | 138.125           | 2.35             | 138.629         | 1.99          |
|                  | 298.15| 144.200          | 146.126           | 1.34             | 146.651         | 1.70          |
|                  | 303.15| 153.077          | 155.729           | 1.73             | 156.142         | 2.00          |
|                  | 308.15| 170.964          | 166.935           | 2.36             | 167.248         | 2.17          |
|                  | 313.15| 179.563          | 179.743           | 0.10             | 180.147         | 0.33          |
|                  | 315.15| 184.660          | 185.315           | 0.35             | 185.855         | 0.65          |

The RDs were calculated according to:

$$RD = \frac{X_{exp} - X_{cal}}{X_{exp}}$$  \hspace{1cm} (2)$$

Where $X_{exp}$ and $X_{cal}$ represent the experimental and calculated solubility values, respectively.

The relationship between temperature and mole fraction solubility in the different solvents was described by the Empirical equation and the Apelblat equation.
Table 5. Mole fraction solubility data and correlation results of (±)-GA in four organic solvents.

| Solvents      | T/K   | \(10^3x_{exp}\) | \(10^3x_{cal}\) | \(10^3RD\) | \(10^3x_{cal}\) | \(10^3RD\) |
|---------------|-------|-----------------|-----------------|-----------|-----------------|-----------|
|               |       |                 | Empirical equation |            | Apelblat equation |            |
|               |       |                 | \(10^3x_{cal}\) | \(10^3RD\) | \(10^3x_{cal}\) | \(10^3RD\) |
| Isopropyl alcohol | 288.15 | 6.182           | 6.687           | 8.16      | 6.269           | 1.41     |
|                | 289.15 | 6.278           | 6.653           | 5.97      | 6.241           | 0.59     |
|                | 293.15 | 6.328           | 6.620           | 4.61      | 6.225           | 1.63     |
|                | 298.15 | 6.459           | 6.815           | 5.51      | 6.410           | 0.76     |
|                | 303.15 | 6.749           | 7.275           | 7.79      | 6.829           | 1.18     |
|                | 308.15 | 7.487           | 7.993           | 6.76      | 7.510           | 0.30     |
|                | 313.15 | 8.532           | 8.984           | 5.30      | 8.507           | 0.29     |
|                | 315.15 | 9.031           | 9.452           | 4.66      | 9.013           | 0.20     |
| Ethanol       | 288.15 | 11.629          | 11.714          | 0.73      | 11.593          | 0.31     |
|                | 289.15 | 11.684          | 11.895          | 1.81      | 11.769          | 0.73     |
|                | 293.15 | 12.616          | 12.612          | 0.03      | 12.473          | 1.13     |
|                | 298.15 | 13.546          | 13.493          | 0.39      | 13.347          | 1.47     |
|                | 303.15 | 14.192          | 14.357          | 1.16      | 14.211          | 0.14     |
|                | 308.15 | 14.934          | 15.205          | 1.81      | 15.060          | 0.84     |
|                | 313.15 | 15.992          | 16.035          | 0.33      | 15.887          | 0.66     |
|                | 315.15 | 16.301          | 16.362          | 0.37      | 16.211          | 0.55     |
| Acetic acid   | 288.15 | 0.147           | 0.169           | 14.97     | 0.141           | 4.08     |
|                | 289.15 | 0.156           | 0.175           | 12.18     | 0.150           | 3.85     |
|                | 293.15 | 0.206           | 0.212           | 2.91      | 0.194           | 5.83     |
|                | 298.15 | 0.251           | 0.280           | 11.55     | 0.265           | 5.58     |
|                | 303.15 | 0.340           | 0.374           | 10.00     | 0.356           | 4.71     |
|                | 308.15 | 0.491           | 0.495           | 0.81      | 0.471           | 4.07     |
|                | 313.15 | 0.618           | 0.641           | 3.72      | 0.617           | 0.16     |
|                | 315.15 | 0.679           | 0.706           | 3.98      | 0.685           | 0.88     |
| Ethyl acetate | 288.15 | 100.376         | 96.450          | 3.91      | 100.726         | 0.35     |
|                | 289.15 | 101.315         | 96.428          | 4.82      | 100.803         | 0.51     |
|                | 293.15 | 102.694         | 97.274          | 5.28      | 101.953         | 0.72     |
|                | 298.15 | 104.987         | 100.428         | 4.34      | 105.272         | 0.27     |
|                | 303.15 | 110.080         | 105.912         | 3.79      | 110.739         | 0.60     |
|                | 308.15 | 119.776         | 113.726         | 5.05      | 118.533         | 1.04     |
|                | 313.15 | 128.851         | 123.870         | 3.87      | 128.952         | 0.08     |
|                | 315.15 | 134.070         | 128.580         | 4.10      | 133.948         | 0.09     |

The effect of the temperature on solubility of (±)-GA in the selected solvents can be correlated by the Empirical equation [14],

\[
X = A_1 + A_2 T + A_3 T^2
\]  

where \(X\) is the mole fraction solubility of (±)-GA or (±)-GA, \(T\) is the corresponding temperature in Kelvin, and \(A_1, A_2\) and \(A_3\) are the parameters of this equation. Values of the parameters \(A_1, A_2\) and \(A_3\) were listed in table 6 and table 7.
Table 6. Parameters of Empirical equation for (-)-GA in four organic solvents.

| Solvents       | A₁    | A₂     | A₃     | R²   | RMSD | RAD  |
|----------------|-------|--------|--------|------|------|------|
| Isopropyl alcohol | 960.688 | -6.458 | 1.1×10⁻² | 0.9523 | 0.342 | 0.022 |
| Ethanol        | 656.337 | -4.431 | 7.621×10⁻³ | 0.9776 | 0.243 | 0.012 |
| Acetic acid    | 69.042  | -0.471 | 8.083×10⁻⁴ | 0.9828 | 0.021 | 0.025 |
| Ethyl acetate  | 2470.29 | -17.351 | 3.205×10⁻² | 0.9825 | 2.211 | 0.011 |

Table 7. Parameters of Empirical equation for (+)-GA in four organic solvents.

| Solvents       | A₁    | A₂     | A₃     | R²   | RMSD | RAD  |
|----------------|-------|--------|--------|------|------|------|
| Isopropyl alcohol | 455.755 | -3.077 | 5.270×10⁻³ | 0.9927 | 0.436 | 0.061 |
| Ethanol        | -68.877 | 0.378  | -3.412×10⁻⁴ | 0.9776 | 0.142 | 0.008 |
| Acetic acid    | 41.382  | -0.292 | 5.167×10⁻⁴ | 0.9828 | 0.023 | 0.075 |
| Ethyl acetate  | 3985.313 | -26.923 | 4.660×10⁻² | 0.9969 | 4.979 | 0.044 |

Table 8. Parameters of Apelblat equation for (-)-GA in four organic solvents.

| Solvents       | A  | B     | C     | R²   | RMSD | RAD  |
|----------------|----|-------|-------|------|------|------|
| Isopropyl alcohol | -1016.901 | 44813.185 | 152.526 | 0.9626 | 0.295 | 0.019 |
| Ethanol        | -636.791 | 27810.049 | 95.840  | 0.9813 | 0.142 | 0.008 |
| Acetic acid    | -1115.635 | 48355.278 | 167.258 | 0.9837 | 0.023 | 0.075 |
| Ethyl acetate  | -192.569 | 7893.617  | 30.027  | 0.9969 | 4.979 | 0.044 |

Table 9. Parameters of Apelblat equation for (+)-GA in four organic solvents.

| Solvents       | A   | B     | C     | R²   | RMSD | RAD  |
|----------------|-----|-------|-------|------|------|------|
| Isopropyl alcohol | -841.151 | 36821.309 | 126.283 | 0.9952 | 0.061 | 0.008 |
| Ethanol        | 74.513 | -4187.661 | -10.158 | 0.9813 | 0.200 | 0.011 |
| Acetic acid    | 105.003 | -9301.701 | -13.187 | 0.9837 | 0.021 | 0.024 |
| Ethyl acetate  | -468.851 | 20450.330 | 71.068  | 0.9967 | 0.614 | 0.005 |

The root-mean-square deviation (RMSD), together with the relative average deviation (RAD) for the Empirical equation and the Apelblat equation, are also listed in table 6, and table 7, respectively. The RMSD are calculated according to:

\[
RMSD = \sqrt{\frac{1}{N} \sum_{i=1}^{N} \left( \frac{x_i - x_{cal}}{x_{exp}} \right)^2}
\]

Where N is the number of experiment times and x_{cal} and x_{exp} represent the calculated and experimental solubility values. The RADs are calculated by:
As we can see from tables 5, 6, 8, and 9, the calculated data of solubility of (-)-GA and (±)-GA in the four pure organic solvents showed good agreement with the experimental data with small RMSD. Comparing the absolute values of the RD among all the values by two equations, the RD calculated by the Apelblat equation was lower than that calculated by the Empirical equation. The calculated data of (-)-GA solubility were fitted by the Apelblat equation. The absolute values of the RD were lower than 4.85%, which indicated that the Apelblat equation was more suitable for correlating the solubility data of (-)-GA in the selected pure solvents. The same conclusion also applied to (±)-GA. Consequently, the Apelblat equation is more accurate than the Empirical equation for these systems. The results mentioned above suggested that the experimental data and the correlations in this work are essential for modeling the purification process of (-)-GA.

The graph of solubility of (-)-GA and (±)-GA in the selected solvent systems was shown in figures 2 and 3. It can be seen clearly from this figure that the solubility of (-)-GA and (±)-GA in each of the selected solvents would increase with the temperature range from 288.15 to 315.15 K. But the increment of solubility with temperature is different in each of the pure solvents. The solubility of (-)-GA in these solvents follows the order that Ethyl acetate > Ethanol > Isopropyl alcohol > Acetic acid. Among these solvents, the solubility of (-)-GA was highest in Ethyl acetate and lowest in Acetic acid. This result shows that the polarity of the solvents is not the only factor in determining the solubility of (-)-GA in the solvents. The selected four solvents are commonly used and essential for modeling the purification process.

In Ethyl acetate, (-)-GA had high solubility at the initial temperature studied (288.15K), and the increment of solubility with temperature is increasing quickly. Therefore, we can estimate that after recrystallization, (-)-GA can be obtained with higher yield in Ethyl acetate than in the other selected solvents. Thus, Ethyl acetate has the potential to be a solvent in purification and recrystallization process of (-)-GA.

\[
RAD = \left( \frac{1}{N} \sum_{i=1}^{N} \left| \frac{X_i^{cal} - X_i^{exp}}{X_i^{exp}} \right| \right)
\]  (6)

Figure 2. Solubility of (-)-GA in four organic solvents.

Figure 3. Solubility of (±)-GA in four organic solvents.
4. Conclusions
The equilibrium method was used to measure the solubility of gossypol acetic acid and gossypol acetic acid of optical activity in isopropyl alcohol, ethanol, acetic acid and ethyl acetate at temperature from 288.15 to 315.15 K. We can draw the following conclusions: (1) the solubility of gossypol acetic acid of optical activity in all of the selected pure solvents increases with temperature; (2) the solubility data can be correlated using the Empirical equation and the Apelblat equation, and the Apelblat equation is more accurate than the Empirical equation; (3) the experimental solubility data and the corresponding parameters can be used for optimizing the process of purification of gossypol acetic acid of optical activity in industry; (4) Ethyl acetate has the potential to be a solvent in recrystallization process of (-)-GA and (±)-GA.

Acknowledgments
This work was funded by the National Natural Science Foundation of China (No. 81260627 and No. 81660641 ) and the Science and technology attack plan project of Xinjiang Production and Construction Corps (No. 2016AD008)

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