# Solubility of Desmosterol in Five Organic Solvents

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The solubilities of desmosterol in methanol, ethanol, acetone, ethyl acetate, and acetonitrile were measured. The solubilities of desmosterol in these solvents increased with temperature and varied greatly with the solvent from a minimum of 0.1151 mol·$L^{-1}$ in acetonitrile at 300.8 K to a maximum of 1.102 mol·$L^{-1}$ in ethyl acetate at 332.8 K. The solubility values were correlated with the modified Apelblat equation. The calculated solubilities for all solvents showed good agreement with the experimental data in the temperature range studied.

## Introduction

Desmosterol ($C_{27}H_{44}O$; molecular weight 384.64; CAS Registry Number 313-04-2; Figure 1) is a biosynthetic precursor of cholesterol and has been identified in hamster, monkey, and human spermatozoa.[1−3] Current pharmacological studies have proposed desmosterol as a biochemical marker of puberty in monkey testis[4] and demonstrated that desmosterol may have the same functions as cholesterol in determining membrane structure, dynamics, and biophysical functions.[5] Apart from its intrinsic interest, desmosterol may be considered as an interesting starting material for the partial synthesis of certain physiologically active steroids, some of which have become very important in recent years (e.g., 25-hydroxycholesterol and active vitamin $D_3$).[6] In the past, desmosterol was partially synthesized,[7] but the produce remained very expensive due to the lack of large quantities of a relatively cheap starting material. Nowadays, desmosterol has been isolated from certain marine mollusks[8] and the plant red algae[9,10] followed by recrystallization from solution. Therefore, knowledge of the solubility data of desmosterol in organic solvents (such as methanol, ethanol, acetone, etc.) is important for its preparation and purification. Desmosterol and cholesterol are structurally quite similar, varying only by a double bond in carbon 24. Solubility data of cholesterol in different solvents have been widely reported, while solubility of desmosterol has not been reported. In this study, the solubility data of desmosterol in methanol, ethanol, acetone, ethyl acetate, and acetonitrile at different temperatures have been measured and reported.

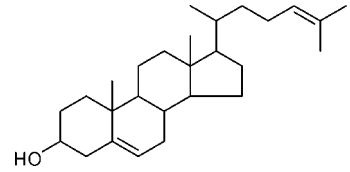


**Figure 1.** Structure of desmosterol.

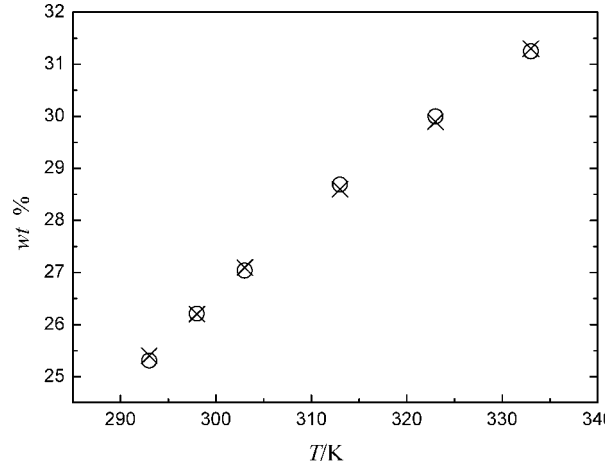


**Figure 2.** Mass fraction solubility of potassium chloride in water at different temperatures: ○, this work; ×, literature value.[11]

## Experimental Section

***Materials.*** Desmosterol with a minimum mass fraction purity of 98.0 %, determined by HPLC, was prepared in the National Laboratory of Secondary Resources Chemical Engineering of Zhejiang University, China. The melting point of desmosterol, measured by a WRS-1B digital melting-point apparatus, was (393.2 to 394.2) K. All the organic solvents used were analytical grade and obtained from Hangzhou Chemical Reagent Co., Ltd. The mass fraction of all the solvents was greater than 99.5 %.

***Apparatus and Procedure.*** The solubility was measured by a static equilibrium method at atmospheric pressure. The experiments were carried out in a magnetically stirred, jacketed glass vessel (50 $cm^3$). A constant temperature was maintained by circulating water through the outer jacket from a thermostatically controlled water bath. The actual value of temperature in the vessel was measured by a microthermometer (uncertainty of ± 0.1 K). A condenser was connected with the vessel to prevent the solvent from evaporating. The saturated solution was allowed to reach equilibrium with an excess amount of desmosterol added to the solvent in the jacketed glass vessel. The mixture was constantly stirred for 12 h to attain equilibrium. Then it was settled for 2 h. The sample of the upper portion was withdrawn, filtered through a 0.45 $\mu$m membrane filter, appropriately diluted, and analyzed for desmosterol by HPLC. A Waters 1525 HPLC pump, a Waters 717 plus autosampler, and a Waters 2487 UV detector were used for analysis of samples.

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10.1021/je8006088 CCC: $40.75 
Published on Web 10/22/2008

**Table 1. Solubility *c* of Desmosterol in Five Organic Solvents**

| *T*/K | $c$/mol·L$^{-1}$ | $(c - c^{calcd})/c$ | *T*/K | $c$/mol·L$^{-1}$ | $(c - c^{calcd})/c$ |
|---|---|---|---|---|---|
| | | methanol | | | |
| 299.8 | 0.03140 ± 0.00026 | 0.054777 | 315.1 | 0.05588 ± 0.00049 | −0.01235 |
| 303.3 | 0.03447 ± 0.00023 | −0.00116 | 318.8 | 0.06704 ± 0.00061 | 0.019093 |
| 307.8 | 0.04064 ± 0.00035 | −0.02805 | 322.5 | 0.07695 ± 0.00024 | 0.008577 |
| 311.5 | 0.04556 ± 0.00013 | −0.07024 | 327.0 | 0.09344 ± 0.00051 | 0.024508 |
| | | ethanol | | | |
| 300.6 | 0.1086 ± 0.0006 | 0.094936 | 320.6 | 0.2345 ± 0.0022 | −0.07122 |
| 304.0 | 0.1175 ± 0.0004 | 0.014468 | 323.6 | 0.2575 ± 0.0021 | −0.11689 |
| 307.8 | 0.1336 ± 0.0013 | −0.03892 | 327.7 | 0.3675 ± 0.0029 | 0.06068 |
| 311.8 | 0.1625 ± 0.0003 | −0.03138 | 332.2 | 0.4689 ± 0.0045 | 0.102794 |
| 315.7 | 0.1937 ± 0.0016 | −0.03665 | | | |
| | | acetone | | | |
| 300.7 | 0.1491 ± 0.0012 | 0.084507 | 315.4 | 0.2911 ± 0.0027 | −0.05943 |
| 304.0 | 0.1591 ± 0.0012 | −0.03394 | 319.2 | 0.3768 ± 0.0013 | −0.00398 |
| 308.0 | 0.2024 ± 0.0018 | −0.01581 | 323.0 | 0.4952 ± 0.0036 | 0.065024 |
| 311.5 | 0.2389 ± 0.0010 | −0.04395 | | | |
| | | ethyl acetate | | | |
| 301.2 | 0.3374 ± 0.0031 | −0.00326 | 319.2 | 0.6758 ± 0.0058 | −0.00459 |
| 304.4 | 0.3733 ± 0.0033 | −0.0292 | 323.0 | 0.7871 ± 0.0069 | 0.005717 |
| 307.7 | 0.4433 ± 0.0041 | 0.01376 | 327.1 | 0.9170 ± 0.0060 | 0.00687 |
| 311.5 | 0.5159 ± 0.0007 | 0.018221 | 332.8 | 1.102 ± 0.0072 | −0.01724 |
| 315.5 | 0.5951 ± 0.0051 | 0.008066 | | | |
| | | acetonitrile | | | |
| 300.8 | 0.01151 ± 0.00010 | 0.041703 | 317.9 | 0.01989 ± 0.00003 | −0.04223 |
| 303.8 | 0.01251 ± 0.00005 | 0.01199 | 322.7 | 0.02501 ± 0.00017 | 0.017993 |
| 308.0 | 0.01407 ± 0.00011 | −0.02772 | 327.1 | 0.02967 ± 0.00014 | 0.036063 |
| 313.8 | 0.01711 ± 0.00001 | −0.04559 | 333.2 | 0.03531 ± 0.00034 | 0.003398 |

**Table 2. Parameters of Equation 1 for Desmosterol in Selected Solvents**

| solvent | *A* | *B* | *C* | rmsd |
|---|---|---|---|---|
| methanol | −63.940 | −614.033 | 10.9542 | 0.00167 |
| ethanol | −77.7457 | −353.0954 | 13.4255 | 0.02015 |
| acetone | −106.8413 | 378.4219 | 18.1559 | 0.0157 |
| ethyl acetate | −72.2956 | 127.515 | 12.4027 | 0.00886 |
| acetonitrile | −48.1124 | −988.5551 | 8.2176 | 0.000621 |

Different dissolution times were tested to determine a suitable equilibrium time. It was found that 12 h was enough for desmosterol in all solvents to reach equilibrium. All the solubility experiments were conducted 3 times to check the reproducibility, and an average value is given. The deviations of solubility data were all less than 1.0 %.

***Sample Analysis.*** To determine the concentration of desmosterol, an HPLC system mentioned above was used with a wavelength of detection set at 205 nm. All chromatographic separations were carried out at 35 °C using a Symmetry C18 column (150 × 3.9 mm, 4 $\mu$m, Waters). The mobile phase was methanol with a flow rate 0.7 mL·min$^{-1}$ and injection volume being 10 $\mu$L. The calibration curves of desmosterol were established by using the standard solutions in the appropriate concentration range.

## Results and Discussion

To validate the method of solubility measurement, the solubility of potassium chloride in pure water was determined at the temperature range of (293.0 to 333.0) K. As shown in Figure 2, the solubilities are similar with respect to the values reported in the literature.[11] The uncertainty of the results for at least three measurements was less than 0.35 %. The repeatability of this method was examined by three parallel experiments separately measuring the solubility in methanol at 303.3 K, 315.1 K, and 322.5 K. The deviations of solubility data were 2.6 %, 1.9 %, and 0.9 %, respectively. Data obtained by this method are repeatable and credible.

The solubility values of desmosterol in methanol, ethanol, acetone, ethyl acetate, and acetonitrile were measured and summarized in Table 1. The solubility of desmosterol in different solvents is in the order: acetonitrile < methanol < ethanol < acetone < ethyl acetate. From the results, we found that the solubility of desmosterol decreased with increasing polarity of the solvents to some degree. The solubilities in strongly polar acetonitrile and methanol (relative permittivity of 37.5 and 32.6,[12] respectively, 293.15 K) were lower than in ethanol and acetone (relative permittivity of 22.4 and 21.4,[12] respectively, 293.15 K). The solubility in ethyl acetate was obviously higher than those in other solvents which may due to the low polarity of ethyl acetate with relative permittivity 6.02[12] at 293.15 K. However, the polarity of the solvent is not an absolute yardstick to determine the solubility; for example, the solubility of desmosterol in nonpolar solvent, such as hexane, as we know, is quite small. The solubility behavior may be explained by discussing the interaction between the solute and the solvent molecules in solution.

In addition to polarity, chemical structure also influences the dissolution of the solute, which is reflected in the empirical rule "like dissolves like". If the interactions in the solute and solvent are similar, then the dissolution is easier. Desmosterol has a hydrophobic steroid skeleton moiety, and the addition of a hydroxyl group makes the whole steroid molecule weakly polar. The main interaction in ethyl acetate and acetone may be through the van der Waals force which may improve the dissolution of the title compound. On the other hand, the hydrogen bond is another interaction in the solute. If the solvent has a hydrogen bond, the solvation of the title compound is easier. From the results, we found that the solubilities in methanol and ethanol were much higher than that in acetonitrile. Acetonitrile is highly polar which may increase the repulsion between the solvent molecule and the steroid skeleton, which leads to a low solubility of desmosterol.

In the purification process of desmosterol, cholesterol is the most important impurity since desmosterol and cholesterol are

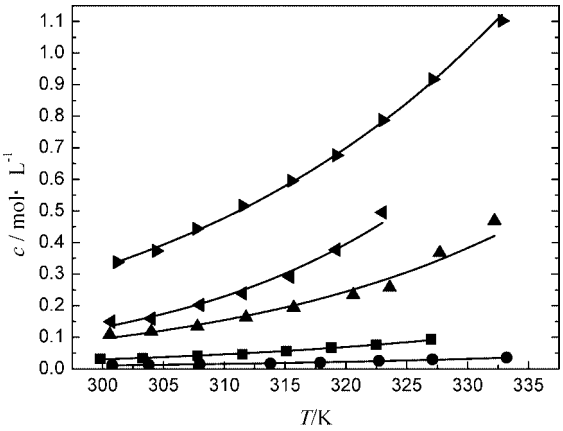


**Figure 3.** Solubilities of desmosterol at different temperatures in five solvents: ●, acetonitrile; ▲, ethanol; solid triangle pointing left, acetone; solid triangle pointing right, ethyl acetate; the corresponding lines are from the calculated values by eq 1.

structurally quite similar. Compared with cholesterol, desmosterol varies by only a double bond in carbon 24. Solubilities of cholesterol in acetonitrile, methanol, ethanol, and acetone[13] are all smaller than solubilities of desmosterol in these solvents, which may be due to the difference between the chemical structures of the two steroids. Compared to cholesterol, with an additional double bond in carbon 24, desmosterol has a stronger interaction with the solvent molecules, which may lead to a higher solubility. Moreover, the difference between the solubilities of the steroids indicates that the separation of the two steroids can be achieved by crystallization with selected solvents.

The experimental solubility of desmosterol increases with an increase in temperature (Figure 3). Thus, the solubility as a function of temperature in a single solvent is correlated by the modified Apelblat equation[14−17]

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

where $A$, $B$, and $C$ are the parameters; $T$ is the absolute temperature; and $c$ is the solubility of desmosterol. The experimental solubility values have been correlated with eq 1 by the least-squares method. The regressed values of the parameters $A$, $B$, and $C$ for the modified Apelblat equation are listed in Table 2 together with the root-mean-square deviation (rmsd), which is defined as the following

$$\mathrm{rmsd} = \left[\frac{1}{N}\sum_{i=1}^{N}\left(c_i - c_i^{\mathrm{calcd}}\right)^2\right]^{1/2} \qquad (2)$$

where $N$ is the number of experimental points and $c_i$ and $c_i^{\mathrm{calcd}}$ denote the experimental and calculated values of solubility, respectively. As shown in Figure 3, the calculated solubilities of desmosterol at different temperatures in all solvents are in accordance with the experimental data.

From Tables 1 and 2, it can be seen that the calculated solubilities showed good agreement with experimental values indicating the modified Apelblat equation can be applied to correlate the solubility data of desmosterol in five organic solvents. The modified Apelblat equation with the parameters is appropriate to describe the temperature dependence of the solubility of desmosterol and may be used as the essential data in purification and crystallization of desmosterol.

## Conclusions

The solubilities of desmosterol in methanol, ethanol, acetone, acetonitrile, and ethyl acetate were measured. Raising the temperature increased the solubility of desmosterol. The solubility of desmosterol decreased with the increasing polarity of the tested solvents to some degree. The dissolution of desmosterol might be easier if the interaction in the solvent might be through either van der Waals force or hydrogen bond force. The solubility of desmosterol was the highest in ethyl acetate in all studied solvents. Acetonitrile presented the lowest solubility in all studied solvents. The temperature dependence of desmosterol solubilities in different solvents can be well-correlated by the modified Apelblat equation.

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Received for review August 3, 2008. Accepted September 24, 2008. The authors are grateful for financial support from the Ministry of Science and Technology of the People's Republic of China (project Nos. 2006BAD27B03 and 2007AA100404) and the Zhejiang Provincial Natural Science Foundation of China (No. Y405102).

JE8006088