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Solubility and Thermodynamics of Dissolution of 13,17−Di−*N*−(2−aminoethyl)amide of Deuteroporphyrin−IX in Aqueous HCl and Tetraoxalate Buffer at 288−328 K

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This paper describes the thermodynamic study of aqueous solutions of 13,17-di-N-(2-aminoethyl)amide of deuteroporphyrin-IX (DDA) which is considered as a potential red natural dye and a perspective substrate for creating new water-soluble sensitizers for photodynamic therapy (PDT). Solubility of the macrocycle in highly aqueous solutions of hydrochloric acid and standard tetraoxalate buffer has been determined in the temperature range from T=288 K to T=328 K. Solubility values are found to decrease with the temperature in both cases due to the weakening of porphyrin basicity at elevated temperatures. Free energies, enthalpies and entropies of solution have been computed from solubility data via the Clark and Glue equation. Enthalpies and entropies of solution are negative and constant for both cases in the temperature range studied. The negative enthalpic term favors DDA dissolution, but large and negative entropies of solution induce the overall unfavorable free energy change.

Keywords: Deuteroporphyrin-IX, aqueous solutions, acid-base interactions, solubility, thermodynamics of dissolution.

Растворимость и термодинамика растворения 13,17−ди−*N*−(2−аминоэтил)амида дейтеропорфирина−IX в водных растворах HCl и тетраоксалатном буфере в температурном интервале 288−328 K

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Проведено термодинамическое исследование водных растворов 13,17-ди-N-(2-аминоэтил)амида дейтеропорфирина-IX (ДДА), который рассматривается в качестве потенциального красного красителя и перспективного субстрата для создания новых водорастворимых сенсибилизаторов для фотодинамической терапии (ФДТ). Растворимость макроцикла в водных растворах соляной кислоты и стандартном тетраоксалатном буфере была определена в интервале температур T=288–328 K. В обоих случаях обнаружено уменьшение растворимости с ростом температуры по причине снижения основности порфирина. С помощью уравнения Кларка и Глю из данных по растворимости были рассчитаны свободные энергии, энтальпии и энтропии растворения. Энтальпии и энтропии растворения отрицательны и постоянны для обоих случаев в исследованном температурном

интервале. Отрицательный энтальпийный вклад способствует растворению ДДА, однако, большие отрицательные энтропии процесса приводят к неблагоприятному изменению свободной энергии в целом.

Ключевые слова: Дейтеропорфирин IX, водные растворы, кислотно-основные взаимодействия, растворимость, термодинамика растворения.

Introduction

Natural porphyrins and related compounds are known to be of great interest for medicine as fluorescence diagnostic labels and drugs for an earlier treatment of different tumors and bacterial infections.[1,2] They also provide an extremely versatile synthetic base for a variety of industrial applications such as the development of non-linear optical materials, natural dyes, different sensors and catalysts *etc*.[3] Many application fields mentioned above require the information about the macrocycle behavior in a liquid phase at different temperatures and concentrations. Solubility values appear to be one of the most important quantities in this sense. These are, however, scarce and restricted by several organic solvents or their mixtures with water mostly at the standard temperature of 298 K.[4-6]

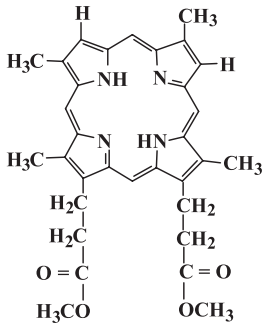
During the last several years we have been involved in the intensive and continuing study (see, for example,[7-9] and references therein) dealing with the behavior of natural macrocycles in individual and mixed organic solvents modeling their biological environment. Here, we focus on thermodynamics of diamino derivative of deuteroporphyrin-IX in highly aqueous acidic solutions, where protonation of the macrocycle and functional amino groups provides sufficient solubility in water to employ the solute as a natural dye for synthetic or semi-synthetic fabrics. This porphyrin can be also used as perspective substrate to synthesize new hydrophilic water-soluble sensitizers for PDT using a quaternization reaction.

Experimental

13,17-Di-*N*-(2-aminoethyl)amide of deuteroporphyrin-IX (**1**) was synthesized using nucleophilic substitution reaction between previously obtained deuteroporphyrin-IX dimethyl ester (**2**) and a freshly distilled 70 % aqueous solution of ethylenediamine. 10 ml of an ethylenediamine solution was added to 0.95 g (1.61 mmol) of deuteroporphyrin-IX dimethyl ester. The reaction mixture was heated at reflux for 3 hours, then diluted by water, neutralized with a dilute aqueous solution of acetic acid to p*H*=7 and filtered with the Schott filter N 16. The porphyrin precipitate was distilled with chloroform to extract possible porphyrin side-products, then filtered again, washed with water and anhydrous acetone and then dried under reduced pressure at T=353 K for usually 72 hours. Mass-spectrum (MALDI) *m/z* for ($C_{34}H_{42}N_8O_2$) calcd. 594.76, found 595.15. Spectra ^{1}H NMR of compounds **1** and **2** are compared in Table 1.

Distilled water was treated with basic potassium permanganate and then distilled twice to reach the electric conductivity of $\sigma=1\cdot10^{-5}$ S·m^{-1}. Standard tetraoxalate buffer (*m*=0.05 mol·kg^{-1}, p*H*=1.68 at 293 K) and a HCl solution (*m*=0.01002 mol·kg^{-1}, p*H*=2.2 at 293 K) were prepared by dissolving or mixing special high purity forms (Reachem) with a necessary amount of bidistilled water.

Solubility measurements were performed with the method of isothermal saturation.[7] Weighed amounts of the porphyrin (~6–7 mg) and an aqueous solution of HCl or tetraoxalate buffer (~45 ml) were placed into a 50 ml glass hermetic cell and



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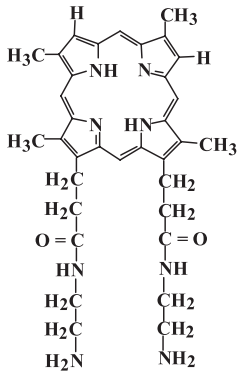

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Table 1. Assignment of protons of 13,17-di-(*N*-(2-aminoethyl)amide of deuteroporphyrin-IX (10 % D_2SO_4 in D_2O) and deuteroporphyrin IX dimethyl ester ($CDCl_3$) in ^{1}H NMR spectra.

Atoms or groups	^{1}H NMR signals	
	Comp. **1**	Comp. **2**
meso-H H(5,10,15,20)	10.06s, 10.04s, 9.99s (1×2×1H)	10.52s, 10.44s, 10.31s (1×2×1H)
3,8-H	9.07s (2H)	9.00s (2H)
13-(2),17-(2)-CH_2	4.41m (4H)	3.98m (4H)
13(3),17(3)-OCH_3	3.74s (6H)	–
2,7,12,18-CH_3	3.70s, 3.69s, 3.62s (3×6×3H)	3.20s, 3.16s, 3.11s, 3.09s (4×3H)
13-(1),17-(1)-CH_2	3.30m (4H)	2.56m (4H)
13(3),17(3)-NH(CH_2)$_2$$NH_2$	–	2.42t, 2.19t (2×4H, *J* = 6.0 Hz)
^{21,23}NH	–3.98bs (2H)	-
Impurities	7.28 (solvent); 1.51, 0.14 (small amounts of solvent impurities)	4.91 (solvent); 2.79, 1.49 (impurities - small amounts)

intensively stirred with a magnetic stirrer usually for two days. The temperature instability inside the cell during the experiment was ±0.05 K. When equilibrium was reached, the stirrer was switched off and one or two milliliters of the liquid content were quickly taken up with a thermostated syringe equipped with a filter (the pore size of 0.45 μm), weighed in a hermetic vessel with analytical balances and diluted by an appropriate solution at a room temperature for a spectrophotometric control. This procedure was repeated from ten to fifteen times and the mean value of the absorbance coefficient was selected to compute solubility values. To evaluate the equilibrium time achievement we have specially performed long-term measurements continuing up to seven days. Our results did indicate that one day was quite enough to achieve saturation after that the porphyrin concentration in a liquid phase remained constant.

DSC measurements were carried out with the differential heat flow calorimeter of DSC 204 F1 Phoenix "NETZSCH". The "NETZSCH" instrument was calibrated for temperature and heat effects with six standards including cyclohexane, indium, biphenyl, mercury, tin and bismuth. The sample of the dried porphyrin (m≈5 mg) placed in an aluminum crucible was run from 253 K to 513 K in an argon atmosphere with sapphire as a standard. The heating rate of the sample was equal to 5 K/min. The uncertainty of the peak positions and heat effects was estimated to be 0.1 and 3 %, respectively.

Results and Discussion

The absorption spectra of the porphyrin in 0.01 and 0.1 *m* HCl aqueous solutions are illustrated in Figure 1. The absorption spectrum contains the intensive Soret band at λ=396 nm and also a less intensive peak at λ=547 nm.

The latter is convenient to evaluate DDA (comp. **1**) concentration in a saturated solution. The absorbance value at λ=547 nm is found to be linearly dependent on the porphyrin concentration. However, Figure 1 highlights the presence of a small shoulder at λ=369 nm in a 0.01 *m* HCl solution which disappears in more acidic media such as tetraoxalate buffer (p*H*=1.68) or 0.1 *m* HCl (p*H*=1.3). This indicates that acid-base equilibria in more acidic aqueous solutions of DDA:

$$H_2DP(NH_2)_2 \overset{+2H^+}{\rightleftharpoons} \{H_2DP(NH_3)_2\}^{2+} \overset{+2H^+}{\rightleftharpoons} \{H_4DP(NH_3)_2\}^{4+} \quad (1),$$

are shifted to the fully protonated form. In contrast, for a 0.01 *m* solution there is some fraction of the porphyrin containing non-protonated nitrogen atoms in the macrocycle (see eq. (1)). It induces the appearance of a shoulder at λ=369 nm shifted hypsochromically to the main Soret band. This behavior is typical for porphyrins with non-protonated nitrogen atoms in the macrocycle.[11]

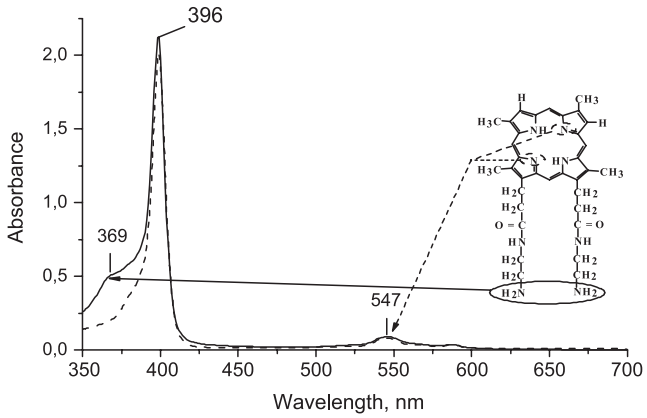

Figure 1. The absorption spectra of DDA in 0.01 and 0.1 *m* HCl solutions at 293 K. DDA molality is of 10^{-5} mol·kg^{-1}.

Since in a 0.1 *m* HCl solution this fraction disappears and DDA molecules are fully protonated, it allows to evaluate the absorption molar coefficient at λ=547 nm and then compute the concentration of the $\{H_4DP(NH_3)_2\}^{4+}$ form in a 0.01 *m* solution. The content of the $\{H_2DP(NH_3)_2\}^{2+}$ fraction absorbing at 369 nm can be simply evaluated as the difference between the analytical concentration of DDA and the $\{H_4DP(NH_3)_2\}^{4+}$ content. Thus, for the calculation of solubility in 0.01 *m* HCl solutions we have exploited two linear calibration curves obtained at 293 K for the absorption bands with λ=369 and 547 nm. The concentration of a fully protonated form $\{H_4DP(NH_3)_2\}^{4+}$ at a room temperature is found to be about ten times as higher as the concentration of $\{H_2DP(NH_3)_2\}^{2+}$.

Solubility values for DDA in buffer and HCl solutions at different temperatures are given in Table 2.

Table 2. Solubility of DDA in 0.01 *m* aqueous HCl and 0.05 *m* tetraoxalate buffer between T=288.15 and T=328.15 K.

Temperature, K	$m_{sat}\cdot10^5$ [a]	$m_{sat}\cdot10^5$
	HCl	Tetraoxalate buffer
288.15	11.32±0.06 [b]	13.26±0.02
293.15	10.51±0.06	12.11±0.04
298.15	10.03±0.04	11.29±0.04
308.15	9.59±0.06	9.93±0.06
313.15	9.37±0.07	8.56±0.04
318.15	9.22±0.06	7.39±0.03
328.15	8.89±0.03	6.26±0.04

[a]The unit is molality of a DDA saturated solution (mol of DDA molecular form per 1 kg of the solvent);
[b]The uncertainty represents the twice standard deviation.

These quantities are seen to be of the same order and reveal similar temperature changes. As the temperature is increased the solubility values decrease monotonously in both cases, the effect being pronounced stronger in more acidic buffer solutions. This observation is rather surprising since solubility of a molecular form of porphyrins in organic solvents increases with the temperature.[8,10] However, the situation here is significantly complicated by protonation of amino groups and central nitrogen atoms and also ion-ion interactions in an aqueous phase. Since porphyrin basicity significantly decreases with the temperature[11] it leads to weaker solute-solvent specific interactions. This may induce the increase of the porphyrin fraction with non-protonated nitrogen atoms in the macrocycle (see eq. (1)), which results in decreasing solubility at elevated temperatures.

The experimental pressure from here on is 101.33 kPa. Standard uncertainties for the experimental determination of temperature and pressure are $u(T)$=±0.01 K, $u(p)$=±0.5 kPa, respectively.

Parameters of eq. (2) at 298 K are $\Delta_{sol}G^0$=(22.83±0.01) kJ·mol^{-1}, $\Delta_{sol}H^0$ =(–3.27±0.08) kJ·mol^{-1}, s_f=0.02 for a HCl

solution (from T=298 to T=328 K); $\Delta_{sol}G^0$=(22.56±0.05) kJ·mol^{-1}, $\Delta_{sol}H^0$=(–14.76±1.1) kJ·mol^{-1}, s_f= 0.38 for tetraoxalate buffer (from T=288 to T=328 K); s_f is the standard deviation of the fit.

To evaluate standard thermodynamic quantities from solubility data we use here the approach originally proposed by Clark and Glew[12] in the simple form assuming the temperature independent enthalpy and entropy change:

$$R\ln m/m_0 = -\frac{\Delta_{sol}G^0}{298.15} + \Delta_{sol}H^0\left[\frac{1}{298.15} - \frac{1}{T}\right] \quad (2),$$

where m is molality of a saturated solution, m_0 is molality of the standard solution of 1 mol/kg with properties of an infinitely diluted solution, 298.15 K is the reference temperature, $\Delta_{sol}G^0$ and $\Delta_{sol}H^0$ are the standard free energy and enthalpy of solution at the reference temperature, respectively. The entropic term $-T\Delta_{sol}S^0$ is simply calculated *via* the Gibbs-Helmholtz equation:

$$-T\Delta_{sol}S^0 = \Delta_{sol}G^0 - \Delta_{sol}H^0 \quad (3)$$

The coefficients of eq. (2) are given in the footnote to Table 2 for both systems. We see from Figure 2 that eq. (2) reproduces well both experimental curves excepting lower temperatures for highly aqueous HCl. In fact, the $R\ln m/m_0$ values decrease linearly with the reciprocal temperature in hot HCl solutions, whereas at room temperatures the experimental points deviate from the calculated ones. It indicates that the heat capacity of solution does not approach zero in cold HCl solutions. We assume that structural changes in a solid phase dealing with packing effects of DDA functional side-chains could be responsible for such a behavior. In fact, our DSC study of the solid porphyrin reveals two broad peaks between 250 and 470 K. Comparing Figures 2, 3 we see that the solubility curve is successfully described by eq. (2) between 298 and 328 K, where structural changes dealing with increasing mobility of functional groups in a solid state take place. In contrast, for T<298 K, where this effect does not already occur (see Figure 3), the solubility shows a much more pronounced temperature dependence.

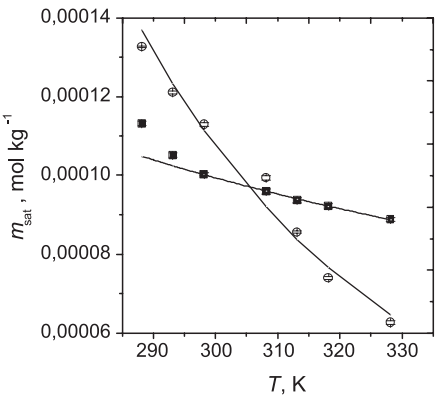

Figure 2. Experimental (points) and calculated by eq. (2) (lines) solubility values of DDA in 0.01 *m* HCl (■) and a standard 0.05 *m* tetraoxalate buffer (○). Uncertainties represent the twice standard deviation.

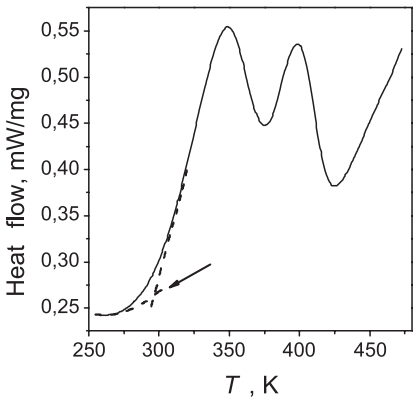

Figure 3. DSC trace for solid DDA. The heating rate is equal to 5 K/min; the arrow shows the intercept of extrapolated DSC curves indicating the beginning of the endothermic phase transition at T_{onset}=297.4 K. The estimated enthalpies of two phase transitions are 31 and 10 kJ·mol^{-1} for the first (T_{max}=350.4 K) and second (T_{max}=381.8 K) peaks on the DSC curve, respectively.

For a more acidic buffer solution, where the porphyrin is fully protonated, the experimental and computed solubility values are in a good agreement in the temperature range studied (see Figure 2) indicating the temperature independent enthalpy and entropy of solution. Perhaps the structural changes mentioned above do not appear in this case due to the presence of multiatomic oxalate-ions in a solid phase. These bulk ions strongly interact with positively charged amino side-chains and significantly reduce their conformational motions providing more dense packing than in the case of the interaction with chloride-ions.

Standard free energies of dissolution are expectedly positive in both cases (see the footnote to Table 2) and increase linearly with the temperature. The enthalpic term favors DDA dissolution, it being much stronger pronounced in more acidic tetraoxalate buffer. However, large and negative entropies of solution induce the overall unfavorable free energy change. This unfavorable entropic term can be attributed to a restricted motion of charged amino side-chains in aqueous solutions due to intensive ion-solvent interactions. Chloride and oxalate anions provide an additional structural effect forming strong H-bonds with surrounding water molecules.

Conclusions

We have synthesized, identified and studied thermodynamics of dissolution of a potential natural dye and a photosensitizer of 13,17-di-*N*-(2-aminoethyl)amide of deuteroporphyrin-IX (DDA) in two acidic media at different temperatures. This water insoluble porphyrin reveals sufficient solubility for the applied goals in an acidic solution. As acidity of a solvent is increased, the solubility rises rapidly giving a possibility to obtain various shades of red on fabrics. HCl solutions have some advantage in this sense due to their low cost. Solubility, however, decreases linearly with the reciprocal temperature for buffer solutions mainly due to the weakening of porphyrin basicity at elevated temperatures. In contrast,

for HCl solutions there is a deviation from linearity at room temperatures dealing with the packing of the porphyrin functional groups in a solid phase. This effect, however, is not observed for a more acidic tetraoxalate buffer.

The thermodynamic analysis of the porphyrin behavior does indicate that enthalpies and entropies of dissolution are negative for both acidic media. The negative enthalpic term favors DDA dissolution, whereas large and negative entropies of dissolution induce the overall unfavorable free energy change that leads to low solubility values.

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