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Thermodynamics of solution and partition of dioxidine in water and the water/1-octanol biphasic system

Andrey V. Kustov *, Nataliya L. Smirnova

United Physico-Chemical Centre of Solutions of G.A., Krestov Institute of Solution Chemistry of Russian Academy of Sciences, Akademicheskaya str. 1, 153045 Ivanovo, Russian Federation
Ivanovo State University of Chemistry and Technology, Sheremetev av. 7,153012 Ivanovo, Russian Federation

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ABSTRACT

We have studied for the first time the thermodynamic behavior of the well-established antimicrobial drug – dioxidine (2,3-dihydroxymethylquinoxaline-1,4-dioxide) in water and the water/1-octanol biphasic system. Enthalpies of solution and solubility of dioxidine were experimentally determined and exploited to compute thermodynamic functions of solution and the solubility of the drug in a wide temperature range. Partition coefficients between water and 1-octanol were also obtained to evaluate lipophilicity of dioxidine and used to compute thermodynamic functions of the solute transfer from water to aqueous octanol. The results are discussed in terms of solute-solvent interactions occurring in water-like and lipid-like phases.

1. Introduction

Quinoxaline and its various derivatives are known to be of great interest as an extremely versatile synthetic base for a variety of applications such as the development of new dyes, fluorescent materials, organic semiconductors *etc.* [1–3]. However, the most important field is biomedicine since many of these heterocycles reveal antiviral, antimicrobial, antifungal and anticancer activity [4,5]. Dioxidine is a synthetic broad-spectrum antimicrobial agent which is extremely important for treating both anaerobic and mixed anaerobic-aerobic infections caused by multiresistent strains [6,7]. The mechanism of dioxidine action is not fully understood but it is believed that this *N*-oxide selectively inhibits DNA chain formation in a bacterial cell and also induces membrane damage provoking formation of reactive oxygen species. However, due to its rather high toxicity dioxidine is often considered as a reserve drug for treating multiresistent nosocomial microflora.

During the last three years we are involved in the extensive and continuing study dealing with the development of new photosensitizers (PS) for antimicrobial and antitumor photodynamic therapy (PDT) based on a porthyrin or chlorine platform [8–11]. PS are able to absorb irradiated photon energy, transfer it to nearby oxygen molecules producing reactive oxygen species such as singlet oxygen $^1O_2$, different radical forms, superoxide anion *etc.* These species induce a significant toxic effect leading to cell death *via* apoptosis or necrosis [12]. In principal, dioxidine may be also considered as a photosensitizer because it absorbs light in a UV- and visible region (see the spectrum shown in Fig. 1) and often reveals residual skin phototoxicity [6,7]. However, violet or blue light penetrates least efficiently through tissue, which significantly reduces phototoxicity of the drug. The conjugation of dioxidine with appropriate chlorine appears to achieve some advantage. It increases solubility of macroheterocycle, may provide better membrane penetration and additional toxicity towards pathogenic microflora. Our preliminary results have indicated [11] that such PSs being soluble in aqueous micelle solutions provide a high killing effect towards both Gram-positive and Gram-negative bacterial microflora. However, the information about dioxidine solubility, its affinity to lipid membranes and interactions with appropriate targets in cells or their models is very scarce. Here, our efforts are mainly directed towards obtaining the experimental thermodynamic information about such interactions in a physiological temperature range both in water-like and lipid-like phases modeling by water and 1-octanol in the hope that it allows to highlight some important features of the drug activity *in vivo*.

2. Experimental section and results

Water was distilled twice to reach the electric conductivity of $1 \cdot 10^{-5}$ S·m^{-1}. 1-octanol (Reachem, chemical purity) was distilled under reduced pressure at 360 K. Karl Fisher titration showed that final water content was 0.2 mass%. Dioxidine (2,3-dihydroxymethylquinoxaline-1,4-dioxide, $C_{10}H_{10}N_2O_4$) was obtained from its 1% aqueous solution provided by "Borisovskiy zavod medicinskikh

* Corresponding author at: United Physico-Chemical Centre of Solutions of G.A., Krestov Institute of Solution Chemistry of Russian Academy of Sciences, Akademicheskaya str. 1, 153045 Ivanovo, Russian Federation.
E-mail address: kustov@isuct.ru (A.V. Kustov).

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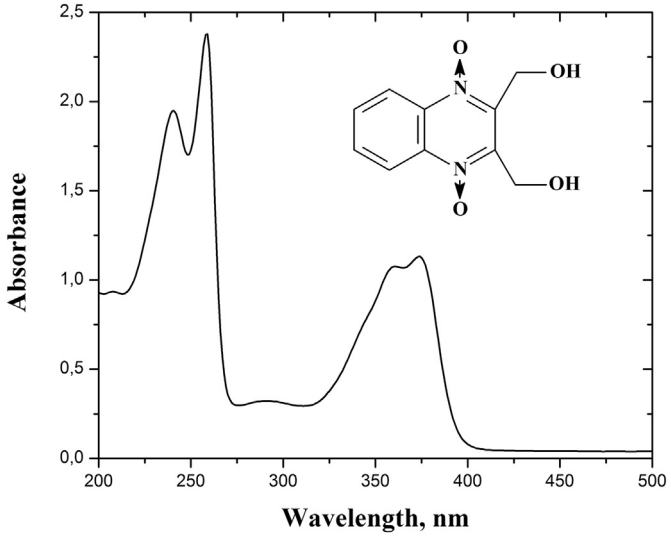

Fig. 1. Chemical structure and absorption spectrum of dioxidine in water.

preparatov" (Borisovskiy ZMP Belarus). The solution was cooled to about 275 K, dioxidine crystals was separated from a solution using the Schott filters, washed by cold water several times and then dried under reduced pressure at 353 K for several days. Purity of the solute was checked with UV-, visible and ^{1}H NMR (see Fig. 1 and Supplementary material).

2.1. Calorimetric measurements

Enthalpies of solution were measured with the self-built isoperibol automated ampoule calorimeter described several times previously [13,14]. The detection limit of the apparatus was 10 μK. The temperature instability in the thermostat was less than 1 mK for all temperatures studied. Enthalpies of solution were measured by a comparative method with the digital Standard Temperature Measuring Instrument. The calorimeter was tested by measuring enthalpies of solution of 1-propanol in water at 298.15 K. Our result $\Delta H^0_{sol} = -10.20 \pm 0.06$ kJ mol^{-1} was in a fair with the recommended value of -10.16 ± 0.02 kJ mol^{-1} [15]. Duration of dioxidine dissolution in water in our cell equipped with effective stirring was equal to about five minutes.

The experimental ΔH^m_{sol} values at different temperatures and concentrations are given in Table 1. We see that experimental values do not depend on solute molality. It allows to compute the standard enthalpy of solution or the enthalpy of solution at infinite dilution ΔH^0_{sol} as the mean value in the range of experimental results. It also indicates that solute-solute correlations are too small to influence ΔH^m_{sol} values in the concentration range studied.

Our attempts to determine the ΔH^m_{sol} value in pure OctOH or OctOH saturated by water were not successful. The dissolution process at the standard temperature was too long to obtain reliable quantities directly. Some amount of the drug was found to be in a solid state even after one hour of the experiment at high dilution of 0.0002 mol kg^{-1}. Nevertheless, we have estimated that the enthalpy of solution should be of 35 kJ mol^{-1}.

2.2. Solubility and partition coefficient measurements

Solubility measurements were performed with the method of isothermal saturation which was nearly identical to that used in our previous studies [8,13]. Weighed amounts of the solute and water were placed into the 50-ml glass hermetic cell and stirred with a magnetic stirrer usually for 24 h. The temperature of the cell was maintained equal to 288.15 ± 0.05 K with the HAAKE DC 10 thermostat. 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) and weighed in a hermetic vessel with analytical balances. Then the liquid content was diluted by water at a room temperature for a spectrophotometric control. The previously obtained calibration plot at λ = 259 nm was used to compute a solubility value. Additionally, we have applied the dry weight method [16] to determine the drug content in a saturated solution. Table 2 shows that both methods lead to identical results. Our analysis of a solid phase has indicated that dioxidine seems to be partially hydrated (see Supplementary material file). Water molecules seem to influence the packing of dioxidine molecules but the interaction with the solute is rather weak. Most of solvent molecules are removed below the boiling point of water indicating that the solvent fills cavities in the solid structure of the drug. We have considered that hydration in a solid phase has negligible effect on the free energy values and do not take it into account in the thermodynamic manipulations.

Partition coefficients (P) were determined with a similar technique [9]. Weighed amounts of an aqueous dioxidine solution with the concentration of 50–80 μmol kg^{-1} and pure OctOH with the volume ratio of 60:40 were placed into a similar cell and intensively stirred usually for one day. When equilibrium was reached, the stirrer was switched off to achieve phase separation. Then, the equilibrium concentration of the drug in an aqueous phase was analyzed with the spectrophometric method mentioned above. The dioxidine concentration in an OctOH phase was computed as the difference between the equilibrium and initial concentrations. The P values given in Table 2 were computed here in a molality scale as follows [9]:

$$P = m_{OctOH}/m_{aq}, \quad (1)$$

Table 1
Experimental (ΔH^m_{sol}) and standard (ΔH^0_{sol}) enthalpies of solution (kJ mol^{-1}) of dioxidine in water at 288.15–338.15 K.

m[a]	ΔH^m_{sol}	m	ΔH^m_{sol}	m	ΔH^m_{sol}
$T = 288.15$ K		$T = 293.15$ K		$T = 298.15$ K	
0.000023	17.98	0.003420	19.20	0.001890	20.20
0.000058	17.96	0.002529	19.15	0.001992	20.22
0.000030	18.01	0.001742	19.18	0.003658	20.15
0.000038	18.09	0.001456	19.20	0.004853	20.12
$\Delta H^0_{sol} = 18.01 \pm 0.03$[b]		$\Delta H^0_{sol} = 19.18 \pm 0.01$		$\Delta H^0_{sol} = 20.17 \pm 0.02$	
7.24		6.31			
$T = 303.15$ K		$T = 308.15$ K		$T = 313.15$ K	
0.001369	21.56	0.000780	22.60	0.002052	24.16
0.001859	21.60	0.001105	22.65	0.001603	23.92
0.001482	21.52	0.000940	22.58	0.003075	24.08
0.001570	21.64	0.002251	22.63	0.001680	23.99
$\Delta H^0_{sol} = 21.58 \pm 0.03$		$\Delta H^0_{sol} = 22.62 \pm 0.02$		$\Delta H^0_{sol} = 24.04 \pm 0.05$	
$T = 318.15$ K		$T = 328.15$ K		$T = 338.15$ K	
0.000940	25.70	0.001283	28.72	0.001223	31.40
0.002019	25.50	0.001685	28.91	0.000912	31.63
0.001881	25.69	0.001970	28.75	0.001485	31.60
0.002214	25.53	0.002218	28.60	0.002943	31.53
$\Delta H^0_{sol} = 25.61 \pm 0.05$		$\Delta H^0_{sol} = 28.75 \pm 0.06$		$\Delta H^0_{sol} = 31.54 \pm 0.05$	

[a] Dioxidine molality.
[b] Errors from here on represent the twice standard deviation.

Table 2
Partition coefficients and solubility values for dioxidine in water.

P[a]		
298.15 K	308.15 K	318.15 K
2.94 ± 0.05	3.85 ± 0.28	5.55 ± 0.29
S, g/100 g of water, $T = 288.15$ K		
Spectrophotometry	Dry weight method	
0.72 ± 0.04	0.74 ± 0.02	

[a] Coefficients of Eq. (7) are $\Delta G^0_t = -2.64 \pm 0.19$ kJ mol^{-1}, $\Delta H^0_t = 25.00 \pm 5$ kJ mol^{-1}, $R^2 = 0.990$, $s_f = 0.38$ J mol^{-1} K^{-1}.