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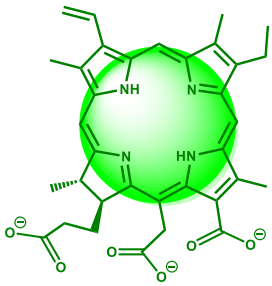
Chlorin–chlorin intermolecular interaction in water: an investigation using calorimetry and dynamic light scattering

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In this paper, we present the results of the first accurate calorimetric study of chlorin e_6 trisodium salt self-association in water using titration calorimetry and dynamic light scattering. The enthalpies of dilution of the photosensitizer were treated in terms of the virial expansion approach and the enthalpic virial parameters obtained were compared with those for organic and inorganic solutes in water.



Keywords: chlorin e_6, water, enthalpies of dilution, pair interaction, photosensitizer self-association.

Photosensitizers (PSs) based on the chlorin e_6 platform (see Figure 1) are still the most popular agents in antitumor and antimicrobial photodynamic therapy.[1-4] The light-sensitive agents "Photolon", "Radachlorin" and "Talaporfin" have proved their high efficacy in treating both different superficial malignancies and tumors of hollow organs.[5-8]

However, many macrocyclic photosensitizing agents exhibit a strong tendency to hydrophobic self-association[9-11] which often negatively affects drug bioavailability and weakens singlet oxygen generation.[9,11] Being a polyanionic and consequently hydrophilic enough solute at physiological conditions[12] , chlorin e_6 (Chl e_6) is a much more soluble and less associated photosensitizing agent compared to cationic chlorins of a similar structure.[10,13] The latter ones form large aggregates in water even at m < 1 mmol/kg.[10]

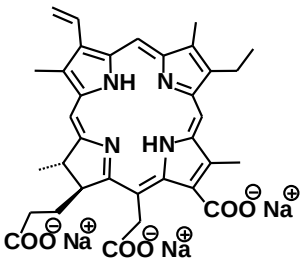


Figure 1. The molecular structure of chlorin e_6 trisodium salt.

In this communication, we present the first attempt to study the interaction between chlorin molecules in water using high precision titration calorimetry and dynamic light scattering (DSC). The thermodynamic formalism used here to extract information about solute-solute interactions from the experimental enthalpies of dilution is based on the excess function concept or virial expansion approach.[14-18] The key quantity of this technique is the second virial coefficient which is related to the potential of mean force (PMF) occurring between two solute species.[14-16] The virial enthalpic parameters considered here are related to the temperature derivative of the integral of PMF.

It should be stressed that we studied highly diluted aqueous solutions where the final PS concentration is between 2-30 μM. This requires to use a highly sensitive and stable device for the detection of small heat effects of 1 mJ or smaller. The self-built twin heat conduction calorimeter recently described elsewhere[19] seems to be one of the few devices capable of obtaining these effects. Figure 2 shows several experimental curves associated with the heat effects of dilution of Chl e_6 solutions and Table S1 gives the numerical $\Delta_{dil}H^m$ values (see Online Supplementary Materials).

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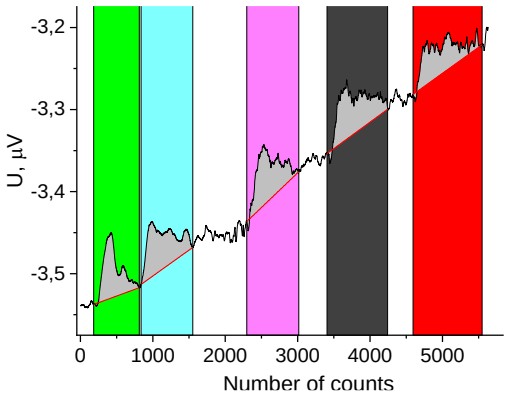


Figure 2. The calorimetric curves for the series of dilution of 40 μl of a Chl e_6 aqueous solution with m_i = 0.00512 mol/kg in water at 298.15 K.

The description of the chemicals as well as the details of the calorimetric and DLS measurements are also given in Online Supplementary Materials. Figures 3 illustrates the $\Delta_{dil}H^m$ *vs* m_f-m_i curve and compares it with the values obtained in terms of the Debye-Hückel limiting law[20,21]:

$$a_H = -\left(\frac{\vartheta}{2}\right)^{0.5} \cdot |z_+ z_-|^{1.5} \cdot \vartheta \cdot RT^2 \cdot A \cdot \left(\frac{1}{T} + \frac{1}{\varepsilon}\frac{\partial \varepsilon}{\partial T} - \frac{1}{3\rho}\frac{\partial \rho}{\partial T}\right) \quad (1),$$

where the A coefficient is defined as follows

$$A = \frac{4.201 \cdot 10^6 \cdot \rho^{0.5}}{(\varepsilon T)^{1.5}} \quad (2)$$

In eqs (1, 2) z and ϑ are the charge and number of corresponding ions after full dissociation of Chl e_6 as an 3:1 electrolyte; ρ and ε are density and dielectric permittivity of water, T=298.15 K.

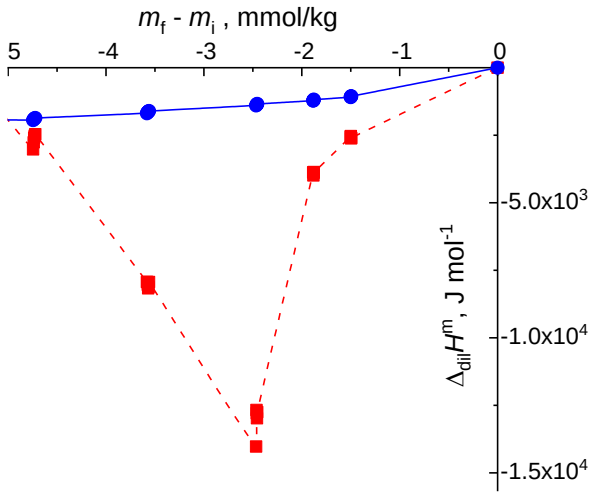


Figure 3. The concentration dependence of $\Delta_{dil}H^m$ values (■) for Chl e_6 in water and the corresponding Debye`s term for 3:1 electrolyte (●). Lines are spline functions, m_f and m_i are final and initial solute molalities, respectively.

We see that the Debye's term is small enough and, therefore, the Coulomb forces are not responsible for the large and negative change in the enthalpy of dilution. This Chl e_6 behavior occurring in a highly diluted aqueous solution has no analogues among known inorganic and organic electrolytes.[22-26] $\Delta_{dil}H^m$ <<0 shows that solvation of Chl e_6 is more exothermic in highly diluted aqueous solutions mainly due to the weakening of the chlorin-chlorin interactions. To evaluate the effect of this interaction on the experimental quantities we applied the formalism based on the excess function concept mentioned above[14-18], where the deviation from the limiting Debye`s law was considered as the sum of pair, triplet and high-order solute-solute interactions[17,18,26]:

$$\Delta_{dil}H^m = a_H \cdot (m_f^{0.5} - m_i^{0.5}) + b \cdot (m_f - m_i) + c \cdot (m_f^{1.5} - m_i^{1.5}) + d \cdot (m_f^2 - m_i^2) + \ldots, \quad (3),$$

where b=h_{22} is the enthalpic virial parameter of the solute-solute pair interaction taking into account the cation-cation, cation-anion and anion-anion contacts.

The numerical results are found to be strongly dependent of the concentration range studied. In Table 1 we give two h_{22} values obtained in the chlorin concentration range between 0.002-1.9 and 0.002-2.47 mmol·kg^{-1} using the linear and quadratic form of eq (3), respectively. Inspection of the data obtained shows that both quantities are much larger than the h_{22} values for simple ionic solutes. We see that the pair interaction between hydrophilic solutes such as $CaCl_2$ or PC is enthalpically attractive due to favorable cation-anion association. In contrast, for hydrophobic but well-hydrated Bu_4NBr the pair interaction is strongly enthalpically repulsive due to the cation-cation term. The latter is large and positive due to the overlapping of hydrophobic hydration shells of tetrabutylammonium ions and expulsion of some waters to a bulk solvent.[15,16]

Table 1. Enthalpic virial parameters of pair interaction (kJ kg·mol^{-2}) for Chl e_6 and several electrolytes[26] in water

Chl e_6	$CaCl_2$	Et_4NBr	Bu_4NBr	PC
1269±75 64875±6633	-6.1±0.2	-5.2±0.2	12.6±0.5	-35.7±0.5

Note. The uncertainties are standard errors. The parameters of eq (3) are a_H =29.2 kJ kg$^{0.5}$·mol$^{-1.5}$, b=1268.8±75.2 kJ kg·mol^{-2}, R^2= 0.995, s_d=0.36 kJ/mol, n=9 (m=0.002-1.9 mmol·kg^{-1}); a_H =29.2 kJ kg$^{0.5}$·mol$^{-1.5}$, b=64875±6633 kJ kg·mol^{-2}, c= -3194120±298076 kJ kg$^{1.5}$·mol$^{-2.5}$, d=4000500±3328070 kJ kg^2·mol^{-3}, s_d=0.27 kJ/mol, R^2= 0.997, n=13 (m=0.002-2.47 mmol·kg^{-1}). PC denotes Prospidii chloride.

For Chl e_6 both h_{22} values are abnormally large and positive indicating that the interaction between two chlorin molecules in water is strongly enthalpically repulsive. Thus, the total interaction monitored by the free energy is entropically driven, which is consistent with a classical view of hydrophobic interaction, where the h_{22} and Ts_{22} values are large and positive due to the overlapping of PS hydration shells.[15] The high order coefficients in eq (3) are alternating in sign (see the footnote to Table 1) indicating the convergence of the virial expansion in Chl e_6 solutions. However, the equation is not able to describe the minimum on the $\Delta_{dil}H^m$ *vs* m_f-m_i curve, after that the Debye`s and experimental quantities become comparable.

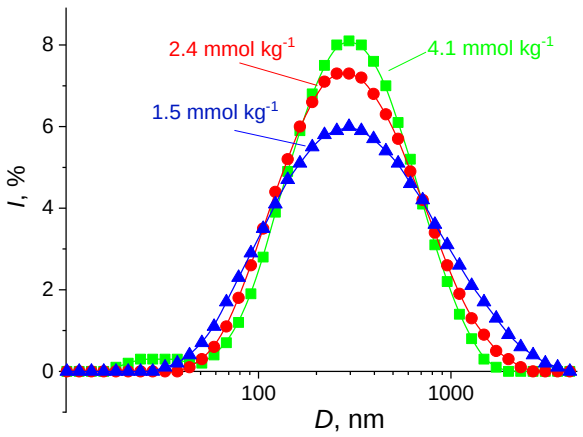


Figure 4. The DLS study of aqueous solutions of Chl e_6.

There are at least two important features in the results obtained which are worthy of note. Firstly, eq (3) operates three coefficients associated with the interaction between two, three and four solute molecules, respectively.[17,18] This indicates that

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according to this approach the minimum in Figure 3 results from the interaction between a larger number of chlorin molecules, *i.e.* it is associated with the formation of the cluster containing at least five or more hydrated PS species. Taking into account that the Chl e_6 molecule contains a poorly hydrated hydrophobic fragment and three hydrophilic carboxyl groups in the 13th, 15th and 17th positions of the macrocycle (see Figure 1), we assume that this finding is correct enough. Secondly, it is known[27-29] that a planar hydrophobic surface is poorly hydrated and the number density of water molecules is depleted in its nearest vicinity. This phenomenon often leads to a pressure imbalance and strong enthalpically-driven attraction between two purely hydrophobic plates in water.[27] However, the anomalously positive h_{22} value (see Table 1) indicates that the correlations between two Chl e_6 molecules in water extend over large distances and the chlorin-chlorin interaction affects a very large number of water molecules. These waters partially loss their hydrogen bonding, which is accompanied by a large enthalpic effect. Thus, the size of a chlorin macrocycle is still not sufficient to induce a significant disruption of the water structure in its nearest vicinity. The Van der Waals and Coulomb interactions of Chl e_6 with surrounding water molecules stabilize a PS hydration shell[27] providing the PS solubility sufficient for clinical utilization. Similar but weaker pronounced manifestations of hydrophobic interaction were found in aqueous solutions of tetraalkylammonium ions using small angle x-ray scattering data and molecular dynamic simulations.[30,31]

To get more information about Chl e_6 association in water we studied the scattering of transmitted red light in three millimolar PS solutions. Figure 4 illustrates the existence of large concentration and density fluctuations. The distribution is found to be monomodal and becomes slightly narrow with the increase in the solute concentration with the constant D_{mean} value of 300 nm. Thus, hydrated Chl e_6 molecules form large clusters which disappear at micromolar concentrations.

In this work, we have set out of obtaining for the first time the thermodynamic measure of the interaction between Chl e_6 molecules in water using a recently built twin heat conduction calorimeter. Most of our efforts have been directed toward obtaining the pair and high-order virial interaction parameters in the hope that these quantities illuminate some of the features which contribute to Chl e_6 association in water. The results are, however, twofold. The experimental quantities are found to be abnormal for highly diluted aqueous solutions. The relative enthalpic pair interaction parameter is very large and positive compared to the simple solutes studied before. Unfortunately, similar quantities for other macrocycles in water still wait to be determined to make correct conclusions. Nevertheless, our results clearly indicate a strong tendency of chlorin molecules to hydrophobic association even at very high dilution. This water-induced effect leads to formation of large clusters containing both PS and water molecules at millimolar Chl e_6 concentrations.

Online Supplementary Materials

Supplementary Materials associated with this article can be found in the online version at doi: 10.71267/mencom.8149.

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