Mutual and self-diffusion of charged porphyrines in aqueous solutions

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ABSTRACT

We have investigated the diffusion properties for an ionic porphyrin in water. Specifically, for the {tetra-sodium tetraphenylporphyrintetrasulfonate (Na$_4$TPPS) + water} binary system, the self-diffusion coefficients of TPPS^{4-} and Na$^+$, and the mutual diffusion coefficients were experimentally determined as a function of Na$_4$TPPS concentration from (0 to 4) $\cdot$ 10^{-3} mol $\cdot$ dm^{-3} at T = 298.15 K. Absorption spectra for this system were obtained over the same concentration range. Molecular mechanics were used to compute size and shape of the TPPS^{4-} porphyrin. We have found that, at low solute concentrations (<0.5 $\cdot$ 10^{-3} mol $\cdot$ dm^{-3}), the mutual diffusion coefficient sharply decreases as the concentration increases. This can be related to both the ionic nature of the porphyrin and complex associative processes in solution. Our experimental results are discussed on the basis of the Nernst equation, Onsager–Fuoss theory and porphyrin metal ion association. In addition, self-diffusion of TPPS^{4-} was used, together with the Stokes–Einstein equation, to determine the equivalent hydrodynamic radius of TPPS^{4-}. By approximating this porphyrin to a disk, we have estimated structural parameters of TPPS^{4-}. These were found to be in good agreement with those obtained using molecular mechanics. Our work shows how the self-diffusion coefficient of an ionic porphyrin in water is substantially different from the corresponding mutual-diffusion coefficient in both magnitude and concentration dependence. This aspect should be taken into account when diffusion-based transport is modelled for *in vitro* and *in vivo* applications of pharmaceutical relevance.

1. Introduction

Porphyrins are promising photosensitizers for photodynamic therapy (PDT) due to their high affinity and phototoxicity to tumour cells [1–9]. Consequently, aqueous solutions of porphyrins and related compounds (*e.g.*, metal-complexes) have become important systems in PDT. Moreover, they are also involved in a wide range of other purposes related to analytical catalysis [10], sensor applications [2–11], optical applications [1,12], biological systems [13] and pharmaceutical chemistry [4,14].

Tetrasodium tetraphenylporphyrintetrasulfonate (Na$_4$TPPS) has interesting photophysical properties, such as high hydrophylicity and high ability to generate singlet oxygen, which can be suitable to PDT applications [3–9]. This porphyrin is one of the few water soluble porphyrins. In fact, the large aromatic tetra-pyrrolic system makes almost all porphyrins only soluble in organic solvents. Therefore, Na$_4$TPPS has a particular importance in the determination of the porphyrin's behaviour in aqueous solutions. The four negative charges on the sulphonic groups result in complex behaviour in aqueous solutions. That is, in aqueous solutions Na$_4$TPPS would be present in several fully ionised, partially ionised and aggregated or non-aggregated forms, depending on concentration, temperature, pH (it is known that at pH 4, the TPPS ions aggregate) and nature of the metallic counter ions [2], added salts or surfactants present.

The characterisation of the diffusion coefficients of porphyrin solutions is important, helping us to understand the properties and behaviour of such chemical systems in the human body. In this sense, we are particularly interested in the characterisation of the Na$_4$TPPS self and mutual diffusion in aqueous solutions at different conditions.

It is very common in the scientific literature to find misunderstandings concerning the meaning of both parameters, frequently just denoted indistinctly by D and referred as "*diffusion coefficient*"

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[15,16]. It is necessary to distinguish between two distinct processes: self-diffusion (also named as intra diffusion, tracer diffusion, single ion diffusion or ionic diffusion) and mutual diffusion (also known as inter diffusion, collective diffusion, concentration diffusion or salt diffusion). Methods such as those based on PGSE based NMR methods, polarography, and capillary-tube techniques with radioactive isotopes measure self-diffusion coefficients, while Taylor dispersion, interferometric techniques, MRI, and dynamic light scattering measure mutual diffusion coefficients.

The mutual diffusion coefficient, D, is the appropriate kinetic parameter describing diffusion-based bulk transport in the presence of concentration gradients. However, self-diffusion coefficients, D^*, are more directly related to the size and shape of ions in solution. Since current theoretical relationships between self-diffusion and mutual diffusion coefficients have had a limited success for the estimations of D (as well as theoretical equations for the calculation of D), the determination of experimental mutual- and self-diffusion coefficients is absolutely necessary.

Due to the ionic nature of Na$_4$TPPS, mutual and self-diffusion of this porphyrin are expected to behave very differently. This is mainly related to the important role of metal counterions on the mutual diffusion coefficient. Specifically, the mutual diffusion rate of an ionic porphyrin is expected to be significantly larger than that predicted from its self-diffusion coefficient due to an electrostatic dragging effect exerted by its smaller counterions. This aspect is very difficult to quantitatively predict even at very low concentrations. Nonetheless, it should be taken into account when pharmacokinetics, and mass transport in general, of pharmaceutical compounds, such as the investigated porphyrin, are modelled for *in vitro* and *in vivo* applications relevant to the pharmaceutical industry. This well justifies our efforts in accurate measurements of these transport properties. Furthermore, our study will provide the basis for other related diffusion studies aimed at understanding the effects of pH, physiological salts and transition metal ions. These are important for examining the roles of electrostatic dragging effects, porphyrin self association [9] and ion complexation on the diffusion rate of this porphyrin.

To our knowledge, no data on self-diffusion or mutual-diffusion coefficients of Na$_4$TPPS aqueous solutions have been previously published. Thus, the present paper intends to fulfil this gap reporting experimental data of mutual-diffusion coefficient and the two ion self-diffusion coefficients, $D^*_{TPPS^{4-}}$ and $D^*_{Na^+}$, for the binary (Na$_4$TPPS + water) system in the concentration range from (0 to 4)$\cdot 10^{-3}$ mol $\cdot$ dm^{-3} at T = 298.15 K, through the Taylor dispersion method (mutual diffusion) [15,17–25] and the Pulsed Gradient Spin-Echo (PGSE) NMR technique previously used for other systems [15,16,26]. Further although the mutual diffusion coefficient is the parameter of interest for describing bulk transport, it is the self-diffusion coefficient that is most easily measured non-invasively in biological systems using MRI (*Magnetic Resonance Imaging*) incorporating PGSE subsequences.

Secondly, molecular mechanics will be used to compute size and shape of the TPPS^{4-} porphyrin. In addition, by the analysis of the absorption spectra on this system, in the same concentration range, will aim at clarifying the possible interaction mechanisms in the referred systems (mainly metal-porphyrin association). Thus, our investigation may contribute to a better understanding of the structure and properties of these important pharmaceutical systems.

Details of the experimental setup and data analysis for the self-diffusion and mutual diffusion measurements together with absorption measurements and details of molecular modelling are given in Section 2. In Section 3 the results of the self-diffusion and mutual diffusion measurements are given and contrasted with the results of the molecular modelling and predictions from the Nernst equation.

2. Experimental section

2.1. Materials

The Na$_4$TPPS supplied by (Sigma–Aldrich, *pro analysi* mass fraction purity > 0.990) was used as supplied, without further purification.

2.2. Self-diffusion coefficients, D*

For the self-diffusion measurements, a stock solution was prepared by weighing 7.14 mg of Na$_4$TPPS (Sigma–Aldrich) into a vial and then adding 1.4 cm^3 of Milli Q water (resistivity = 18.2 MΩ $\cdot$ cm) giving a concentration of Na$_4$TPPS of 4.99 $\cdot 10^{-3}$ mol $\cdot$ dm^{-3} (assuming the Na$_4$TPPS was anhydrous, Mw = 1022.92 g $\cdot$ mol^{-1}). Na$_4$TPPS solutions of 0.499 $\cdot 10^{-3}$ mol $\cdot$ dm^{-3}, 0.998 $\cdot 10^{-3}$ mol $\cdot$ dm^{-3}, 1.99 $\cdot 10^{-3}$ mol $\cdot$ dm^{-3}, 2.99 $\cdot 10^{-3}$ mol $\cdot$ dm^{-3} and 3.99 $\cdot 10^{-3}$ mol $\cdot$ dm^{-3} were then prepared by dilution.

The ^{1}H NMR diffusion experiments were performed at T = 298.15 K on a Bruker Avance 400 MHz with 5 mm broadband probe equipped with a z-axis gradient. The temperature was calibrated using an ethylene glycol sample [27–29]. All data fittings were performed with OriginPro 8 (OriginLab Corporation) software using the Levenberg–Marquardt algorithm. The pulsed gradient spin-echo (PGSE)-WATERGATE pulse sequence [30] was used to evaluate the diffusion coefficient of TPPS^{4-} (*i.e.* ^{1}H signals) and the standard Hahn-echo based PGSE sequence was used to obtain the diffusion coefficient of Na$^+$. The attenuation measured with the Stejskal and Tanner sequence (a modified Hahn Spin-Echo) [26] is given by [16,30]

$$E(g^2, \Delta) = \exp\left(-\gamma^2 g^2 D^* \delta^2 \left(\Delta - \frac{\delta}{3}\right)\right) = \exp(-bD^*), \qquad (1)$$

where $E(g, \Delta)$ is the signal (normalised) with attenuation from diffusion, δ is the duration of the gradient pulse in seconds, γ is the gyromagnetic ratio of the observed nucleus, g is the gradient strength (T $\cdot$ m^{-1}), and Δ is the diffusion time in seconds. Typical acquisition parameters were: recycle delay time between diffusion experiments, 7.5 s to 9 s for the ^{1}H measurements and 0.4 s for the ^{23}Na measurements (NB these values were sufficient to allow complete longitudinal relaxation); δ, 3 ms (^{1}H) and 6 ms (^{23}Na); Δ 0.05 s (^{1}H) and 0.04 s (^{23}Na); the gradient strength was initially 0 T $\cdot$ m^{-1} and then varied from (0.011 to 0.498) T $\cdot$ m^{-1} in increments of 0.030 T $\cdot$ m^{-1} (a total of 18 data points for each attenuation curve). The data from the ^{1}H measurements were normalised to the value corresponding to 0.011 T $\cdot$ m^{-1} gradient strength, since at 0 T $\cdot$ m^{-1} there is no water suppression. The data from the ^{23}Na measurements were normalised to the 0 T $\cdot$ m^{-1} gradient strength spectrum. Non-linear regression with a mono-exponential function (*i.e.*, $E(g, \Delta) = A \exp(-bD^*)$) was used to calculate the diffusion coefficients. For almost all of the data, including those sets normalised to the intensity of the $g = 0$ T $\cdot$ m^{-1} spectrum, A was set = 1 in the fitting (the function then directly corresponded to equation (1)). For the remaining data sets it was sometimes necessary to float the variable A during the fitting to partially correct for minor intensity disturbances in spectra acquired at lower gradient strengths due to radiation damping or incomplete water suppression [31]. Nevertheless, the resulting value of A was always close to 1 (*i.e.*, 1.01 to 1.10).

Each sample was measured in duplicate and the weighted average self-diffusion coefficient and the corresponding error calculated from the results of the duplicate measurements. Note that after including factors like inherent gradient inhomogeneity, a variation of the order of a few percent is expected for duplicate measurements.

2.3. Mutual diffusion coefficients, D

For the mutual diffusion measurements, Na$_4$TPPS solutions (their concentration, in molarity) were prepared from a Na$_4$TPPS stock solution $6 \cdot 10^{-3}$ mol $\cdot$ dm^{-3}. This was prepared by dissolving the appropriate amount of Na$_4$TPPS in bi-distilled water (resistivity = 3.1 M$\Omega \cdot$ cm) using calibrated volumetric flasks and de-aerated during $\sim$30 min, before use (concentration uncertainty less than ± 0.1%). The concentrations of the injected solutions ($c_j + \Delta c$) and the carrier solutions (c_j) differed by $4 \cdot 10^{-3}$ mol $\cdot$ dm^{-3} or less.

Solutions of different composition were injected into each carrier solution to confirm that the obtained diffusion coefficient values were independent of the initial concentration difference and therefore represent the differential value of D at the carrier-stream composition.

The theory of the Taylor dispersion technique is well described in the literature [15,17–25,32–35], and only the salient points for the experimental determination of binary diffusion coefficients and ternary diffusion coefficients are discussed.

Dispersion methods for diffusion measurements are based on the dispersion of small amounts of solution injected into laminar carrier streams of solvent or solution of different composition, flowing through a long capillary tube with the length of 3.2799 ($\pm0.0001) \cdot 10^3$ cm. The radius of the tube, 0.05570 (±0.00003) cm, was calculated from the tube volume obtained by accurately weighing (resolution 0.1 mg) the tube when empty and when filled with distilled water of known density.

At the start of each run, a 6-port Teflon injection valve (Rheodyne, model 5020) was used to introduce 0.063 cm^3 of solution into the laminar carrier stream of slightly different composition. A flow rate of 0.17 cm$^3 \cdot$ min^{-1} was maintained by a metering pump (Gilson model Minipuls 3) to give retention times of about $8 \cdot 10^3$ s. The dispersion tube and the injection valve were kept at temperatures 298.15 K and 303.15 K (±0.01 K) in an air thermostat.

Dispersion of the injected samples was monitored using a differential refractometer (Waters model 2410) at the outlet of the dispersion tube. Detector voltages, $V(t)$, were measured at accurately timed 5 s intervals with a digital voltmeter (Agilent 34401 A) with an IEEE interface. Binary diffusion coefficients were evaluated by fitting the dispersion equation [17]

$$V(t) = V_0 + V_1 t + V_{max}(t_R/t)^{1/2} \exp[-12D(t-t_R)/r^2 t] \quad (2)$$

to the detector voltages. The additional fitting parameters were the mean sample retention time t_R, peak height V_{max}, baseline voltage V_0, and baseline slope V_1.

2.4. Absorption spectra

Absorption spectra (wavelength range: 480 nm to 740 nm) were obtained at room temperature (295.15 K with a Beckman DU 800 using a cuvette with path length: $b = 0.1$ cm. A $4.0 \cdot 10^{-3}$ mol $\cdot$ dm^{-3} stock solution of aqueous Na$_4$TPPS was prepared by weight. Solutions at concentrations c were obtained by dilution of this stock solution. The solution pH was measured and found to be slightly higher than its neutral value in all cases. The obtained results were reported as normalised absorption spectra by calculating the molar absorption coefficient: $\varepsilon = A/(b\,c)$, where A is the corresponding absorbance value.

2.5. Molecular modelling studies

The software used in the molecular studies was the ChemBio 3D Ultra v.12 package, 2009, from Cambridge Soft, USA [35]. This software allows performing molecular mechanics calculations using the MM force field. MM [36] is a standard method within the

Molecular Mechanics family of force fields widely used for calculations on small molecules, parameterized to fit values obtained through electron diffraction.

3. Results and discussion

3.1. Self-diffusion coefficients and molecular modelling studies

Self-diffusion coefficients for TPPS^{4-}, $D^*_{TPPS^{4-}}$, and Na$^+$, $D^*_{Na^+}$, for the (Na$_4$TPPS + water) system at $T = 298.15$ K are reported in table 1.

As it is well-known, the Stokes–Einstein equation for spherical particles [15]

$$D_P^0 = \frac{k_B T}{6\pi\eta R_h} \quad (3)$$

can be used to extract the size of solute molecules treated as Brownian particles immersed in a continuum fluid, provided that the solute particle is at infinitesimal concentration and large compared to solvent molecules. Equation (3) (where η is the macroscopic viscosity value of the solvent [37], T the absolute temperature and k_B the Boltzmann constant) establishes a link between the hydrodynamic radius of an equivalent spherical particle, R_h, and its self-diffusion coefficient at infinitesimal concentration, D_P^0, also known as tracer diffusion coefficient.

This relation can only be considered as an approximated one, (mainly arising from the fact that the structure of both the solute kinetic species and the solvent are disregarded). However, since porphyrins are relatively large compared to water molecules, it can be used to derive some valuable information on the relation between porphyrin size, shape, and self-diffusion coefficient at infinitesimal concentration.

In figure 1, the behaviour of TPPS^{4-}, self-diffusion coefficient, $D^*_{TPPS^{4-}}$, as a function of solute concentration is shown. These data were used to determine the TPPS^{4-} tracer diffusion coefficient, $D^0_{TPPS^{4-}} = 0.31 \cdot 10^{-9}$ m$^2 \cdot$ s^{-1}, in water at $T = 298.15$ K. By applying the Stokes–Einstein equation (equation (3)) to $D^0_{TPPS^{4-}}$ with $\eta = 0.890 \cdot 10^{-3}$ kg $\cdot$ m$^{-1} \cdot$ s^{-1}, we obtain the equivalent hydrodynamic radius: $R_h = 0.79$ nm.

A comparison between the extracted value of equivalent hydrodynamic radius and the actual porphyrin size can be performed if the molecular shape of TPPS^{4-} is known or can be modelled. After applying a molecular model for TPPS^{4-} (after MM2 energy minimisation in vacuum), we obtain the structure shown in figure 2. According to the corresponding structural parameters (see figure 2), we have chosen to approximately describe TPPS^{4-} as a disk with a height(l)-to-diameter(d) ratio, $p = l/d = 0.2$.

The following equation can be used to describe the relationship between R_h and p for a disk [38],

$$\frac{R_h}{l} = \frac{1}{2}p^{-2/3}[a_1 + a_2(\ln p) + a_3(\ln p)^2 + a_4(\ln p)^3] \quad \text{with}$$

$$0.1 \leqslant p \leqslant 20, \quad \text{and} \quad a_1 = 1.155; \; a_2 = 1.597 \cdot 10^{-2};$$

$$a_3 = 9.020 \cdot 10^{-2}; \; a_4 = 6.914 \cdot 10^{-3}. \quad (4)$$

TABLE 1

Self-diffusion coefficients, $D^*_{TPPS^{4-}}$ and $D^*_{Na^+}$, together with their respective standard deviations, S^*_D, obtained from ^{1}H and ^{23}Na measurements at $T = 298.15$ K.

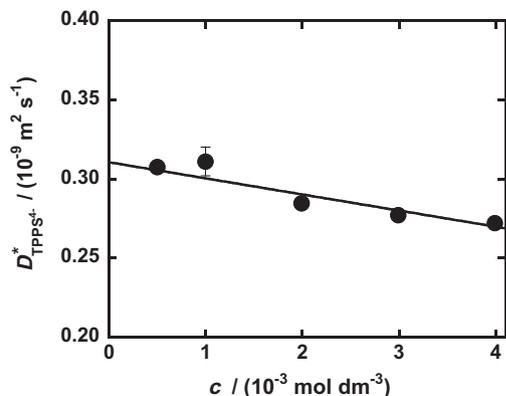| $c/$ (10^{-3} mol $\cdot$ dm^{-3})	$D^*_{TPPS^{4-}} \pm S^*_D/(10^{-9}$ m$^2 \cdot$ s$^{-1})$	$D^*_{Na^+} \pm S^*_D/(10^{-9}$ m$^2 \cdot$ s$^{-1})$
0.499	0.308 ± 0.002	1.263 ± 0.012
0.988	0.311 ± 0.009	1.258 ± 0.008
1.99	0.285 ± 0.002	1.241 ± 0.005
2.99	0.277 ± 0.003	1.219 ± 0.005
3.99	0.272 ± 0.002	1.208 ± 0.004

FIGURE 1. Self-diffusion coefficients of TPPS^{4-}, $D^*_{TPPS^{4-}}$, as a function of solute concentration, c.
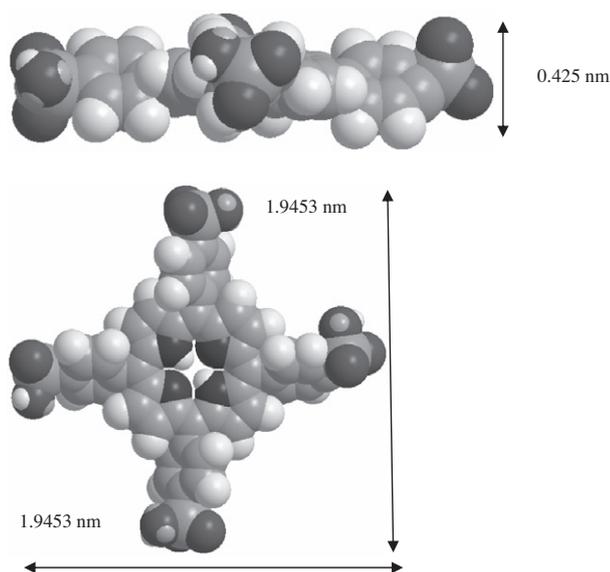

FIGURE 2. Molecular model for TPPS^{4-}.

For $p = 0.2$ and $R_h = 0.79$ nm, we calculate $l = 0.41$ nm and $d = 2.05$ nm. These values are in good agreement with the molecular-size parameters shown in figure 2. Our results indicate that self-diffusion coefficient of this porphyrin TPPS^{4-} can be estimated through the use of the Stokes–Einstein by modelling these molecules as disks with $p = 0.2$. Thus, having in mind that the predicted hydrodynamic size fits well with the molecular model, we can assume that porhyrin is not in some associated state (*i.e.*, dimerised).

3.2. Mutual-diffusion coefficient at infinitesimal concentration from Nernst equation

The mutual diffusion coefficient at infinitesimal concentration, D^0, for the (Na$_4$TPPS + water) system can be calculated using the Nernst equation [15,37,39–43],

$$D^0 = \frac{5D^0_{TPPS^{4-}}D^0_{Na^+}}{(4D^0_{TPPS^{4-}} + D^0_{Na^+})}, \tag{5}$$

where $D^0_{TPPS^{4-}}$ and $D^0_{Na^+}$ represent the tracer diffusion coefficients of TPPS^{4-} $(0.31 \cdot 10^{-9}$ m$^2 \cdot$ s$^{-1})$ and of the sodium ion $(1.33 \cdot 10^{-9}$ m$^2 \cdot$ s$^{-1})$ [15], respectively. Therefore, we obtained $D^0 = (0.80 \cdot 10^{-9}$ m$^2 \cdot$ s$^{-1})$ from equation (5). Our calculation shows that the mutual diffusion coefficient of this system at infinitesimal

concentration is significantly larger (2.6 times) than that the corresponding tracer diffusion coefficient. This increase characterises the electrostatic dragging effect of sodium ions on TPPS^{4-}.

We note that the Taylor dispersion method may be used to determine mutual diffusion coefficients at infinitesimal concentration provided that the carrier stream is pure solvent. However, in our case, we have found that the observed limiting mutual diffusion coefficient strongly decreases as the concentration of the injected solution increases. This behaviour is related to the ionic nature of our system. Due to data steepness and our relatively narrow range of accessible injected-solution concentrations, it is difficult to unambiguously obtain accurate D^0 values by extrapolation to zero concentration of the injected solution. Nonetheless, the value of D^0 obtained from equation (5) fits well with these mutual-diffusion data, provided that the chosen fitting equation is based on the concentration square root.

3.3. Concentration dependence of the mutual diffusion coefficient

Mutual diffusion coefficients, D, for Na$_4$TPPS in aqueous solutions at $T = 298.15$ K are reported in table 2 as a function of solute concentration, c. They were determined from, at least, four profiles generated by injecting samples above and below the concentration of that carrier solution.

In table 2, we can see that D decreases as solute concentration increases. The value for $0.5 \cdot 10^{-3}$ mol $\cdot$ dm^{-3} is significantly lower (25%) than the value at infinitesimal concentration predicted from the Nernst equation. This strong concentration dependence was corroborated by the Taylor-dispersion experiments at zero carrier concentration.

As a first attempt, we have examined the strong dependence of D on c, using the Onsager–Fuoss model [37,39–43], for strong electrolytes. Based on non-equilibrium thermodynamics, the mutual diffusion coefficient at constant temperature and pressure can be written as the product:

$$D = F_M \cdot F_T, \tag{6}$$

where $F_M \equiv RT(v/c)L$ is the molar mobility coefficient, and $F_T \equiv (c/v)(d\mu/dc)/RT$ is the thermodynamic factor, v is the number of ions produced by the dissociation of one solute molecule ($v = 5$ for Na$_4$TPPS), L the Onsager transport coefficient, μ the solute chemical potential, and R the ideal-gas constant. According to the Onsager–Fuoss theory, we can write for our system to first order:

$$F_M = D^0\left[1 - \frac{4}{5}\frac{(D^0_{Na^+} - D^0_{TPPS^{4-}})^2}{(D^0_{Na^+} + 4D^0_{TPPS^{4-}})D^0_{Na^+}D^0_{TPPS^{4-}}}\frac{k_BT}{6\pi\eta}\frac{\kappa}{1+\kappa a}\right], \tag{7}$$

where a is the mean distance of closest approach of ions [41], and κ is the reciprocal average radius of the ionic atmosphere (see *e.g.* [41]), which is directly proportional to $(I)^{1/2}$, where I is the solution ionic strength. In our case, we have: $\kappa/(I)^{1/2} = 3.2898$ nm^{-1} and $I = 10(c/c^0)$, where $c^0 = 1$ mol $\cdot$ dm^{-3}. Equation (7) describes the role of the electrophoretic effect on mutual diffusion of electrolytes.

According to Debye–Hückel theory, we can write for our system:

TABLE 2
Mutual diffusion coefficients, D, of Na$_4$TPPS in aqueous solutions at various concentrations, c, at $T = 298.15$ K and the standard deviations of the means, S_D.

$c/(10^{-3}$ mol $\cdot$ dm$^{-3})$	$D^a \pm S_D{}^b/(10^{-9} \cdot$ m$^2 \cdot$ s$^{-1})$
0.5	0.609 ± 0.007
1.0	0.596 ± 0.014
2.0	0.571 ± 0.017
3.0	0.569 ± 0.019
4.0	0.567 ± 0.007

[a] D is the mean diffusion coefficient value from 4–6 experiments.
[b] S_D is the standard deviation of that mean.

$$F_T = 1 - \frac{2A(I)^{1/2}}{(1 + Ba(I)^{1/2})^2}, \tag{8}$$

where $A = 1.1762$ and $B = 0.32898 \cdot 10^8\,\mathrm{m}^{-1}$ [40].

We have used Eqs. (5)–(8) combined with the values of η for water, $D^0_{\mathrm{Na}^+}$ and $D^0_{\mathrm{TPPS}^{4-}}$ reported above to compute $D(c)$ for several values of the ion-ion distance a (from 0 to $2 \cdot 10^{-10}$ m (0 Å to 2 Å). Our results are shown in figure 3.

As we can see in the figure, Onsager–Fuoss theory predicts a significant decrease of D, which can be related to the relatively large ionic strength. However, equations (5) to (8) fail to quantitatively predict the very steep decrease of the experimental $D(c)$. This discrepancy may be mainly related to complexation and/or ion association, porphyrin self association and related hydration effects [37,39–43], which are not considered in the proposed Onsager–Fuoss model. Nonetheless, Onsager–Fuoss theory can be used to estimate the relative importance of F_T and F_M. According to equations (5) to (8), we can write to first order in $(I)^{1/2}$: $F_T = 1 - 2.35(I)^{1/2} + \cdots$ and $F_M = D^0(1 - 0.63(I)^{1/2} + \cdots)$. This numerical analysis allows us to estimate that the contribution of the thermodynamic factor to the decrease of D is 3.7 times larger than that of the mobility factor at low solute concentrations. In other words, the variation in D is mainly due to the variation of F_T (attributed to the non-ideality in the thermodynamic behaviour), compared to the electrophoretic effect in the mobility factor, F_M. This is typical of electrolyte systems [15].

As mentioned above, deviations between experimental and calculated results could be related to an electrostatically-driven TPPS–Na association, porphyrin dimerization or more complex aggregation mechanisms. Since associative equilibria can be probed by spectrophotometry, we have obtained absorbance spectra in the same concentration range as our diffusion measurements. These absorbance spectra, which are shown in figure 4, are consistent with previous spectrophotometric results obtained on similar systems [44]. In this figure, we observe that the normalised spectra do not overlap and exhibit isosbestic points. Based on this experimental observation, it is reasonable to assume the existence of two chemical species in chemical equilibrium. This could involve either a TPPS–Na association or a porphyrin dimerization process.

In relation to mutual diffusion, porphyrin-sodium association is expected to reduce the electrostatic dragging effects of the small sodium ions on TPPS^{4-}. This effect can be qualitatively examined using the Nernst equation (equation (5)). For example, in the limiting case that one sodium ion completely associates from TPPS^{4-},
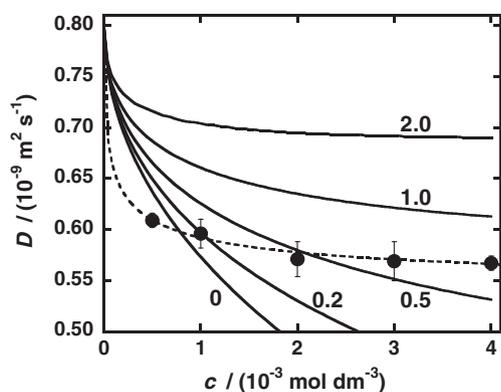

FIGURE 3. Experimental mutual diffusion coefficients, D, as a function of solute concentration, c (solid circles). The dashed curve is a fit through the data using the empirical equation: $D = D^0(1 + a_1(c)^{1/2})/(1 + a_2(c)^{1/2})$. The solid curves represent $D(c)$ calculated using equations (5) to (8) for several values of a. The numbers associated with each curve identify the corresponding values of a (in nm).
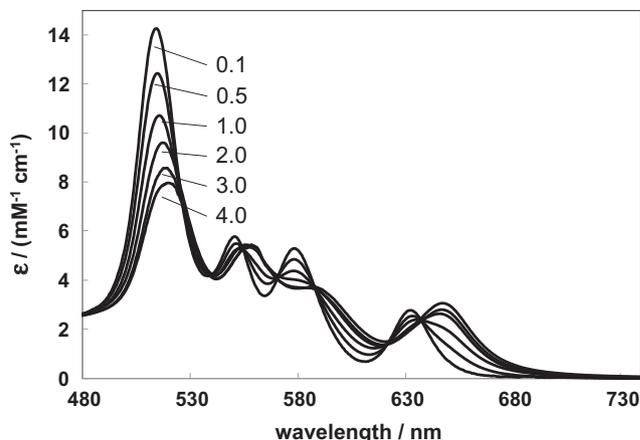

FIGURE 4. Normalised absorption spectra of a series of Na$_4$TPPS aqueous solutions with solute concentration ranging from (0.1 to 4.0) $\cdot 10^{-3}$ mol $\cdot$ dm^{-3}.

the Nernst equation predicts about a 10% decrease of the mutual-diffusion coefficient. This estimation is obtained by setting $D^0 = 4D^0_{\mathrm{TPPSNa}^{3-}}D^0_{\mathrm{Na}^+}/(3D^0_{\mathrm{TPPSNa}^{3-}} + D^0_{\mathrm{Na}^+}) = 0.73 \cdot 10^{-9}\,\mathrm{m}^2\,\mathrm{s}^{-1}$ where we have assumed $D^0_{\mathrm{TPPSNa}^{3-}} = D^0_{\mathrm{TPPS}^{4-}}$ for the tracer diffusion coefficient of the TPPSNa^{3-} species. Thus, complexation is predicted to contribute to the decrease of D as solute concentration increases. The tracer diffusion coefficient of the relatively larger dimers is expected to be lower than that of the monomeric porphyrin. For example, if we assume that a dimer can be described as a disk with $p = 0.4$ and same diameter as that of the monomer (for which $p = 0.2$), we can estimate the tracer diffusion coefficient of the dimer, $(\mathrm{TTPS}^{4-})_2$ to be $D^0_{(\mathrm{TPPS}^{4-})_2} = 0.87 D^0_{\mathrm{TPPS}^{4-}}$ using Eqs. (3) and (4). However the net effect on mutual diffusion is complicated by the ionic nature of TTPS^{4-}. Indeed, according to our qualitative considerations based on the Nernst equation, the association of two ionic species will increase the overall electrostatic dragging effect of small ions thereby producing an increase of the observed mutual diffusion coefficient. For example, in the limiting case that porphyrins completely associates as dimers and counterion association can be neglected, the Nernst equation predicts about a 15% increase of the mutual-diffusion coefficient. This estimation is obtained by setting $D^0 = 9D^0_{(\mathrm{TPPS}^{4-})_2}D^0_{\mathrm{Na}^+}/(8D^0_{(\mathrm{TPPS}^{4-})_2} + D^0_{\mathrm{Na}^+}) = 0.93 \cdot 10^{-9}\,\mathrm{m}^2\,\mathrm{s}^{-1}$.

The TPPS self-association is known to be significant at pH 4 [45]. However, at this pH, the magnitude of the TPPS mean charge is about 50% of that at pH 7 due to acid-base equilibria [2]. Although porphyrin self-association has been extensively described in literature and sodium is known to only weakly bind to porphyrins, long-range electrostatic forces should favour TPPS^{4-} association with the oppositely charged sodium ions and disfavour dimerization of TPPS^{4-} molecules at pH 7. This hypothesis is consistent with the work of Andrade et al. [9], which indicates the formation of a TPPS-sodium complex based on analogous spectrophotometric results. Hence, we have examined the effect of TPPS-sodium association on the behaviour of mutual diffusion, neglecting other aggregation processes.

We shall introduce a simple model that can describe metal-ion association to charged porphyrins. Consistent with the hypothesis of an electrostatically-driven association, we describe thermodynamic equilibrium by including ion activity coefficients, γ, based on the limiting Debye–Hückel theory and neglect ion-size effects. In other words, we expect TPPS-sodium association to be important at low ionic strengths. For the reversible chemical reaction:
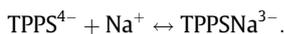
$$\mathrm{TPPS}^{4-} + \mathrm{Na}^+ \leftrightarrow \mathrm{TPPSNa}^{3-}.$$

The corresponding association constant is introduced by

$$\beta = \frac{[TPPSNa^{3-}]c^0}{[TPPS^{4-}][Na^+]} \frac{\gamma_{TPPSNa^{3-}}}{\gamma_{TPPS^{4-}}\gamma_{Na^+}}, \qquad (9)$$

where $c^0 \equiv 1$ M, $\ln\gamma_{TPPSNa^{3-}} = -9A(I)^{1/2}$, $\ln\gamma_{TPPS^{4-}} = -16A(I)^{1/2}$ and $\ln\gamma_{Na^+} = -A(I)^{1/2}$. For a given value of solute concentration, c, and association constant β, the concentration of the three ionic species can be numerically calculated by considering the mass balance:

$$c = [TPPS^{4-}] + [TPPSNa^{3-}]. \qquad (10)$$

The electroneutrality condition (at pH 7):

$$[Na^+] = 4[TPPS^{4-}] + 3[TPPSNa^{3-}] \qquad (11)$$

and that the equilibrium condition can be rewritten in the following way:

$$[TPPSNa^{3-}] = \beta[TPPS^{4-}][Na^+]\exp(-8A(I)^{1/2}), \qquad (12)$$

where the ionic strength is related to the concentration of the three species by:

$$I = \frac{1}{2}(16[TPPS^{4-}] + 9[TPPSNa^{3-}] + [Na^+]) \qquad (13)$$

The TPPS–Na association is expected to affect the behaviour of the sodium self-diffusion coefficient. Indeed the observed $D^*_{Na^+}(c)$ can be expressed as a weighted average between the self-diffusion coefficients of the two states: free and bound sodium. If we assume that these two self-diffusion coefficients are constant and equal to $D^0_{Na^+}$ and the tracer diffusion coefficient of TPPSNa^{3-}, $D^0_{TPPSNa^{3-}}$, respectively, we can write:

$$D^*_{Na^+} = \alpha_f D^0_{Na^+} + (1 - \alpha_f)D^0_{TPPSNa^{3-}}, \qquad (14)$$

where $\alpha_f = [Na^+]/(4c)$ is the fraction of free sodium ions. In relation to the bound sodium, we expect the tracer diffusion coefficient of TPPSNa^{3-}, $D^0_{TPPSNa^{3-}}$, to be approximately equal to that of the free porphyrin since the association of a small ion and related hydration effects should not significantly change the size and shape of the particle. We therefore set: $D^0_{TPPSNa^{3-}} = D^0_{TPPS^{4-}}$. On the other hand, $D^0_{Na^+}$ will be significantly higher than $D^0_{TPPSNa^{3-}}$. We have computed $D^*_{Na^+}(c)$ using equation (14) for several values of β and compared with our experimental results reported in table 1. Our comparison is shown in figure 5.

This figure shows that the hypothesis of TPPS–Na association is consistent with the behaviour of our experimental sodium self-diffusion coefficient. The value of $\beta = 300$ provides the best fit to the experimental data.

We note that a similar analysis can be performed on the absorption spectra shown in figure 4. Specifically each normalised spectrum can be expressed as a weighted average of the spectra of the two states: free TPPS and TPPS–Na states. However, while the spectra associated with the free TPPS can be obtained by extrapolation to $c = 0$, that of the pure TPPS–Na state is difficult to estimate by extrapolation to $c \to \infty$. Nonetheless, we have found that $\beta = 300$ is consistent with the observed spectra changes. For example, the behaviour of $\varepsilon(c)$ at $\lambda = 516$ nm is well described using $\beta = 300$ by setting $\varepsilon(0) = 15$ mM cm^{-1} and $\varepsilon(\infty) = 0$, while that at $\lambda = 648$ nm by setting $\varepsilon(0) = 0.8$ mM·cm^{-1} and $\varepsilon(0) = 5.5$ mM·cm^{-1} (data not shown).

For the mutual diffusion coefficient we shall derive an expression for both the mobility F_M and thermodynamic F_T factors. According to non-equilibrium thermodynamics, the mobility factor can be introduced through the following linear relation between the solute molar flux J and the corresponding gradient of chemical potential $\nabla\mu$:

$$-J = \frac{c}{v}F_M\frac{\nabla\mu}{RT} \qquad (15)$$

with $v = 5$. A similar relation can be also written for each ionic species in the limit of infinitesimal concentration:
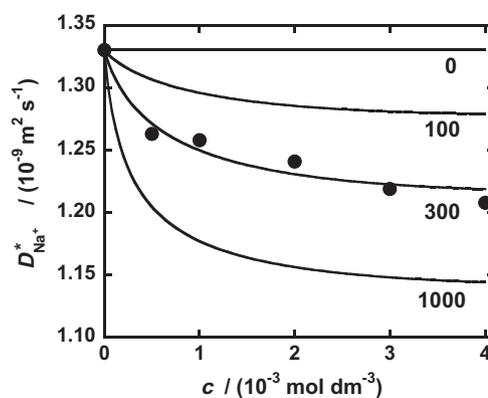

FIGURE 5. Experimental self diffusion coefficient of sodium, $D^*_{Na^+}$, as a function of solute concentration, c (solid circles). The solid curves represent $D^*_{Na^+}(c)$ calculated using equation (14) for several values of β. The numbers associated with each curve identify the corresponding values of β.

$$-J_i = [i]D^0_i\frac{\nabla\tilde{\mu}_i}{RT}, \qquad (16)$$

where i = Na$^+$, TPPS^{4-} and TPPSNa^{3-}, and $\tilde{\mu}_i$ is the electrochemical potential of the ionic species i. As an approximation, we shall assume that equation (16) is valid within our experimental concentration range. This implies that we will neglect electrophoretic effects (those described by Onsager–Fuoss theory for binary electrolyte systems) and assume that the gradient of electrochemical potential of one species does not affect the flux of another species (non-interacting fluxes). The chemical potential of a dissociating solute can be expressed as a linear combination of the electrochemical potentials of the constituent ionic species. In matrix form, we can write:

$$\nabla\mu = (1 \quad 4)\cdot\begin{pmatrix}\nabla\tilde{\mu}_{TPPS^{4-}} \\ \nabla\tilde{\mu}_{Na^+}\end{pmatrix}, \qquad (17)$$

where the coefficients of the linear combination are the corresponding stoichiometric coefficients. We now note that F_M can be expressed as a function of the tracer diffusion coefficients D^0_i, if we obtain a link between equations (15) and (16). Thus, we first relate the solute flux to the flux of the constituent species using the following mass balance:

$$J\begin{pmatrix}1 \\ 4\end{pmatrix} = \begin{pmatrix}J_{TPPS^{4-}} + J_{TPPSNa^{3-}} \\ J_{Na^+} + J_{TPPSNa^{3-}}\end{pmatrix}. \qquad (18)$$

We then insert the expressions for the species fluxes (equation (16)) into the right side of equation (18), and remove $\nabla\tilde{\mu}_{TPPSNa^{3-}}$ by applying the equilibrium condition: $\nabla\tilde{\mu}_{TPPSNa^{3-}} = \nabla\tilde{\mu}_{TPPS^{4-}} + \nabla\tilde{\mu}_{Na^+}$. We finally obtain:

$$-J\begin{pmatrix}1 \\ 4\end{pmatrix} = \frac{l}{RT}\cdot\begin{pmatrix}\nabla\tilde{\mu}_{TPPS^{4-}} \\ \nabla\tilde{\mu}_{Na^+}\end{pmatrix}, \qquad (19)$$

where

$$l \equiv \begin{pmatrix}([TPPS^{4-}] + [TPPSNa^{3-}])D^0_{TPPS^{4-}} & [TPPSNa^{3-}]D^0_{TPPS^{4-}} \\ [TPPSNa^{3-}]D^0_{TPPS^{4-}} & [Na^+]D^0_{Na^+} + [TPPSNa^{3-}]D^0_{TPPS^{4-}}\end{pmatrix}.$$

It is important to note that the use of the equilibrium conditions for $\nabla\tilde{\mu}_{TPPSNa^{3-}}$ is based on the reasonable assumption that porphyrin-ion association is fast compared to diffusion.

We now solve equation (19) with respect to the vector of electrochemical-potential gradients by applying the inverse matrix, l^{-1} to both sides of equation (19). By substituting our result into equation (17), a new expression for $\nabla\mu$ is obtained, which is then inserted into equation (15) yields:
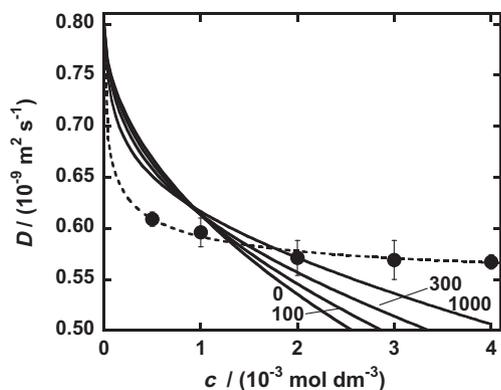
FIGURE 6. Experimental mutual diffusion coefficient, D, as a function of solute concentration, c (solid circles). The dashed curve is a fit through the data using the empirical equation: $D = D^0(1 + a_1(c)^{1/2})/(1 + a_2(c)^{1/2})$. The solid curves represent $D(c)$ calculated using equations (6), (20), (21) for several values of β. The numbers associated with each curve identify the corresponding values of β.

$$F_M = \frac{v}{c}\left[(1 \quad 4)\cdot l^{-1}\cdot\begin{pmatrix}1\\4\end{pmatrix}\right]^{-1}. \tag{20}$$

The expression for the thermodynamic factor can be obtained by considering the limiting Debye–Hückel theory:

$$F_T = \frac{1}{v}\frac{d}{d\ln c}(\ln[\text{TPPS}^{4-}] + 4\ln[\text{Na}^+] - 20A(I)^{1/2}). \tag{21}$$

Provided that the concentration of each ionic species is known as a function of solute concentration, the thermodynamic factor can be numerically computed. The comparison between the experimental mutual-diffusion data and the proposed model is shown in figure 6.

As shown in figure 6, the calculated $D(c)$ curves fail to describe quantitatively our experimental results. This can be related to the several assumptions made for developing the proposed model. Nonetheless, it is important to observe that our model qualitatively predicts that porphyrin–metal ion association increases the initial steepness of $D(c)$. Clearly, the behaviour of the mutual diffusion coefficient for ionic porphyrins in water is very complex even at very low concentrations, including those relevant to physiological conditions. Further experimental and theoretical investigations are needed in order to derive accurate models that can quantitatively predict the observed behaviour of this transport property. This description is consistent with the previous observation based on Onsager–Fuoss theory that non-ideality thermodynamic effects described by Debye–Hückel theory are important.

4. Conclusions

The self-diffusion coefficients of the cation and anion and the mutual diffusion coefficient of an aqueous solution of Na_4TPPS were measured over the concentration range (0 to $4)\cdot 10^{-3}$ mol·dm^{-3} at $T = 298.15$ K. These data, together with complementary molecular mechanics and absorption spectra studies, provided deep insight into the structure of this binary system and its thermodynamic behaviour. For example, the hypothesis of TPPS–Na association was consistent with the behaviour of our experimental self-diffusion coefficients and also with the analysis performed on the absorption spectra. However, from the complexity of the behaviour of the mutual diffusion coefficient for ionic porphyrins in water, even at very low concentrations, we conclude that the probable porphyrin–metal ion interaction increases the initial steepness of D. However, our model of TPPS–Na association predicts a more significant decrease in the diffusion coefficient at low concentrations and a weaker decrease at higher concentrations

when compared to the strong electrolyte case (*i.e.*, $\beta = 0$, see equation (9). This is qualitatively (not quantitatively) in agreement with experimental results.

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