International Journal of Thermophysics, Vol. 12, No. 2, 1991

Diffusion Coefficients of Tetrazolium Blue in Homogeneous and Micellar Solutions

M. I. Viseu,[1] C. A. Nieto de Castro,[1] and S. M. B. Costa[1]

Received April 27, 1990

Diffusion coefficients of the electron acceptor dye tetrazolium blue were measured by the Taylor dispersion method, with an accuracy better than 4%, in two solvents: (i) a homogeneous one—aqueous phosphate buffer, 0.1 *M*, pH = 7.0 (medium I); and (ii) a heterogeneous one—nonionic micelles of Triton X-100, 2.0 mM (where M stands for $mol \cdot dm^{-3}$), in the same aqueous phosphate buffer (medium II). The values obtained were $D_{12}^{I} = 3.64 \times 10^{-10}\ m^2 \cdot s^{-1}$ and $D_{12}^{II} = 3.01 \times 10^{-10}\ m^2 \cdot s^{-1}$. D_{12}^{II} has the meaning of a "macroscopic" or "average" diffusion coefficient, in which the partition coefficient of tetrazolium blue between micelles and water, as well as the diffusion coefficients of this dye and of the micelles in the aqueous phase, are involved.

KEY WORDS: diffusion; Taylor dispersion method; tetrazolium blue; Triton X-100 micelles.

1. INTRODUCTION

The knowledge of the diffusion coefficients of organic compounds is very limited, even for liquid phases composed of very simple molecules. The experimental data are difficult to obtain, and the existing predictive methods are questionable. Only empirical estimation techniques are available, and their application is normally restricted to the mixtures for which they have been developed [1, 2].

The problem of obtaining diffusion coefficients in complex micellar solutions, like the ones used in this work, is still in its infancy, because the experimental measurements are even more difficult and the estimation techniques nonexistent.

[1] Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, 1096 Lisboa Codex, Portugal.

0195-928X/91/0300-0323$06.50/0

In the photochemical research currently performed in our laboratory [3], the dye tetrazolium blue acts as a good electron acceptor, in both homogeneous and heterogeneous media. The need for the knowledge of some redox properties of tetrazolium blue led to the application of a series of electroanalytical techniques to this dye. Calculation of electrochemical parameters for complex solutions is hampered by a lack of accurate experimental values for the diffusion coefficients. The error in these coefficients is, by far, the largest contributing factor to the error in the electrochemical parameters evaluated. Consequently, we were forced to attempt the accurate measurement of the diffusion coefficients of tetrazolium blue in the solutions under study: a homogeneous, aqueous, medium (medium I) and a Triton X-100 micellar one (medium II).

A convenient method of measurement was found to be the chromatographic dispersion technique, based on the work of Taylor [4]. The same technique has been previously applied by Weinheimer et al. [5] and by Evans et al. [6] to measure the diffusion coefficients of micelles in aqueous solution (Triton X-100 [5], sodium dodecyl sulfate [5, 6], and tetradecyltrimethylamonium bromide [6] micelles). The first group of authors [5] also measured the diffusion coefficient, in Triton X-100 solution, of a dye (methyl yellow) which is exclusively solubilized in the micellar pseudophase and, therefore, obtained a value close to that of the micelles themselves in aqueous solution.

An additional complicating factor in micellar solutions arises when a solute is partitioned between the micelles and the surrounding water. In this case, the measured diffusion coefficient is expected to differ from the diffusion coefficient of the micelles, since it must also take into account the diffusion coefficient of the dye in the intermicellar phase, as well as its partition coefficient between the two pseudophases [7–9].

The aim of the present paper is to report the experimental measurement of the diffusion coefficient of tetrazolium blue (which, in Triton X-100 solutions, is partitioned between the micelles and the aqueous phase [3, 10]), in the homogeneous and heterogeneous media mentioned above.

2. THE TAYLOR DISPERSION METHOD

The Taylor method for the measurement of diffusion coefficients in liquid solutions is based on a chromatographic dispersion technique, in which the sample, in the solvent under study, is injected in the same solvent, flowing in a long, circular tube, in a laminar regime.

The dispersion profile of the sample is obtained at the exit of the tube. The observed broadening of the profile is due to the molecular diffusion of the sample through the solvent, enhanced by the convection of the solvent

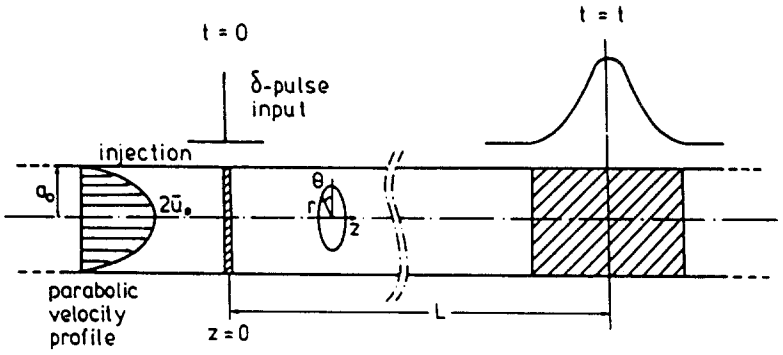

Fig. 1. Schematic diagram of the dispersion profile of the sample through the chromatographic tube, in the ideal Taylor dispersion experiment. From Ref. 11.

itself (in a parabolic velocity profile). For a δ-pulse input, the dispersion profile is a Gaussian distribution (see Fig. 1). The diffusion coefficient of the sample in the solvent under study (D_{12}) is evaluated from the first two temporal moments of the distribution [first moment or time of residence ($\bar{t}$) and second central moment or variance (σ^2)].

The theoretical principles of the method, first described by Taylor [4], have been developed by Alizadeh et al. in 1980 [11]. References 12–15 describe some applications of the method, using the same experimental apparatus used here. For these reasons, only the experimental conditions and the equations strictly needed to the calculations are presented here.

The mutual diffusion coefficient of a solute 1 in a solvent 2 (D_{12}), as described in Ref. 11, can be evaluated by the following equation:

$$D_{12} = \frac{a_0^2}{24\bar{t}_{id}} \left\{ \frac{\sqrt{1+4\sigma_{id}^2/\bar{t}_{id}^2}+3}{\sqrt{1+4\sigma_{id}^2/\bar{t}_{id}^2}+2\sigma_{id}^2/\bar{t}_{id}^2-1} \right\} \left\{ \frac{1}{2}+\frac{1}{2}\sqrt{1-\delta_a} \right\} \tag{1.a}$$

with

$$\delta_a = 12.800\xi_0 \tag{1.b}$$

and

$$\xi_0 = \frac{2\sigma_{id}^2 - \bar{t}_{id}^2 + \sqrt{\bar{t}_{id}^4 + 4\bar{t}_{id}^2\sigma_{id}^2}}{8\bar{t}_{id}^2 - 4\sigma_{id}^2} \tag{1.c}$$

where a_0 stands for the internal radius of the diffusion tube; and $\bar{t}_{id}$ and σ_{id}^2 are, respectively, the first and second temporal moments obtained in an ideal experiment.[2]

[2] This ideal experiment would involve the injection of an infinitesimal volume of the sample, as a δ-pulse; a laminar flow regime of the chromatographic solvent; an infinitely long tube, perfectly straight, with a uniform circular cross section; a concentration detector with infinitesimal volume; and a fluid (solute + solvent) with physical properties independent of the composition, particularly D_{12}.

The two (ideal) temporal moments are related to the respective experimentally measured moments ($\bar{t}_{exp}$, σ^2_{exp}) by the equations

$$\bar{t}_{id} = \bar{t}_{exp} + \sum_i \delta\bar{t}_i \tag{2.a}$$

$$\sigma^2_{id} = \sigma^2_{exp} + \sum_i \delta\sigma^2_i \tag{2.b}$$

where $\delta\bar{t}_i$ and $\delta\sigma^2_i$ are small corrections, that take into account the following factors:

(1) a finite volume of the injection pulse (instead of a δ-pulse, the injection is approximately a rectangular pulse);

(2) a finite volume of the concentration monitor (which is located at the exit of the diffusion tube, a distance L from the injection point); and

(3) the existence of a connecting tube between the diffusion tube and the concentration monitor.

These factors, encountered in any nonideal experiment, cannot be eliminated by the design of the apparatus [11, 12]. The calculation of these corrections is described in Ref. 11.

A final correction is needed for the dependence of the physical properties of the mixture (in this case D_{12}) with its composition [11]. The evaluated value of D_{12} does not correspond to the real concentration (C_{1f}) of the sample injected, but to a reference concentration (C_{1r}). These are related by

$$C_{1r} = C_{1f} + \delta C_1 \tag{3}$$

in which the small correction (δC_1) is a function of the number of extra moles injected (N_1).

3. EXPERIMENTAL

Tetrazolium blue [3,3′-dianisole-4,4′-bis(2,5-diphenyltetrazolium) chloride] was obtained from Fluka, in a p.a. grade, for bacteriology. Triton X-100 [iso-octylphenoxy-poly(oxyethylene)-glycol, containing a mean of 10 oxyethylene units per molecule] was purchased from BDH, in a scintillation grade. The other chemicals were of analytical grade. All the commercial reagents and solvents were used without further purification.

Triton X-100 solutions, with a surfactant concentration of 2.00 mM[3]

[3] M stands for mol · dm^{-3}.

Table I. Diffusion Tube [13] and Sample Characterization

Diffusion-tube length, L	13.1337 m
Diffusion-tube internal radius, a_0	3.904×10^{-4} m
Coil radius, R_c	0.1608 m
Injection volume, V_i	2×10^{-8} m^3
Detector volume, V_d	1×10^{-8} m^3
Length of connecting tube, l	0.3584 m
Internal radius of connecting tube, a_c	1.143×10^{-4} m

Sample	$[TB^{2+}]$ (mM)[a]	Solvent
I	0.500	Phosphate buffer, 0.1 M, pH = 7.0/water
II	0.500	Triton X-100, 2 mM/phosphate buffer/water

[a] M stands for mol · dm^{-3}.

(micellar concentration of 12.2 μM), were prepared by dissolution of the surfactant in a 0.10 M phosphate buffer, at pH = 7.00. Tetrazolium blue, at a concentration of 500 μM, was directly dissolved in these buffer or micellar solutions [3]. For medium II, a mean occupation number of 7.8 can be calculated for tetrazolium blue in Triton X-100 micelles. At these concentrations, fluorescent methods suggest that the micellar structure of Triton X-100 (size, shape, etc.) is not appreciably altered [3, 10].

Table I summarizes the experimental conditions used: the samples and the characteristics of the diffusion apparatus. The concentration of the samples at the end of the diffusion tube was monitored by a differential refractometer (Waters, Model R-401), with a resolution of 4×10^{-4} refraction index units, in the scale used. The dispersion profiles were obtained in an $X-t$ recorder. For each sample, a set of at least five experiments was performed.

4. RESULTS

Figure 2 shows one of the dispersion profiles obtained for tetrazolium blue (TB^{2+}) in the micellar medium referred to above (medium II). These profiles are approximately Gaussian, so the experimentally measured parameters—residence time, $\bar{t}_{exp}$, and width at half-height of the peak, $W_{1/2}$—can be related, respectively, to the mean velocity of the flow ($\bar{u}_0$) and to the standard deviation (σ), by Eqs. (4) and (5):

$$\bar{u}_0 = \frac{L}{\bar{t}_{exp}} \tag{4}$$

$$W_{1/2} = 2.354\sigma_{exp} \tag{5}$$

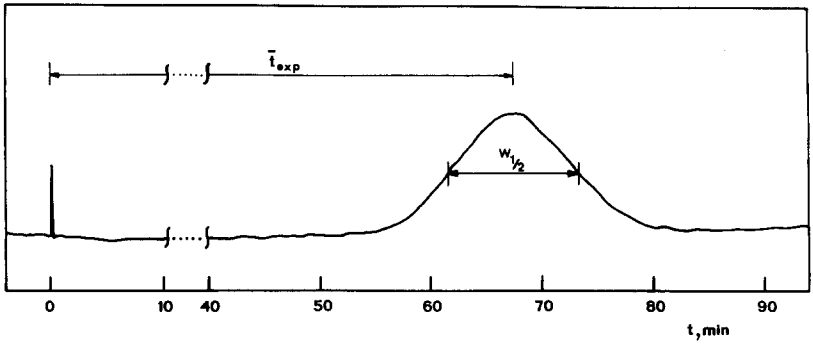

Fig. 2. Typical dispersion profile for tetrazolium blue in Triton X-100 micelles (sample II).

For each profile, D_{12} was calculated by means of Eqs. (1) and (2), with the corrections in the real parameters ($\bar{t}_{exp}$, σ^2_{exp}) previously evaluated. The relative error obtained in the sum of all the three correction factors is typically of the order of 0.2 to 0.3%.

Table II illustrates the experimental results obtained for one experiment in Sample I (homogeneous medium) and one experiment in Sample II (micellar medium). The ideal moments of the dispersion profiles, and the final value, D_{12}, are therein displayed. A set of five experiments with sample I and a set of six experiments with sample II were performed. The mean values of D_{12} obtained in both media are indicated in Table III. Herein are also shown the values of the reference concentration, $[TB^{2+}]_r$, corrected for the extra moles of tetrazolium blue injected, by means of Eq. (3).

Table II. Typical Runs for Samples I and II

	Sample I	Sample II
T (K)	298.25	298.23
$\bar{t}_{exp}$ (s)	3960.0	3900.0
$10^{-3}\bar{u}_0$ (m · s^{-1})	3.317	3.368
$W_{1/2}$ (s)	620.0	680.0
σ^2_{exp} (s^2)	69367.0	83442.0
$\bar{t}_{id}$ (s)	3970.0	3912.0
σ^2_{id} (s^2)	69476.0	83581.0
$10^{10}D_{12}$ (m^2 · s^{-1})	3.62	2.96

Table III. Average Diffusion Coefficients for Samples I and II

Sample	$[TB^{2+}]_r$ (mM)[a]	T (K)	$10^{10}D_{12}$ ($m^2 \cdot s^{-1}$)
I	0.506	298.25	3.64 ± 0.13
II	0.506	298.22	3.01 ± 0.10

[a] M stands for $mol \cdot dm^{-3}$.

5. DISCUSSION

As expected, the order of magnitude of the diffusion coefficients determined for both samples is the same, but D_{12}^{I} is larger than D_{12}^{II}, as the long polyoxyethylene chains in the Triton X-100 surfactant molecules should slow down the diffusional movement of tetrazolium blue ions (TB^{2+}), as compared to water and phosphate ions.

As tetrazolium blue exists in a partition equilibrium between the micellar and the intermicellar pseudophases [3], D_{12}^{II} is in fact a "macroscopic" or "average" diffusion coefficient, in which this equilibrium (in terms of its partition coefficient), and the diffusion coefficients of tetrazolium blue and of the micelles in the aqueous intermicellar phase, must be taken into account. This equilibrium should also decrease D_{12}^{II} relatively to the diffusion coefficient of tetrazolium blue in the same intermicellar medium, as the diffusion of the micelles themselves is expected to be much slower than the diffusion of TB^{2+} ions, when the same medium is considered.

The present results can therefore be compared with those of Weinheimer et al. [5] for Triton X-100 micelle and monomer diffusion. In a range of surfactant concentrations (1.6 to 2.7 mM) above the critical micelle concentration (CMC = 0.25 mM for Triton X-100 [16]) and similar to ours (2.00 mM), these authors obtained a value of $6.9 \times 10^{-11}\ m^2 \cdot s^{-1}$ for the diffusion coefficient of Triton X-100 micelles in aqueous solution; for a surfactant concentration below the CMC (0.03 mM, where Triton X-100 is mainly in the monomeric form), they obtained a value of $8.3 \times 10^{-11}\ m^2 \cdot s^{-1}$. In the present work, the diffusion coefficient of tetrazolium blue in the Triton X-100 micellar medium ($D_{12}^{II} = 3.01 \times 10^{-10}\ m^2 \cdot s^{-1}$) was found to be larger than both of these values. This result seems to confirm the expected behavior of mass transport in this type of complex solutions, as the movement of the long surfactant molecules (either aggregated in micelles or not) should be restricted, compared to the movement of TB^{2+} ions in the same micellar solutions.

From the work of Burkey et al. [7] and of Amstrong et al. [8, 9], the observed diffusion coefficient of an organic solute partitioned between a micellar and an aqueous pseudophase (D_{obs}) is related to both of the diffusion coefficients of the micelle (D_m^{aq}) and of the solute (D_s^{aq}) in the intermicellar medium (aqueous surfactant solution just below the CMC), by means of the equation

$$D_{obs} = fD_m^{aq} + (1-f)\, D_s^{aq} \tag{6.a}$$

where f (the molar fraction of the solute in the micellar pseudophase) is given by

$$f = \left\{1 + \frac{1 - V_M[M]}{K_p V_M[M]}\right\}^{-1} \tag{6.b}$$

with [M] (the concentration of the micelles) given by

$$[M] = \frac{[S] - CMC}{v} \tag{6.c}$$

In these equations, K_p is the partition coefficient of the solute between the micellar and the aqueous pseudophases, V_M is the molar micellar volume ($200.6\ M^{-1}$ for Triton X-100 [17]), [S] is the surfactant concentration, CMC is the critical micellar concentration, as stated before, and v is the mean aggregation number (143 for Triton X-100 [17]).

From the accurate measurement of the diffusion coefficients, D_{obs} (which in our case would be D_{12}^{II}), D_m^{aq}, and D_s^{aq}, the partition coefficient of the solute between the two pseudophases can therefore be evaluated.

In our work, the partition coefficients of tetrazolium blue (K_p) as a function of the mean occupation number ($\bar{n}$) were determined independently by a fluorescent method [3, 10]. A value of $D_{12}^{II} = 3.09 \times 10^{-10}\ m^2 \cdot s^{-1}$ can then be evaluated by Eqs. (6) above, for the solutions studied in this work (with $\bar{n} = 7.8$, $K_p = 94$, and $f = 0.188$), if we take the result of Weinheimer et al. [5] ($D_m^{aq} = 6.9 \times 10^{-11}\ m^2 \cdot s^{-1}$) for the diffusion coefficient of the Triton X-100 micelles, and our own value of D_{12}^{I} ($3.64 \times 10^{-10}\ m^2 \cdot s^{-1}$) as an approximation for D_s^{aq} (these values should not be very different: $D_{12}^{I} \gtrsim D_s^{aq}$). This result is in excellent agreement with our own experimental value, $D_{12}^{II} = 3.01 \times 10^{-10}\ m^2 \cdot s^{-1}$ (less than 3% error).

As shown in the work of Burkey et al. [7] and Amstrong et al. [8–9], and in our own work, the Taylor's technique (or any other that accurately measures diffusion coefficients) is therefore a valuable tool for determining partition coefficients of solutes in micellar solutions or, conversely, for checking these values when determined by independent methods.

The values now obtained were used in connection with polarographic and cyclic voltametric data to improve the accuracy of the electrochemical parameters evaluated for tetrazolium blue [18].

ACKNOWLEDGMENT

This work was supported by the Instituto Nacional de Investigação Científica (INIC/CQE).

REFERENCES

1. H. J. V. Tyrrell and K. R. Harris, *Diffusion in Liquids* (Butherworths, London, 1984).
2. R. C. Reid, J. M. Prausnitz, and T. K. Sherwood, *The Properties of Gases and Liquids*, 3rd ed. (McGraw–Hill, New York, 1977).
3. M. I. Viseu, *Transferência Electrónica Fotossensiblizada pela Clorofila a em Meio Micelar de Triton X-100*, Ph.D. thesis (Instituto Superior Técnico, Lisboa, 1988) (in Portuguese, with English abstract).
4. G. I. Taylor, *Proc. Roy. Soc. Lond. A* **219**:186 (1953).
5. R. M. Weinheimer, D. F. Evans, and E. L. Cussler, *J. Colloid Interface Sci.* **80**:357 (1981).
6. D. F. Evans, S. Mukherjee, D. J. Mitchell, and B. W. Ninham, *J. Colloid Interface Sci.* **93**:184 (1983).
7. T. J. Burkey, D. Griller, D. A. Lindsey, and J. C. Scaiano, *J. Am. Chem. Soc.* **106**:1983 (1984).
8. D. W. Amstrong, T. J. Ward, and A. Berthod, *Anal. Chem.* **58**:579 (1986).
9. D. W. Amstrong, R. A. Menges, and S. M. Han, *J. Colloid Interface Sci.* **126**:239 (1988).
10. M. I. Viseu and S. M. B. Costa, *Chem. Phys. Lett.* (in press).
11. A. Alizadeh, C. A. Nieto de Castro, and W. A. Wakeham, *Int. J. Thermophys.* **1**:243 (1980).
12. M. L. S. Matos Lopes and C. A. Nieto de Castro, *High Temp. High Press.* **17**:599 (1985).
13. M. L. S. Matos Lopes and C. A. Nieto de Castro, *Int. J. Thermophys.* **7**:699 (1986).
14. M. L. S. Matos Lopes, C. A. Nieto de Castro, and C. M. Padrel de Oliveira, *Fluid Phase Equil.* **36**:195 (1987).
15. C. M. Padrel de Oliveira, J. M. N. A. Fareleira, and C. A. Nieto de Castro, *Int. J. Thermophys.* **10**:973 (1989).
16. C.-U. Herrmann and M. Kahlweit, *J. Phys. Chem.* **84**:1536 (1980).
17. R. J. Robson and E. A. Dennis, *J. Phys. Chem.* **81**:1075 (1977).
18. M. I. Viseu, M. L. S. Simões Gonçalves, S. M. B. Costa, and M. I. C. Ferreira, *J. Electroanal. Chem.* **282**:201 (1990); **282**:215 (1990).