A Light Scattering Theory Applicable to Ions Which Aggregate in Solution and Possess Two Charge Centers of One Sign and One Charge Center of the Opposite Sign: Aggregation of BTA-243 in an Aqueous Medium

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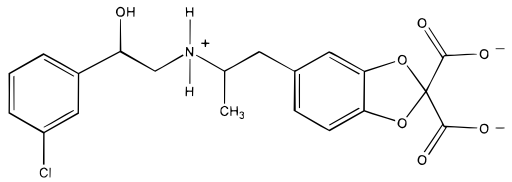
In a slightly acidic aqueous medium, the drug BTA-243 produces ions which aggregate in a manner similar to that shown by surfactant ions. Each of these ions has a positive charge center and two negative charge centers. A new procedure developed to handle light scattering data collected from this unique system indicates that in a 0.2 M sodium acetate buffer, the drug has an aggregation number between 80 and 105.

Introduction

The drug BTA-243 (a benzodioxole containing phenethanolamine with the formula $Na_2C_{20}H_{18}NO_7Cl$) acts as a selective agonist of beta$_3$-adrenergic receptors and is of potential use in the treatment of obesity. When dissolved in slightly acidified water (pH 4.7−6.5), the drug produces ions with the structure



These triply charged ions (referred to as D^- ions in the remainder of the paper) behave in a manner similar to that exhibited by surfactant ions. Below a certain concentration, most of the D^- ions present in solution are unaggregated. Above this concentration, aggregation becomes significant. As in the case of surfactant solutions, we shall refer to aggregates of drug ions as micelles and to the concentration at which micellization becomes discernible as the critical micelle concentration (cmc).

The primary goal of our research was to learn something about the size of the drug micelles by light scattering. Since these micelles have the ability to bind both small cations and anions, existing methods used to treat light scattering data obtained with surfactant solutions were not applicable. We therefore have developed expressions based on a model that approximates our system.

Theory

Our objective is to relate drug aggregation number and micellar charge to solution turbidity. To do this, we first need to introduce some terms and symbols. We let B_2D represent a drug like BTA-243, i.e., B^+ stand for Na^+ and D^{2-} stand for the ion $C_{20}H_{18}NO_7Cl^{2-}$. If we add a little acid to a solution containing B_2D, each D^{2-} ion acquires a proton and becomes the triply charged D^- ion depicted in the Introduction. We will regard solutions of our drug B_2D in the acid HX as solutions of the "drug" B_2DX, where $D^{2-} + H^+ = D^-$.

Although our theory will be couched in terms of drug ions like D^-, it will be applicable to drug ions that have one negative and two positive unit charge centers per ion. One need only switch charge signs mentally when performing calculations.

Consider an aqueous solution of the drug B_2DX and the salt BX. The molality of B_2DX is m' and that of BX is m_3. Below m'_0, the drug's critical micelle concentration (cmc), only B^+, D^-, and X^- ions are assumed to be present in solution. Each drug ion D^- has one positive charge and two negative charge centers. A consequence is that when m' is made greater than m'_0 and aggregation occurs, small ions B^+ and X^- can be incorporated into the micelles that form.

$$bB^+ + mD^- + xX^- \rightarrow B_bD_mX_x^{z-} \qquad (1)$$

The aggregation number is m and the micellar charge is $-z$. We assume that x has an integral value in the range 0 to m, i.e.

$$x = rm$$

r is therefore the ratio of the number of small ions X^- to small ions B^+ that are incorporated in (bound to) the micelle. Since there are twice as many negative charge centers in the micelle as there are positive charge centers, it is unlikely that r will ever be greater than 1, i.e., $0 \le r \le 1$.

Since $-z = -m - x + b = -m - rm + b$

$$b = (1 + r)m - z$$

This permits us to write (1) as

$$[(1 + r)m - z]B^+ + mD^- + rmX^- \rightarrow B_{(1+r)m-z}D_mX_{rm}^{z-}$$

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We assume that the molality of unaggregated drug ions in all solutions above the cmc is m'_0 and that the molality of micelles is given by

$$m_2 = (m' - m'_0)/m$$

If we let m'_2 represent the molality of drug ions which are in micellar form, we have

$$m'_2 = mm_2 = m' - m_0$$

We make the following component choices:

component 1 (solvent)	H_2O
component 2 (micellar salt)	$(BD)_m(BX)_{rm}$
component 3 (supporting electrolyte)	$(BD)_{m'_0/m_3}(BX)_{[m'_0+(1-r)m'_2+m_3]/m_3}$

Components 2 and 3 dissociate as follows:

$$(BD)_m(BX)_{rm} \rightarrow B_{(1+r)m-z}D_mX_{rm}{}^{z-} + zB^+ \quad (2)$$

$$(BD)_{m'_0/m_3}(BX)_{[m'_0+(1-r)m'_2+m_3]/m_3} \rightarrow [2m'_0 + (1-r)m'_2 + m_3]/m_3B^+ + m'_0/m_3D^- + [m'_0 + (1-r)m'_2 + m_3]/m_3X^- \quad (3)$$

If ions resulting from the ionization of the solvent and from the addition of HX (above that required to convert B_2D to B_2DX) to adjust the solution's pH are neglected, the total number of particles in volume V^0 of the solution which contains 1 kg of H_2O is given by

$$\sum = N_1 + N_2 + N_{B^+} + N_{X^-} + N_D \quad (4)$$

Here N_1 is the number of water molecules, N_2 the number of micelles, N_{B^+} the number of free B^+ ions, N_{X^-} the number of free X^- ions, and N_{D^-} the number of unaggregated D^- ions.

Since there are 55.506 mol of water in 1 kg, we can write

$$N_1 = 55.506L \quad (5)$$

where L is Avogadro's number. We can also write

$$N_2 = m'_2L/m = m_2L \quad (6)$$

We let N_3 represent the number of component 3 formula units in V^0, i.e.

$$N_3 = m_3L \quad (7)$$

Dissociations (2) and (3) permit us to write

$$N_{B^+} = zN_2 + [2m'_0 + (1-r)m'_2 + m_3]N_3/m_3 \quad (8)$$

$$N_{X^-} = [m'_0 + (1-r)m'_2 + m_3]N_3/m_3 \quad (9)$$

$$N_{D^-} = m'_0N_3/m_3 \quad (10)$$

Assuming the critical micelle concentration to be a linear function of the supporting electrolyte concentration, we write

$$m'_0 = C - Qm_3 = C - QN_3/L \quad (11)$$

C and Q are determined experimentally in real situations for which our model applies. We combine relationships (4)−(11) to obtain

$$\sum = N_1 + [1 + z + 2(1-r)m]N_2 + [2 - 4Q]N_3 + 4LC \quad (12)$$

We now attempt to associate turbidities with our model solutions. According to Stockmayer,[1] the turbidity of a three-component system in excess of that of the solvent (water in the present case) and in the absence of dissymmetry and depolarization is given by

$$\tau' = [32\pi^3KTn^2V^0(n_2{}^2G_{33} + n_3{}^2G_{22} - 2n_2n_3G_{23})]/3\lambda^4(G_{22}G_{33} - G_{23}{}^2) \quad (13)$$

Here n is the solution refractive index, K Boltzmann's constant, T the absolute temperature, V^0 the volume of solution containing 1 kg of solvent, λ the wavelength of incident light, and $n_i = (\partial n/\partial N_i)_{T,P,N_k}$; N_i is the number of formula units of component i in V^0, P is the pressure, $G_{ij} = (\partial^2 G/\partial N_i \partial N_j)_{T,P,N_k}$, and G is the Gibbs free energy of solution.

Since $(BD)_m(BX)_{rm}$ is equivalent to $(B_2DX)_m(BX)_{-(1-r)m}$, we can write for component 2

$$n_2 = (\partial n/\partial N_2)_{T,P,N_k} = L^{-1}(\partial n/\partial m_2)_{T,P,m_k} = (m/L)(\partial n/\partial m_{B_2DX})_{T,P,m_k} - (1-r)mL^{-1}(\partial n/\partial m_{BX})_{T,P,m_k} \quad (14)$$

For component 3, we can write

$$(BD)_{m'_0/m_3}(BX)_{[m'_0(1-r)m'_2+m_3]/m_3} = (B_2DX)_{m'_0/m_3}(BX)_{[(1-r)m'_2+m_3]/m_3}$$

Hence

$$n_3 = (\partial n/\partial N_3)_{T,P,N_k} = L^{-1}(\partial n/\partial m_3)_{T,P,m_k} = L^{-1}[(m'_0/m_3)(\partial n/\partial m_{B_2DX})_{T,P,m_k} + \{(1-r)m'_2 + m_3\}m_3{}^{-1}(\partial n/\partial m_{BX})_{T,P,m_k}] \quad (15)$$

The solution refractive index is given by

$$n = n_{H_2O} + m'(\partial n/\partial m_{B_2DX})_{T,P,m_k} + m_3(\partial n/\partial m_{BX})_{T,P,m_k} \quad (16)$$

where $(\partial n/\partial m_{B_2DX})_{T,P,m_k}$ and $(\partial n/\partial m_{BX})_{T,P,m_k}$ are refractive index increments for the drug B_2DX and salt BX, respectively.

We now tackle the G_{ij}'s. The chemical potentials of components 2 and 3 are

$$\mu_2 = (\partial G/\partial N_2)_{T,P,N_1,N_3} = \mu_{2D} + z\mu_B \quad (17)$$

$$\mu_3 = (\partial G/\partial N_3)_{T,P,N_1,N_2} = [2m'_0 + (1-r)m'_2 + m_3]m_3{}^{-1}\mu_B + m'_0m_3{}^{-1}\mu_D + [m'_0 + (1-r)m'_2 + m_3]m_3{}^{-1}\mu_X \quad (18)$$

where μ_{2D} is the chemical potential of the micelle $B_{(1+r)m-z}D_mX_{rm}{}^{-z}$, μ_B is the chemical potential of the ion B^+, μ_D is the chemical potential of the ion D^-, and μ_X is the chemical potential of the ion X^-. In terms of particle fractions, we write for these potentials

$$\mu_{2D} = KT \ln \gamma_{2D} N_2/\Sigma + \mu_{2D}^\circ \quad (19)$$

$$\mu_B = KT \ln \gamma_B [zN_2 + \{2m'_0 + (1-r)m'_2 + m_3\} m_3^{-1} N_3]/\Sigma + \mu_B^\circ \quad (20)$$

$$\mu_D = KT \ln \gamma_D [m'_0 N_3 m_3^{-1}]/\Sigma + \mu_D^\circ \quad (21)$$

$$\mu_X = KT \ln \gamma_X [\{m'_0 + (1-r)m'_2 + m_3\} m_3^{-1} N_3]/\Sigma + \mu_X^\circ \quad (22)$$

where the γ denotes activity coefficient and the μ° is the standard chemical potential.

Substitution of (19) and (20) into (17); use of (12) for Σ; substitution of (20), (21), and (22) into (18); employment of (6), (7), and (11); and differentiation (assuming activity coefficients to be constants and terms involving N_3^{-2} to be negligible) leads to

$$G_{22} = KT/N_2 - (1+z) \times KT[1 + z + 2(1-r)m]/\Sigma + zKT[z + (1-r)m]/[\{z + (1-r)m\}N_2 + (1-2Q)N_3 + 2CL] \quad (23)$$

$$G_{23} = (1+z)(4Q-2)KT/\Sigma + (1-2Q)zKT/[\{z + (1-r)m\}N_2 + (1-2Q)N_3 + 2CL] \quad (24)$$

$$G_{33} = [2CL/N_3 - 2Q + (1-r)mN_2/N_3 + 1][KT(1-2Q)/\{zN_2 + 2CL - 2QN_3 + (1-r)mN_2 + N_3\} - KT(2-4Q)/\Sigma] - [CL/N_3 - Q][QKT/(CL - QN_3) + KT(2-4Q)/\Sigma] + [CL/N_3 - Q + (1-r)mN_2/N_3 + 1] \times [KT(1-Q)/\{CL - QN_3 + (1-r)mN_2 + N_3\} - KT(2-4Q)/\Sigma] \quad (25)$$

τ' can now be calculated by using (14), (15), (16), (23), (24), and (25) to calculate n_2, n_3, n, G_{22}, G_{23}, and G_{33} and then substituting the results into (13).

We now develop expressions that can be used to calculate the turbidity in excess of that of water of the solution corresponding to the critical micelle concentration. We choose water to be component 1, the drug B_2DX to be component 2, and the salt BX to be component 3. We let N_1 = number of water molecules, N_2 = number of B_2DX formula units, and N_3 = number of BX formula units in volume V^0 of the solution at the cmc which contains 1 kg of water. The dissociation of component 2 leads to $2N_2$ B^+ ions, N_2 D^- ions, and N_2 X^- ions. The dissociation of component 3 yields N_3 B^+ and N_3 X^- ions. Hence, the total number of particles in volume V^0 is

$$\Sigma = N_1 + 4N_2 + 2N_3 \quad (26)$$

The chemical potentials of components 2 and 3 are

$$\mu_2 = (\partial G/\partial N_2)_{T,P,N_1,N_3} = 2\mu_B + \mu_D + \mu_X \quad (27)$$

$$\mu_3 = (\partial G/\partial N_3)_{T,P,N_1,N_2} = \mu_B + \mu_X \quad (28)$$

Chemical potentials of the various ions are

$$\mu_B = KT \ln \gamma_B (2N_2 + N_3)/\Sigma + \mu_B^\circ \quad (29)$$

$$\mu_X = KT \ln \gamma_X (N_2 + N_3)/\Sigma + \mu_X^\circ \quad (30)$$

$$\mu_D = KT \ln \gamma_D N_2/\Sigma + \mu_D^\circ \quad (31)$$

Substitution of (29), (30) and (31) into (27) and (28), employment of (26), and differentiation (activity coefficients assumed to be constant) leads to

$$G_{22} = KT[1/N_2 - 16/(N_1 + 4N_2 + 2N_3) + 4/(2N_2 + N_3) + 1/(N_2 + N_3)] \quad (32)$$

$$G_{23} = KT[2/(2N_2 + N_3) + 1/(N_2 + N_3) - 8/(N_1 + 4N_2 + 2N_3)] \quad (33)$$

$$G_{33} = KT[1/(2N_2 + N_3) + 1/(N_2 + N_3) - 4/(N_1 + 4N_2 + 2N_3)] \quad (34)$$

For refractive index increments and the solution refractive index at the cmc, we have

$$n_2 = (\partial n/\partial N_2)_{T,P,N_k} = L^{-1}(\partial n/\partial m_{B_2DX})_{T,P,m_k} \quad (35)$$

$$n_3 = (\partial n/\partial N_3)_{T,P,N_k} = L^{-1}(\partial n/\partial m_{BX})_{T,P,m_k} \quad (36)$$

$$n = n_{H_2O} + m'_0(\partial n/\partial m_{B_2DX})_{T,P,m_k} + m_3(\partial n/\partial m_{BX})_{T,P,m_k} \quad (37)$$

The turbidity at the cmc in excess of that of water, τ'_0, may now be determined by using (32) to (37) to calculate G_{22}, G_{23}, G_{33}, n_2, n_3, and n and then substituting the results into (13).

We employ the above expressions in the following way. We assume a particular value for r. Then, for each drug solution whose turbidity in excess of that at the cmc has been measured, i.e., $(\tau' - \tau'_0)_{exp}$, we calculate from the above equations a "theoretical" difference $(\tau' - \tau'_0)_{theo}$ based upon an assumed set of m and z values. Then we calculate the sum of the squares of the differences between $(\tau' - \tau'_0)_{exp}$ and $(\tau' - \tau'_0)_{theo}$ pairs. We repeat the process with a different set of m and z values. Finally, we use as a criterion for the best aggregation number m and charge $-z$ for a particular r, the combination that produces the smallest sum of the squares of the differences between $(\tau' - \tau'_0)_{exp}$ and $(\tau' - \tau'_0)_{theo}$.

We wrote and used a computer program to perform the required computations.

Experimental Section

The BTA-243 was donated by Wyeth-Ayerst Research and used as supplied. The water used to prepare solutions was doubly distilled, deionized, and degassed. Sodium acetate was AnalaR grade. Acetic acid, glacial (Sigma), was >99% pure. Potentiometric titration of BTA-243 gave a pK_a value of 8.29 ± 0.06 for the amine group. Observation of the chemical shift changes of protons close to the ionizable groups of BTA-243 with change of apparent pH in D_2O at 25 °C using NMR techniques (Varian VXR400S 400 MHz spectrometer equipped with Sun 4/110 host computers) showed no change in the ionization of the two acidic groups above pH 4. Hence, although the pH varied between 4.57 and 6.0 over the range of measurements of the micellar properties because of the limitations of the buffer capacity, this did not affect the ionization (pK_a values always more than 2 pH units away).

Static light scattering measurements were made at 22 °C in a 0.2 M sodium acetate buffer close to a pH of 5 with a Malvern PCS 100 light scattering instrument. Equipment used and experimental procedures followed were those of earlier research.[2] The vertically polarized light of wavelength 488 nm used was supplied by a 2 W argon ion laser (Coherent Innova 90). Solutions were clarified by ultrafiltration through 0.1 μm

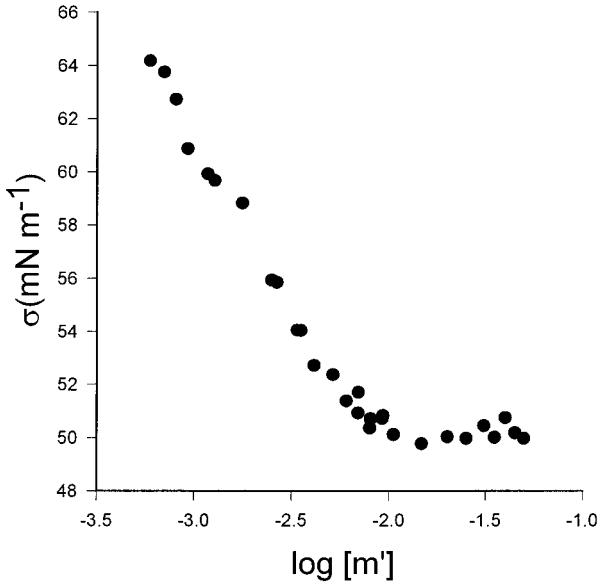

Figure 1. Surface tension σ as a function of logarithm of molality of BTA-243 in 0.2 M sodium acetate buffer at pH $\approx$ 5.

TABLE 1

substance	increment/kg mol^{-1}
sodium acetate	0.00896
BTA-243	0.0985
acetic acid	0.00412

filters. The refractive index increments were measured with an Abbe 60/ED precision refractometer (Bellingham and Stanley Ltd.).

Surface tension measurements were made by the Wilhelmy plate method using a Kruss K-12 surface tension equipment, equipped with a processor to acquire the data automatically. The equipment was connected to a circulating water bath to keep the temperature constant at 25 °C to within ±0.01 °C. Solutions of BTA-243 in 0.2 M acetate buffer were progressively diluted with buffer solution using an automatic pump (Dosimat 665 Metrohm). The usual precautions were taken to ensure cleanliness.

Results

Although BTA-243 does not display the clear separation of hydrophobic and hydrophilic regions characteristic of conventional amphiphiles,[3] it is clear from the plot of σ against log m' (where σ is surface tension) that it is a surface active drug (see Figure 1). Aggregation commenced at a well-defined critical micelle concentration of 0.010 mol kg^{-1}. Moreover, simple dye solubilization tests on aqueous solutions of the drug showed an absence of solubilization of the oil-soluble dye Sudan III at a drug concentration of 0.002 mol kg^{-1}. In contrast, the colored solution obtained at a concentration of 0.020 mol kg^{-1} is indicative of appreciable dye solubilization and suggests the presence of hydrophobic domains in the micelle.

Refractive index increments used in the calculations of micelle size from light scattering data are shown in Table 1. For our program we took the drug to be a one-to-one combination of BTA-243 and $HC_2H_3O_2$ with a refractive index increment of 0.1026 kg mol^{-1}. We used a value of 1.338 50 (obtained by interpolation from indices at 404.41 and 589.00 nm) for the refractive index of water.

Values of $(\tau' - \tau'_0)_{exp}$, i.e., excess turbidity, are plotted against drug molality in Figure 2. Measured cmc's of the drug were

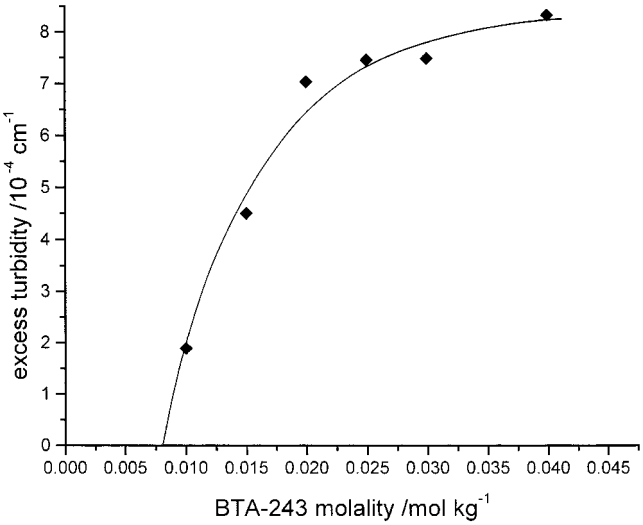

Figure 2. Scattering of BTA-243 in 0.2 M sodium acetate buffer at pH $\approx$ 5. Turbidities (cm^{-1}) are in excess of the turbidities at the cmc.

TABLE 2

assumed m	assumed z	$[(\tau' - \tau'_0)_{exp} - (\tau' - \tau'_0)_{theo}]^2 \times 10^8$
93	43	1.328
93	44	1.099
93	45	1.149
91	44	1.197
91	43	1.090
91	42	1.268
89	43	1.266
89	42	1.099
89	41	1.223

TABLE 3

r	m	z
0.0	102	31
0.5	91	43
1.0	87	71

0.008 mol kg^{-1} in the 0.2 M sodium acetate buffer and 0.010 mol kg^{-1} in water (pH adjusted). The value in buffer was in reasonable agreement to that from surface tension measurements.

The results of a few calculations from our program for an assumed r value of 0.5 are tabulated in Table 2. According to the criterion used, an aggregation number of 91 and a micellar charge of 43 are the best choices for an r value of 0.5.

The "best" m and z calculated for assumed r values of 0, 0.5, and 1.0 are shown in Table 3. In the case of $r = 1.0$, it is possible to apply eqs 6, 9, and 10 from an older work[4] to calculate charge and aggregation numbers for the current system. When this is done, values $m = 77$ and $z = 61$ are obtained.

Summary

Equations were derived which enable one to estimate from light scattering data the size of aggregates in solution of ions each of which has two charge centers of one sign and one charge center of the opposite sign. To reduce the uncertainty associated with the aggregation number of such an ion, one would have to determine by another procedure the charge carried by the aggregate. The light scattering data collected in this study indicates that the micelles which BTA-243 forms in 0.2 M $Na_2C_2H_3O_2$ have an aggregation number between 77 and 102.

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References and Notes

(1) Stockmayer, W. H. *J. Chem. Phys.* **1950**, *18*, 58.
(2) Mosquera, V.; del Río, J. M.; Attwood, D.; García, M.; Jones, M. N.; Prieto, G.; Suarez, M. J.; Sarmiento, F. *J. Colloid Interface Sci.* **1998**, *206*, 66.
(3) Attwood, D.; Florence, A. T. *Surfactant Systems*; Chapman and Hall: New York. 1983 Chapter 4.
(4) Anacker, E. W.; Westwell, A. E. *J. Phys. Chem.* **1964**, *68*, 3490.