Biophysical Chemistry 3 (1975) 355–361

STUDIES ON TRITON X-100 DETERGENT MICELLES *

C. Jayne BIASELLE and David B. MILLAR
Laboratory of Physical Biochemistry, Environmental Biosciences Department, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20014, USA

Triton X-100 micelle formation at 25°C was studied by use of sedimentation equilibrium and fluorescence spectroscopic techniques. The apparent molecular weight of the major Triton X-100 micelle was found to be 81 250, indicating a micelle number of 125. A micelle number of 121 was obtained with fluorescence titration experiments, which showed one molecule of 1-anilino-8-naphthalene sulfonate binding per micelle with an apparent association constant of 0.9×10^5 M. The fluorescent titration experiments also indicated the presence of another TX-100 binding species of variable size.

1. Introduction

Currently, the non-ionic detergent, Triton X-100 (an isoethyl phenoxy polyethoxy ethanol) is very frequently used in the isolation and study of membrane bound proteins and water insoluble lipids [1–8]. This interaction of membrane proteins with TX-100 monomers and micelles can be used to yield information on the mechanism whereby they are attached within membranes [1,8]. For these reasons, an accurate value of the micelle molecular weight of TX-100 would be useful. Previous estimations, however, are extremely disparate ranging from about 65 000 to 150 000 [2,8–13]. Part of this wide variation may result from the different techniques and conditions used. Additional factors to be considered are possible changes in detergent composition and the dependence of molecular weight upon concentration expected for a micellar system. It may be noted that attempting a rigorous thermodynamic analysis of the commercially available TX-100 preparations is, strictly speaking, ill advised due to the distribution of chain lengths [14]. However, as most membrane investigators use the product without further purification, and in view of the lack of agreement between reported micelle molecular weights upon such preparations, a redetermination of the apparent micelle molecular weight has merit. This report presents such a determination by sedimentation equilibrium methods and by fluorescence methods employing the familiar probe, 1-anilino-8-naphthalene sulfonate (ANS).

2. Materials and methods

Triton X-100 (TX-100) was purchased from Rohm and Haas and used without further purification. There are approximately equal numbers of 10 and 9 carbon oxyethylene chains in commercially available TX-100. Accordingly, the manufacturer's suggested average monomer molecular weight of 650 for TX-100 was used in all calculations (Rohm and Haas product circular). The TX-100 was made up (w/v) in 0.01 M sodium phosphate buffer, pH 7.0. The magnesium salt of 1-anilino-8-naphthalene sulfonate (ANS) was prepared from the ammonium salt and recrystallized twice from distilled water. Concentrations of stock solutions were determined spectroscopically using a Cary Model 14 spectrophotometer at room temperature. An extinction coefficient for ANS of 5000 cm^2/mole at 350 nm was employed in all calculations [15].

* From the Bureau of Medicine and Surgery, Navy Department, Research Subtask MR041.06.01.0005AOCK. The opinions and statements contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

2.1. Sedimentation equilibrium studies

Weight-average molecular weight studies were performed at 25°C using the Beckman-Spinco Model E analytical ultracentrifuge. The patterns were photographed on either Kodak metallographic or II G plates and the images read on a Nikon Model 6C profile projector. At TX-100 concentrations below 1 mg/ml, molecular weights were calculated using the meniscus depletion technique of Yphantis [16] with interference optics. Even with 7 mm solution columns and using the highest speeds practicable, the low monomer molecular weights were calculated using the meniscus detion at the meniscus. Since layering cells were used in the depletion techniques, fringe shift at the meniscus and hence meniscus concentration could easily be followed as equilibrium was obtained (< 24 hours). The resulting correction was minimal and had little effect upon calculated molecular weights at the higher concentrations. At detergent concentrations greater than 1 mg/ml, the Yphantis small column method was used [17] with both Schlieren and interference optics. Molecular weights were calculated as before [18] except that calculations were carried out on a Wang 2200-B calculator.

A value of 0.916 ml/g was used for the partial specific volume ($\bar{v}$) of TX-100 (11). At TX-100 concentrations greater than 1%, the C_0 Schlieren pattern became so large that by the time diffusion had decreased the peak height to a measurable size, the base line had become curvilinear. We overcame this difficulty by layering 1% TX-100 on top of 2% TX-100 and adding the 1% C_0 value to the differential value. This procedure of layering the lower concentration to the next higher concentration was successful as evidenced by the fact that a plot of C_0 versus concentration was linear from 0% to 10% TX-100, with a correlation coefficient of 0.999. With Schlieren optics, C_0 was found to equal 7.22×10^{-3} cm^2/mg/ml TX-100 for an 80° bar angle at 25°C. With interference optics, J_0 was found to equal 2.85_3 fringes/mg/ml at 25°C.

2.2. Fluorescence studies

Fluorescence measurements (25°C) were performed using an Aminco-Bowman uncompensated fluorometer with a 15 nm band pass. The fluorescent emission was corrected for ANS absorption [18] and for dilution effects. The amount of ANS bound to TX-100 micelles in the fluorescence studies was calculated in the following manner: E_{max}, the maximum fluorescence occurring for a quantity of totally bound dye was determined by measuring the fluorescence (E_{max}) of 1.12×10^{-5} M ANS in the presence of a 10% (w/v) solution of TX-100 and/or by titrating a 1.2×10^{-5} M ANS solution with TX-100 and plotting $1/F$ versus $1/$(TX-100). The intercept at the ordinate is $1/E_{max}$. The estimates by the two techniques agreed within 5%. The bound ANS (ANS_b) was then calculated using the relation $ANS_b = F/(E_{max}/1.2 \times 10^{-5})$ where F was the observed fluorescence for any given total ANS concentration. Instrument settings were maintained constant for any E_{max} determination and fluorescent titration.

Fluorescent lifetimes (25°C) were measured using a TRW Model 31A spectral source and decay time computer employing Corning filters # 9863 and # 3385 to isolate the exciting and emission bands, respectively. Reported values were the average of at least twenty readings. We initially assumed that the lifetimes measured represent only one binding domain. As will be seen below, the validity of this assumption is not critical to our conclusion.

3. Results

3.1. Sedimentation equilibrium studies

The attainment of sedimentation equilibrium in the small column runs were determined by following the height of the Schlieren gradient with time [17]. For a 1% solution of TX-100, equilibrium was attained in 64 minutes. An equilibrium time determination of 10% TX-100 agreed with this value. In general, however, photographs were taken after much longer times of speed – circa three hours. Fig. 1 shows the variation of $M_{w,app}$ with total TX-100 concentration. The data was first analyzed assuming that the self-association of TX-100 could be represented as a monomer–n-mer equilibrium with the monomer and n-mer having the same partial specific volume. For an ideal monomer–n-mer association, the dependence of the molecular weight on concentration is described by eq. (1):

$$M_w = M_1(c_1 + nKc_1^n)/c. \quad (1)$$

The nonideality apparent in fig. 1 can be corrected by

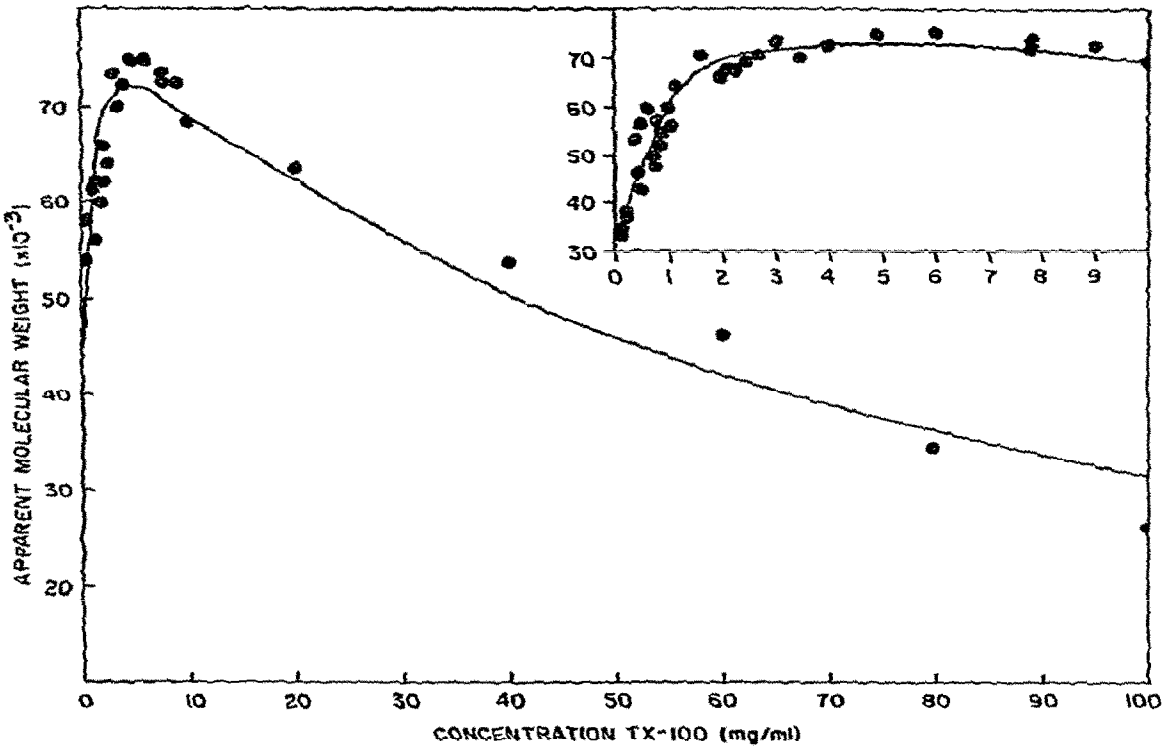

Fig. 1. Molecular weight of TX-100 as a function of concentration. The maximum in the plot indicates nonideality. Experiments were performed at 25°C in 0.01 M sodium phosphate buffer, pH 7.0. Circles represent experimental data; the line drawn through the data points represents a theoretical model system (details in text). The insert shows the detailed behavior of MW_{app} versus low TX-100 concentration.

the use of eq. (2):

$$M_1/M_{w,app} = M_1/M_w + BM_1 c. \qquad (2)$$

In these equations, M_w and $M_{w,app}$ are the true and apparent molecular weight respectively, M_1 is the monomer molecular weight, c_1 is the monomer concentration, n is the number of monomer units in the micelle, K is the equilibrium association constant, c is the total detergent concentration, and B is the virial coefficient (all on the mg/ml scale). The line drawn through fig. 1 is calculated with $K = 5 \times 10^{76}$, $n = 125$, and $BM_1 = 1.2 \times 10^{-4}$ ml/mg. The fit of the calculated line to the data is reasonably good. Calculating the weight fractions of monomer and n-mer, and defining the CMC as the point at which the line describing the variation of n-mer with total concentration intersects the x-axis, an apparent CMC of 2.6×10^{-4} M can be obtained. This estimate is in good agreement with other values obtained by different techniques [1,3]. Two other possible association schemes were investigated to see if they could fit the data shown in fig. 1. The first was the isodesmic association [20]. Such an association is characterized by the following equations:

$$K_{1,2} = [c_2]/[c_1]^2, \qquad (3a)$$

$$K_{2,3} = [c_3]/[c_2][c_1] = [c_3]/K_{1,2}[c_1]^3, \qquad (3b)$$

and so on.

If the molar concentrations in eqs. (3a) and (3b) are expressed in the milligram/milliliter scale, then:

$$c_2 = 2(K/M_1)c_1^2 = 2k\,c_1^2, \qquad (4a)$$

and

$$c_3 = 3(K/M_1)c_1^3 = 3k^2 c_1^3. \qquad (4b)$$

The intrinsic self-association constant k is thus equal for all the endless number of association steps. Programming eq. (4) (carried out for n = 1 to 50 000) * and the appropriate analogues of 1 and 2 we attempted to fit an isodesmic association to the data of fig. 1. We failed to make a satisfactory fit (chiefly at low detergent concentration) due to the molecular weight lowering effect of low degree n-mers (dimers, trimers, quatramers, etc.).

We finally tried a situation in which the main micelle (n = 125) dimerizes. Eq. (5) describes this system:

$$M_w = (c_1 M_1 + K c_1^n M + K_2 (K c_1^n)^2 M_2/c, \qquad (5)$$

where K is the micellization constant, M_2 is the dimer molecular weight, K_2 is the dimerization constant and the other symbols have their previous meanings. With $K = 2 \times 10^{47}$ and $K_2 = 6 \times 10^{94}$ and the virial coefficient † the same as in the simple monomer-micelle case, a fit slightly less perfect than for the simple micelle case is obtained. More complicated cases did not fit as well. While a straightforward isodesmic association can be ruled out, the choice between a simple monomer–n-mer association, a monomer–n-mer association followed by further self-association of the n-mer or further slow growth of the main micelle may be difficult. However, the fit of the simple monomer–n-mer case is superior to all these possibilities. Expressing goodness of fit as the minimum of sums of squares of error between calculated model data and experimental data, the monomer–n-mer case always fits better by 5% to 10%.

3.2. Fluorescence studies

As an alternate means of determining the apparent micelle size we utilized the dye ANS. In aqueous solutions, ANS fluoresces only in environments where water moderated quenching conformations cannot be attained. In this connection, Rubalcava et al. have shown that ANS fluoresces when added to TX-100 solutions [21]. The fluorescence accompanying penetration of the dye into the interior of the micelle can then be used as a method of directly assaying micelle size. With E_{max} (see section 2) known, titration of a solution of TX-100 with ANS yields fluorescent data as a function of ANS concentration from which the required data for the Scatchard equation [22] are obtained. Fig. 2 shows an experiment from which E_{max} can be obtained. Note that the rate of fluorescent emission increase begins to level off at about 1% TX-100 (paralleling the molecular weight results). Accordingly, we performed several titrations of 1% TX-100 by ANS. A typical Scatchard analysis is shown in fig. 3 which is a plot of R/C versus R. R is moles ANS bound/mole TX-100 (TX-100 expressed as monomer concentration), and C is free ANS. The x axis intercept at $R/C = 0$ is n, the maximum value of R. The slope is $-K$, the association constant. For five such titrations the average value of $1/n$ (moles of TX-100/mole of ANS, i.e., the micelle number) was 121 ± 2.5. The average K was $0.9 \times 10^5 \pm 0.4 \times 10^5$. By comparison, Rubalcava et al. [21] report a higher micelle number of 200 and an association constant similar to ours. Fluorescent titrations at 5°C and 40°C show little change in n at 5°C but about a 10% decrease at 40°C. (These values are corrected for the decrease in quantum yield of ANS at 40°C by means of a 5°C or 40°C E_{max} determination). The above titrations were carried out at levels of ANS reaching 15×10^5. If the titrations were repeated using ten fold lower concentrations of ANS, curvilinear Scatchard plots resulted. One portion of the line extrapolated to a micelle number of 129. The more severely sloping portion yielded a value of n equal to 6.7×10^4 and a K of 4×10^5. Similar results were obtained for 5% and 10% TX-100 solutions although in these cases the extreme values of n reached about 1 to 2×10^5. Rubalcava et al. [21] did not report such studies. We do not place any reliance on the absolute values of the higher values of n in view of the ease with which difference intercepts could be drawn. However, in all titrations of this type, curvilinear behavior and n values of similar magnitude were noted. This apparent variation in n with TX-100 concentration and temperature is accompanied by significant changes in the interior of the micelles as is reflected by changes in the fluorescent lifetime of ANS (table 1).

4. Discussion

The ultracentrifuge and fluorometric results leave little doubt that the chief micelle species (at least up to 1% TX-100) is one with a micelle number of about

* In principle n has no limit but employing values of $n >$ 50 000 had no detectable effect on the calculations.
† The virial coefficient was obtained via curve fitting.

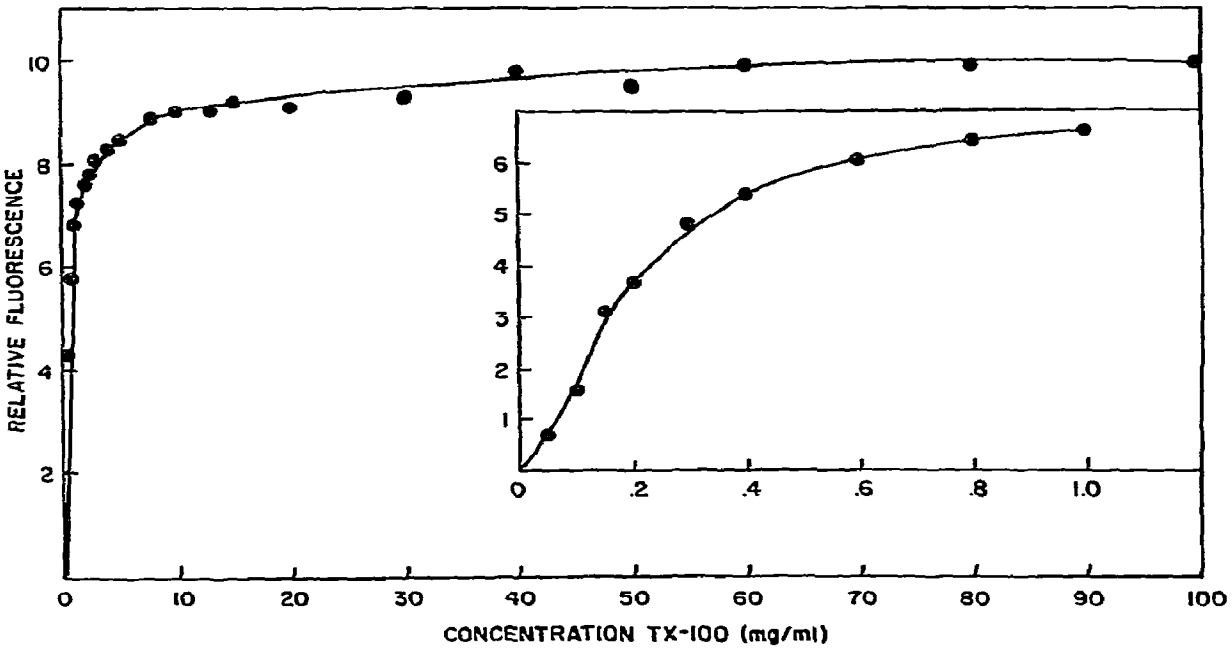

Fig. 2. Plot of relative fluorescence at 495 nm against TX-100 concentration. [ANS] = 1.2×10^{-5}; excitation wavelength = 380 nm. The insert shows the data at TX-100 concentration of 0.1% and below. Buffer as in fig. 1.

125. This conclusion is in excellent agreement with the recent sedimentation and diffusion results of Yedgar et al. [2] carried out at 20°C. However, Lewis et al. [8] report a value of 65 520 at 5°C suggesting a possible temperature dependence of the micellization process.

Under particular conditions, the fluorometric data indicates the TX-100 system to be complex. The exceedingly large n values obtained fluorometrically at low ANS concentrations are difficult to interpret as super micelles with molecular weights in the millions would result. Such species are not indicated by the centrifuge results and therefore could exist only in small amounts. An alternate explanation is the presence of a small amount of high molecular weight impurity which can bind ANS relatively tightly as compared to TX-100 micelles. If such species are present in small concentrations, titrations at high ANS levels would fail to reveal them.

Table 1
Fluorescent lifetime measurements [a]

% TX-100	Temperature (°C)	τ (nanoseconds)
0.0116	20	2.6 ± 0.5
0.33	20	2.8 ± 0.5
1	5	3.9 ± 0.4
1	20	3.3 ± 0.4
1	40	1.8 ± 0.3
5	20	3.4 ± 0.2
10	20	2.9 ± 0.2

[a] [ANS] = 1.22×10^{-4} M.

The slight variation in n with TX-100 concentration is difficult to explain in the above context, however. Possibly, there are concentration dependent changes taking place in the geometry and configuration of the micelle which effect the micelle interior. A micelle geometry in which ANS binds with lowered quantum yield would be consistent with the lifetime changes shown in table 1. The drop in τ_{app} as the concentration is lowered from 1% TX-100 almost certainly reflects the increasing presence of lower order or incomplete

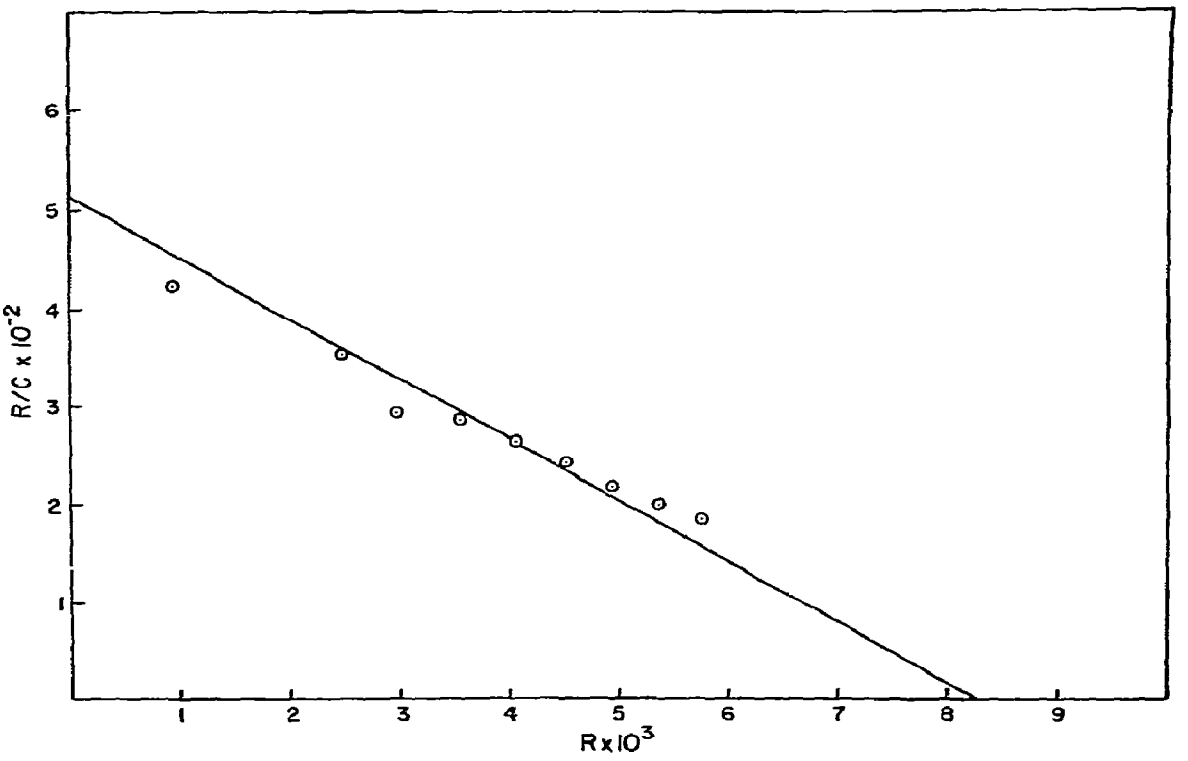

Fig. 3. Scatchard plot resulting from the titration of 1% TX-100 by ANS. Buffer as in fig. 1. $T = 25^{\circ}C$.

(leaky) micelles. In such micelles, the aqueous quenching formation of ANS is apparently more easily obtained. The small drop in τ_{app} at 10% TX-100 may then indicate the existence of a different geometry micelle which is more stable at high TX-100 concentrations [23] and in which the ANS binding domain is susceptible to quenching conformations/conditions. These results suggest that fluorescent lifetime measurements of micelle bound dye may be useful tools to probe the interior of micelles. Further, the data presented here indicate that simple association schemes may only be adequate to fit data obtained at relatively low concentrations and that wide concentration range and temperature studies be undertaken prior to selecting an association scheme for TX-100 micelle formation.

Acknowledgements

We thank T. Mallinson for expert technical assistance and Dr. M. Lewis, National Institutes of Health, for helpful criticism.

References

[1] S. Makino, J.A. Reynolds and C. Tanford, J. Biol. Chem. 248 (1973) 4926–4932.
[2] S. Yedgar, Y. Barenholz and V.G. Cooper, Biochim. Biophys. Acta 363 (1974) 98–111.
[3] N.C. Robinson and C. Tanford, Biochemistry 14 (1975) 369–378.
[4] A.A. Ribeiro and E.A. Dennis, Biochim. Biophys. Acta 332 (1974) 26–35.
[5] E.A. Dennis, Arch. Biochem. Biophys. 165 (1974) 764–773.
[6] R. Srinivasan, A. Karczmar and J. Berhsohn, Biochim. Biophys. Acta 284 (1972) 349–354.

[7] D.B. Millar, M.A. Grafius, D.A. Palmer and G. Millar, Eur. J. Biochem. 37 (1973) 425–433.
[8] M.S. Lewis, L.C. Krieg and D.W. Kirk, Exp. Eye Res. 18 (1974) 29–40.
[9] A.M. Mankowich, J. Phys. Chem. 58 (1954) 1027–1030.
[10] L.M. Kushner and W.D. Hubbard, J. Phys. Chem. 58 (1954) 1163–1167.
[11] C.W. Dwiggins, R.J. Bolen and H.N. Dunning, J. Phys. Chem. 64 (1960) 1175–1178.
[12] K. Kuriyama, Kolloid. Z. 181 (1962) 144–149.
[13] J.M. Korkill and T. Walker, J. Coll. Inter. Sci. 39 (1972) 621–631.
[14] Rohm and Hass, Surfactants, Handbook of Physical Properties CS-16 G/Cd, Rohm and Haas, Philadelphia, Pennsylvania (1971) p. 6.
[15] L. Stryer, J. Mol. Biol. 13 (1965) 482–495.
[16] D.A. Yphantis, Biochemistry 3 (1964) 297–317.
[17] D.A. Yphantis, Ann. N.Y. Acad. Sci. 88 (1960) 586–601.
[18] D.B. Millar, G.E. Willick, R.F. Steiner and V. Frattali, J. Biol. Chem. 244 (1969) 281–284.
[19] C.A. Parker and W.J. Barnes, Analyst 82 (1957) 606–609.
[20] E.T. Adams, Jr., Fractions 3 (1967) 1–18.
[21] B. Rubalcava, D.M. de Munõz and C. Gitler, Biochemistry 8 (1969) 2742–2747.
[22] G. Scatchard, Ann. N.Y. Acad Sci. 51 (1949) 660–672.
[23] C. Tanford, J. Phys. Chem. 78 (1974) 2469–2479.