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Phase behavior of synthetic amphiphile vesicles investigated by calorimetry and fluorescence methods[1]

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Abstract

The understanding of biological membranes may be improved by investigating physical properties of vesicles from natural or synthetic amphiphiles. The application of vesicles as mimetic agents depends on the knowledgment of their structure and properties. Vesicles having different curvature and size may be obtained using different preparation protocols. We have used differential scanning calorimetry (DSC) and steady-state fluorescence to investigate the gel to liquid-crystal phase transition of vesicles prepared by sonication (SUV) and non-sonication (GUV) of the synthetic dioctadecyldimethylammonium bromide (DODAB) in aqueous solution. DSC thermograms for a non-sonicated dispersion show a well-defined pre- and main transition corresponding to two narrow peaks at 36 and 45°C in the first upscan, while in a second upscan, only the main peak was observed. The sharpness of the peaks indicate a cooperative phase behavior for GUV. For a sonicated DODAB dispersion, the first upscan shows a third peak at 40.3°C, whereas for the second upscan the peaks are not well-defined, indicating a less cooperative phase behavior. Alternatively, the fluorescence quantum yield (Φ_f) and the anisotropy (r) of *trans, trans, trans*-1-[4-(3-carboxypropyl)-phenyl]-6-[4-butylphenyl]-1,3,5-hexatriene (4H4A) and the ratio I_1/I_3 of the first to the third vibronic peaks of the pyrene emission spectrum as function of temperature are used as well to describe the phase behavior of DODAB sonicated and non-sonicated dispersions. It is in good agreement with the DSC results that the cooperativity of the thermotropic process is diminished under sonication of the DODAB dispersion, meaning that sonication changes from homogeneous to heterogeneous populations of the amphiphile aggregates. The pre- and main transitions obtained from these techniques are in fairly good accord with results from the literature. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Differential scanning calorimetry; Steady-state fluorescence; Melting temperature; Thermotropic phase behavior; Synthetic amphiphile vesicles

1. Introduction

Vesicles or liposomes consist of closed bilayers formed from hydrated natural or synthetic amphiphiles. Small, large or giant unilamellar vesicles are easily obtained using appropriate methods such as sonication or extrusion [1]. The vesicle structure depends on the preparation protocol, as well as on the amount and nature of the amphiphiles and additives [1].

DODAX (dioctadecyldimethylammonium halide) molecules are double, long-chained, synthetic vesicle-forming amphiphiles, widely used in studies of

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association colloid systems [2–9]. In the presence of water, they assemble as small (SUV) or large vesicles (LUV) when treated, respectively, with sonication or chloroform-injection [2]. Recently, we reported that within the diluted regime, DODAB molecules self-assemble spontaneously as a bipopulation of large and giant unilamellar vesicles (GUV) [3,4]. At higher concentrations, however, the vesicle structure becomes multilamellar [5]. The mean hydrodynamic radii of SUV, LUV and GUV measured by dynamic light scattering are, respectively, 22–33 [4], 275 [6] and 337 nm [3].

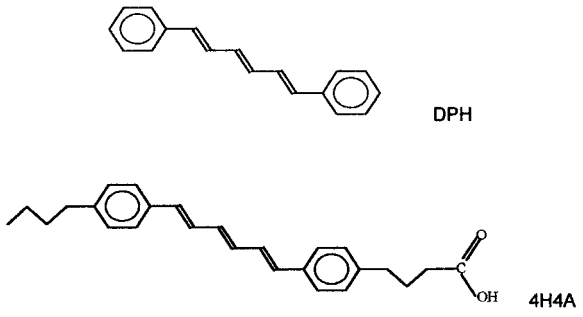
The thermotropic phase behavior of DODAB vesicles is not well understood. Different values for the melting temperature of DODAB (or derivatives) in water solution are found in the literature [4,7–10] and, in addition, pre-transition temperatures are not usually detected for these vesicular systems [8,9]. Fluorescence [10–12], light scattering [3,4], EPR [7] and calorimetry [8,9] are the most commonly used methods for these type of studies. In general, the melting temperature (T_M), around which the gel to liquid-crystal phase transition occurs, depends on the particular experimental method used to determine it. Typical T_M values for DODAB vesicles are 36 and 45°C for the pre- and main transition, respectively.

We have used differential scanning calorimetry (DSC) and steady-state fluorescence methods to investigate the thermotropic phase behavior in vesicle systems prepared by sonication or by simply mixing (non-sonication) of DODAB–water dispersions above the amphiphile T_M. Using these techniques, pre- and main transitions of about 36 and 45°C were detected and the cooperativity of the phase behavior is dependent on the vesicle curvature or preparation protocol.

2. Materials and methods

2.1. Chemicals

DODAB (Eastman Kodak), pyrene (Sigma) and *trans, trans, trans*-1,6-diphenyl-1,3,5-hexatriene (DPH) (Aldrich) were recrystallized twice from acetone. *Trans, trans, trans*-1-[4-(3-carboxypropyl)-phenyl]-6-[4-butylphenyl]-1,3,5-hexatriene (4H4A) was prepared as reported previously [11,12]. The chemical structures of DPH and 4H4A are shown below.



2.2. Vesicle preparation

Giant unilamellar vesicles (GUV) from DODAB were prepared by mixing the DODAB–water mixture at 55°C (above T_M) till the amphiphile present at low concentration (up to ca. 1.0 mM) was completely dissolved. To prepare small unilamellar vesicles (DODAB), the non-sonicated DODAB dispersion was sonicated at 55°C until it became optically clear. A bath-type cell disrupter (Microsonic, SX-10) was used in the sonication process. After preparation the DODAB stock, dispersions were cooled to room temperature and stored at it for the experiments. The vesicles so prepared are stable for months [3,4]. The properties of giant and small DODAB vesicles are described elsewhere [3,4,6]. The term 'giant' is used here to distinguish the non-sonicated vesicles from those large ones obtained by chloroform injection [6].

2.3. Probed vesicle preparation

A small amount of 1.0 mM 4H4A/$CHCl_3$, or pyrene/$CHCl_3$ solution was placed on the bottom of a cylindrical glass flask and the chloroform evaporated under a constant N_2 flux. The sonicated or non-sonicated sample was than added to the flask and the solution was stirred for about 24 h in a light protected container.

2.4. Differential Scanning Calorimetry (DSC)

The DSC measurements were made in a high sensitive differential microcalorimeter (Microcal, MC-2) using twin 1.2 ml total-fill cells to heat the sample and the reference (water) simultaneously and

independently. At a phase transition, heat is applied to the DODAB sample (endothermic process) to maintain the same temperature in both the sample and reference. The samples were scanned at the rate of 1°C/min. The collection and analysis of the data were made with the ORIGIN™ (version 2.9) software supplied by the manufacturer.

2.5. Fluorescence

All measurements were performed using a Hitachi 4500 fluorescence spectrofluorimeter. The temperature of the water-jacketed cell holder was controlled with a circulating bath and monitored by a thermistor probe. The fluorescence quantum yield was determined using DPH in cyclohexane as reference ($\Phi_{f(ref)} = 0.80$) [12]. The reference solution was prepared with the same optical density as the probe-containing vesicle dispersion and its integrated emission band compared at the same wavelength region [11].

The fluorescence anisotropy results were determined using a polarization accessory (right-angle geometry) through the relationship [13]:

$$r = \frac{I_{\parallel} - GI_{\perp}}{I_{\parallel} + 2GI_{\perp}} \quad (1)$$

where $G = i_{\perp}/i_{\parallel}$ is an instrumental correction factor for the equipment set up, and the fluorescence intensity parameters (i and I) account for the ordinary polarization of the excitation and emission light. The subscript symbols $\parallel$ and $\perp$ refer to the position of the polarizers, whether parallel or perpendicular, respectively, to the plane of polarization of the excitation or emission light.

We have obtained the ratio between the intensities of the first and the third vibronic peaks of the emission spectrum of pyrene (I_1/I_3), which is highly sensitive to the polarity of the probe microenvironment [13], to monitor the DODAB vesicle bilayer core. Pyrene was excited at 310 nm and the emission spectra detected at a right angle to the excitation radiation.

3. Results and discussion

3.1. DSC results

All the DSC and fluorescence measurements were made at least 24 h after the sample preparation to ensure the kinetic and mechanical equilibrium of the vesicle suspensions. Fig. 1 (A) shows, after a first scan, the effect of increasing temperature on the heat excess capacity (ΔC_P) of the DODAB (1.0 mM) non-sonicated vesicle sample within the range of 25–55°C. It shows two main peaks, one at 36 and the other at 45°C, corresponding respectively to the pre- and main transition, in good agreement with DSC results from the literature [8,9]. After the first upscan, the sample was quickly cooled and then heated again. The second DSC heating thermogram made about 1.5 h after the first scan shows only the main transition at 45°C (Fig. 1 (B)), suggesting that the first peak in Fig. 1 (A) corresponds to a pre-transition which is a kinetically slow process.

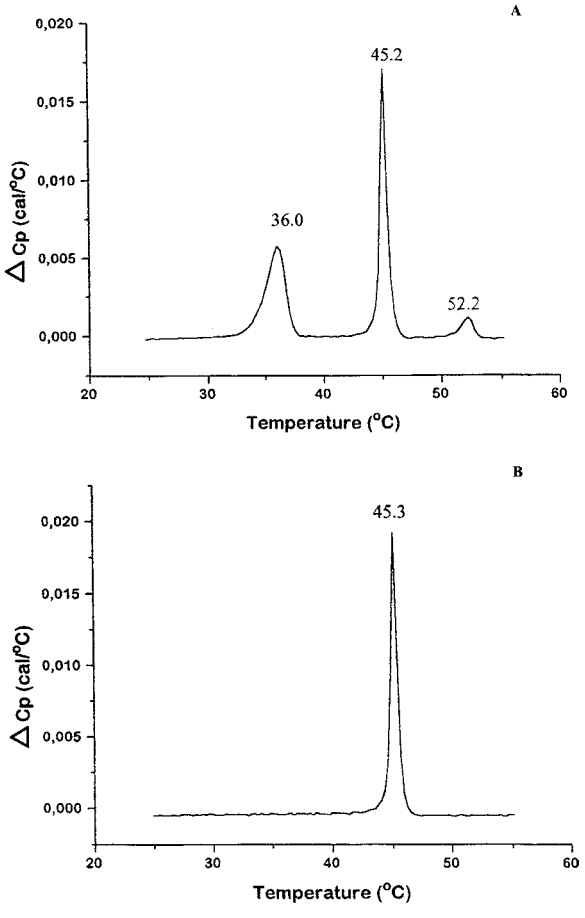


Fig. 1. Change in excess heat capacity with increasing temperature of 1.0 mM DODAB non-sonicated vesicles in water. (A) First upscan, and (B) second upscan. The scan rate was 1°C/min.

The origin of the pre-transition either in lipid or synthetic amphiphile vesicles is not clear. It has been attributed either to changes in the chain conformations [14] or to structural transformations within the amphiphile head groups [15]. Blandamer et al. [8,9], on the other hand, using DSC reported that the addition of ionic surfactants to a non-sonicated DODAB vesicle dispersion eliminates the pre-transition peak and linked it to intervesicular interactions. In this work, the time (1.5 h) to cooling and reheat the sample was not enough for the vesicles to attain the kinetic equilibrium, since the second upscan does not reveal the pre-transition peak.

After 60 min sonication of the non-sonicated DODAB dispersion, the DSC thermograms change considerably in comparison to those obtained before sonication (Fig. 2 (A) and Fig. 2 (B)). The pre-transition peak at 36°C is considerably weakened and the main transition temperature is lowered by about one degree ($T_M \approx 44.4$°C) relative to the non-sonicated sample (Fig. 2 (A)). The sonicated dispersion presents a third intermediate peak at 40.3°C probably due to the co-existing populations of structurally different aggregates, such as bilayer fragments and vesicles as already reported in a previous communication [4]. A second upscan reveals a broad peak which dominates the thermogram indicating that the gel to liquid-crystalline phase transition is not well-defined (Fig. 2 (B)). The mixture of two populations of DODAB supramolecular aggregates having different structures (fragments and vesicles) for the sonicated dispersion is probably responsible for the loss of cooperativity relative to the non-sonicated dispersion which consist not only of two populations but also of vesicle structure with differing size [3].

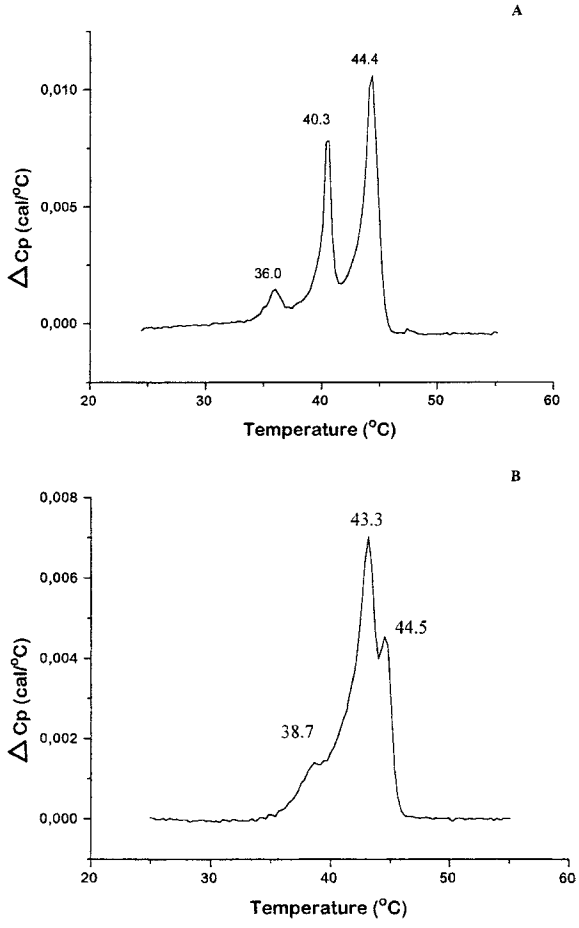


Fig. 2. Change in excess heat capacity with increasing temperature of 1.0 mM 90 min sonicated DODAB vesicles in water. (A) First upscan and (B) second upscan. The scan rate was 1°C/min.

3.2. Fluorescence results

The fluorescence results corroborate the calorimetric ones shown above. 4H4A is a very sensitive amphiphile-like probe suitable to investigate the properties of natural or synthetic amphiphile like micelles or vesicles [11,12]. Fig. 3 shows the effect of increasing the temperature on the fluorescence quantum yield (Φ_f) for 4H4A solubilized in a small (sonicated) and giant (non-sonicated) DODAB unilamellar vesicle in water. The curve for non-sonicated vesicles have two break points, at about 35 and 42°C, suggesting that both a pre- and main transitions can be detected from the Φ_f curve for 4H4A. Sonicated vesicles, on the other hand, show a single less defined break point around 43°C, characteristic of a non-cooperative phase transition as observed from the DSC experiments. Assuming that the 4H4A is solubilized inside the vesicle bilayer because of the amphiphilic characteristics of the prove, as already observed for phospholipid vesicles [11], the existence of intervesicular interactions could slightly change the packing state of the DODAB bilayer causing the appearance of the first (pre-transition) break point in non-sonicated vesicles.

Fig. 4 illustrates typical emission spectra for pyrene in water and in a 1.0 mM DODAB dispersion showing the vibronic peaks. For comparison, it also shows the

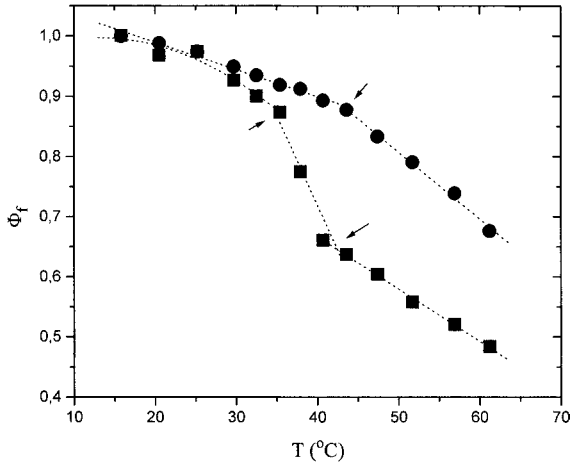


Fig. 3. Dependence of the fluorescence quantum yield for 4H4A (2.2 μM) on the sample heating temperature for 1.0 mM DODAB 90 min. sonicated (●) and non-sonicated (■) dispersions. The arrows indicate the phase transitions.

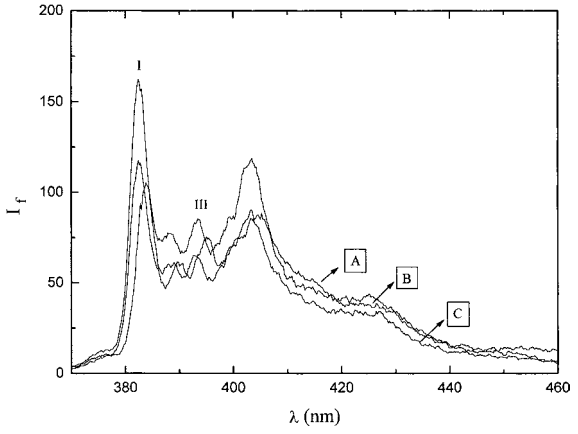


Fig. 4. Emission spectra for pyrene in (A) pure water, (B) 1.0 mM DODAB and (C)NaBr solutions showing the first (I) and third (III) vibronic peaks.

spectrum for a 1.0 mM NaBr aqueous solution. Note that 1.0 mM NaBr does not change the pyrene I_1/I_3 (≈1.85, from Fig. 4) in pure water but it has a stronger quenching effect than the same amount of DODAB molecules because only the bound Br^- counterions (ca. 80% of the whole amount of the amphiphile) quench the fluorescence signal. Pyrene incorporated either in sonicated or non-sonicated vesicles decreases Φ_f relative to that in pure water and slightly shifts the vibronic peaks to a higher wavelength. Such a beha-

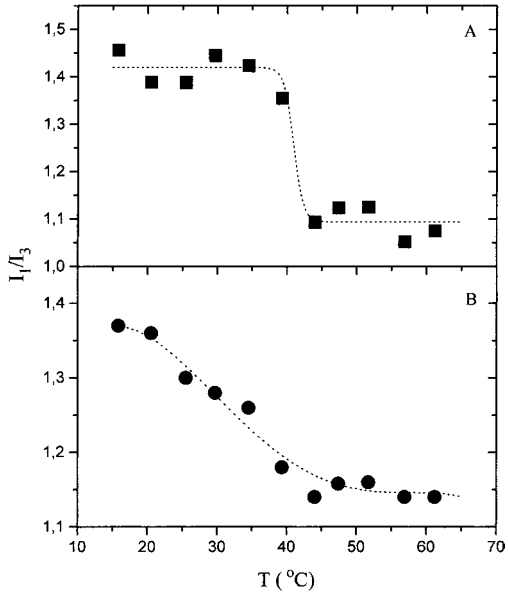


Fig. 5. Dependence of the pyrene I_1/I_3 ratio on the sample heating temperature for (A) 1.0 mM DODAB non-sonicated and (B) bath-sonicated dispersions. [pyrene] = 1.0 μM.

vior is expected, owing to the fluorescence quenching effect of the bromide counterions bound and close to the vesicle interfaces. This conclusion is based as well on the very small pyrene concentration used and the absence of the pyrene excimer emission band (Fig. 4).

Fig. 5 (A) and Fig. 5 (B) show changes in the ratio I_1/I_3 of the intensities of the first and third vibronic peaks of the pyrene fluorescence emission spectra for increasing temperature. The I_1/I_3 value at 20°C ($I_1/I_3 \approx 1.4$) is similar to that for the micelle-forming cationic surfactants like hexadecyltrimethylammonium bromide (CTAB) or dodecyltrimethylammonium chloride (DTAC) [16], indicating that pyrene is placed near the interface where water molecules are present to some extent. The ratio I_1/I_3 as a function of temperature gives a slightly lower T_M value (ca. 40.3°C) for non-sonicated vesicles (Fig. 5 (A)). The sharpness of the curve around T_M indicates a cooperative process for the non-sonicated but not for the sonicated DODAB vesicles (Fig. 5 (B)). Fig. 5 (B) also stresses that T_M for sonicated DODAB vesicles is not well defined and a value of about 43°C is obtained, corroborating the DSC and Φ_f results.

Fig. 6 shows the effect of heating and cooling the sonicated DODAB vesicle on the fluorescence aniso-

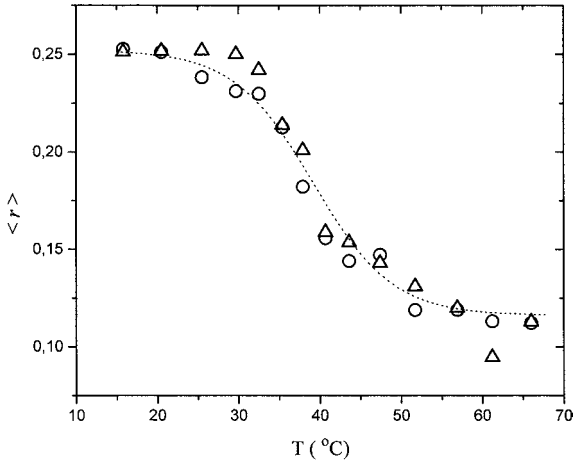


Fig. 6. Dependence of the 4H4A probe fluorescence anisotropy on sample temperature for 90 min. sonicated DODAB aqueous dispersion under heating (O) and cooling (Δ), as shown. [4H4A] = 2.5 μM.

tropy (r). The overlapping of these curves shows that the transition is fully reversible. A mean value for T_M of about 38.2°C is obtained from these curves. Since the curves show no sharp change around T_M, the transition is non-cooperative in good accord with the the DSC, Φ_f and I_1/I_3 results shown above. A more cooperative transition was observed from the anisotropy versus temperature curve for non-sonicated DODAB dispersions without changing considerably T_M, as shown in another communication [10].

4. Conclusions

Different experimental methods show that sonicated and non-sonicated DODAB vesicles have different thermotropic phase behavior. The gel to liquid crystal phase transition is cooperative for giant (non-sonicated) vesicles and non-cooperative for small (sonicated) vesicles as the sonicated dispersions consist of mixed aggregate structures in suspensions, whereas the non-sonicated dispersions contain only vesicle structures. For kinetically equilibrated giant DODAB vesicles in water, the DSC thermograms reveal a pre- and a main transition, whereas pyrene fluorescence shows only the main transition corresponding to the melting temperature (T_M). Quantum yield measurements for 4H4A, on the other hand, detected both the pre- and main transitions. For small sonicated DODAB vesicles, a third peak was detected between the pre- and main transitions and the peaks are not well-defined, showing a less cooperative phase behavior which is in good agreement with fluorescence measurements which shows that the cooperativity of the phase behavior decreases as the vesicle curvature increases.

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References

[1] M. Rosoff (Ed.), Vesicles: Surfactant Science Series, vol. 62, Marcel Dekker, New York, 1996, p. 151.
[2] A.M. Carmona-Ribeiro, Chem. Soc. Rev. 21 (1992) 209.
[3] E. Feitosa, K. Edwards, G. Karlsson, manuscript in preparation.
[4] E. Feitosa, W. Brown, Langmuir 13 (1997) 4810.
[5] E. Feitosa, L.Q. Amaral, Condensed Matter Physics Meeting, Caxambu, MG, Brazil, 1998.
[6] I.M. Cuccovia, E. Feitosa, H. Chaimovich, L. Sepulveda, W.F. Reed, J. Phys. Chem. 94 (1990) 3722.
[7] R.B. Pansu, B. Arrio, J. Roncin, J. Faure, J. Phys. Chem. 94 (1990) 796.
[8] M.J. Blandamer, B. Briggs, P.M. Cullis, J.B.F.N. Engberts, D. Hoekstra, J. Chem. Soc., Faraday Trans. 90 (1994) 1905.
[9] M.J. Blandamer, B. Briggs, P.M. Cullis, J.B.F.N. Engberts, A. Kacperska, J. Chem. Soc., Faraday Trans. 91 (1995) 4275.
[10] C.R. Benatti, M.J. Tiera, E. Feitosa, manuscript in preparation.
[11] M. T Allen, L. Miola, D.G. Whitten, J. Am. Chem. Soc. 110 (1988) 3198.
[12] M.T. Allen, L. Miola, D.G. Whitten, J. Phys. Chem. 24 (1991) 6099.
[13] J. Lakowics, Principles of Fluorescence Spectroscopy. Plenum Press, New York, 1983.
[14] R.P. Rand, D. Chapman, K. Larsson, Biophys. J. 15 (1975) 1117.
[15] M.J. Janiak, D.M. Small, G.G. Shipley, Biochemistry 15 (1976) 4575.
[16] K. Kalyanasundaram, Photochemistry in Microheterogeneous Systems, Academic Press, New York, 1987, p. 40.