Relationship between Pluronic Block Copolymer Structure, Critical Micellization Concentration and Partitioning Coefficients of Low Molecular Mass Solutes

Mikhail Yu. Kozlov,[†,‡] Nikolai S. Melik-Nubarov,[†] Elena V. Batrakova,[‡] and Alexander V. Kabanov*[,‡]

Department of Polymer Science, M.V. Lomonosov Moscow State University, Leniskie Gory, Moscow V-234, 119899, Russia; and College of Pharmacy, Department of Pharmaceutical Sciences, 986025 University of Nebraska Medical Center, Omaha, Nebraska 68198-6025

Received September 27, 1999; Revised Manuscript Received February 23, 2000

ABSTRACT: Using pyrene and homologous alkyl derivatives of fluorescein as fluorescent probes, this work examines the partitioning coefficients of hydrophobic solutes in aqueous dispersions of Pluronic block copolymers (poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide)). An incremental approach is developed, allowing measurement of the free energy of transfer of a methylene group from aqueous media into the micelles. Effects of variation of length of the ethylene oxide (EO) and the propylene oxide (PO) blocks in Pluronic molecules on the partitioning characteristics of the solutes are established. A simple reciprocal relationship between partitioning coefficients of the solute and critical micellization concentration is demonstrated.

Introduction

Amphiphilic block copolymers have attracted significant attention in studies on drug delivery and drug targeting.[1−3] In particular, micelles formed by Pluronic block copolymers (poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide), $EO_{m/2}-PO_n-EO_{m/2}$) were used as "microcontainers" to incorporate drug molecules and transport drugs into the cell and within the body.[4,5] Critical micellization concentration (cmc) and drug partitioning between the aqueous and micellar phases are of particular significance to the optimization of pharmaceutical formulations using Pluronic block copolymers.[6,7] The cmc determines thermodynamic stability of the micelles against possible dilution of the drug delivery system in body fluids.[2,6] Further, cmc is an important parameter in view of biological response modifying effects of Pluronic block copolymers in cancer and other cells since it determines the maximum achievable concentration of the polymer single chains ("unimers") that cause those effects in cells.[7] The partitioning coefficient, *P*, determines the portion of drug incorporated into the micelle and provides thermodynamic characterization for the stability of the drug-micelle complex during dilution within the body fluids.[2,6] A variety of Pluronic block copolymers differing in the lengths of the poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) blocks are available for formulation with pharmaceutical drugs. By changing the lengths of the chain segments, a remarkable variability in the values of cmc and *P* can be achieved, which is a decisive factor in the optimization of corresponding pharmaceutical formulations.[7]

A number of studies characterizing structure parameters of Pluronic micelles such as micelle size, copolymer aggregation number, dimensions of hydrophobic core, and hydrophilic corona, have been reported in the literature.[3,8−14] Comprehensive work is available relating the cmc and structure of Pluronic block copolymers,[15,16] and several attempts were made to develop a theoretical basis for micelle formation and the incorporation of hydrophobic substances in the micelle core.[17−22] Present study characterizes partitioning coefficients of hydrophobic solutes in aqueous dispersions of Pluronic block copolymers. Homologous alkyl derivatives of fluorescein are synthesized and used as model compounds to elucidate the effects of the molecular parameters of Pluronic block copolymers on the partitioning characteristics of hydrophobic solutes. This study determines the free energy of transfer of a methylene group from aqueous media into the micelle and relates it to the lengths of PEO and PPO blocks of Pluronic molecules. Using a broad panel of Pluronic block copolymers, a simple reciprocal relationship between partitioning coefficients of the solute and cmc is established. Overall, this work advances characterization of micellization and solubilization phenomena in aqueous dispersions of Pluronic block copolymers. The results of the work are useful for predictions of solubilization and partitioning of pharmaceutical drugs in the block copolymer micelles and development of block copolymer based formulations for drug delivery.

Materials and Methods

Materials. Fluorescein isothiocyanate isomer I (FITC) purchased from Aldrich Chemical Co. (Milwaukee, WI) and pyrene (benzo[*def*]phenantrene) purchased from Sigma Chemical Co. (St. Louis, MO) were used as received. Propylamine, pentylamine, hexylamine, heptylamine, and octylamine (all from Aldrich Chemical Co.) and butylamine (Fisher Scientific, Fairlawn, NJ) were dried by distillation over sodium hydroxide. Pluronic block copolymers (BASF Corp., Parsipanny, NJ) were used without additional purification. The molecular characteristics of the block copolymers used in this study are presented in Table 1. Here and below Pluronic block copolymers are designated by their indexes. e.g., "P85" means "Pluronic P85".

* Corresponding author. Telephone: (402) 559−9364. Fax (402) 559−9543. E-mail: akabanov@unmc.edu.
[†] M.V. Lomonosov Moscow State University.
[‡] University of Nebraska Medical Center.

10.1021/ma991634x CCC: $19.00
Published on Web 04/06/2000

Table 1. Characteristics of Pluronic Block Copolymers Studied for This Work

copolymer	MW[a]	av no. of PO units (N_{PO})[b]	av no. of EO units (N_{EO})[b]	HLB[c]	CMC, M[d]	lot no.
L35	1900	16.38	21.59	19	5.3×10^{-3}	WPMQ-592D
L43	1850	22.33	12.61	12	2.2×10^{-3}	WPMS-508B
L44	2200	22.76	20.00	16	3.6×10^{-3}	WPAR-5368
L61	2000	31.03	4.55	3	1.1×10^{-4}	WPYQ-533D
L62	2500	34.48	11.36	7	4.0×10^{-4}	WPCS-502B
L64	2900	30.00	26.36	15	4.8×10^{-4}	WPAQ-561B
F68	8400	28.97	152.73	29	4.8×10^{-4}	WPOP-590B
L81	2750	42.67	6.25	2	2.3×10^{-5}	WSOO-83457
P84	4200	43.45	38.18	14	7.1×10^{-5}	WPMP-547B
P85	4600	39.66	52.27	16	6.5×10^{-5}	WPOP-587A
F87	7700	39.83	122.50	24	9.1×10^{-5}	WPHM-629B
F88	11400	39.31	207.27	28	2.5×10^{-4}	WPAS-575B
L92	3650	50.34	16.59	6	8.8×10^{-5}	WPMR-535B
F98	13000	44.83	236.36	28	7.7×10^{-5}	WPAS-569B
L101	3800	58.97	8.64	1	2.1×10^{-6}	WPHP-547B
P103	4950	59.74	33.75	9	6.1×10^{-6}	WPWQ-557B
P104	5900	61.03	53.64	13	3.4×10^{-6}	WPWQ-505B
P105	6500	56.03	73.86	15	6.2×10^{-6}	WPER-598B
F108	14600	50.34	265.45	27	2.2×10^{-5}	WPON-522C
L121	4400	68.28	10.00	1	1.0×10^{-6}	WPAC-550B
P123	5750	69.40	39.20	8	4.4×10^{-6}	WPIP-620B
F127	12600	65.17	200.45	22	2.8×10^{-6}	WPMN-581B

[a] Average molecular weights provided by the manufacturer. [b] The average numbers of EO and PO units were calculated using the average molecular weights. [c] HLB (hydrophilic−lipophilic balance) values of the copolymers were determined by manufacturer. [d] The cmc values were determined previously using the pyrene probe.[7]

Synthesis of Alkylfluorescein Homologues (CH_3−$(CH_2)_N$−Flu). FITC was dissolved in dry dimethylformamide and allowed to react with a 3-fold molar excess of the corresponding alkylamine. Reaction mixtures were incubated for 20 h at room temperature, and fluorescein derivatives were isolated by preparative thin-layer chromatography on silica gel plates using a mixture of ethanol with chloroform (1:5) as the mobile phase. These conditions allow separation of the product from excess of alkylamine. A fluorescein-containing spot was extracted with methanol, the solvent was evaporated, and the amount of recovered product was determined. In all cases the yields of alkylfluorescein homologues ranged from ca. 40 to 50%. These compounds were analyzed with adsorption chromatography silica gel plates (ethanol−chloroform mixture, 1:5) and mass spectrometry, which revealed no compound admixtures other than the desired alkylfluorescein products.

Partition Coefficient Measurements. All Pluronic block copolymer solutions were prepared by dissolving dry polymer in PBS (phosphate buffered saline, 10 mM phosphate, 150 mM NaCl, pH 7.4) and diluted to the desired concentration (ranging from 10^{-5} to 30% w/v). Both pyrene and CH_3−$(CH_2)_N$−Flu probe solutions were prepared by adding acetone solution of a corresponding probe into empty dry glass vials. After evaporation of acetone, solutions of Pluronic block copolymers of various concentrations were added to the probes. The final concentrations of the probes were 3×10^{-7} M for pyrene and 10^{-6} M for CH_3−$(CH_2)_N$−Flu. Samples were placed in disposable methacrylate fluorescent cuvetts (Fisher Scientific, Pittsburgh, PA) and equilibrated in the dark at 37 °C at least 1 h (3 h for CH_3−$(CH_2)_N$−Flu probes). Fluorescent spectra were recorded at 37 °C using Shimadzu RF5000U spectroluorophotometer at excitation wavelengths 333 nm for pyrene (emission range of 370−390 nm) and 496 nm for CH_3−$(CH_2)_N$−Flu probes (emission range of 500−530 nm). Temperature control within 0.5 °C was achieved using a refrigerated bath/circulator (Fisher Scientific).

Partial Specific Volume. The partial specific volumes of Pluronic block copolymers in PBS were determined for 1% solutions at 37 °C using a pycnometer.

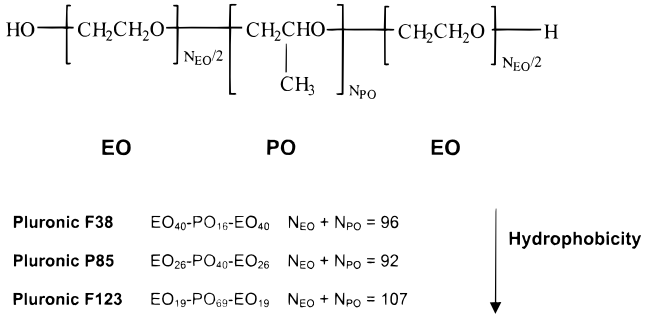


Figure 1. Pluronic block copolymers containing two PEO segments and a PPO segment.

Results and Discussion

Pluronic Block Copolymers. Pluronic block copolymers used for this study are listed in Table 1. These molecules differ in the lengths of the hydrophilic EO and hydrophobic PO segments, as defined by the number of corresponding repeating units, N_{EO} and N_{PO}. The polymers having long PO and short EO chains are hydrophobic and vice versa; polymers with short PO and long EO chains are hydrophilic as schematically shown in Figure 1. The ratio of the lengths of the EO and PO segments determines the hydrophilic−lipophilic balance (HLB). Specifically, the HLB of Pluronic block copolymers is inversely proportional to the portion of the PO segment repeating units and is expressed by the following empirical equation:[7,30]

$$\mathrm{HLB} = -36.0 \frac{N_{PO}}{N_{PO} + N_{EO}} + 33.2 \qquad (1)$$

Determination of Partitioning Coefficients. To describe interactions between micelles of Pluronic block copolymers and low molecular weight solutes, we measured partitioning coefficients of several substances in the system Pluronic/water. By definition, partitioning coefficient stands for the ratio

$$P = \frac{C_m}{C_w} \qquad (2)$$

where C_m and C_w are the solute concentrations in the micelle microphase and aqueous phase, respectively.

Partitioning coefficients of pyrene and alkylfluorescein homologues, CH_3−$(CH_2)_N$−Flu, were determined using previously described techniques.[6,23] For this purpose, fluorescence spectra of the probes in the aqueous solutions containing various concentrations of Pluronic block copolymers were recorded. Fluorescence emission intensities of these probes are sensitive to the polarity of the probe microenvironment. Particularly, upon incorporation of pyrene into the micelles, its fluorescence intensity at 374 nm (usually referred to as I_1) increases.[6] In contrast the fluorescence intensity of CH_3−$(CH_2)_N$−Flu probes at 515 nm gradually decreases in Pluronic solutions upon increase in the block copolymer concentration. Using dependencies of fluorescence intensity of these probes on Pluronic concentration the partitioning coefficients were calculated as described in Appendix 1 for various copolymers listed in Table 1.

Pyrene Partitioning Coefficients. Figure 2 presents the data on pyrene partitioning in aqueous dispersions of various Pluronic block copolymers as a function of log P on the length of the hydrophobic segment

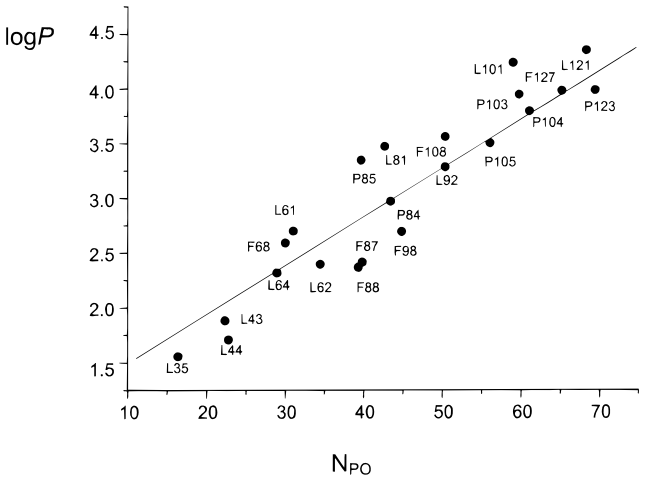


Figure 2. Dependence of the partitioning coefficients of pyrene on the number of PO units in the molecule of Pluronic block copolymer.

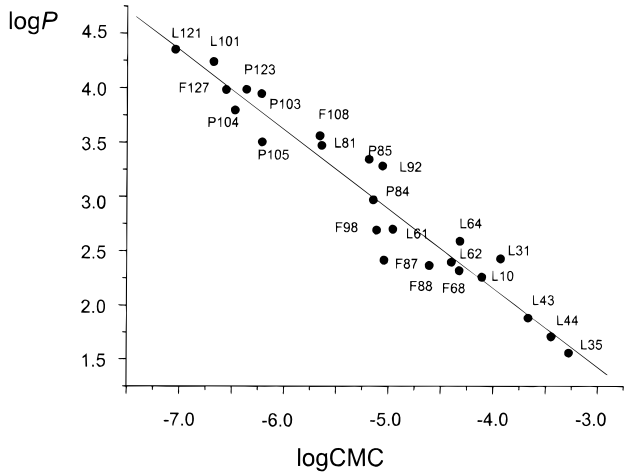


Figure 3. Relationship between the partitioning coefficients of pyrene and cmc in Pluronic block copolymer systems.

(number of PO units, N_{PO}) in the copolymer molecule. This dependence is expressed by the following empirical equation:

$$\log P = 0.05 \log N_{PO} + 0.081 \quad (3)$$

The partitioning coefficient is a thermodynamic parameter, and its value is determined by the properties of the probe and phases between which this probe is partitioned. In our case there were no changes in the properties of the bulk aqueous phase. Therefore, variations in the partitioning coefficients for different Pluronic copolymers were entirely due to the changes in the properties of the micellar microphase. In other words, a strong dependency of pyrene partitioning coefficient on the number of PO units observed in Figure 2 suggests that the micelles undergo structural changes upon variation of this parameter. Indeed, previous studies demonstrated that the lengths of PEO and PPO blocks determine the structure of Pluronic micelles.[17,18,21] On the basis of these reports the influence of the hydrophobic PPO block appears to be more pronounced. Usually the increase in the PPO length is accompanied by the increase in the aggregation number, hydrophobic core size, and solubilization of hydrophobic substances. At the same time, copolymers with a higher fraction of hydrophilic PEO block tend to form micelles having a lower aggregation number, smaller PPO core, and higher concentration of water in the core.[17] As a result, an increase in the PEO content in the block copolymer usually lowers the solubilization of hydrophobes.[17] However, our measurements revealed no clear correlation between log *P* values and the number of EO units in Pluronic molecules (data not presented). One possible reason for this is a lack of sensitivity of our partitioning coefficient measurements as discussed in the next section.

The log *P* vs log Cmc Relationship. Interestingly enough this work establishes a simple linear reciprocal relationship between log *P* and log cmc for various Pluronic copolymers (Figure 3). This relationship is quantitatively expressed as

$$\log P = -0.67 \log \mathrm{cmc} + 0.26 \quad (4)$$

where cmc is expressed in mol/L.

The correlation suggests that both micellization and solubilization of hydrophobes in Pluronic systems are favored in a similar fashion by common factors, particularly, the size of the hydrophobic block. Indeed, experimental relationships between the values of cmc and lengths of the blocks of Pluronic copolymers available in the literature suggest that the number of PO units is a primary factor in the micellization process.[15,16] According to the previous studies log cmc linearly decreases with elevation in the number of PO units; i.e., copolymers with higher content of hydrophobic chains form micelles at lower concentrations. Effects of the PEO block length on micellization is less pronounced and consists of a small increase in cmc with increasing number of EO units. Our measurements support previous conclusions regarding cmc dependencies on N_{PO} and N_{EO} (these dependencies are not presented in figures) and for the first time establish an empirical eq 4 relating log *P* and log cmc. The similarity of the effects of the Pluronic structure on the cmc and partitioning coefficient appears to be one reason for log *P* vs log cmc correlation reported in this study. Since both cmc and partitioning coefficient determine the properties of the pharmaceutical compositions on the base of Pluronic copolymers the empirical relationship (eq 4) might be useful in the optimization of these compositions.[7]

Effect of Partial Specific Volume on the Partitioning Coefficient Determination. The above-described approach based on comparison of the partitioning coefficients for different block copolymers has one major deficiency. As is shown in Appendix 1, to calculate the value of a partitioning coefficient, one should know the partial specific volume (ν) of a given copolymer determined in a separate experiment. Appearance of the partial specific volume in the equations used for determining partitioning coefficients is based on the microphase theory considering Pluronic micelles as a uniform microphase, which does not account for the actual volumes of the micelle core and corona.[6] As is seen in Table 2, the values of the partial specific volume do not differ significantly for Pluronic copolymers having different lengths of PEO and PPO blocks. At the same time, the results available in the literature suggest that Pluronic copolymers of different composition can produce micelles having distinct structures, particularly different volumes of the core and corona.[17−21] Therefore, the partial specific volume generally does not

Table 2. Partial Specific Volumes of Select Pluronic Copolymers

pluronic	L64	F68	L81	P84	P85	F87	P104	F108	P123	F127
ν, cm^3/g	0.8126	0.8726	0.9639	0.952	0.9054	0.8875	0.9061	0.8239	0.9639	0.8787

reflect the actual volume of the hydrophobic microphase where the probe is solubilized. Particularly, in the case of block copolymers with high content of EO units and/or low content of PO units, the volume of the solubilizing microphase can be significantly lower than the net micelle volume. As a result, the use of the partial specific volume can mask the effects of the lengths of the EO and PO blocks on the partitioning characteristics.

Incremental Approach to Characterization of Partitioning. To obtain partitioning parameters independent of the effects on the partial specific volume let us express P as a function of the slope (S) of the curve α^{-1} vs $(C_{Pl} - \text{cmc})^{-1}$ as discussed in Appendix 1:

$$P = (0.01\nu S)^{-1} \tag{5}$$

The logarithmic form of this equation is as follows:

$$\log P = \log \nu - \log(0.01S) \tag{6}$$

In this equation, the first term is entirely determined by the copolymer and is independent of the probe structure.[31] The second term depends on the structure of both the probe and micelle. By varying the solute in the system containing micelles of a given copolymer, one can characterize incremental changes in the second term depending on the solute structure. This will yield thermodynamic characteristics of the partitioning of the solute, which are independent of the partial specific volume of the block copolymer or any similar parameter accounting for the volume of the solubilizing microphase.

Generally, this approach can be applied with a wide range of structurally diverse solutes. In combination with molecular modeling techniques, it might result in establishing comprehensive relationships between parameters characterizing solubilization, structure of block copolymers, and solutes. In this work, we will illustrate this approach using a specific example of homologous alkylfluorescein probes to characterize hydrophobic interactions upon transfer of a methylene group from aqueous solution into the micelle core. Hydrophobic interactions have a major impact in both micellization and solubilization processes in the studied systems.[17−21] A widely accepted standard measure for the hydrophobic interactions is the increment of the free energy of the transfer of a methylene group from water into an organic solvent ($\Delta\mu^{\theta}_{CH_2}$). For water-immiscible organic solvents, this increment usually is about − 3.1 kJ/mol at room-temperature regardless of the nature of the solvent.[24] We postulate that if the micelle core contains a considerable amount of water that can hydrate a methylene group, the $\Delta\mu^{\theta}_{CH_2}$ absolute value should decrease depending on the quantity of water available for hydration. Thus, by measuring the increment of a methylene group transfer from water into the micelle one can evaluate the net "hydrophobicity" of the core.

On the basis of these assumptions, we have chosen a homologous set of $CH_3-(CH_2)_N-$Flu probes as model compounds for studying partitioning characteristics. Calculations of $\Delta\mu^{\theta}_{CH_2}$ values in this case are similar to those applied to characterize partitioning of organic molecules between water and the organic phases.[24] For the ideal solution the chemical potential of the solute is expressed as follows:

$$\mu(T,P,X) = \mu\theta(T,P) + RT \ln X \tag{7}$$

Here, μ^{θ} is the chemical potential of the pure solute (assuming that the solution remains ideal at all dilutions), and X is its molar fraction. For the dilute solutions, expression 7 can be modified

$$\mu(T,P,X) = \mu^{\theta}(T,P) + RT \ln \bar{V}^{o}_{s} + RT \ln C \tag{8}$$

where C is the molar concentration of the solute and $\bar{V}^{o}_{s}$ is the molar volume of the solvent.

Assuming that the net chemical potential of a solute is a sum of the free energy contributions of its hydrophilic groups μ^{θ}(h) and lipophilic groups μ^{θ}(l) and considering the micellar solution as a two-phase system, one can write down

$$\mu_w{}^{\theta} = \mu_w{}^{\theta}(h) + \mu_w{}^{\theta}(l) \tag{9}$$

$$\mu_m{}^{\theta} = \mu_m{}^{\theta}(h) + \mu_m{}^{\theta}(l) \tag{10}$$

where indexes "w" and "m" designate aqueous phase and micelle microphase, respectively.

Combining eqs 9, 10, and 8 yields

$$\mu_w = \mu_w{}^{\theta}(h) + \mu_w{}^{\theta}(l) + RT \ln \bar{V}^{o}_{w} + RT \ln C_w \tag{11}$$

$$\mu_m = \mu_m{}^{\theta}(h) + \mu_m{}^{\theta}(l) + RT \ln \bar{V}^{o}_{m} + RT \ln C_m \tag{12}$$

Since at the equilibrium $\mu_w = \mu_m$

$$RT \ln[\bar{V}^{o}_{w}/\bar{V}^{o}_{m}] - [\mu_m{}^{\theta}(h) - \mu_w{}^{\theta}(h)] - [\mu_m{}^{\theta}(l) - \mu_w{}^{\theta}(l)] = RT \ln P \tag{13}$$

where P is the partitioning coefficient as determined by eq 2.

In the case of aliphatic homologous solutes, e.g., $CH_3-(CH_2)_N-$Flu probes, one can express the chemical potential of a hydrocarbon radical as a sum of the free energy increments for methylene groups ($\mu^{\theta}_{CH_2}$) and the terminal methyl group ($\mu^{\theta}_{CH_3}$):

$$\mu^{\theta}(l) = N\,\mu^{\theta}_{CH_2} + \mu^{\theta}_{CH_3} \tag{14}$$

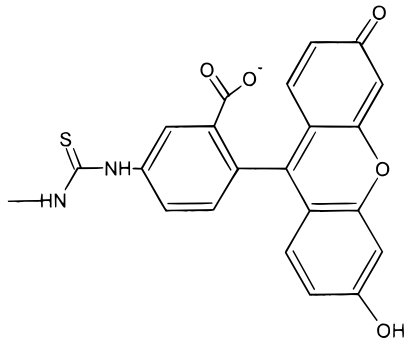
Further, if we designate $\Delta\mu = \mu_m - \mu_w$

$$\log P = \log \frac{\bar{V}^{o}_{w}}{\bar{V}^{o}_{m}} - N \frac{\Delta\mu^{\theta}_{CH_2}}{2.3RT} - \frac{\Delta\mu^{\theta}_{CH_3}}{2.3RT} - \frac{\Delta\mu^{\theta}(h)}{2.3RT} \tag{15}$$

This suggests that the plot of log P vs N is a straight line with a slope equal to $\Delta\mu^{\theta}_{CH_2}/2.3RT$. Thus, by performing measurements on a set of homologues $CH_3-(CH_2)_N-$Flu, one can determine the $\Delta\mu^{\theta}_{CH_2}$ value.

$CH_3-(CH_2)_N-$Flu Homologues. We synthesized several fluorescent probes of the common formula $CH_3-(CH_2)_N-$Flu, where N varies from 2 to 7, and Flu designates the following fluorescent residue:

Flu=



Fluorescence excitation and emission spectra of these probes in aqueous solutions as well as in organic solvents (e.g., methyl and ethyl alcohol) were independent of the hydrophobic radical length (data not shown). As is seen in Figure 4, the fluorescence intensity of the probe decreased with a decrease in the polarity of the solvent, reaching zero in such solvents as octanol-1 and PPO (MW 2000). Decrease in the fluorescence intensity is due to a decrease in excitation efficacy of the fluorophore in a nonpolar environment.[25] Likewise, no fluorescence was observed in 30% (w/v) and more concentrated aqueous solutions of P85 for all $CH_3-(CH_2)_N-$Flu probes studied in this work. However, in less concentrated solutions of the block copolymer, for example, 1% P85, the fluorescence of the probes was registered. In these systems there was a strong dependency of fluorescence on the length of the alkyl radical of the probe. As is seen in Figure 5, fluorescence intensity of the probe decreased when the length of hydrophobic radical was increased from propyl to heptyl. This effect is obviously due to incorporation of the probes in the micelles accompanied by a decrease in the fluorescence. The more hydrophobic probe ($CH_3-(CH_2)_6-$Flu) has higher partitioning coefficient than the less hydrophobic one ($CH_3-(CH_2)_2-$Flu). As a result, a larger portion of this probe becomes entrapped in the micelles at a given concentration of the block copolymer. Figure 6 shows a typical dependency of the fluorescence intensity on the block copolymer concentration observed for $CH_3-(CH_2)_N-$Flu probes. The portion of the probe incorporated in the micelles increases when the concentration of the surfactant (and the volume fraction of the micelles) elevates inhibiting the fluorescence.[6] In most cases studied the concentration of the block copolymers corresponding to a complete fluorescence inhibition (i.e., complete incorporation of the probe in the PPO microphase) was too high for a micelle solution to exist. Under these conditions, Pluronic gels were formed. On the basis of the fluorescence measurements performed in PPO, we assumed that the fluorescence is completely inhibited upon incorporation of the probe in the PPO core of the micelles. This assumption allowed setting the fluorescence of the micelle-incorporated probe to zero and was used in calculations of the partitioning coefficients according to Appendix 1. As discussed in the next section, in certain cases the micelle interior could be too hydrophilic to cause changes in the fluorescence of the probe, which results in a limitation of the use of the described approach.

Incremental Changes in Partitioning of $CH_3-(CH_2)_N-$Flu Probes in Pluronic Systems. Figure 7 presents typical dependencies of the partitioning coefficients of $CH_3-(CH_2)_N-$Flu probes on the length of the hydrocarbon radical. As predicted by eq 15, straight lines should describe these dependencies. In reality,

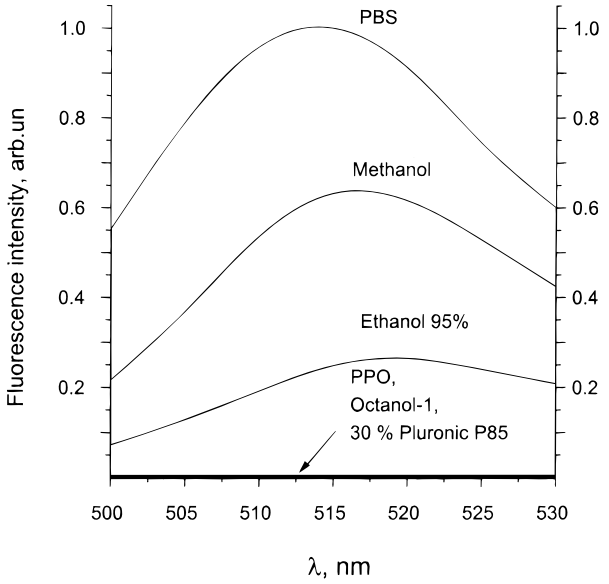


Figure 4. Emission spectra of $CH_3-(CH_2)_3-$Flu probe in PBS, methanol, 95% ethanol, propylene glycol (MW 2000), octanol-1, and 30% P85 in PBS. Excitation wavelength: 496 nm.

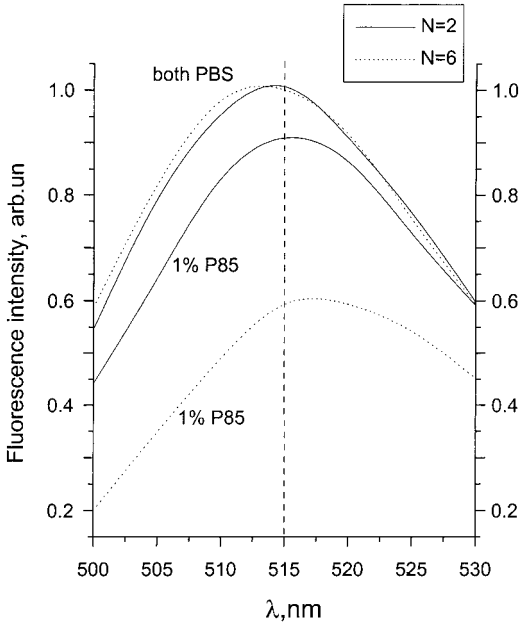


Figure 5. Figure emission spectra of $CH_3-(CH_2)_2-$Flu and $CH_3-(CH_2)_6-$Flu probes in PBS and 1% P85 solution in PBS.

however, linear dependencies were observed for a limited range of aliphatic segment lengths, which was dependent on the type of the block copolymer studied (Figure 7). This is summarized in the schematic presented in Figure 8 dividing Pluronic block copolymers in four groups. In the first group (part a), which includes relatively hydrophobic copolymers (i.e., HLB from ca. 8 to 13) having long PPO segments, the linearity was observed for all probes with N ranging from 2 to 7. In the second group (part b), including copolymers with long or intermediate PPO segments and HLB ranging from 15 to 22, probes with $2 \leq N \leq 6$ fitted the linear dependence, while the probe with $N = 7$ deviated from it. In the third group (part c), including hydrophilic copolymers (i.e., HLB 27−28) with long PPO segments, the probes with short ($N = 2$) and long ($N = 7$) alkyl tails did not fit linear dependence, while intermediate

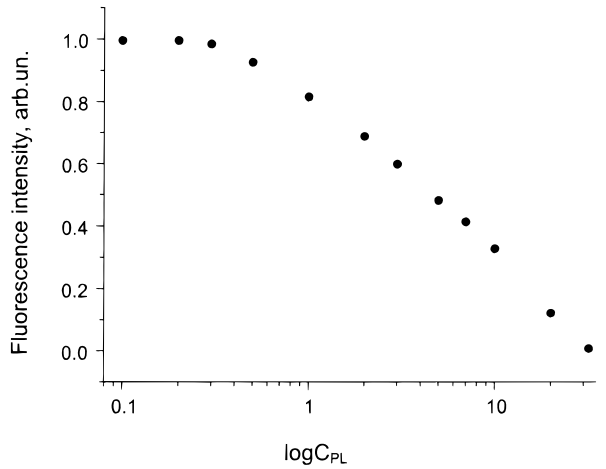


Figure 6. Dependence of fluorescence intensity of $CH_3-(CH_2)_2-Flu$ probe on the concentration of P85 in PBS.

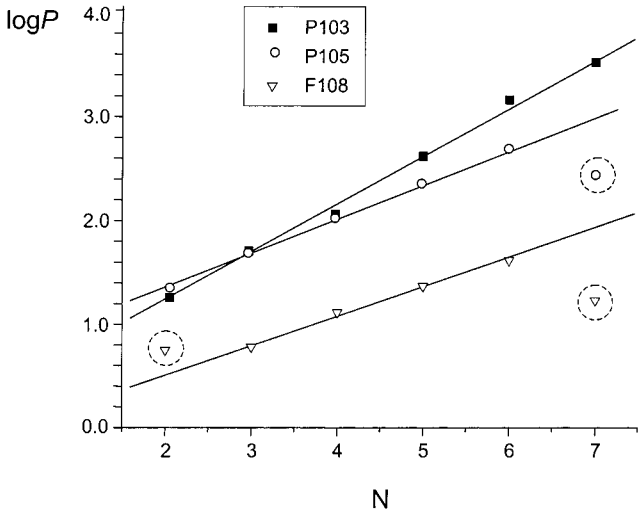


Figure 7. Partitioning coefficients of $CH_3-(CH_2)_N-Flu$ probes vs the number of methylene groups in the alkyl radical obtained for P103, P105, and F108.

probes with $3 \leq N \leq 6$ did. Finally, in the fourth group (part d), including the block copolymer with the shortest PPO segments or hydrophilic copolymers with PPO segments of intermediate length, no changes in fluorescence of $CH_3-(CH_2)_N-Flu$ probes was observed in the copolymer solutions compared to copolymer-free solutions.

Anomalous behavior of $CH_3-(CH_2)_7-Flu$ with the copolymers of second and third groups cannot be attributed to self-assembly of the probe molecule into micelles in aqueous solution. Indeed, the concentration of this probe in the fluorescence studies was 10^{-6} M, which is much less than cmc of anionic surfactants having the same alkyl radicals (e.g., sodium octyl sulfate cmc $= 1.3 \times 10^{-1}$ M[26]). Furthermore, absence of deviations from linearity in the case of the block copolymers of the first group indicates that those effects are related to the structure of the Pluronic micelles, rather than properties of the probe alone. It is evident from Figure 8 that those deviations increase when the portion of EO units in the copolymer increases (i.e., more hydrophilic copolymers) or when the PO segments become shorter. According to ref 22, copolymers of second and third groups form spherical micelles with a smaller core size and/or larger corona size than the copolymers of the first

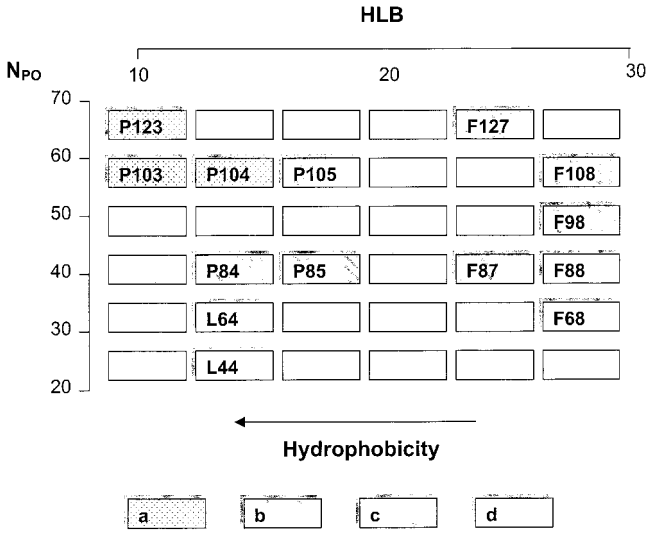


Figure 8. Incremental changes of log *P* upon variation of the alkyl radical length: (a) linearity was observed for all probes with *N* ranging from 2 to 7; (b) probes with $2 \leq N \leq 6$ fitted the linear dependence, while the probe with $N = 7$ deviated from it; (c) the probes with short ($N = 2$) and long ($N = 7$) alkyl tails did not fit the linear dependence, while the probes with $3 \leq N \leq 6$ did; (d) no partitioning of $CH_3-(CH_2)_N-Flu$ probes in the micelles was observed.

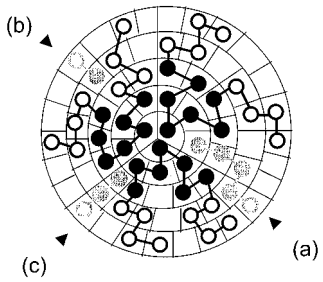


Figure 9. Schematic presentation of Pluronic micelle using spherical lattice model described in refs 17−19. Hydrophobic PO units (black filled circles) localize in the central part of the micelle, while hydrophilic EO units (black empty circles) and water molecules (empty cells) fill external layers. Parts a−c show incorporation of a solute (probe) having alkyl radicals of varying length (gray filled circles) and a polar fluorescent group (gray empty circle). Note unfavorable contacts between hydrophobic groups of the solute and water molecules or EO units in the case of the probes with shorter radicals.

group, which form either spherical or cylindrical micelles. Clearly "core" and "corona" are conditional terms, particularly in the case of Pluronic block copolymers where hydrophobicity and polarity of segments are not that dramatically different as, for example, in the case of polystyrene-*b*-PEO.[27] EO and PO oxide units of the block copolymer are distributed within the micelle which also contains water molecules (Figure 9). Hydrophobic PO units are distributed closer to the center of the micelle (core), while hydrophilic EO units tend to be localized in the external layers (corona). The portion of water molecules increases from the core to the surface. However, the core is also hydrated and in certain cases volume fraction of water in the central layers can be as much as 40%.[17−19]

We hypothesize that if the core is too small the probes with long alkyl radicals ($N = 7$) experience steric difficulties in accommodating in it and become exposed in the corona hydrophilic areas coming in contact with EO and water (Figure 9a). Also, it is possible that the

Table 3. Increment of the Free Energy of Methylene Group Transfer from Aqueous Solution to Pluronic Micelle, $\Delta\mu^{\theta}_{CH_2}$

pluronic	$\Delta\mu^{\theta}_{CH_2}$, kJ/mol	pluronic	$\Delta\mu^{\theta}_{CH_2}$, kJ/mol
L44	n.a.[a]	P98	-1.75 ± 0.12
L64	-1.31 ± 0.12	P103	-3.00 ± 0.14
F68	n.a.	P104	-2.53 ± 0.08
P84	-2.10 ± 0.09	P105	-2.10 ± 0.10
P85	-2.00 ± 0.07	F108	-1.87 ± 0.18
F87	-1.60 ± 0.13	P123	-2.75 ± 0.11
F88	n.a.	F127	-2.55 ± 0.06

[a] Not available.

core of cylindrical micelles (i.e., P103 and P123) is better suited for accommodation of long alkyl groups than that of spherical micelles. This could explain apparent decrease in the partitioning coefficients of $CH_3-(CH_2)_7-$Flu in the case of copolymers of second and third group.

On the other hand, if the corona is too large the probes with short alkyl radicals ($N = 2$) cannot incorporate into the micelle without placing a portion of the alkyl chain in a relatively hydrophilic area and/or inserting charged fluorescein group in relatively hydrophobic areas (Figure 9b). If this is the case, then initial increases in the length of the alkyl chains should not produce a marked increase in the partitioning coefficient because extra methylene groups will be needed to stretch the probe molecule from hydrophilic to hydrophobic layers. However, when the alkyl radical becomes long enough every added methylene group incorporates in the core resulting in an incremental increase in the partitioning coefficient (Figure 9c).

Finally, in the case of hydrophilic block copolymers of the fourth group the size of the core may be insufficient to incorporate any of the $CH_3-(CH_2)_N-$Flu probes studied. Indeed, one copolymer of this group, L44, has the lowest molecular weight (2200) and the highest cmc (=0.79%) of all block copolymers studied in this work. Most likely L44 forms micelles which can incorporate very hydrophobic probes such as pyrene (as shown by changes in pyrene fluorescence), but do not incorporate less hydrophobic $CH_3-(CH_2)_N-$Flu probes. Two other representatives of the fourth group are the very hydrophilic copolymers F68 and F88. This study suggests that the partitioning coefficients observed with these copolymers are low even in the case of pyrene (Figure 2) and may be too low to allow solubilization of $CH_3-(CH_2)_N-$Flu probes. This conclusion is consistent with the earlier reported study on solubilization of 1,6-diphenyl-1,3,5-hexatriene in Pluronic dispersions suggesting that F68 forms premicellar aggregates which do not have well-defined hydrophobic cores necessary for incorporation of nonpolar compounds.[21] Furthermore, in the case of the copolymers of the fourth group the entire micelle interior may be too hydrophilic to cause significant changes in the probe fluorescence even if the probe incorporates into the core. As a result, for those copolymers this method is not applicable.

Increment of One Methylene Group Transfer. Despite the limitations of the incremental approach described in the previous section, the values of $\Delta\mu^{\theta}_{CH_2}$ can be easily determined for the copolymers of the first three groups from the linear portions of the partitioning coefficient dependence on the length of the alkyl radical. These values are presented in Table 3. In all cases, absolute values of $\Delta\mu^{\theta}_{CH_2}$ are lower than that of the increment of the free energy of transfer of a methylene group from water to octanol-1 ($\Delta\mu^{\theta}_{CH_2} = -3.1$ kJ/mol). This suggests that the cores of Pluronic micelles are less "hydrophobic" than the environment of a typical nonpolar solvent. At the same time, these values are lower than the free energy of transfer of one PO group from aqueous medium into a micelle core, which is in the range of -0.8 to -0.5 kJ/mol as estimated based on the data from ref 8. This is consistent with the conclusion that the hydrophobicity of a methylene group is several times higher than that of a PO group.[8]

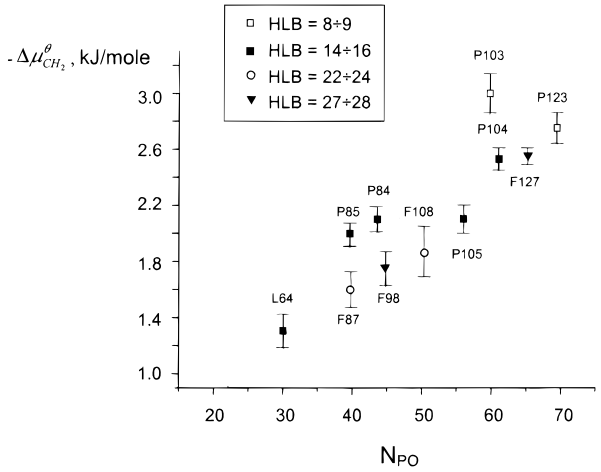


Figure 10. Dependence of the increment of methylene group transfer from water into the micelle ($\Delta\mu^{\theta}_{CH_2}$) on the length of PPO block for groups of block copolymers having comparable HLB.

Another conclusion is that the $\Delta\mu^{\theta}_{CH_2}$ value strongly depends on the chemical structure of the block copolymer, particularly on the lengths of PPO segments. Figure 10 presents the dependence of $-\Delta\mu^{\theta}_{CH_2}$ on the number of PO units in the Pluronic block copolymers. One general tendency observed is that the absolute value of free energy increment increases with the length of PPO segments. In other words, micelles formed by copolymers with longer hydrophobic chains exhibit greater potency for solubilization of a methylene group. This tendency is seen for the group of copolymers having the same or close values of HLB, such as L64, P85, P84, P105, P104 (HLB = 14−16), F87, F108 (HLB = 22−24), and F98, F127 (HLB = 27−28). The only exception is the pair of more hydrophobic copolymers P103 and P123 (HLB = 8−9), for which $\Delta\mu^{\theta}_{CH_2}$ slightly decreased when the length of the PPO segments increased.

Figure 11 illustrates the effect of the length of the PEO segment on $-\Delta\mu^{\theta}_{CH_2}$ for three groups of copolymers having comparable lengths of PPO blocks: P84, P85, P87 ($N_{PO} = 40-43$); P103, P104, P105 ($N_{PO} = 56-61$) and P123 and P127 ($N_{PO} = 65-69$). These data show that $-\Delta\mu^{\theta}_{CH_2}$ decreases with the increase in the hydrophilic segment length, although this dependence is less dramatic than $-\Delta\mu^{\theta}_{CH_2}$ vs N_{PO} dependence. Smallest changes in $-\Delta\mu^{\theta}_{CH_2}$ were observed for the copolymers having the longest PPO chains, P123 and P127.

Overall, these data suggest that transfer of the methylene group from water into the micelle becomes more favorable when the length of the PPO chain increases or the length of PEO decreases. This conclusion is consistent with the model presented in Figure 9. An increase in PPO chain length leads to an increase in the size of the core and a decrease in the amount of

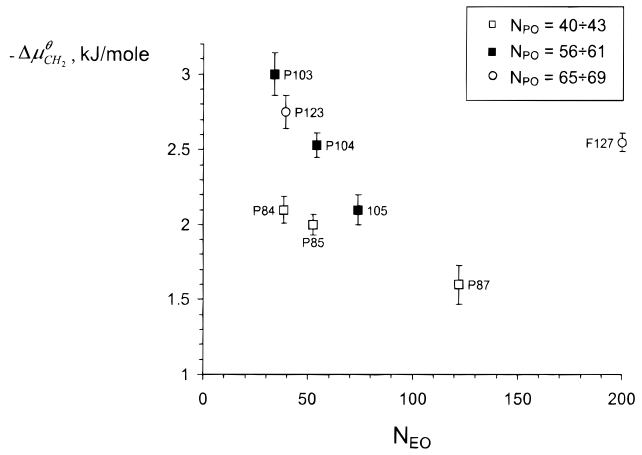


Figure 11. Dependence of the increment of methylene group transfer from water into the micelle ($\Delta\mu^{\theta}_{CH_2}$) on the length of PEO block for groups of block copolymers having comparable lengths of PPO block.

water in the inner layers of the micelle.[17−19] As a result, the environment of the methylene group of the solute becomes more hydrophobic enhancing solubilization. It is possible that these effects are less pronounced for the cylindrical micelles formed by P103 and P123. Indeed the size of the micelle core in this group changes less than 10% while the size changes in other groups are more substantial.[22] Conversely to the effect of the PPO block, increases in PEO length enhances distribution of EO units and water molecules into the core of the micelles. As a result the environment of the methylene group becomes more hydrophilic, which decreases solubilization. In the case of copolymers having the longest PPO segment, P123 and P127, the effect of an increase in the PPO chain length on the core size is much less pronounced than with two other copolymer groups presented in Figure 11.[22] As a result there is practically no difference in the free energy of solubilization.

In conclusion, the results of this study concerning the hydrophobicity of micelle core size are consistent with previous theoretical and experimental work studying solution behavior and solubilization in Pluronic systems.[15−22] The model of Pluronic micelle described in those studies includes the core, intermediate region, and hydrophilic corona. Theory is developed allowing prediction of the distribution functions of PO, EO units, and water molecules in the micelle layers, which depend on the molecular structure of the block copolymer. This experimental work supports those theoretical considerations. There is a fundamental relationship between the partitioning of the hydrophobic solute and micelle formation in these systems. Both processes are favored by the hydrophobic interactions in the micelle inner layers, which increase when PPO length increases or PEO length decreases.

Acknowledgment. M.Y.K. work on this project in UNMC (Omaha, NE) was supported by the Presidential Fellowship from the Russian Federation. This study was supported in parts by NIH Grant R01 NS36229-01A1, NSF Grant DMR-9502807 and Nebraska Research Initiative Drug Delivery Program. Discussion of the results between international group of collaborators was greatly facilitated by NSF International Collaboration Grant DMR-9617837. Pluronic block copolymers were a generous gift of BASF Corp. (Parsipanny, NJ). Authors are pleased to thank Dr. T. Bronich (Omaha, NE) for valuable and stimulating discussions. The Nebraska Center for Mass Spectrometry (Lincoln, NE) is acknowledged for carrying out analyses of the probes.

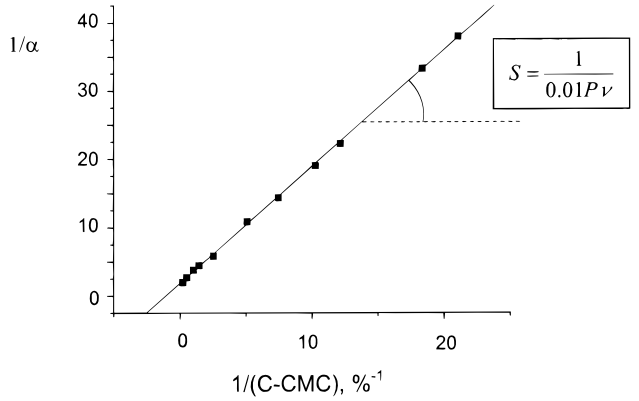


Figure 12. Linear 1/α vs 1(C_{PL} − cmc) plot used for determining the partitioning coefficient, *P*.

Appendix: Determination of Partitioning Coefficients

Partitioning coefficients were determined using previously described method.[6] For this purpose, the portion of the probe incorporated in micelles (α) was determined as

$$\alpha = \frac{I - I_0}{I_\infty - I_0} \tag{16}$$

where I stands for the current value of fluorescence intensity, I_o is the initial level of fluorescence (no polymer), and I_∞ is the fluorescence at saturating concentrations of the polymer. (Fluorescence intensities are measured at emission wavelengths of 374 and 515 nm for pyrene and $CH_3-(CH_2)_N-$Flu probes respectively.)[32]

At the same time α can be expressed as

$$\alpha = \frac{P\Theta}{P\Theta + 1 - \Theta} \tag{17}$$

where P is the partitioning coefficient and Θ is the volume fraction of the micelle phase in solution at a given concentration. The volume fraction can be expressed using partial specific volume of a given copolymer, ν

$$\Theta = 0.01(C_{Pl} - \text{cmc})\nu \tag{18}$$

where C_{Pl} is total Pluronic concentration and cmc is the critical micelle concentration, respectively, both expressed in % w/v.

Partial specific volume ν is a thermodynamic parameter, which defines various intermolecular interactions.[28] It is usually calculated from density measurements at a given copolymer concentration C_{Pl}:

$$\nu = 0.01\rho_{\text{solvent}}{}^{-1}[1 - (\rho_{\text{solution}} - \rho_{\text{solvent}})/C_{Pl}] \tag{19}$$

Combining expressions 17 and 18, one can obtain the following equation:

$$\frac{1}{\alpha} = \frac{1}{0.01P\nu(C_{P1} - \mathrm{cmc})} + 1 - \frac{1}{P} \qquad (20)$$

Plotting dependence of α vs $C_{Pl} - \mathrm{cmc}$ in double inverse coordinates yields a straight line with the slope equal to $1/(0.01P\nu)$, which is used to determine the partitioning coefficient (Figure 12).

References and Notes

(1) Cammas, S.; Kataoka, K. In *Solvents and Self-Organization of Polymers*; Webber S. E., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1996; pp 83−113.
(2) Alakhov, V.Yu; Kabanov, A. V. *Expert Op. Invest. Drugs* **1998**, *7*, 1453−1473.
(3) Allen, C.; Maysinger, D.; Eisenberg, A. *Colloids Surf., B: Biomater.*, in press.
(4) Kabanov, A. V.; Chekhonin, V. P.; Alakhov, V. Yu.; Batrakova. E. V.; Lebedev, A. S.; Melik-Nubarov, N. S.; Arzhakov, S. A.; Levahov, A. V.; Morozov, G. V.; Severin, E. S.; Kabanov, V. A. *FEBS Lett.* **1989**, *258*, 343−345.
(5) Batrakova, E. V.; Han, H.-Y.; Miller, D. W.; Kabanov, A. V. *Pharm. Res.* **1998**, *15*, 1525−1532.
(6) Kabanov, A. V.; Nazarova, I. R.; Astafieva, I. V.; Batrakova, E. V.; Alakhov, V. Yu.; Yaroslavov A. A.; Kabanov V. A. *Macromolecules* **1995**, *28*, 2303−2314.
(7) Batrakova, E. V.; Lee, S.; Li, S.; Venne, A.; Alakhov, V. Yu.; Kabanov, A. V. *Pharm. Res.* **1999**, *16*, 1375−1381.
(8) Wanka, G.; Hoffmann, H.; Ulbricht, W. *Macromolecules* **1994**, *27*, 4145−4159.
(9) Mortensen, K.; Pedersen, J. S. *Macromolecules* **1993**, *26*, 805−812.
(10) Mortensen, K. *Macromolecules* **1993**, *26*, 4128−4135.
(11) Schillen, K.; Brown, W.; Johnsen, R. M. *Macromolecules* **1994**, *27*, 4825−4832.
(12) Schmolka, I. R. *J. Am. Oil Chem. Soc.* **1977**, *54*, 110−116.
(13) Zhou, Z.; Chu, B. *J. Colloid Interface Sci.* **1988**, *126*, 171−180.
(14) Zhou, Z.; Chu, B. *Macromolecules* **1994**, *27*, 2025−2033.
(15) Alexandridis, P.; Holzwarth, J. F.; Hatton, T. A. *Macromolecules* **1994**, *27*, 2414−2425.
(16) Alexandridis, P.; Athanassiou, V.; Fukuda, S.; Hatton T. A. *Langmuir* **1994**, *10*, 2604−2612.
(17) Hurter, P. N.; Scheutjens, J. M. H. M.; Hatton, T. A. *Macromolecules* **1993**, *26*, 5592−5601.
(18) Hurter, P. N.; Scheutjens, J. M. H. M.; Hatton, T. A. *Macromolecules* **1993**, *26*, 5030−5040.
(19) (a) Linse, P. *Macromolecules* **1993**, *26*, 4437−4449. (b) Linse, P. *Macromolecules* **1994**, *27*, 2685−2693.
(20) Nagarajan, R.; Ganesh, K. *Macromolecules* **1989**, *22*, 4312−4325.
(21) Hurter, P. N.; Hatton, T. A. *Langmuir* **1992**, *8*, 1291−1299.
(22) Nagarajan; et al. *Colloids Surf., B: Biomater.*, in press.
(23) Alakhov, V. Yu.; Moskaleva, E. Yu.; Batrakova, E. V.; Kabanov, A. V. *Bioconjugate Chem.* **1996**, *7*, 209−216.
(24) Leo, A.; Hansch, C.; Elkins, D. *Chem. Rev.* **1971**, *71*, 525−616.
(25) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983.
(26) Ananthapadmanabhan, K. P. In *Interactions of Surfactants with Polymers and Proteins*; Goddard, E. D., Ananthapadmanabhan, K. P., Eds.; CRC Press: Boca Raton, FL, 1993; pp 5−58.
(27) Wilhelm, M.; Zhao. C.-L.; Wang, Y.; Xu, R.; Winnik, M. A. *Macromolecules* **1991**, *24*, 1033−1040.
(28) Armstrong, J. K.; Parsonage, J.; Chowdhry, B.; Leharne, S.; Mitchell, J.; Beezer, A.; Löhner, K.; Laggner, P. *J. Phys. Chem.* **1993**, *97*, 3904−3909.
(29) Zhao, J.; Allen, C.; Eisenberg, A. *Macromolecules* **1997**, *30*, 7143−7150.
(30) This is not a standard definition of HLB. However, we found that HLB of Pluronic block copolymers provided by the manufacturer is well approximated by eq 1.
(31) It is noteworthy that the probe concentration used in this work was chosen low enough to avoid possible effects of the probe on the micelle structure. The concentration was 10^{-6} M or less, which usually corresponded to less than one molecule of the probe per micelle.
(32) Some authors use the I_3/I_1 ratio to determine pyrene partitioning in the micelles.[27] However, α can be easily expressed as a function of a physical parameter discriminating free and micellar probe only in the case if this parameter is linearly proportional to the concentration of the probe. While fluorescence intensity of pyrene satisfies this requirement in the conditions of the experiment,[6] the ratio of I_3/I_1 does not. Therefore, strictly speaking, the use of I_3/I_1 for determination of α in expressions similar to eq 16 is not correct. However, the error could be relatively small since in a certain range of copolymer concentration I_3 elevates much faster that I_1, which allows to consider I_3/I_1 as some "normalized fluorescence intensity" proportional to the probe concentration as an approximation.[29]

MA991634X