Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Photochemistry and Photobiology A: Chemistry 168 (2004) 217–226
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

New 4-amino-*N*-alkylphthalimides as fluorescence probes for β-cyclodextrin inclusion complexes and hydrophobic microdomains of amphiphilic systems

Véronique Wintgens*, Catherine Amiel

Laboratoire de Recherche sur les Polymères, LRP, CNRS, UMR 7581, 2-8 rue Henri Dunant, 94320 Thiais, France

Received 5 May 2004; accepted 9 June 2004
Available online 23 July 2004

Abstract

The synthesis and photophysical behaviour of a series of 4-amino-*N*-alkylphthalimides have been described. The complexation between β-cyclodextrin and the different phthalimides has been studied by steady-state fluorescence. The association constant *K* depends strongly on the hydrophobicity of the alkyl substituent, and the *K* values vary between 115 M^{-1} and 19,000 M^{-1}. The studied compounds have been used as fluorescent probes to determine the first and second association constants of surfactants with β-cyclodextrin from competitive binding data. The results are compared with those given by other authors, and limitations of the method are discussed. 4-Amino-*N*-*tert*-butylphthalimide is also used as a sensor for following micellar aggregation process of surfactants and autoassociation of hydrophobically modified polymers. Values of critical micellar concentration (cmc) and critical aggregation concentration (cac) are determined, and comparison is made with pyrene.

Keywords: Fluorescence probe; 4-Amino-*N*-alkylphthalimide; β-Cyclodextrin; Surfactant; Hydrophobically modified polymer

1. Introduction

A large number of fluorescent dyes are used as probes of microenvironments in biological systems or in simpler-organized systems such as micelles and cyclodextrins. Fluorescent electron donor–acceptor molecules display large sensitivity of their emission properties to the polarity of the medium due to the intramolecular charge transfer (ICT) nature of their lowest singlet state; therefore, these compounds are good candidates as fluorescent probes. 4-Amino-phthalimide (AP) shows such an intramolecular charge transfer excited state, and stabilization of the ICT state in polar solvents leads to an important Stokes shift of the fluorescence maximum [1,2]. The fluorescence properties of AP are even more sensitive to the hydrogen bonding properties of the solvents [3]. Therefore, the solvatochromism and hydrogen bonding interaction have made AP and AP derivatives interesting probes to follow the micellar aggregation process [4–6] and the solvation dynamics in micelles [7,8], microemulsion and in the vicinity of a protein [9]. AP binds to α- and β-cyclodextrins with low association constants (respectively, 92 M^{-1} and 208 M^{-1}) [2], and the complex formation leads to a change of the AP fluorescence due to the elimination of water molecules from the surrounding of the probe [2,10]. The solvation dynamics of dimethylformamide inside the nanocavity have also been studied with AP as a probe [11].

Cyclodextrins form inclusion complexes with a wide variety of hydrophobic and amphiphilic species in water solutions. Different methods are used to determine the association constants: conductimetry, electrochemistry, microcalorimetry, NMR spectroscopy, absorption and fluorescence spectroscopies. The last two methods require the presence of a chromophore, but cyclodextrins, and generally the guests, do not bear one. In such cases, an external fluorescent probe is used, and the association constants are obtained by a competitive method. Usual probes (i.e. 1-anilinonaphthalene-8-sulfonate, (aminostyryl)-1-methylpyridinium) carry either a negative or a positive charge, which hinder their use with a guest having the opposite charge, due to electrostatic interactions between the probe and the guest. In order to determine by fluorimetric methods the association constants of cationic, anionic and neutral

* Corresponding author. Tel.: +33 1 49781230; fax: +33 1 49781208.
E-mail address: wintgens@glvt-cnrs.fr (V. Wintgens).

1010-6030/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2004.06.002

guests with cyclodextrins using the same probe, one needs a neutral fluorescent probe; AP could be suggested but its association constant with cyclodextrins is too low. One way to enhance the association constant of AP is to increase the hydrophobicity of the molecule. In this paper, we present the properties of several new 4-amino-*N*-alkylphthalimides, for which the hydrophobicity of the alkyl group leads to a large enhancement (two orders of magnitude) of the association constant with β-cyclodextrin (βCD). We also report the association constant for the 1:1 and 1:2 complexes formed between βCD and different surfactants, using three AP derivatives as fluorescent probes, and we discuss the results in comparison with data in the literature, where different probes have been used depending on the respective charges of the probe and the surfactant [12,13]. On the other hand, we show that one of the compounds, 4-amino-*N*-*tert*-butylphthalimide, is easily used to determine the critical micellar concentration (cmc) of various surfactants, and the critical aggregation concentration (cac) of hydrophobically modified polymers. The results are compared with those obtained with pyrene as a probe.

2. Experimental

2.1. Materials

The different alkyl amines, 4-nitrophthalic acid, 1-octanol (C_8OH), decyltrimethylammonium bromide (DoTAB), dodecyltrimethylammonium chloride (DTAC), sodium dodecyl sulfate (SDS), tetradecyltrimethylammonium bromide (TTAB), hexadecyltrimethyl ammonium bromide (CTAB), Triton X-100 (TX100), pyrene and β-cyclodextrin (βCD) are used as received either from Aldrich or Fluka. All the solvents used are of spectroscopic grade, and water is of deionized quality. The dodecyl modified dextran (DMC12) was synthesized in the LRP laboratory by an esterification reaction between dextran ($M_w = 40{,}000$) and lauroyl chloride in dimethylformamide [14].

2.2. Synthesis

AP derivatives were prepared in a two-step reaction (Scheme 1). 4-Nitrophthalic acid is maintained overnight under reflux in acetic acid with an excess of the alkylamine [15]. The mixture is poured onto water and the obtained nitro derivative is collected by filtration. The reduction is done with stannous chloride in hydrochloric acid [16,17]. The mixture is partly neutralized until the amino derivative precipitates. All the amino derivatives were purified by column chromatography (SiO_2, eluant CH_2Cl_2). Several physical properties and abbreviation names of the AP derivatives are reported in Table 1.

2.3. Sample preparation

For all the complexation experiments, a stock solution of the probe in water (around $2–5 \times 10^{-5}$ M) is prepared and used to make a 10^{-2} M βCD solution in order to have the same probe concentration; then mixtures of these two solutions are used to record the fluorescence at different βCD concentrations (with excitation wavelength located at the isosbestic point to avoid any optical density variation). For the competition experiments, a stock solution of the probe with appropriate βCD concentration is prepared and used to make a concentrated solution of the competitor; again mixtures of these two solutions are used to record the fluorescence at different competitor concentrations. The same procedure with stock solutions is used for the surfactant studies.

For fluorescence quantum yields measurements, solutions of studied compounds in the different solvents are prepared with the same optical density (OD = 0.1) at 360 nm and quinine sulphate is used as a reference ($\Phi_f = 0.55$ in H_2SO_4 1N [18]).

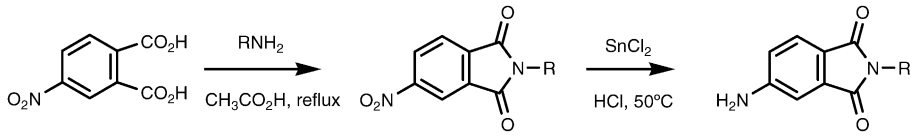
2.4. Instrumentation

The melting points were determined using a Mel-Temp II. The NMR spectra were recorded in deuterated chloroform with a Bruker 200 MHz. The absorption and fluorescence spectra were, respectively, recorded with a Varian Cary 50Bio and a SLM Aminco 8100; the temperature of the samples was maintained at 23 °C using water-jacketed cell holders coupled with a thermocryostat Lauda RM6 bath circulating.

3. Results and discussion

3.1. Properties in homogeneous media

Measured absorption and emission wavelengths of the different AP derivatives in acetonitrile are reported in Table 1. The substituents have almost no influence on the spectroscopic properties; only AP-tBut and AP-1Ada show lower absorption and emission wavelengths. This is attributed to a bulky effect of the amino substituents, which reduce the



Scheme 1. Reaction scheme.

Table 1
Physical characteristic of the studied AP derivatives

Substituent (name)	Yield	Melting point (°C)	RMN $CDCl_3$	λ_{max}^{abs} (nm) CH_3CN	λ_{max}^{fluo} (nm) CH_3CN	Φ_{fluo} CH_3CN
Methyl (AP-Me)	0.46	239–240 247 [17]	3.1 (s,3H); 4.3 (s,2H); 6.75 (dd,1H); 6.95 (d,1H); 7.5 (d,1H)	365	477	0.55
n-Propyl (AP-nPr)	0.67	126–127 120 [33]	0.85 (t,3H); 1.6 (m,2H); 3.55 (t,2H); 4.3 (s,2H); 6.75 (dd,1H); 6.95 (d,1H); 7.5 (d,1H)	366	475	0.56
Isobutyl (AP-isBut)	0.72	135–137	0.85 (d,2H); 2.05 (m,1H); 3.4 (d,2H); 4.3 (s,2H); 6.8 (dd,1H); 7.0 (d,1H); 7.5 (d,1H)	366	474	0.55
tert-Butyl (AP-tBut)	0.20	159–160	1.6 (s, 9H); 4.2 (s,2H); 6.75(dd,1H); 6.9 (d,1H); 7.45 (d,1H)	362	467	0.52
Cyclohexyl (AP-cHex)	0.76	190–191	1.25 (m,3H); 1.7 (m,5H); 2.1 (m,2H); 4.0 (m,1H); 4.3 (s,2H); 6.75 (dd,1H); 6.95 (d,1H); 7.45 (d,1H)	365	475	0.55
1-Adamantyl (AP-1Ada)	0.29	193–194	1.65 (m,6H); 2.05 (s,3H); 2.4 (d,6H); 4.2 (s,2H); 6.75(dd,1H); 6.85 (d,1H); 7.45 (d,1H)	363	467	0.53

planarity of the diimide group. The fluorescence quantum yield is almost constant for the different derivatives in acetonitrile. It is comparable to the one of AP, for which values between 0.55 [3] and 0.63 [1] have been reported in acetonitrile. An important Stokes shift (around 100 nm) is noticed, which is related to a large change in the charge distribution between the ground and the excited states. This indicates an internal charge transfer excited state.

In the case of AP-tBut, we studied the effect of polarity and hydrogen bonding interaction of the solvents on the spectroscopic properties, and results are reported in Table 2. Increase of the polarity of aprotic solvents (scaled by the Reichardt constant $E_T(30)$ in Table 2) leads to a red shift of the emission wavelengths, from 451 nm in 1,4-dioxane to 472 nm in acetonitrile. The effect is less important on the absorption maximum than on the emission maximum, which suggests an excited state more polar than the ground state. The change in charge distribution following the excitation may be quantified by determination of the dipole moment variation between the ground state and the excited state using the Lippert–Mataga equation [19,20]:

$$\nu_{abs} - \nu_{fluo} = \frac{2(\mu_e - \mu_g)^2}{hca^3}\left[\frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}\right] \quad (1)$$

where $(\nu_{abs} - \nu_{fluo})$ is the energy difference between the absorption and fluorescence maxima, $(\mu_e - \mu_g)$ is the difference between the excited and ground state dipole moments, a is the Onsager cavity radius, ε and n are, respectively, the dielectric constant and the refractive index of the solvent. Taking a value of 3.35 Å for the Onsager cavity radius, as in the case of AP [1], the dipole moment variation can roughly be estimated to be 2.5 D. In the case of AP, a value of 3.6 D has been reported [1]. The charge transfer occurs from the amino group to the diimide, and the *tert*-butyl group is more electron donating than the hydrogen atom; therefore a lower value for the dipole moment of AP-tBut than for AP is expected. Almost no change of the fluorescence quantum yield with the polarity is observed in aprotic solvents (average of 0.56) as in the case of AP. In hydroxylated solvents, such as ethanol and water, an important red shift (Table 2) of the emission wavelength is noticed compared to polar and aprotic solvents, for instance, $\lambda_{max}^{fluo} = 520$ nm in ethanol and $\lambda_{max}^{fluo} = 472$ nm in acetonitrile, although acetonitrile and ethanol have almost the same polarity. On the other hand, the unexpected large Stokes shift observed in water (nearly 200 nm) is not attributed to the increase of the solvent polarity, but to the hydrogen bonding interaction between the

Table 2
Spectroscopic properties of AP-tBut in different solvents

Solvent	$E_T(30)$[a]	λ_{max}^{abs} (nm)	λ_{max}^{fluo} (nm)	Φ_{fluo}
1,4-Dioxane	36.0	359	451	0.60
Tetrahydrofuran	37.5	363.5	455	0.55
Dichloromethane	40.7	353.5	462	0.62
Acetone	43.0	364	463	0.52
Acetonitrile	45.6	362	472	0.52
Ethanol	51.9	375	520	0.36
Water	63.1	369.5	569	0.035

[a] Reference [34].

Table 3
Spectroscopic properties of AP derivatives in water and in 10^{-2} M βCD solution and values of the association constants with β-CD

Compound	λ_{max}^{abs} H_2O (nm)	λ_{max}^{abs} β-CD (nm)	$\lambda_{max}^{isosbestic}$ (nm)	λ_{max}^{fluo} H_2O (nm)	λ_{max}^{fluo} β-CD (nm)	$\Phi_{\beta\text{-CD}}/\Phi_{H_2O}$	K (M^{-1})
AP-Me	378	379	376	583	539	5.4	115
AP-nPr	378	378	379	583	535	6.9	480
AP-isBut	379	374	379	583	540	5.4	1400
AP-tBut	370	364	372	568	540	3.2	3100
AP-cHex	377	371	391	578	543	4.2	6200
AP-1Ada		365			540		19,000

carbonyl groups of the phthalimide (which act as hydrogen bonding acceptor) and the hydroxylated solvents (which act as hydrogen bond donor). This interaction is also responsible of the decrease of the fluorescence quantum yield. In water, Φ_{fluo} is almost 20 times less than in the aprotic solvents. Non-radiative deactivation of the excited state through hydrogen bonding [3] has been suggested to explain this specific behaviour in protic solvents.

3.2. Complexation with β-cyclodextrin

AP and AP derivatives are sensitive to the solvent polarity; therefore, the change of the spectroscopic properties can be used to follow encapsulation into cyclodextrin cavities where the interior's polarity is lower than the exterior's one. Complexation of AP with βCD has been studied [2,10] but a low association constant, $K = 208\,M^{-1}$, was reported. Introducing a hydrophobic substituent on the molecule should favour the complexation and should lead to higher association constants.

Addition of various amounts of βCD in aqueous solution of the studied AP derivatives leads to a weak modification of the absorption; but, each series of spectra shows isosbestic points, which indicate formation of a 1:1 complex. The positions of the absorption maxima and of the isosbestic point located at long wavelength in water and in 10^{-2} M βCD solution are given in Table 3. Contrary to the absorption, changes of the fluorescence properties in presence of βCD are marked. A blue shift of the fluorescence spectrum (around 30–50 nm) is observed with the increase of βCD concentration in the aqueous solution. Fluorescence maxima in water and in 10^{-2} M βCD solution are also given in Table 3. Fig. 1 shows the fluorescence spectra of AP-tBut recorded at different βCD concentrations. The fluorescence quantum yields in 10^{-2} M βCD are higher by a factor of three to seven than in water for the different derivatives. This clearly indicates that AP derivatives form inclusion complexes with βCD; in the complex, the probe is in a surrounding that is poor in water molecules, and this causes both the blue shift of the fluorescence and the increase of its intensity.

From the variation of the fluorescence intensity in function of βCD concentration (Insert in Fig. 1) and on the basis of the usual equations for a 1:1 complex:

$$\text{Probe} + \beta\text{CD} \rightleftarrows \text{CD-P}; \quad K = \frac{[\text{CD-P}]}{[\text{Probe}][\beta\text{CD}]_{free}} \qquad (2)$$

where [Probe], $[\beta CD]_{free}$ and [CD-P] are, respectively, the concentrations of the fluorescence probe, the uncomplexed βCD and the probe/βCD complex, the association constants K for the different compounds were determined (Eq. (A.10) in Annexe A) and the values are reported in Table 3. As expected, increase of the bulky character and of the hydrophobicity of the alkyl groups leads to a stronger interaction between the probe and the cavity; there is more than a factor of 50 between the association constants of AP-Me ($115\,M^{-1}$) and AP-cHex ($6200\,M^{-1}$). Such an increase of the association constant was reported in the literature for a series of alkyl alcohols [21]. We suggest that the part of the molecule lying inside the cavity is the one carrying the alkyl group, which is the most hydrophobic part. In the case of AP, Soujanya et al. [10] have proposed that the amino group with the phenyl ring lies inside the cavity, and that the imido part stays outside. Our results show the reverse, but increasing the hydrophobicity of the imido part may changes the way the molecule lies inside the cavity. The 1-adamantyl group is known to well fit the βCD cavity. Even if AP-1Ada is unfortunately insoluble in water, it can be solubilized in presence of βCD. Following the increase of its absorption, or of its fluorescence, in function of βCD concentration allows the determination of the association constant. A value around $19{,}000\,M^{-1}$ is evaluated; this is the highest association constant of the studied compounds, and this again reflects the entry of the bulky alkyl substituent in the βCD cage.

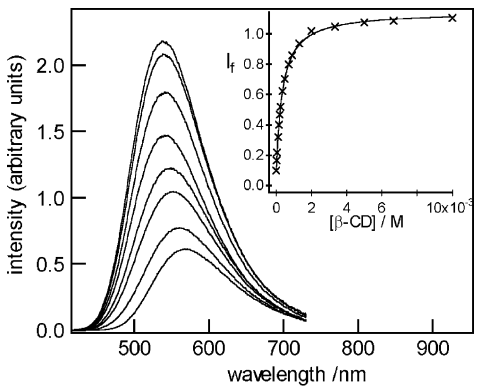


Fig. 1. Change on the AP-tBut emission spectrum on addition of βCD in water. Insert: variation of the fluorescence intensity measured at 500 nm in function of βCD concentration; the solid line represents the fit of experimental data (×) using equation A.10.

3.3. Association constants of non-fluorescent guests with β-cyclodextrin

Indirect spectroscopic methods can be used to determine the association constant of a guest (also called competitor), which does not have a chromophore, such as alkyl alcohols, alkyltrimethylammonium and alkylsulphate ions. In this case, one uses a probe that bears a chromophore and associates with βCD; by competition between the association of the probe and the guest, one determines the association constant of the competitor. Most of the used probe are dyes and exhibit either a positive (ASP [13]) or a negative (ANS [12]) charge; a charged probe cannot be used with a competitor having the opposite charge due to electrostatic attraction, which will disturb the measurements. In this work, the studied probes are neutral, so they can be used whatever the nature of the guest is.

In this type of experiment, one usually works at fixed concentrations of βCD (around 1/*K*) and of the fluorescent probe (in this study, between 2×10^{-5} M and 5×10^{-5} M). Adding the competitor leads to a decrease of the fluorescence intensity of the probe. This decrease is due to complex formation between the competitor and βCD, which results in a depletion of the free βCD concentration, and therefore in the dissociation of the fluorescent probe–βCD complex. Fig. 2 shows the variation of AP-tBut fluorescence intensity in function of the concentration of different competitors. From the fluorescence intensity measurements, and on the basis of both the Eq. (2) and the following one for a 1:1 complex between the competitor and βCD:

$$\text{Comp} + \beta\text{CD} \rightleftarrows \text{CD-1}; \quad K_1 = \frac{[\text{CD-1}]}{[\text{Comp}][\beta\text{CD}]_{\text{free}}} \qquad (3)$$

where [Comp], $[\beta\text{CD}]_{\text{free}}$ and [CD-1] are, respectively, the concentrations of the competitor, the uncomplexed βCD and the competitor/βCD complex, $[\beta\text{CD}]_{\text{free}}$ and [CD-1] can be obtained at each competitor concentration (see Annexe B). The concentration of the competitor/βCD complex, [CD-1], is usually noted as the bonded β-cyclodextrin, $[\beta\text{CD}]_{\text{bond}}$. Then, the association constant K_1 of the competitor with βCD is obtained from the slope of the plot $[\beta\text{CD}]_{\text{bond}}/[\beta\text{CD}]_{\text{free}}$ in function of [Comp] − $[\text{CD}]_{\text{bond}}$ (Eq. (B.5) in Annexe B). Fig. 3 shows the plots obtained for several competitors using AP-tBut as a probe. A straight line crossing the (0,0) point is obtained for guests with an alkyl chain containing less than 12 carbon atoms, and the K_1 values are listed in Table 4; they vary between $1.7 \times 10^3\,\text{M}^{-1}$ and $1.4 \times 10^4\,\text{M}^{-1}$ for C_8OH to DTAC using AP-tBut as a probe. There is a relatively good agreement with the literature data also reported in Table 4, referring only to fluorimetric determinations (data from other methods are available in the cited references). For compounds with an alkyl chain containing more than 12 carbon atoms, the amount of bonded βCD to the competitor is higher than the competitor concentration at the low competitor concentration: the difference $[\text{Comp}]_{\text{tot}} - [\text{CD}]_{\text{bond}}$ is negative as shown in the case of TTAB in Fig. 3. This indicates formation of complexes with different stoichiometries than the presumed 1:1, usually 1:2, but 1:3 and 2:2 have also been proposed [12].

On the basis of the Eqs. (2) and (3) and the following one for a 1:2 complex between the competitor and the βCD:

$$\text{CD-1} + \beta\text{CD} \rightleftarrows \text{CD-2}; \quad K_2 = \frac{[\text{CD-2}]}{[\text{CD-1}][\beta\text{CD}]_{\text{free}}} \qquad (4)$$

where [CD-2] is the concentration competitor/βCD complex with a 1:2 stoichiometry (Annexe C), the K_1 and K_2 association constants can be obtained by nonlinear least-squares regression analysis of the plot of the total competitor concentration, $[\text{Comp}]_{\text{tot}}$, versus $[\text{CD}]_{\text{free}}$ (Eq. (C.10) in Annexe C). Fig. 4 shows the case of TTAB and the obtained K_1 and K_2 values for the different competitors with three different probes are listed in Table 4.

Before further comments on these K_1 and K_2 data, we would like to point out that the choice of the probe is

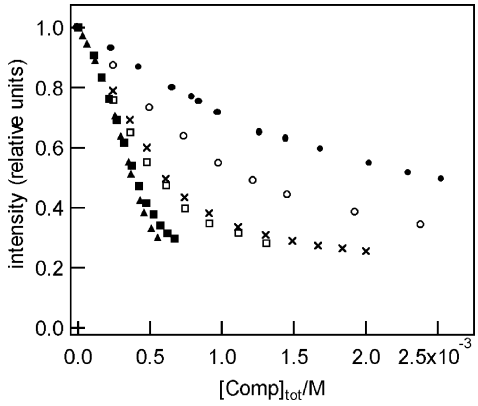


Fig. 2. Variation of AP-tBut fluorescence intensity measured at 500 nm in function of the competitor concentration (●) C_8OH; (○) DoTAB; (×) DTAC; (□) SDS; (■) TTAB; (▲) CTAB.

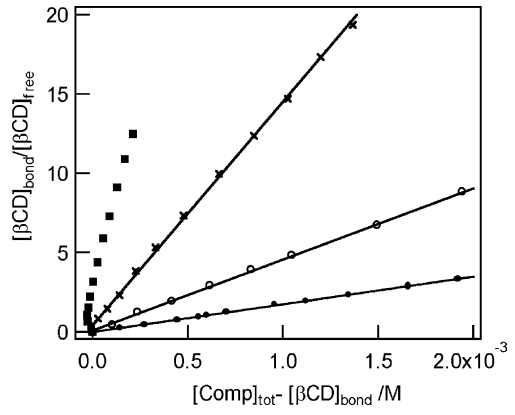


Fig. 3. Variation of the ratio $[\beta\text{CD}]_{\text{bond}}/[\beta\text{CD}]_{\text{free}}$ in function of the difference ($[\text{Comp}]_{\text{tot}} - [\beta\text{CD}]_{\text{bond}}$) at 5×10^{-4} M βCD concentration and with AP-tBut as a probe for C_8OH (●), DoTABr (○), DTAC (×), TTAB (■); the solid lines represent the fits of experimental data using equation B5.

Table 4
K_1 and K_2 values of the studied surfactants determined in the different experimental conditions

Probe [β-CD] (M)		C_8OH	DoTAB	SDS	DTAC	TTAB	CTAB
AP-isBut 10^{-3}	K_1	1920	5100		13,200	59,000	–[b]
	K_2	–[b]	230		550	780	1200
AP-tBut 5×10^{-4}	K_1[a]	1750	4500	18,600	14,500	–[b]	–[b]
	K_1	1750	4500	17,800	14,500	47,000	117,000
	K_2	–[b]	165	640	310	1100	590
AP-cHex 2×10^{-4}	K_1		4900		15,000	43,000	87,000
	K_2		–[b]		620	1500	1700
Literature	K_1	1630[c]	3770[c]	25,600[d]	22,100[c]	44,000[c]	59,800[c]
	K_2		<16[c]	69–200[d]	52[c]	118[c]	390[c]

[a] Determined from plots such as those of Fig. 3, otherwise K_1 and K_2 are determined from plots as those of Fig. 4.
[b] No reliable data, see text.
[c] Reference [13].
[d] Reference [12].

indeed crucial for this competition method. Since one usually works with a βCD concentration around $1/K$, then the βCD concentration will depend on the chosen probe and will be as high as K is weak. On the other hand, for the same competitor, experiments done with a higher βCD concentration in the solution will favour formation of complexes with stoichiometry higher than 1:1, and mainly at low competitor concentrations. This is shown in Fig. 5 where $[\beta CD]_{bond}$ is higher than [DTAC] at the low DTAC concentrations and especially for $[\beta CD] = 1 \times 10^{-3}$ M with AP-isBut as probe. Complexes with stoichiometries higher than 1:1 are formed, and the experimental conditions are favourable for K_2 determination (AP-isBut as probe and $[\beta CD] = 1 \times 10^{-3}$ M). At the reverse, working with a lower βCD concentration (AP-cHex as probe and $[\beta CD] = 2 \times 10^{-4}$ M) is suitable for K_1 determination. To get reliable K_1 and K_2 data, it could be necessary to do experiments with different probes.

In the case of C_8OH, no reliable value can be obtained for K_2 even by using the general equation C.10. The alkyl chain is not long enough to give 1:2 complexes. An average value of $1830 \pm 100\,M^{-1}$ is deduced for K_1 from the measurements with two different probes. These results are in good agreement with the literature data.

In the case of DoTABr, SDS and DTAB, average K_1 values of $4800 \pm 300\,M^{-1}$, $18{,}200 \pm 400\,M^{-1}$ and $14{,}200 \pm 800\,M^{-1}$ are obtained from the experiments done with the three different probes. They are in the same range as the reported ones; this is not the case for data obtained for K_2, which are higher by almost one order of magnitude. One of the reasons may be due to our more accurate determinations. We always took into account the concentration of the probe/βCD complex, [CD-P], to calculate $[CD]_{bond}$; this is not done in the cited papers and this could induce errors, since K_2 is determined mainly by the experimental data at low competitor concentration when [CD-P] is not neglected.

In the case of TTAB, the K_1 value is much higher with AP-isBut as probe than with the two others. This is due to the lack of accuracy on these data; the experimental conditions are not optimized for such a determination and the situation is reverse for K_2 (see before). We reasonably suggest average data of $45{,}000 \pm 2000\,M^{-1}$ for K_1 using AP-tBut

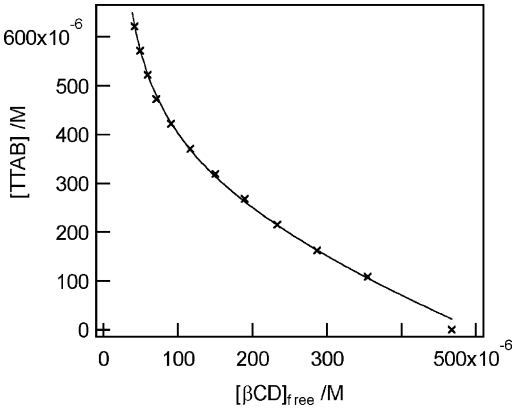


Fig. 4. Plot of TTAB concentration vs. $[CD]_{free}$ at 5×10^{-4} M βCD concentration and with AP-tBut as probe; the solid line represents the fit of experimental data (×) using equation C.10.

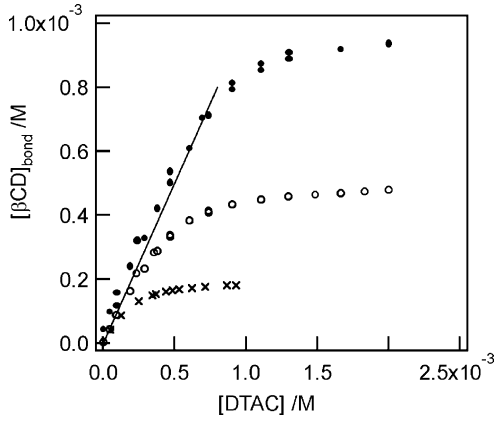


Fig. 5. Plot of $[\beta CD]_{bond}$ vs. [DTAC] in the case of AP-isBut (●) with $[\beta CD] = 1 \times 10^{-3}$ M, AP-tBut (○) with $[\beta CD] = 5 \times 10^{-4}$ M, AP-cHex (×) with $[\beta CD] = 2 \times 10^{-4}$ M.

Table 5
Spectroscopic properties and equilibrium constants of AP-tBut in different surfactants, and values of the surfactants cmc

Surfactant	Concentration (M)	λ_{max}^{fluo} (nm)	Φsurfactant/Φeau	cmc (M)		K_{eq} (M^{-1})
				Measured	Literature	
DTAC	0.10	536	4.3	0.020	0.020[a]	14,000
SDS	0.07	537	4.3	0.0075	0.0080[b]	29,000
CTAB	0.08	528	6.2	0.0008	0.00092[b]	38,000
TX100	0.13	523	7.1	0.00026	0.00026[b]	59,000

[a] Reference [35].
[b] Reference [23].

and AP-cHex as probes, and 950 ± 150 M^{-1} for K_2 using AP-isBut and AP-tBut as probes.

The case of CTAB is even trickier. With AP-isBut, a K_2 value around 1350 ± 150 M^{-1} is obtained with K_1 varying between 1.1 × 10^{-5} M and 4 × 10^5 M^{-1}; this shows that if K_2 can be determined using AP-isBut as probe, no reliable K_1 data can be deduced. On the other hand, the K_1 and K_2 values obtained with the two other probes differ largely; one reason is the formation of complexes with stoichiometries other than 1:1 and 1:2 as suggested by Park and Song [12]. In this case, it is almost impossible to get reliable K_1 and K_2 data if there are more than two stoichiometries for the complexes. Even if other equilibrium equations were added to fit the experimental results, this would lead to more unknown parameters and loss of reliability. One should notice that K_1 data for CTAB is close to 10^5 M^{-1}, a value slightly higher than those previously reported.

3.4. Behaviour in micellar environment

Aggregation of surfactant molecules into micelles can be followed by fluorescence using a probe that responds differently to solvents' polarity. It has been reported that AP shows a sharp change of its spectroscopic properties around the critical micellar concentration [4]. An important blue shift is observed and the fluorescence intensity strongly increases. As AP is a neutral probe, it has been used to study micelles formation of SDS, CTAB, and TX100. Its binding constants to the micelles were reported, they are of the same order of magnitude, 3400–5600 M^{-1}, for these different surfactants. By quenching studies, it has also been deduced that AP is localized at the micelle–water interface. AP derivatives with long fatty acids were also used as probes [5,6]. Due to their properties similar to those of AP, our AP derivatives can also be used to study micelle formation. We report here the results obtained with AP-tBut.

In aqueous solutions of surfactants at concentration larger than cmc, AP-tBut shows a blue shift (29–45 nm) of its fluorescence maximum compared to the value of 569 nm in water. The localization of the probe in a less polar surrounding is responsible for the fluorescence blue shift and for the intensity increase. Table 5 reports fluorescence maxima at specified surfactant concentrations, with the ratio of fluorescence quantum yields in surfactant and in water solutions. The fluorescence intensity is almost constant at low surfactant concentrations (probe in water solution) and suddenly increases when the micelles are formed (probe inside the micelles). This sharp variation of the fluorescence intensity with the surfactant concentration allows the determination of the cmc value. Fig. 6a reports the case of SDS, and the values obtained for the different surfactants are reported in Table 5. A good agreement is noticed between our results and the literature data; this confirms the potentiality of AP-tBut as a fluorescent probe to follow micelle formation.

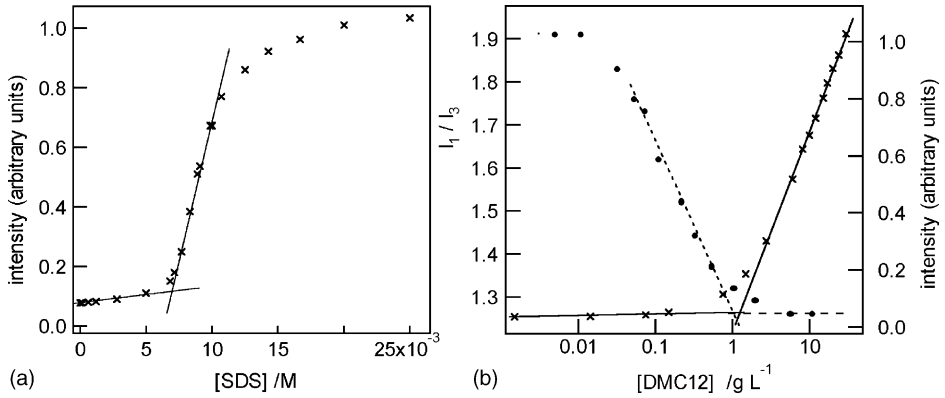


Fig. 6. (a) Variation of AP-tBut fluorescence intensity measured at 500 nm in function of SDS concentration. (b) Variation of AP-tBut fluorescence intensity (×) measured at 500 nm and of the I_1/I_3 ratio of pyrene (●) in function of DMC12 concentration.

An estimation of the equilibrium constants, K_{eq}, for the association of AP-tBut with the surfactants micelle was done using the method suggested by Almgren [22]:

$$\frac{I_\infty - I_0}{I - I_0} = 1 + (K_{eq}[M]^{-1}) \quad (5)$$

where I_∞, I_0 and I are, respectively, the relative fluorescence intensities when all the probe molecules are inside the micelles, in the water or both inside the micelles and in water, [M] is the micelle concentration and is equal to ([surf] − cmc)/N, where [surf] and N are, respectively, the surfactant concentration and the aggregation number of the micelle (we used N values of 60, 62, 60 and 143, respectively, for DTAC, SDS, CTAB and TX100 [23]). The obtained values are reported in Table 5; they vary between $1.4 \times 10^4\ M^{-1}$ and $5.9 \times 10^4\ M^{-1}$. These data are almost one order of magnitude higher than those obtained for AP [4]. This difference is attributed to the higher hydrophobicity of AP-tBut compared to AP; this hydrophobicity of AP-tBut is a determining factor, since it is responsible of the high equilibrium constants with surfactants and of the high association constant with βCD.

Hydrophobically modified polymers usually form hydrophobic microdomains when their concentration increases. Formation of these microdomains can be observed using fluorescent compounds, which have spectroscopic properties depending on the medium. One of these is pyrene, which shows variation of the ratio of the first and third emission bands, I_1/I_3, depending on its surrounding [24,25]. Pyrene properties have been largely used to determine cmc and aggregation number of surfactants [26] and of hydrophobically modified polymers [27]. Some water-soluble dyes, which also respond to the formation of hydrophobic microdomains, have been proposed [28,29].

The spectroscopic properties of AP-tBut allow the detection of hydrophobic microdomains formation. The dextran polymer used in this study owns dodecyl groups (substitution degree around 4%); these hydrophobic groups lead to the formation of polymeric micelles. Fig. 6b reports the fluorescence intensity variation of AP-tBut in function of DMC12 concentration. A strong variation of the intensity is observed at about 1 g/L; this concentration is defined as the critical aggregation concentration corresponding to the onset of microdomains formation [28]. At this concentration, the fluorescence probe is included in the hydrophobic domains. By comparison, an experiment has been done using the ratio I_1/I_3 of pyrene solutions. At low polymer concentrations, the pyrene is located in water as the I_1/I_3 ratio has the same value as in water, around 1.9. With the increase of the polymer concentration, I_1/I_3 stays constant until 0.01 g/L and then decreases over more than two decades of concentration. The transition zone is much broader than the one observed with surfactants, as it is usually observed with amphiphilic copolymers. The cac value is taken at the crossing point between the tangential at the inflexion point and the tangential at the end of the curve [30]; a value around 1 g/L is obtained. There is a fairly good agreement between the cac values determined with the two different probes. However, the sharper transition zone obtained with AP-tBut makes it a better probe for cac determination. Moreover, AP-tBut, compared to pyrene, presents the advantage to be easily solubilized in water solution and samples do not need to be equilibrated for days.

4. Conclusion

In this work, we have shown that substitution of the AP nitrogen by different alkyl substituents does not modify the ICT nature of the excited state but strongly increases the hydrophobicity of the compounds. Therefore, these AP derivatives are promising new fluorescent probes. They form 1:1 complexes with β-cyclodextrin, with values of the association constants covering two orders of magnitude. The neutral character of these probes allows determining the association constants of 1:1 and 1:2 complexes formed between cationic, anionic or neutral surfactants and β-cyclodextrin. It also appears that the choice of the fluorescence probe is a determining factor to get reliable data in the competition method. Nevertheless, the method is not applicable if complexes with more than two stoichiometries are formed, which is the case of surfactants with an alkyl chain containing more than 16 carbon atoms. We have also demonstrated that 4-amino-*N*-*tert*-butylphthalimide is a good sensor of microenvironments and that critical micellar concentration of surfactants and critical aggregation concentration of hydrophobically modified polymers are easily obtained with this sensor.

Appendix A

For a 1:1 complex formation between the probe and βCD, one can write in addition to Eq. (2):

$$[\text{Probe}]_{tot} = [\text{Probe}] + [\text{CD-P}] \quad (A.1)$$

$$[\beta\text{CD}]_{tot} = [\beta\text{CD}]_{free} + [\text{CD-P}] \quad (A.2)$$

where $[\text{Probe}]_{tot}$ and $[\beta\text{CD}]_{tot}$ are, respectively, the total concentration of probe and βCD. In absence of βCD, the initial fluorescence intensity I_0 is proportional to $[\text{Probe}]_{tot}$ and in presence of a large excess of βCD, the fluorescence intensity reaches the limiting value I_∞ where all the probe molecules are complexed. If Φ_{Probe} and $\Phi_{CD\text{-}P}$ are, respectively, the fluorescence quantum yield of the probe and of the 1:1 complex, one can write:

$$I_0 \propto \Phi_{Probe}[\text{Probe}]_{tot} \quad (A.3)$$

$$I_\infty \propto \Phi_{CD\text{-}P}[\text{Probe}]_{tot} \quad (A.4)$$

After each βCD addition, the measured fluorescence intensity I is proportional to:

$$I \propto \Phi_{Probe}[\text{Probe}] + \Phi_{CD\text{-}P}[\text{CD-P}] \quad (A.5)$$

Using the different equations leads to:

$$[\text{CD-P}] = \frac{(I - I_0)}{(I_\infty - I_0)}\,[\text{Probe}]_{\text{tot}} \tag{A.6}$$

$$[\text{Probe}] = \frac{(I_\infty - I)}{(I_\infty - I_0)}\,[\text{Probe}]_{\text{tot}}\,. \tag{A.7}$$

If one may consider $[\text{CD}]_{\text{tot}} \gg [\text{CD-P}]$, the usual Benesi–Hildebrandt equation is obtained [31]:

$$\frac{I - I_0}{[\beta\text{CD}]_{\text{tot}}} = K(I_\infty - I_0) - K(I - I_0) \tag{A.8}$$

If $K > 1000\,\text{M}^{-1}$, this approximation is not valuable and one gets:

$$[\text{CD-P}]^2 - [\text{CD-P}]([\text{Probe}]_{\text{tot}} + [\beta\text{CD}]_{\text{tot}} + 1/K) + [\text{Probe}]_{\text{tot}}[\beta\text{CD}]_{\text{tot}} = 0 \tag{A.9}$$

from which one derives the following relation [32]:

$$I = I_0 + \frac{(I_\infty - I_0)}{2[\text{Probe}]_{\text{tot}}}\left([\text{Probe}]_{\text{tot}} + [\beta\text{CD}]_{\text{tot}} + \frac{1}{K} - \left(\left(4[\text{Probe}]_{\text{tot}} + [\beta\text{CD}]_{\text{tot}} + \frac{1}{K}\right)^2 - 4[\text{Probe}]_{\text{tot}} + [\beta\text{CD}]_{\text{tot}}\right)^{0.5}\right) \tag{A.10}$$

K is obtained by a nonlinear least-squares analysis of the plots, fluorescence intensity versus βCD concentration, keeping I_∞ as a floating parameter; an example of such a fit is given in the insert of Fig. 1.

Appendix B

When a competitor is added to a solution of probe and βCD, one can write in addition to Eqs. (2) and (3):

$$[\beta\text{CD}]_{\text{tot}} = [\beta\text{CD}]_{\text{free}} + [\text{CD-P}] + [\text{CD-1}] \tag{B.1}$$

$$[\text{Comp}]_{\text{tot}} = [\text{Comp}] + [\text{CD-1}] \tag{B.2}$$

where $[\text{Comp}]_{\text{tot}}$ is the total concentration of competitor.

From Eqs. (3), (A.6) and (A.7), $[\text{CD}]_{\text{free}}$ is expressed in function of the fluorescence intensities:

$$[\beta\text{CD}]_{\text{free}} = \frac{[\text{CD-P}]}{K\,[\text{Probe}]} = \frac{(I - I_0)}{K(I_\infty - I)} \tag{B.3}$$

[CD-1], noted as $[\beta\text{CD}]_{\text{bond}}$, is also expressed in function of the fluorescence intensities

$$[\beta\text{CD}]_{\text{bond}} = [\beta\text{CD}]_{\text{tot}} - [\beta\text{CD}]_{\text{free}} - [\text{CD-P}] = [\beta\text{CD}]_{\text{tot}} - \frac{(I - I_0)}{K(I_\infty - I)} - \frac{(I - I_0)}{(I_\infty - I_0)}[\text{Probe}]_{\text{tot}} \tag{B.4}$$

Then one gets:

$$\frac{[\text{CD-1}]}{[\beta\text{CD}]_{\text{free}}} = \frac{[\beta\text{CD}]_{\text{bond}}}{[\beta\text{CD}]_{\text{free}}} = K_1[\text{Comp}] = K_1([\text{Comp}]_{\text{tot}} - [\beta\text{CD}]_{\text{bond}}) \tag{B.5}$$

In most reported studies, [CD-P] is neglected in Eq. (B.4). However, if $K > 1000\,\text{M}^{-1}$, [CD-P] cannot be neglected, especially at low concentration of competitor. In this work, $[\beta\text{CD}]_{\text{bond}}$ has always been calculated using equation B.4. Plot of the ratio $[\beta\text{CD}]_{\text{bond}}/[\beta\text{CD}]_{\text{free}}$ in function of $([\text{Comp}]_{\text{tot}} - [\beta\text{CD}]_{\text{bond}})$ leads to K_1 obtained as the slope of the straight line (see Fig. 2).

Appendix C

If the competitor gives with βCD a mixture of 1:1 (CD-1) and 1:2 (CD-2) complexes, one can write in addition to Eqs. (2)–(4)

$$[\beta\text{CD}]_{\text{tot}} = [\beta\text{CD}]_{\text{free}} + [\text{CD-P}] + [\text{CD-1}] + 2[\text{CD-2}] \tag{C.1}$$

$$[\text{Comp}]_{\text{tot}} = [\text{Comp}] + [\text{CD-1}] + [\text{CD-2}] \tag{C.2}$$

Then

$$[\text{Comp}]_{\text{tot}} = [\text{Comp}] + K_1[\text{Comp}][\beta\text{CD}]_{\text{free}} + K_2(K_1[\text{Comp}][\beta\text{CD}]_{\text{free}})[\beta\text{CD}]_{\text{free}} \tag{C.3}$$

$$[\text{Comp}]_{\text{tot}} = [\text{Comp}](1 + K_1[\beta\text{CD}]_{\text{free}} + K_1K_2[\beta\text{CD}]^2_{\text{free}}) \tag{C.4}$$

$$[\beta\text{CD}]_{\text{bond}} = [\beta\text{CD}]_{\text{tot}} - [\beta\text{CD}]_{\text{free}} - [\text{CD-P}] = [\text{CD-1}] + 2[\text{CD-2}] \tag{C.5}$$

$$[\beta\text{CD}]_{\text{bond}} = [\text{Comp}](K_1[\beta\text{CD}]_{\text{free}} + 2K_1K_2[\beta\text{CD}]^2_{\text{free}}) \tag{C.6}$$

One obtains:

$$[\text{Comp}]_{\text{tot}} = [\beta\text{CD}]_{\text{bond}}\frac{1 + K_1[\beta\text{CD}]_{\text{free}} + K_1K_2[\beta\text{CD}]^2_{\text{free}}}{K_1[\beta\text{CD}]_{\text{free}} + 2K_1K_2[\beta\text{CD}]^2_{\text{free}}} \tag{C.7}$$

$$[\text{Comp}]_{\text{tot}} = ([\beta\text{CD}]_{\text{tot}} - [\beta\text{CD}]_{\text{free}} - [\text{CD-P}]) \times \frac{1 + K_1[\beta\text{CD}]_{\text{free}} + K_1K_2[\beta\text{CD}]^2_{\text{free}}}{K_1[\beta\text{CD}]_{\text{free}} + 2K_1K_2[\beta\text{CD}]^2_{\text{free}}} \tag{C.8}$$

Expressing [CD-P] in function of $[\beta\text{CD}]_{\text{free}}$ leads to a relation where $[\text{Comp}]_{\text{tot}}$ depends only on $[\beta\text{CD}]_{\text{free}}$, which is obtained from the variation of fluorescence intensity; example of such fit is given in Fig. 4.

$$[\text{CD-P}] = K([\text{Probe}]_{\text{tot}} - [\text{CD-P}])[\beta\text{CD}]_{\text{free}} = \frac{K[\text{Probe}]_{\text{tot}}[\beta\text{CD}]_{\text{free}}}{1 + K[\beta\text{CD}]_{\text{free}}} \quad \text{(C.9)}$$

$$[\text{Comp}]_{\text{tot}} = \left([\text{CD}]_{\text{tot}} - [\beta\text{CD}]_{\text{free}} - \frac{K[\text{Probe}]_{\text{tot}}[\beta\text{CD}]_{\text{free}}}{1 + K[\beta\text{CD}]_{\text{free}}}\right) \times \left(\frac{1 + K_1[\beta\text{CD}]_{\text{free}} + K_1K_2[\beta\text{CD}]^2_{\text{free}}}{K_1[\beta\text{CD}]_{\text{free}} + 2K_1K_2[\beta\text{CD}]^2_{\text{free}}}\right) \quad \text{(C.10)}$$

References

[1] T. Soujanya, R.W. Fessenden, A. Samanta, J. Phys. Chem. 100 (1996) 3507–3512.
[2] T. Soujanya, T.S.R. Krishna, A. Samanta, J. Photochem. Photobiol. A: Chem. 66 (1992) 185–192.
[3] A. Morimoito, T. Yatsuhashi, T. Shimada, L. Biczok, D.A. Tryk, H. Inoue, J. Phys. Chem. 105 (2001) 10488–10496.
[4] G. Saroja, A. Samanta, Chem. Phys. Lett. 246 (1995) 506.
[5] G. Saroja, A. Samanta, J. Chem. Soc. Faraday Trans. 92 (1996) 2697–2701.
[6] G. Saroja, B. Ramachandram, S. Saha, A. Samanta, J. Phys. Chem. 103 (1999) 2906–2911.
[7] A. Datta, D. Mandal, S.K. Pal, S. Das, K. Bhattacharya, J. Mol. Liq. 77 (1998) 121–129.
[8] D. Mandal, S. Sen, D. Sukul, K. Bhattacharya, A.K. Mandal, R. Banerjee, S. Roy, J. Phys. Chem. B 106 (2002) 10741–10747.
[9] D. Mandal, S. Sen, D. Sukul, K. Bhattacharya, A.K. Mandal, R. Banerjee, S. Roy, J. Phys. Chem. 106 (2002) 10741–10747.
[10] T. Soujanya, T.S.R. Krishna, A. Samanta, J. Phys. Chem. 96 (1992) 8544.
[11] S. Sen, D. Sukul, P. Dutta, K. Bhattacharya, J. Phys. Chem. A 105 (2001) 10635–10639.
[12] J.W. Park, H.J. Song, J. Phys. Chem. 93 (1989) 6454–6458.
[13] J.W. Park, K.H. Park, J. Inclusion Phenom. Mol. Recognit. Chem. 17 (1994) 277–290.
[14] C. Amiel, C. David, E. Renard, B. Sebille, Polym. Prepr. 40 (1999) 207.
[15] L.R. Caswell, M. Guevara, L.D. Corley, A.V. Martinez, T. Hollis, K. Largess, D.L. Thornley, Synthesis (1992) 823.
[16] L.F. Levy, H. Stephen, J. Chem. Soc. (1931) 79–82.
[17] W. Flitsch, Chem. Ber. 94 (1961) 2494–2501.
[18] W.H. Melhuish, J. Phys. Chem. 65 (1961) 229.
[19] E. Lippert, Z. Naturforsch. 10a (1955) 541–545.
[20] N. Mataga, Y. Kaify, M. Koizumi, Bull. Chem. Soc. Jap. 28 (1955) 690–691.
[21] Y. Matsui, K. Mochida, Bull. Chem. Soc. Jap. 52 (1979) 2808.
[22] M. Almgren, F. Griesser, J.K. Thomas, J. Am. Chem. Soc. 101 (1979) 279–291.
[23] K. Kalyanasundaram, Photochemistry in Microheterogeneous Systems, Academic Press, New York, 1987.
[24] K. Kalyanasundaram, J.K. Thomas, J. Am. Chem. Soc. 99 (1977) 2039–2044.
[25] D.C. Dong, M.A. Winnik, Can. J. Chem. 62 (1984) 2560–2565.
[26] R. Zana, Surfactant solutions: new methods of investigation, Surfactant Science Series 22, New York, 1987, pp. 241.
[27] E. Alami, M. Rawiso, F. Isel, G. Beinert, W. Binana-Limbelé, F. François, Hydrophobic Polymers, Advances in Chemistry Series 248, ACS, Washington DC, 1996.
[28] K. Nakashima, T. Anzai, Y. Fujimoto, Langmuir 10 (1994) 658–661.
[29] M. Hasegawa, T. Sugimura, Y. Suzaki, Y. Shindo, J. Phys. Chem. 98 (1994) 2120–2124.
[30] M. Frindi, B. Michels, R. Zana, J. Phys. Chem 96 (1992) 6095–6102.
[31] H.A. Benesi, H. Hildebrandt, J. Am. Chem. Soc. 71 (1949) 2703.
[32] J. Bourson, J. Pouget, B. Valeur, J. Phys. Chem. 97 (1993) 4552–4557.
[33] G. Saroja, A. Samanta, J. Chem. Soc. Faraday Trans. 94 (1998) 3141–3145.
[34] C. Reichardt, Solvents and Solvent Effects in Organic Chemistry, VCH, Weinheim, 1988.
[35] K.P. Ananthapadmanabhan, Surfactant solutions: absorption and aggregation properties, in: Interactions of Surfactants with Polymers and Proteins, CRC Press, Boca Raton, 1993, pp. 22.