Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Photochemistry and Photobiology A: Chemistry 161 (2004) 125–129
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Photoluminescent properties of Rhodamine 6G in the nanoaggregates of poly(ethylene oxide)-*b*-polymethacrylate in aqueous solutions

Y. Li, L.-J. Ding, Y.-K. Gong, K. Nakashima*

Department of Chemistry, Faculty of Science and Engineering, Saga University, 1 Honjo-machi, Saga 840-8502, Japan

Received 28 February 2003; received in revised form 25 April 2003; accepted 20 May 2003

Abstract

Absorption and fluorescence spectra of Rhodamine 6G (R6G), as well as degree of polarization (*P*) and lifetime of fluorescence, have been measured in order to investigate the usefulness of R6G as a photoluminescent probe for the micelle-like nanoaggregates formed from poly(ethylene oxide)-*b*-polymethacrylate (PEO-*b*-PMA) and counter ions. The counter ions used are cetyltrimethylammonium chloride (CTAC), dibucaine (DC), tetracaine (TC), poly-L-lysine (PLS), and alkaline earth metal ions (Ba^{2+} or Ca^{2+}). The change in the spectrum or *P* value in CTAC/PEO-*b*-PMA and DC/PEO-*b*-PMA systems indicates that R6G is useful for detecting and characterizing the aggregates. However, the fluorescence parameters do not significantly change when TC, PLS, Ba^{2+}, and Ca^{2+} are used as a counter ion. The difference is discussed based on the hydrophobicity of the counter ions and the structures of the aggregates.

Keywords: Rhodamine 6G; Poly(ethylene oxide)-*b*-polymethacrylate; Nanoaggregate; Counter ion; Cetyltrimethylammonium chloride; Anesthetics; Poly-L-lysine; Alkaline earth metal ions

1. Introduction

Photoluminescent properties of dyes in colloidal systems such as surfactant micelles [1–4], amphiphilic block copolymer micelles [5–9], and latex dispersions [10–12] have received much attention for examining the formation and properties of the microheterogeneous structures [13,14]. The use of photoluminescent probe is based on the fact that these microheterogeneous environments provide the probe with the local concentration, distribution, orientation and mobility, which are quite different from those in homogeneous solutions. In the previous works, we have investigated the usefulness of fluorescent dyes for characterizing the polymer micelles and latex particles [7–12]. For example, we found that a remarkable change in the fluorescence parameters (e.g. intensity, degree of polarization, and lifetime) of octadecyl Rhodamine B occurs in the solutions of Pluronic F68 solutions when the polymeric micelles are formed [9].

Recently, we have succeeded in obtaining the micelle-like nanoaggregates of "double-hydrophilic" block copolymer, poly(ethylene oxide)-*b*-polymethacrylate (PEO-*b*-PMA), by insolubilizing PMA block with counter ions, such as alkaline earth metal ions (Ba^{2+} or Ca^{2+}) [15], cetyltrimethylammonium chloride (CTAC) [16], cationic anesthetics dibucaine (DC) and tetracaine (TC) [17], and poly-L-lysine (PLS) [18], etc. Schematic illustration of the aggregate formation is presented in Fig. 1. This kind of aggregate is quite new and seems to be interesting as one of the microheterogeneous systems for photoreactions. In this study, we investigated photoluminescent properties of Rhodamine 6G (R6G) to know the usefulness of this probe for characterizing the micelle-like nanoaggregate of PEO-*b*-PMA.

2. Experimental details

Water was purified with a Milli-Q purification system. R6G (laser grade) was purchased from Aldrich and used as supplied. The concentration of R6G was constant at 2 μM. As the preparation procedure of the nanoaggregate was previously reported in detail [15–18], a brief description about it is given here. Known amounts of PEO-*b*-PMA solutions containing R6G were electrically neutralized by titration with solutions of cations. To show the ratio of added cations to the total amount of carboxylate ion, we

* Corresponding author. Tel.: +81-952-28-8850; fax: +81-952-28-8548.
E-mail addresses: 00ts31@edu.cc.saga-u.ac.jp (Y. Li), nakashik@cc.saga-u.ac.jp (K. Nakashima).

1010-6030/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/S1010-6030(03)00281-8

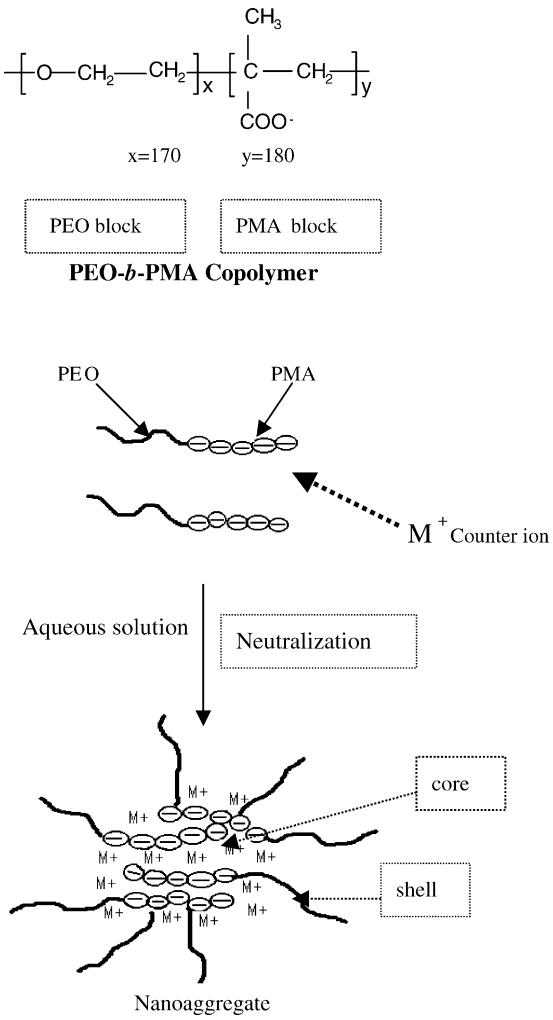

Fig. 1. Structure of PEO-*b*-PMA copolymer and pictorial representation of nanoaggregate formation.

define an "apparent degree of neutralization (DN)" by

$$\mathrm{DN}\ (\%) = \frac{\text{amount of added cations (mol)}}{\text{amount of COO}^- \text{ groups (mol)}} \times 100 \qquad (1)$$

where the cation is CTAC, DC, TC, PLS, Ba^{2+}, or Ca^{2+}. All the experiments were carried out under pH = 6–8, where almost all carboxylic groups of PMA block have an anionic form. The size of the nanoaggregates ranges from 50 to 200 nm and was unchanged for several weeks [15–18].

Absorption spectra were recorded on a Jasco Ubest-50 spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4000 spectrofluorometer. The fluorescence spectra were corrected by the use of a standard tungsten lamp with a known color temperature. Degree of polarization (*P*) was observed with Hitachi F-4000 spectrofluorometer employing a set of film polarizers in the L-format. Fluorescence lifetime measurements were performed with a Horiba NAES 1100 time-resolved spectrofluorometer which uses a time-correlated single photon counting technique.

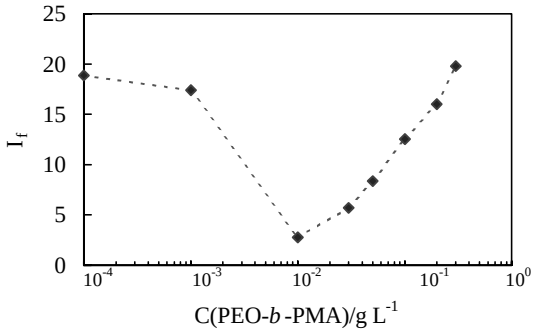

Fig. 2. Dependence of fluorescence intensity of R6G (2 μM) on PEO-*b*-PMA concentration. The samples were excited at 470 nm. The fluorescence intensity is shown by a peak height around 551 nm.

3. Results and discussion

3.1. Effect of PEO-b-PMA on dimer formation of R6G

It is well known that R6G forms a non-fluorescent dimer (or multimer) in solution. Usually the dimer formation is negligible if the concentration of R6G is lower than 10^{-2} M [19]. In the presence of the polymers, however, dimer formation will be enhanced if R6G is bound to the polymers. Fig. 2 shows the dependence of the fluorescence intensity of R6G on PEO-*b*-PMA concentration. At low polymer concentration, most of R6G molecules exist in the aqueous bulk phase, so that fluorescence intensity is nearly equal to that in the aqueous solution. With the increase of the PEO-*b*-PMA concentration, the amount of bound R6G molecules becomes increased. Since R6G and PMA have positive and negative charges, respectively, R6G seems to be preferentially adsorbed on PMA block by electrostatic interaction. On the polymer chains, the R6G undergoes extensive dimer formation due to their higher local concentration. Thus, fluorescence intensity of R6G is decreased, eventually passing through a minimum value at the polymer concentration of 0.01 g/l. If we continue to increase the polymer concentration, the fluorescence intensity is recovered. This suggests that R6G molecules are re-distributed on the polymer chains, resulting in the dissociation of the dimers into the monomers. Concomitantly, the absorption band of R6G monomers increased with decrease in that of the dimers. This also gives us the evidence for the dissociation of dimers into the monomers.

3.2. Photoluminescent properties of R6G in the CTAC/PEO-b-PMA nanoaggregates

Fig. 3 shows absorption spectra of R6G in CTAC/PEO-*b*-PMA aggregates. The absorption due to dimers (490–510 nm) [20] is dominant in the absence of CTAC. When CTAC concentration is increased in the solutions, the monomer absorption (520–540 nm) becomes significant. This suggests

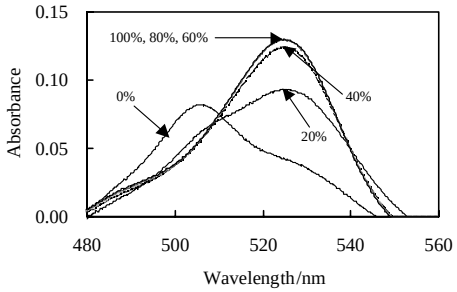

Fig. 3. Absorption spectra of R6G (2 μM) in the solutions of PEO-*b*-PMA at different DN. Counter ion: CTAC. [PEO-*b*-PMA] = 0.01 g/l.

that the dimers are dissociated into monomers on addition of CTAC.

We observed fluorescence spectra of R6G in CTAC/PEO-*b*-PMA aggregates (Fig. 4). It should be noted here that the polymer concentration is constant at 0.01 g/l, which gives minimum fluorescence intensity in Fig. 2. As shown in Fig. 4, the fluorescence intensity increased with increase of DN. When CTAC is added at 30% DN level, the intensity of R6G fluorescence in the nanoaggregates is comparable with that in water, suggesting that R6G dimers are effectively dissociated to the monomers with nanoaggregate formation. However, the intensity is still increased if DN is increased above 30%. We attribute these changes to the increase in the quantum yield of R6G fluorescence due to the increase in viscosity around the probe, which suppresses the deactivation processes such as internal rotation of amino fragments. The increase in the fluorescence intensity above 30% DN is evidence for the fact that the probe molecules are incorporated in the nanoaggregates.

Degree of polarization, *P*, gives information about the micro-viscosity around the probe molecule [21]. It is defined by

$$P = \frac{I_v - I_h}{I_v + I_h} \tag{2}$$

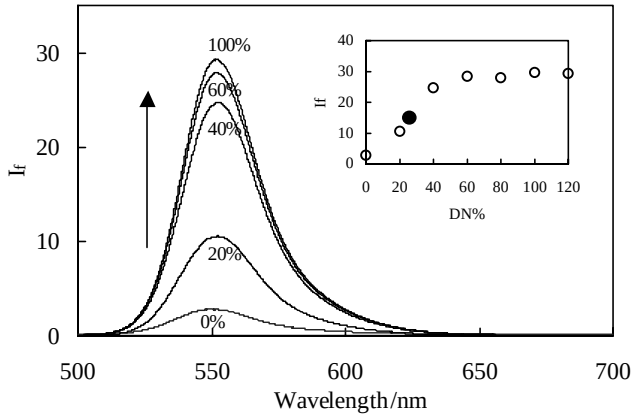

Fig. 4. Fluorescence spectra of R6G (2 μM) in the solutions of PEO-*b*-PMA at different DN with CTAC. [PEO-*b*-PMA] = 0.01 g/l. λ_{ex} = 470 nm. Inset: dependence of fluorescence intensity of R6G (○) on DN in the solutions of PEO-*b*-PMA. [PEO-*b*-PMA] = 0.01 g/l. λ_{ex}=470 nm. The fluorescence intensity of R6G in water (●) is also shown for comparation.

where I_v and I_h are the intensities of the vertically and horizontally polarized emission, respectively, when the sample is excited with vertically polarized light. For a rotating probe molecule in a solution, *P* is related to the viscosity (η) of the environment of the probe as follows:

$$\frac{1}{P} = \frac{1}{P_0} + \left(\frac{1}{P_0} - \frac{1}{3}\right)\left(\frac{kT\tau}{\eta V}\right) \tag{3}$$

where P_0 is the degree of polarization in rigid media, *k* the Boltzmann constant, *T* the absolute temperature, τ fluorescence life time, and *V* is the effective volume of the probe [21]. According to Eq. (2), *P* increases with increase in the viscosity around the probe molecule. *P* also increases with decrease in the fluorescence lifetime of the probe molecule. For the measurements of *P* in the CTAC/PEO-*b*-PMA nanoaggregates, R6G seems to be a suitable probe for two reasons: (1) R6G molecules have been effectively incorporated into the aggregates, and (2) the lifetime of R6G fluorescence is relatively short (ca. 4 ns in water [22]) compared with the rotational diffusion time, resulting in high *P* value. In Fig. 5, we show the dependence of *P* on DN. There is no significant change of *P*, suggesting that CTAC/PEO-*b*-PMA aggregate is loosely packed, so that R6G can rotate as freely as in aqueous bulk phase ($P = 0.02$, in water).

3.3. Photoluminescent properties of R6G in the DC/PEO-b-PMA nanoaggregates

Fluorescence spectra of R6G in DC/PEO-*b*-PMA aggregates are shown in Fig. 6. On adding DC to polymer, the fluorescence intensity is decreased. Above 20% DN, intensity is recovered to some extent. However, it is still lower than that in the absence of DC. This seems to be due to that R6G is quenched by DC. We also note the significant red shift compare with that in CTAC/PEO-*b*-PMA system. This will be discussed later.

To obtain an insight into the quenching of R6G fluorescence by DC, we observed the fluorescence decay curves of R6G in DC/PEO-*b*-PMA systems. The decay curves of R6G in PEO-*b*-PMA solutions show double-exponential functions irrespective of the presence of DC. Such

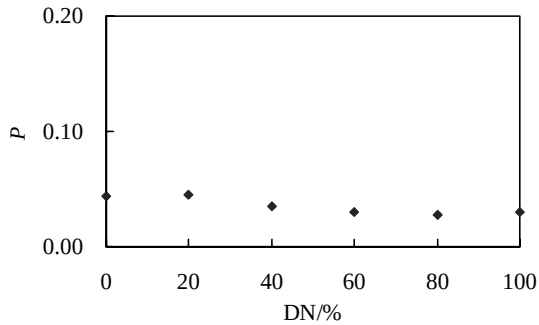

Fig. 5. Degree of polarization of R6G (2 μM) as a function of DN in the solutions of PEO-*b*-PMA and CTAC. [PEO-*b*-PMA] = 0.01 g/l. λ_{ex} = 470 nm, λ_{em} = 553 nm.

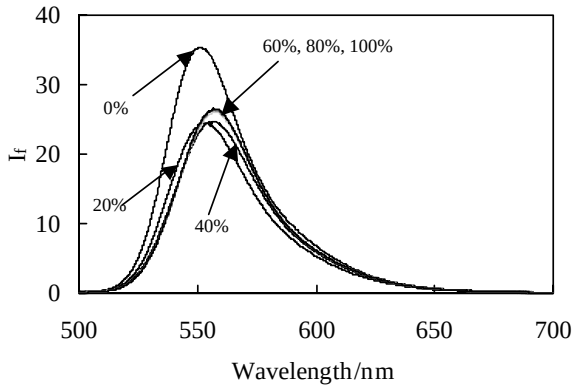

Fig. 6. Fluorescence spectra of R6G (2 μM) in the solutions of PEO-*b*-PMA at different DN with DC. [PEO-*b*-PMA] = 0.05 g/l. λ_{ex} = 470 nm.

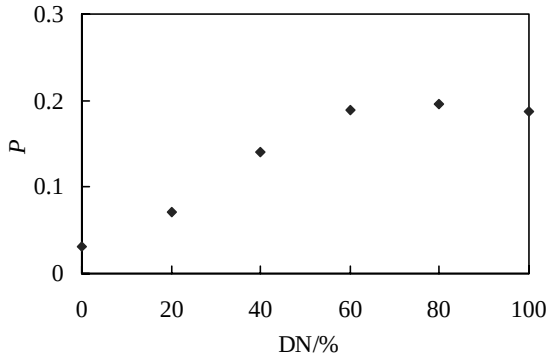

Fig. 8. Degree of polarization of R6G (2 μM) as a function of DN in the solutions of PEO-*b*-PMA and DC. [PEO-*b*-PMA] = 0.05 g/l. λ_{ex} = 470 nm, λ_{em} = 553 nm.

multi-exponential decay is often observed in polymer systems because the probe molecules are distributed among several different sites in the polymers. Therefore, we calculate a mean lifetime of R6G in the aggregates by

$$\langle\tau\rangle = \frac{\sum A_i\tau_i^2}{\sum A_i\tau_i} \quad (i = 1, 2) \tag{4}$$

where A_i and τ_i, respectively, denote the pre-exponential factor and the lifetime of *i*th component. We plot $\langle\tau\rangle$ against DN in Fig. 7. The mean lifetime has no significant change with increase of DN. Therefore, the quenching of the R6G seems to be operated by a static mechanism. This suggests formation of a non-fluorescent complex between R6G and DC.

Fig. 8 shows the dependence of *P* on DN for DC/PEO-*b*-PMA systems. There is a marked increase in *P* when DN is increased from 0 to 60%. As the lifetime of R6G does not much change with increasing DN (see Fig. 7), the increase of *P* can be attributed to the increase of micro-viscosity around R6G molecules. Therefore, the increase in *P* indicates that R6G is transferred into the polymer aggregates from aqueous bulk phase.

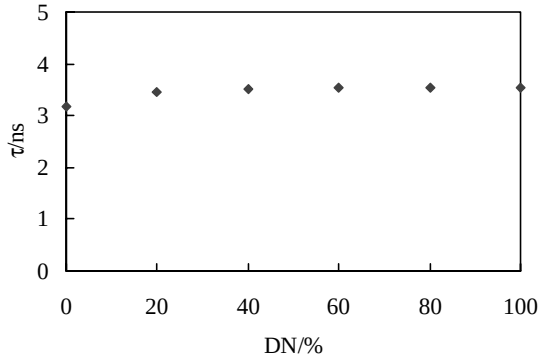

Fig. 7. Fluorescence lifetime of R6G (2 μM) as a function of DN in the solutions of PEO-*b*-PMA and DC. [PEO-*b*-PMA] = 0.05 g/l. The samples were excited at 470 nm. The emission was collected through Y51 cut off filter.

It is interesting to compare the red-shifted spectra of R6G in DC/PEO-*b*-PMA with that in CTAC/PEO-*b*-PMA nanoaggregates. Although solvent effects on the absorption and fluorescence spectra of Rhodamine dyes are complicated and the discussion continues to be controversial [23,24], we may be able to get some information about the location of the probe in the nanoaggregates. The peak positions in the absorption and fluorescence spectra of R6G in CTAC/PEO-*b*-PMA are not much different from those in water, while there is a significant red-shift in the spectra of R6G in DC/PEO-*b*-PMA nanoaggregates. To elucidate the difference, we measured fluorescence and absorption spectra of R6G in various micelles of low-molecular weight surfactants, drug and block copolymer. Wavelengths of maximal intensity (λ_{max}) in the absorption and fluorescence of R6G are summarized in Table 1.

The tendency of the solvent shift in Table 1 is similar to that reported by Arbeloa et al. about R6G in monoalcohols [23]. According to Arbeloa et al., absorption and fluorescence peaks show red-shift with decreasing polarity. As seen in Table 1, the R6G spectra in the micelles of DC, SDS, and Triton X-100 show significant red-shift compared to those in water, implying that R6G is located at the less

Table 1
Wavelengths of maximal intensity in absorption and fluorescence spectra of R6G (2 μM) in water, micelles or nanoaggregates

Medium	Concentration	λ_{max} (nm)	
		ABS[a]	FL[a]
Water	–	525.4	552.0
CTAC (M)	0.003	526.2	553.3
DC (M)	0.1	535.2	561.6
SDS (M)	0.015	532.6	558.9
Triton X-100 ($\times10^{-4}$ M)	5.2	534.0	558.6
Pluronic L64 (g/dl)	18	532.4	559.0
CTAC/PEO-*b*-PMA (g/dl, 100% DN)	0.01	525.4	551.7
DC/PEO-*b*-PMA (g/dl, 100% DN)	0.05	534.6	556.9

[a] The symbols ABS and FL denote absorption and fluorescence spectra, respectively.

polar (i.e. central) domain in the micelles. In contrast, the R6G spectra in the micelles of CTAC show little red-shift. One of the explanations for the little red-shift might be that R6G is not incorporated into the CTAC micelles. However, this is not the case because the fluorescence intensity is significantly enhanced in the CTAC micelles compared with that in aqueous solutions. Therefore, the little red-shift in the CTAC micelles suggests that R6G molecules are accommodated in the domain close to water/micelle interface. Concerning to the red-shift of absorption and fluorescence spectra of R6G, there is correlation between CTAC micelles and CTAC/PEO-*b*-PMA nanoaggregates or between DC micelles and DC/PEO-*b*-PMA nanoaggregates, implying that, in the surfactant/PEO-*b*-PMA nanoaggregates R6G molecules are accommodated in the domains which have environments similar to those in the micelles of low-molecular weight surfactants.

We also investigated the properties of R6G in the nanoaggregates induced by TC, PLS, Ba^{2+}, or Ca^{2+}. There is no significant change in the spectral properties of R6G (peak position, *P* value, etc.) in TC, PLS, Ba^{2+}, and Ca^{2+}-neutralized systems. There seem to be two reasons: (1) R6G is not incorporated into the aggregate because hydrophobic interaction between R6G and the nanoaggregate is also necessary for incorporation of R6G into the aggregate; (2) the core has a loosely packed structure due to the weak hydrophobic interaction between the PMA block and counter ions. Considerable amount of water may exist in the interior of the aggregates, so that the environment around R6G is similar to that in aqueous bulk phase. R6G labeled-polymer is needed for clarifying which is the case. Such experiments will be done in a future work.

4. Conclusions

We have examined the usefulness of R6G for characterizing nanaoaggregates of PEO-*b*-PMA neutralized with counter ions. The shift of peak position in the absorption and fluorescence spectra, the change of fluorescence intensity, and the increase of *P* value demonstrate that R6G is a useful probe for detecting and characterizing the aggregates of CTAC/PEO-*b*-PMA and DC/PEO-*b*-PMA systems. However, in the PEO-*b*-PMA aggregates which are neutralized with TC, PLS, Ba^{2+}, or Ca^{2+}, neither the absorption and fluorescence spectra nor the *P* value show significant change even after the aggregates are formed. This seems to be attributed to the weak hydrophobicity of such counter ions.

References

[1] V.K. Kelkar, B.S. Valaulikar, J.T. Kunjappu, C. Manohar, Photochem. Photobiol. 52 (1990) 717.
[2] J.C. Mialocq, P. Hebert, X. Armand, R. Bonneau, J.P. Morand, J. Photochem. Photobiol. A: Chem. 56 (1991) 323.
[3] Y. Yan, M.L. Myrick, Anal. Chim. Acta 441 (2001) 87.
[4] T. Kawamoto, Y. Morishima, Langmuir 14 (1998) 6669.
[5] C.-L. Zhao, M.A. Winnik, G. Riess, M.D. Croucher, Langmuir 6 (1990) 514.
[6] R. Vogel, M. Harvey, G. Edwards, P. Meredith, N. Heckenberg, M. Trau, H. Rubinsztein-Dunlop, Macromolecules 35 (2002) 2063.
[7] K. Nakashima, Y. Fujimoto, T. Anazi, Photochem. Photobiol. 61 (1995) 592.
[8] K. Nakashima, K. Takeuchi, Appl. Spectrosc. 55 (2001) 1237.
[9] K. Nakashima, T. Anzai, Y. Fujimoto, Langmuir 10 (1994) 658.
[10] K. Nakashima, Y. Fujimoto, N. Kido, Photochem. Photobiol. 62 (1995) 674.
[11] Y.-K. Gong, K. Nakashima, R. Xu, Langmuir 16 (2000) 8546.
[12] Y.-K. Gong, K. Nakashima, Chem. Commun. (2001) 1772.
[13] K. Kalyanasundaram, Photochemistry in Microheterogeneous Systems, Academic Press, New York, 1987.
[14] G. Liu, S. Stewart, Polym. Mater. Sci. Eng. 81 (1999) 10.
[15] Y. Li, Y.-K. Gong, K. Nakashima, Y. Murata, Langmuir 18 (2002) 6727.
[16] Y. Li, K. Nakashima, Langmuir 19 (2003) 548.
[17] Y. Li, S. Ikeda, K. Nakashima, H. Nakamura, Colloid Polym. Sci. 281 (2003) 562.
[18] Y. Li, L.-J. Ding, H. Nakamura, K. Nakashima, J. Colloid Interface Sci. in press.
[19] P.J. Sadkowski, G.R. Fleming, Chem. Phys. Lett. 57 (1978) 526.
[20] V.K. Kelkar, B.S. Valaulikar, J.T. Kunjappu, C. Manohar, Photochem. Photobiol. 52 (1990) 717.
[21] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, second ed., Kluwer Academic/Plenum Publishers, Dordrecht/New York, 1999.
[22] K. Nakashima, J. Duhamel, M.A. Winnik, J. Phys. Chem. 97 (1993) 10702.
[23] T.L. Arbeloa, F.L. Arbeloa, I.L. Arbeloa, A. Costela, I.G. Moreno, J.M. Figuera, F.A. Guerri, R. Sastre, J. Lumin. 75 (1997) 309.
[24] F.L. Arbeloa, T.L. Arbeloa, M.J.T. Estevez, I.L. Arbeloa, J. Phys. Chem. 95 (1991) 2203.