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Association of a Substituted Zinc(II) Phthalocyanine−*N*-(2-Hydroxypropyl)methacrylamide Copolymer Conjugate

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ABSTRACT: The solution properties of a *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer containing Zn(II) 4,9,16,23-tetraaminophthalocyanine (HPMA-TAPC-Zn copolymer) attached to the copolymer via oligopeptide side chains (GFLGG) were studied using light scattering and spectroscopic methods. The light scattering data indicated that the copolymer formed aggregates in aqueous solutions stable down to $c = 2 \times 10^{-5}$ g/mL. The extent of aggregation decreased with increasing concentration of detergents in buffer solutions or organic solvents in mixed solvents as Tris buffer/DMSO. Dramatic changes in the aggregate formation were observed in the vicinity of a mixture composition of 60 vol % of DMSO. The local interactions of hydrophobic TAPC-Zn species were studied by absorption and fluorescence spectrometry. The majority of all TAPC-Zn species are dimerized in aqueous solutions by hydrophobic interactions and hydrogen bonds. The proportion of TAPC-Zn monomers and dimers estimated for Tris buffer/DMSO mixtures from both the absorption and fluorescence spectra was correlated to the aggregation behavior of the copolymer. The copolymer aggregation was explained by the random association model. Mostly point-like contacts formed by TAPC-Zn dimers are supposed for aqueous solutions of HPMA-TAPC-Zn copolymer.

Introduction

Phthalocyanines[1] have been suggested as effective photosensitizers for cancer photodynamic therapy.[2,3] They absorb in the red region of the spectrum, permitting deeper light penetration into tissue as compared to porphyrins or chlorins. They have large extinction coefficients and high quantum yields of triplet formation and are nontoxic in the dark.[2,3] The attachment of photosensitizers to water soluble polymeric carriers can result in the localization of the carrier bound photosensitizer in the lysosomal compartment of cells.[4] Additionally, attachment of targeting moieties, such as antibodies or saccharides, may direct the targetable polymeric drug to a subset of cells. A double targeting effect can be achieved by the subsequent localized application of light.[4] However, upon binding of hydrophobic species (drugs) to a hydrophilic polymeric carrier, a conjugate is obtained having amphiphilic properties in aqueous solution.[5−10] As a result, drug conjugates may associate intramolecularly and intermolecularly, affecting the conformation of single chains and yielding formation of aggregates, respectively.[5,6] The therapeutic effectiveness of macromolecular drug carriers may be altered due to drug-mediated association phenomena.[6,7] We have shown previously that the photosensitizing efficiency of a *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer−chlorin e_6[11] and HPMA copolymer−Zn(II) 4,9,16,23-tetraaminophthalocyanine (TAPC-Zn)[12] conjugates increased upon the release of the photosensitizer (chlorin e_6 or TAPC-Zn) from the polymeric carrier catalyzed by cathepsin B. These results indicated the influence of polymer aggregation on the process of singlet oxygen generation. Therefore, we have extended our study of the solution properties of HPMA-TAPC-Zn copolymers[12] in order to understand their association phenomena in aqueous solutions.

In this paper the solution properties of HPMA-TAPC-Zn copolymer are described using dynamic and static laser light scattering methods to characterize the behavior of the copolymers on the macromolecular and supramolecular levels. UV/vis absorbance as well as fluorescence spectroscopy were used to analyze the aggregation of the polymer-bound TAPC-Zn photosensitizer. The TAPC-Zn species were attached to HPMA copolymers via oligopeptide (GFLGG) side chains. The effect of solvent composition and concentration on the association of the conjugates was studied.

Experimental Section

Synthesis of HPMA Copolymer Conjugate. *N*-Glycyl zinc(II) 4,9,16,23-tetraaminophthalocyanine (G-TAPC-Zn) was synthesized by the reaction of zinc(II) 4,9,16,23-tetraaminophthalocyanine with *N*-(*tert*-butoxycarbonyl)glycine *N*-hydroxybenzotriazole ester, followed by deprotection of the *tert*-butoxycarbonyl group as previously described.[12]

HPMA copolymer−*N*-glycyl zinc(II) 4,9,16,23-tetraaminophthalocyanine conjugate (HPMA-TAPC-Zn copolymer) was synthesized as previously described[12] by a polymeranalogous attachment of G-TAPC-Zn to a copolymer of HPMA with *N*-methacryloylglycylphenylalanylleucylglycine (GFLG) *p*-nitrophenyl ester (polymer precursor). The copolymer (Chart 1) contained 2.06 mol % of side chains terminated in TAPC-Zn (m = 0.0206).

The weight-average molecular weight, M_w, of HPMA-TAPC-Zn copolymer could not be determined directly by GPC because of aggregation. But the weight-average molecular weight and polydispersity of polymer precursor that was used for binding of TAPC-Zn were determined: $M_w = 31\,000$ and $M_w/M_n = 1.5$, respectively. Since both the HPMA-TAPC-Zn copolymer and the polymer precursor should have the same average number of monomers per chain $n \sim 200$, M_w of the HPMA-TAPC-Zn copolymer should be about 34 000. The Fast Protein Liquid

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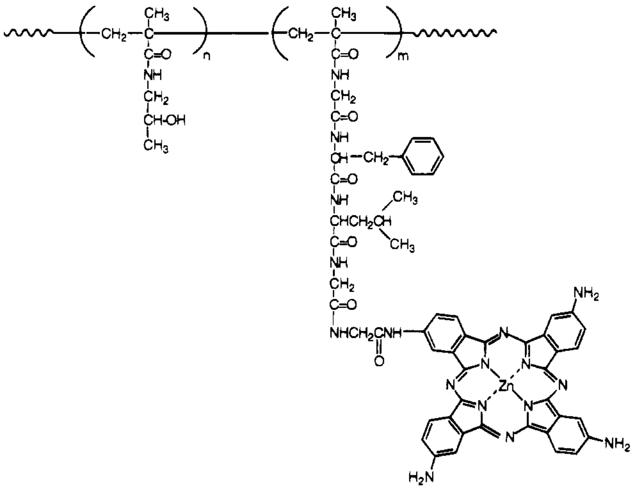
Chart 1. Chemical Structure of the HPMA-TAPC-Zn Copolymer[a]



[a] m is the content of TAPC-Zn species.

Table 1. Characteristics of HPMA-TAPC-Zn Copolymer Aggregates in Aqueous Solutions and DMSO

solvent	R_{ha} (nm)	$\Delta R(0)/Kc^b$ (g mol^{-1})
buffer C	210	5.2×10^7
Tris buffer	220	3.6×10^7
DMSO	21[a]	6.3×10^5

[a] REPES analysis. [b] Data for $c = 7.6 \times 10^{-5}$ g/mL.

Chromatography (Pharmacia) system was used for measurements with Superose 6 column eluted with 0.05 M Tris + 0.5 M NaCl, pH 8.0, buffer. The column was calibrated with poly(HPMA) fractions. The copolymer precursor was aminolyzed before M_w measurements with 1-amino-2-propanol.

Buffers (Aqueous). (a) Tris buffer: 0.05 M Tris, pH 7.4, adjusted by concentrated HCl; (b) buffer C: 0.058 M Na_2HPO_4, 0.005 M citric acid, 0.002 M ethylenediaminetetraacetic acid (EDTA), pH 7.

Static Light Scattering (SLS). Static light scattering measurements were performed with a Brookhaven goniometer equipped with an argon laser (vertically polarized, $\lambda = 514.5$ nm at 24 °C). The data obtained on the copolymer solutions were processed in the form of equation:

$$Kc/\Delta R(0) = 1/M_w + 2A_2c$$

where M_w is the weight-average molecular weight, K is the optical constant which includes the square of the refractive index increment, A_2 is the second virial coefficient, c is concentration, and $\Delta R(0)$ is the excess Rayleigh ratio, proportional to the intensity of light scattered from the copolymer solutions, extrapolated to zero scattering angle. The visible light absorption seriously limited the light scattering measurements. Therefore, the measurements were realized at a copolymer concentration $c = 7.6 \times 10^{-5}$ g/mL only providing sufficient excess light scattering intensity at relatively low incident light absorption.

Since direct measurements of the refractive index increment for HPMA-TAPC-Zn copolymer solutions resulted in a high absorption of visible light, we could not reliably analyze the light scattering data for mixed solvents (a possibility of selective sorption). Therefore, the analysis has been restricted to copolymer solutions in Tris buffer and DMSO only. The refractive index increments of HPMA homopolymer in Tris buffer ((dn/dc) = 0.146 mL/g)[6] and in DMSO ((dn/dc) = 0.056) were used in $\Delta R(0)/Kc$ calculations in Tris and C buffers, and DMSO, respectively, instead of those for HPMA-TAPC-Zn copolymer. Corrections for the light absorption were applied. Apparent molecular weights $\Delta R(0)/Kc$ in buffers and DMSO are shown in Table 1.

Dynamic Light Scattering (DLS). Dynamic light scattering measurements were performed using a standard multiangle Brookhaven Instruments spectrometer with an argon ion laser and a 78 channel BI 2030, multibit, multitau autocorrelator (Brookhaven Instruments). The samples were thermostated in a refractive index-matching liquid (toluene). Two different methods were used to analyze the autocorrelation functions: (1) the method of cumulants using both the measured and floating base-line options and assuming homodyne detection; and (2) inverse Laplace transform using the REPES[13] method of constrained regularization which is similar in many respects to the inversion routine CONTIN[14] to obtain a distribution $A(\tau)$ of decay time τ. However, REPES directly minimizes the sum of the squared differences between the experimental and calculated intensity time correlation functions using nonlinear programming. This method uses an equidistant logarithmic grid with fixed components (here a grid 10 components per decade) and determines their amplitudes. From the characteristic decay times, τ_i (the first cumulant in method 1 or peak positions of $A(\tau)$ in method 2), the apparent diffusion coefficients, D_i^a, were calculated from the equation:

Table 2. Viscosities and Densities of Mixed Solvents Tris Buffer/DMSO at 25 °C

vol % of DMSO	density (g/mL)	viscosity (10^{-3} Pa s)
0	0.997	0.914
20	1.026	1.452
40	1.058	2.424
60	1.085	3.585
80	1.096	3.380
100	1.099	2.022

$$D_i^a = 1/\tau_i q^2$$

where q is the scattering vector. The apparent hydrodynamic radius, R_{hi}^a, was calculated from the Stokes–Einstein relationship $R_{hi}^a = kT/6\pi\eta D_i^a$, where η is the solvent viscosity, T is the absolute temperature, and k is the Boltzmann constant. Viscosities of Tris buffer/DMSO mixed solvents determined by a capillary viscometer are shown together with corresponding solvent densities in Table 2. The hydrodynamic radius, R_{hi}, was calculated at the zero angle and at a concentration limit of D_i^a.

Results

Solution properties of HPMA-TAPC-Zn copolymer are described using dynamic (DLS) and partially static laser light scattering (SLS) to characterize the behavior of the polymer on macromolecular and supramolecular levels and photophysical methods, namely, UV/vis and fluorescence spectroscopy, to analyze interaction of polymer-bound TAPC-Zn species. The effects of an organic solvent (DMSO) and a low-molecular-weight detergent (CTAB) on the random association of the HPMA-TAPC-Zn copolymer were studied.

Light Scattering Measurements. The HPMA-TAPC-Zn copolymer was studied by DLS to obtain evidence for intermolecular association in the mixed solvent system Tris buffer/DMSO. The concentration of copolymer was kept constant throughout this experiment: $c = 7.6 \times 10^{-5}$ g/mL. Intermolecular aggregations of copolymer molecules were observed at all compositions of mixed solvents but on different levels in water-rich and DMSO-rich solvents. Apparent hydrodynamic radius, $R_{ha}^a(90°)$, of aggregates calculated from the first cumulants measured at $\theta = 90°$ using Stokes–Einstein relationship is plotted as a function of DMSO content, x (vol %), in Figure 1.

The light scattering intensity from copolymer solutions in DMSO was expressively lower than in buffer solutions. This is partly due to a decrease of the refractive index increment from about 0.146 mL/g in Tris buffer to 0.056 in DMSO and partly to a decrease

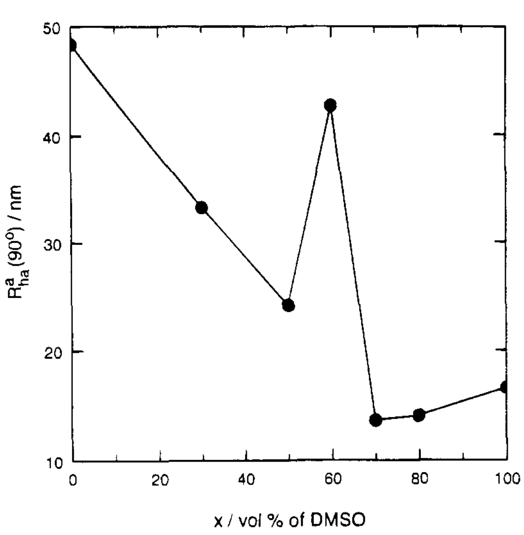


Figure 1. The apparent hydrodynamic radius, $R_{\mathrm{ha}}{}^{\mathrm{a}}(90°)$/nm, of HPMA-TAPC-Zn copolymer as a function of Tris buffer/DMSO mixture composition x/vol %; $c = 7.6 \times 10^{-5}$ g/mL.

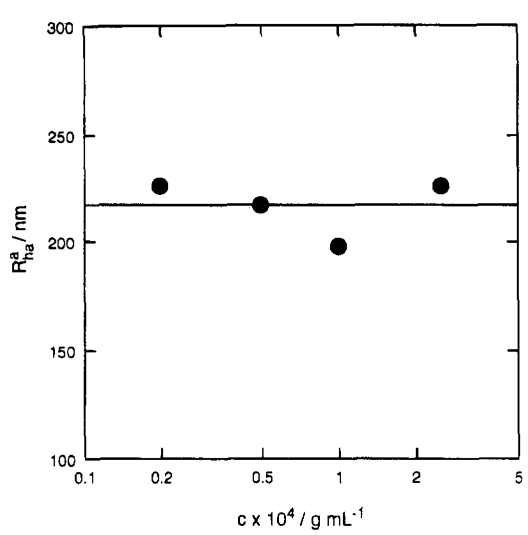


Figure 2. Plot of the apparent hydrodynamic radius, $R_{\mathrm{ha}}{}^{\mathrm{a}}(0)$/nm, as a function of HPMA-TAPC-Zn copolymer concentration, c.

of apparent molecular weight of scatterers (cf. Table 1). The decay time distributions obtained by the inverse Laplace transformation of multisampling time correlation functions for HPMA-TAPC-Zn copolymer solutions repeatedly reveal two band distributions with a small band at short decay times, τ, and about a 10 times stronger one at longer τ. The small shoulder at short τ could be found even in copolymer solutions in mixed solvent with $x = 80\%$ of DMSO and disappeared in the noise at mixed solvents with $x < 80\%$. Thus, we have concluded that the fast mode probably corresponds to polymer coil diffusion and the slow mode to aggregate diffusion. Since the overall scattering intensity from DMSO copolymer solutions was extremely low, we could not evaluate reliable sizes of copolymer coils from DLS data. Values of the hydrodynamic radius, R_{ha}, of intermolecular aggregates in C and Tris buffers and DMSO are shown in Table 1. Since both the coil and aggregate modes were simultaneously observed in mixed solvents with 80% of DMSO and in DMSO, we can conclude by comparing the corresponding relative scattering amplitudes that the total amount of HPMA-TAPC-Zn copolymer molecules detected in aggregates is several orders of magnitude lower than the amount dissolved molecularly. Thus, contrary to aqueous solutions, the coil–aggregate equilibrium in DMSO is shifted toward copolymer coils.

The data in Figure 1 show dramatic changes in size parameters in the vicinity of the mixture composition of $x = 60$ vol %. Similar behavior was observed in solutions of HPMA copolymers with azobenzene-containing side chains in water/ethanol mixtures at the solubility limit.[10] This effect can be explained by formation of weak swollen intramolecular copolymer aggregates attached together by only a minimal number of intermolecularly aggregated TAPC-Zn species. A decrease of the DMSO content in the mixture below 60 vol % of DMSO intensified the aggregation process through an aggregation of TAPC-Zn species, and aggregates became predominant. Therefore, interpolymer aggregates probably become more compact at 50 vol % of DMSO and then grow with decreasing x as observed in DLS scattering results in Figure 1.

To determine the effect of cetyltrimethylammonium bromide (CTAB) detergent on aggregate formation, the solution properties of HPMA-TAPC-Zn copolymer in CTAB-containing buffer C were measured. The cationic CTAB detergent also dissociated the aggregates, as observed by a decrease in aggregate sizes; $R_{\mathrm{ha}}{}^{\mathrm{a}}(90°) = 53$, 46, and 40 nm for 0, 0.35, and 6 mM of CTAB in solution, respectively. Light scattering measurements at higher concentrations of CTAB in solution were strongly influenced by the light scattering of cationic CTAB micelles. Therefore, reliable characteristics of HPMA-TAPC-Zn copolymer aggregates could not be obtained from these data.

The hydrodynamic sizes of copolymer aggregates were practically independent on the concentration with an accuracy of ±7% (see Figure 2) in the experimentally attainable concentration region $c = 2 \times 10^{-5}$–2.5×10^{-4} g/mL. The low concentration limit was given by the sensitivity of the light scattering apparatus and the upper one by the light absorption in copolymer solutions. Thus, the critical aggregate concentration should be below $c = 2 \times 10^{-5}$ g/mL. Since the solutions of the copolymer in both the buffers and in DMSO were prepared several times, we can state that reproducibility of R_{ha} was about ±5%. As for the time stability, a small decrease of aggregate size by about 10% was observed in 14 days.

Absorption and Fluorescence Spectra. The solutions of HPMA-TAPC-Zn copolymer were studied by UV/vis and fluorescence spectroscopy, which are dye-specific optical methods providing information on the formation of hydrophobic domains of TAPC-Zn species. Free tetrasulfophthalocyanines are known to associate in aqueous solutions, forming dimers and higher order aggregates (multimers).[1,15] Water is a solvent that promotes formation of aggregates. It is suggested that the water molecules play a specific role in binding the TAPC-Zn monomers together.[15] The extent of association of free tetrasulfophthalocyanines has been shown to depend on the phthalocyanine concentration, salt concentration, solvent composition, etc.[1,15] The equilibrium between monomer (M) and dimer (D) of tetrasulfophthalocyanines obeys the equilibrium relationship, $K = [\mathrm{D}]/[\mathrm{M}]^2[\mathrm{H_2O}]^n$, where K is the equilibrium constant and $n = 12$.[15] In aqueous solutions of the HPMA-TAPC-Zn copolymer under study, a similar association tendency between TAPC-Zn hydrophobic species may occur and will compete with the solvation effect of the polymer backbone and side chains. This

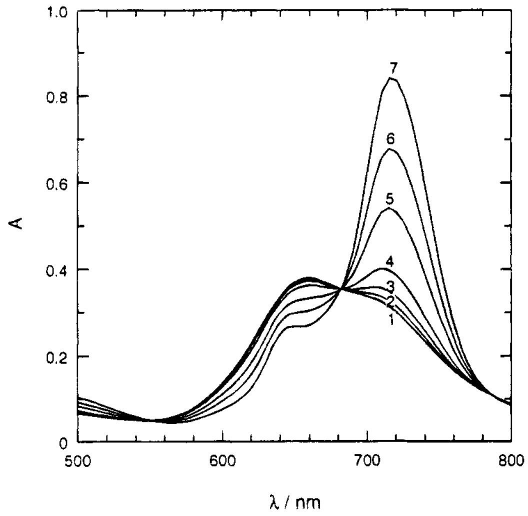


Figure 3. Spectra of HPMA-TAPC-Zn copolymer ($c = 7.6 \times 10^{-5}$ g/mL) in mixtures of Tris buffer and DMSO. DMSO content, x (vol %): **1**, 0; **2**, 30; **3**, 50; **4**, 60; **5**, 70; **6**, 80; **7**, 100. Adapted from ref 12.

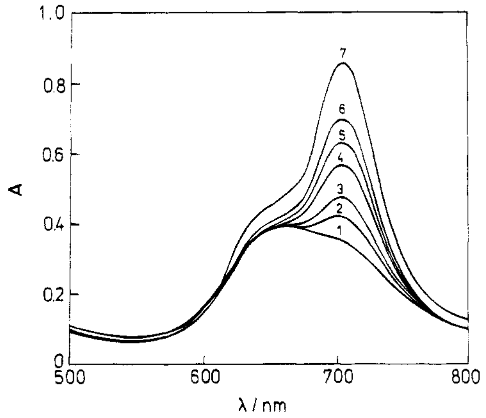


Figure 4. Spectra of HPMA-TAPC-Zn copolymer ($c = 7.6 \times 10^{-5}$ g/mL) in buffer C containing different amounts of CTAB. CTAB content (10^{-3} mol/L): **1**, 0; **2**, 0.32; **3**, 6; **4**, 36; **5**, 72; **6**, 130; **7**, 274. Adapted from ref 12.

polymer solvation effect is decisive, particularly in aqueous solutions of tetraaminophthalocyanines since free tetraaminophthalocyanines are less soluble in water (pH 7) than tetrasulfophthalocyanines. Since the absorption spectra of phthalocyanine monomers, dimers, and higher multimers differ, particularly in the 600–800 nm region (so called Q-band),[1] absorption spectrometry is a favored method to study association phenomena of phthalocyanines.

Figures 3 and 4 show the absorption spectra of polymer-bound TAPC-Zn in mixed solvents of Tris buffer/DMSO and in buffer C containing different amounts of CTAB, as indicated. Practically the same changes of absorption spectra were observed in mixed solvents of buffer C/DMSO.[12] The absorption spectrum with a sharp peak at 720 nm obtained in DMSO is typical for the monomer absorption of Zn(II) phthalocyanines.[16,17] This absorption spectrum has been assigned to the transition between bonding and antibonding orbitals; the most intense peak corresponds to a 0–0 line of the vibronic transition involving the ground state and a $\pi\pi^*$ ligand-centered excited state.[1] The broad, low, double absorption peak with a maximum at 658 nm found in aqueous solutions of HPMA-TAPC-Zn copolymer is characteristic of TAPC-Zn aggregates (multimers).[17,18] The differences in the absorption spectra of TAPC-Zn monomers and multimers are due to strong interactions between the TAPC-Zn components in aggregates. In this context, it has been proposed that the interaction should be described as an overlap between π-electron clouds. The spectra of HPMA-TAPC-Zn copolymer in mixtures of buffers and DMSO and in buffer C containing different amounts of CTAB indicate the presence of both the TAPC-Zn monomers and multimers in solutions. The degree of monomerization evidently increased with an increasing content of the organic solvent (DMSO) and with an increasing content of detergent (CTAB). The effect of other aqueous detergents (cetylpyridinium chloride, sodium dodecyl sulfate, Triton-100) was similar to that in the buffer C/CTAB system.[12] Thus, a high concentration of aqueous detergents or a high concentration of organic solvents is necessary to prevent the aggregation of TAPC-Zn species. These results are in agreement with those observed in solutions of free metal tetrasulfophthalocyanines. It was found that aggregates of Zn(II) phthalocyanines are completely dissociated by aqueous detergents, provided that the concentration of detergents is greater than the dye concentration,[18] and the same effect can be obtained by the addition of small amount (ca. 5% v/v) of organic solvents (e.g., DMF).[17]

The above experimental results gave rise to the question as to what is the degree of association of TAPC-Zn species in aqueous and DMSO solutions of HPMA-TAPC-Zn copolymer. The fraction of monomers, dimers, and higher multimers in solutions is controlled by a dynamic equilibrium between the corresponding components, which is concentration dependent. Thus, the fraction of interpolymer dimers and possibly multimers should increase and the fraction of monomers decrease with increasing concentration of TAPC-Zn species in solution. These changes of TAPC-Zn aggregation can again be analyzed by absorption spectroscopy, taking advantage of differences between the absorption spectra of monomers and dimers as described above, and dimers and higher multimers. The formation of higher multimers (tetramers, etc.) results in a decrease of the apparent extinction coefficient, together with a progressive blue shift of the "dimer" band by 25 nm and loss of isosbestic behavior.[19] Therefore, the concentration dependence of the absorption spectra of TAPC-Zn species attached to the copolymer was measured over the concentration range $c = 2.5 \times 10^{-5}$–5×10^{-4} g/mL in both Tris buffer and DMSO. We have found that spectra of the extinction coefficient, ϵ, in Tris buffer are independent of copolymer concentration within the experimental error (see Figure 5). Moreover, these spectra are close to the spectra of ϵ reported for dimers in aqueous solutions of free Zn(II) tetrasulfophthalocyanines.[17,18] Therefore, it is plausible to suppose that dimers are the major component in aqueous solutions of HPMA-TAPC-Zn copolymer. It means that the monomer–dimer equilibrium is shifted toward dimers. The formation of higher multimers is probably suppressed because of low TAPC-Zn concentration ($\leq 6 \times 10^{-5}$ M). Practically negligible changes of absorption spectra with concentration were observed even in DMSO. Since these absorption spectra, in contrast to those in aqueous solvents, are similar to those published for free Zn(II) tetrasulfophthalocyanines in DMSO[16] and other organic solvents[17] interpreted as monomer spectra, we assume that TAPC-Zn species are dominantly dissociated to monomers. Thus, both the nearly pure monomer and dimer visible Q-band spectra of TAPC-

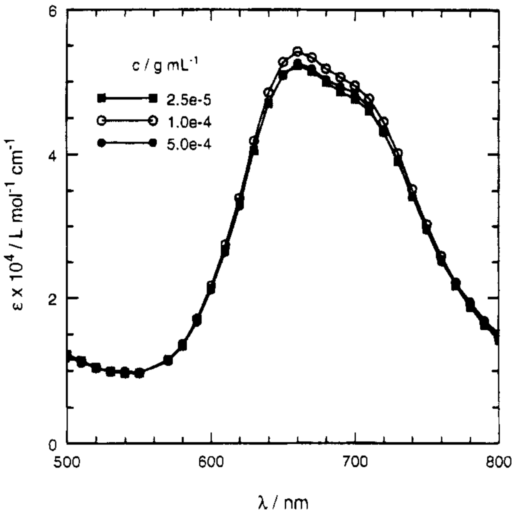


Figure 5. Spectra of the extinction coefficient, ε, of HPMA-TAPC-Zn copolymer measured at three various copolymer concentrations in Tris buffer; copolymer concentrations are indicated.

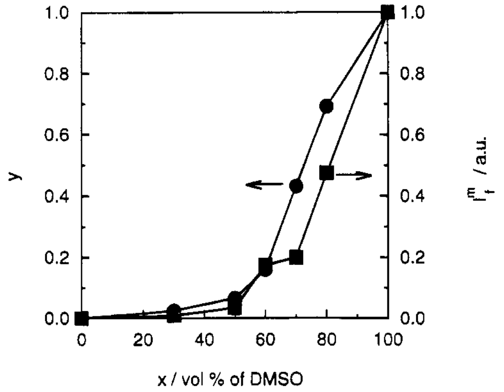


Figure 6. Plot of the fraction of molar concentration of TAPC-Zn monomer, y, and relative fluorescence intensity, I_f^m, as a function of Tris buffer/DMSO mixture composition; concentration of HPMA-TAPC-Zn copolymer $c = 7.6 \times 10^{-5}$ g/mL.

Zn species were found in DMSO and buffer solutions, respectively.

If we suppose, in agreement with others,[20] that the extinction coefficients are the same in water as in DMSO, the monomer–dimer equilibria in Tris buffer/DMSO mixed solvents can be evaluated by analyzing the absorption spectra. We have used a fitting procedure directly comparing a superposition of monomer (DMSO) and dimer (Tris) absorption spectra with those measured in mixed solvents. The resulting fraction of molar concentration of TAPC-Zn present as monomer, y, is given in Figure 6 as a function of the volume fraction of DMSO, x. The value of y strongly decreases with decreasing x down to 50 vol %. The fraction of molar concentration of TAPC-Zn monomer $y \approx 16\%$ was estimated for $x \approx 60$ vol %. Thus, a direct correlation between the dimerization of TAPC-Zn species and the formation of intermolecular aggregates is evident (compare Figures 1 and 6).

As with other aggregated phthalocyanines,[1,2] no fluorescence could be detected in aqueous solutions of HPMA-TAPC-Zn copolymer. In contrast, a single fluorescence peak at 740 nm (uncorrected) (see Figure 7), corresponding to the fluorescence of TAPC-Zn monomers, has been observed in DMSO.[12] Similar fluorescence spectra with half-lives shorter than tens of nanoseconds have been observed in monomeric phthalocyanines with closed shell metal ions.[21-23] The normalized peak value of fluorescence intensity I_f^m is plotted as a function of x in Figure 6 for comparison. Since only monomeric phthalocyanine species present in mixed solvents fluoresce, I_f^m should follow the y vs x dependence. But this is not the case (cf. Figure 6) since I_f^m decreases faster than y with decreasing x; this could be due to an additional fluorescence quenching process active in mixed solvents. Probably, the dimers and multimers formed in mixed solvents could be active as quenchers of monomer fluorescence because they have the necessary overlap of absorption spectra. Weak fluorescence of HPMA-TAPC-Zn copolymer in buffers used strongly supports the interpretation that the relative concentration of TAPC-Zn monomers is very low there.

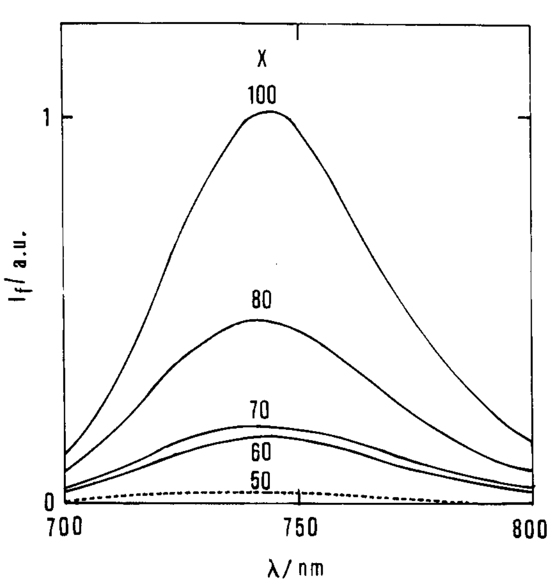


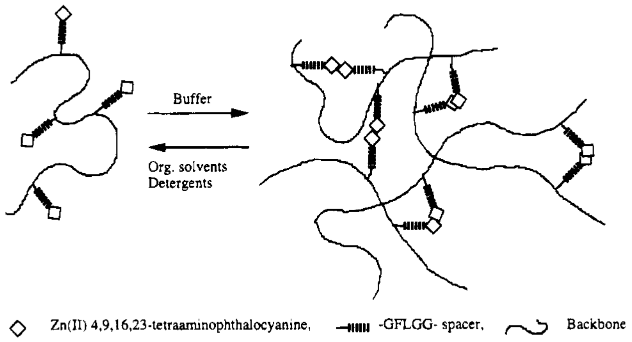
Figure 7. Fluorescence spectra (uncorrected) of HMPA-TAPC-Zn copolymer ($c = 7.6 \times 10^{-5}$ g/mL) in mixtures of Tris buffer and DMSO. DMSO content, x (vol %), is indicated.

The fluorescence of aqueous solutions of HPMA-TAPC-Zn copolymer was also enhanced by aqueous detergents. In buffer C with a high content of CTAB, the HPMA-TAPC-Zn copolymer emitted at 727 nm with an intensity lower than that in DMSO.[12] The fluorescence spectra of TAPC-Zn species in other aqueous detergents were similar to those observed in buffer C with CTAB detergent. The fluorescence data presented above also indicate that the TAPC-Zn species are completely aggregated in aqueous solutions of HPMA-TAPC-Zn copolymer and that the monomer form is at least partially favored in DMSO.

Conclusions

From the data presented above, we can propose a qualitative model for the solution behavior of HPMA-TAPC-Zn copolymer. In aqueous solvents (pH ≈ 7), both absorption and fluorescence spectra indicate that TAPC-Zn species are aggregated, with a major fraction of dimers. Dimers, in addition to the hydrophobic interactions, are also stabilized by interaction with water molecules. Thus, the intermolecular aggregates observed in aqueous solutions by the light scattering methods are randomly associated copolymer chains bound together by point-like contacts (dimers) of TAPC-Zn species (Scheme 1). The addition of organic solvents (e.g., DMSO) causes dissociation and favors the TAPC-Zn monomer. The HPMA-TAPC-Zn copolymer, as con-

Scheme 1. Effect of Organic Solvents and Aqueous Detergents on Association Behavior of HPMA-TAPC-Zn Copolymer



cluded from dynamic light scattering experiments, is predominantly molecularly dissolved in DMSO (Scheme 1). Moreover, aggregates of TAPC-Zn are completely monomerized by aqueous detergents (CTAB), provided that their concentration is substantially greater than the photosensitizer concentration.

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