Synthesis, Characterization, and Degradation of Silicon(IV) Phthalocyanines Conjugated Axially with Poly(sebacic anhydride)

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ABSTRACT: A novel silicon(IV) phthalocyanine with two axial poly(sebacic anhydride) chains has been synthesized by melt condensation of silicon phthalocyanine dihydroxide and oligo(sebacic anhydride). The polymer has been spectroscopically characterized and its molecular weights have been determined by gel permeation chromatography (GPC) and ^{1}H NMR spectroscopy. Nanoparticles with an intensity-average apparent hydrodynamic radius of 65 ± 1 nm have been prepared from this polymer via a microphase inversion method with sodium dodecyl sulfate as the surfactant. The spherical nanoparticles contain loosely aggregated polymer chains, trapping about 90% of the water. On treatment with NaOH, these nanoparticles undergo degradation that has been monitored by laser light scattering and fluorescence spectroscopy. Because of the axial substitution, the change in the aggregation state of the phthalocyanine core of this polymer during nanoparticle formation and degradation is relatively small compared with that of the zinc(II) phthalocyanine analogue reported earlier, in which poly(sebacic anhydride) chains are linked to the periphery of the phthalocyanine ring. © 2005 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 43: 837–843, 2005
Keywords: degradation; light scattering; nanoparticles; phthalocyanines; polyanhydrides

INTRODUCTION

Because of the strong absorption in tissue-penetrating red light, desirable photophysical and photochemical properties, low dark toxicity, and ease of preparation and chemical modification, phthalocyanines have been widely studied for their applications as second-generation photosensitizers for photodynamic therapy (PDT).[1] The large hydrophobic π-systems usually require axial or peripheral substitution with hydrophilic groups to become soluble in biological media. Alternatively, carriers such as polymeric particles, biocompatible emulsifying agents, and proteins are also commonly used to facilitate administration and enhance the cellular uptake.[2] Among the various delivery systems, polymeric particles have their unique advantages and appear to be highly promising. By changing the nature or simply the size of the particles, the biodistribution of the entrapped substance can be altered, which imparts a certain degree of selectivity.[3] The use of biodegradable polymers can also provide a means of controlled drug release.[4] Such an approach has been widely used for the delivery of various chemotherapeutic agents.[5] However, entrapping phthalocyanines in polymeric particles for targeted PDT has only been sporadically reported.[6]

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We have recently reported a series of copolymers of zinc(II) phthalocyanine and sebacic anhydride,[7] which is a common building block of biocompatible and biodegradable polymers. With the aid of surfactants, these polymers form nanoparticles in water, from which phthalocyanines are released during degradation. We describe here an extension of this work, with silicon(IV) phthalocyanine as the core. Having the polymer chains at the axial instead of the peripheral positions, the resulting polymer behaves differently in the degradation and release of phthalocyanine.

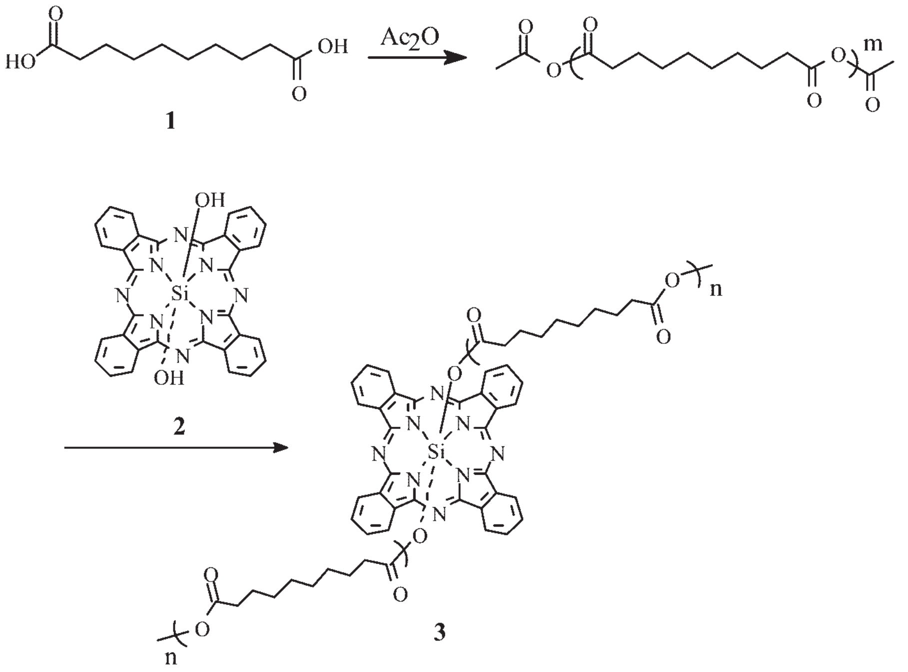
EXPERIMENTAL

Toluene and tetrahydrofuran (THF) were distilled from sodium and sodium benzophenone ketyl, respectively. Sebacic acid (**1**) was recrystallized twice from toluene before use. All other reagents and solvents were of reagent grade and were used as received. Silicon phthalocyanine dihydroxide (**2**) was prepared according to the literature procedure.[8]

^{1}H NMR spectra were recorded on a Bruker DPX 300 spectrometer (300 MHz) in $CDCl_3$. Chemical shifts were relative to internal $SiMe_4$ ($\delta = 0$). UV–visible (UV–vis) and steady-state fluorescence spectra were measured on a Cary 5G UV–vis-near IR spectrophotometer and a Hitachi F-4500 spectrofluorometer, respectively. The fluorescence quantum yields were determined by the equation: $\Phi_{sample} = (F_{sample}/F_{ref})\ (A_{ref}/A_{sample})\ (n^2_{sample}/n^2_{ref})\Phi_{ref}$, where F, A, and n are the measured fluorescence (area under the emission peak), the absorbance at the excitation position (610 nm), and the refractive index of the solvent, respectively; the subscripts "sample" and "ref" denote the sample and the reference, respectively, and $\Phi_{ref} = 0.30$ for unsubstituted zinc(II) phthalocyanine (ZnPc) in 1-chloronaphthalene.[9] Singlet oxygen quantum yields (Φ_Δ) were measured by the method of chemical quenching of 1,3-diphenylisobenzofuran described by Wöhrle et al.,[10] except that the absolute light intensity of our system was not determined. Measurements were performed in *N*,*N*-dimethylformamide (DMF) and referenced to ZnPc (Φ_Δ = 0.55). Molecular weights and distribution were determined by a gel permeation chromatography (GPC) system equipped with an Isco 2350 pump, an Isco V4 UV–vis absorbance detector, a Viscotek 250 viscosity/refractive index dual detector, and two Ultrastyragel columns at 35 °C. THF was used as the eluent at a flow rate of 1 mL min^{-1}. The molecular weights were calculated on the basis of polystyrene standards.

Laser Light Scattering (LLS)

A modified commercial LLS spectrometer (ALV/SP-125) equipped with a multi-τ digital time correlator (ALV-5000) and a solid-state diode laser (Adlas DPY425II, output power $\approx$ 400 mW at λ = 532 nm) was used. The laser beam was properly attenuated to avoid heating of the scattering volume. In static LLS, the angular dependence of the excess absolute time-average scattered intensity, known as the Rayleigh ratio, $R_{vv}(q)$, was measured. It is iworh iot ihat isn-384.ihas istudy-332.1(ihare)]TJT



Scheme 1

Melt Condensation of Silicon(IV) Phthalocyanine Dihydroxide (2) with Oligo(sebacic anhydride)

A mixture of sebacic acid (**1**) (10.0 g, 0.05 mol) in acetic anhydride (100 mL) was heated at reflux for 2 h under a nitrogen atmosphere. The excess acetic anhydride was then removed *in vacuo* and the residual solid was recrystallized twice from toluene to give oligo(sebacic anhydride) as white crystals (9.46 g).[12,13] This oligomer (0.89 g, ca. 10 equiv. of sebacic acid with respect to **2**) was then mixed with silicon(IV) phthalocyanine dihydroxide (**2**) (0.25 g, 0.44 mmol), and the mixture was heated at 180 °C for 1 h *in vacuo*. After cooling, the mixture was diluted with THF (2 mL) and precipitated with MeOH (250 mL) through a cotton filter. The greenish-blue powdery polymer formed was collected by filtration and dried *in vacuo* (0.80 g, 70%).

Nanoparticle Formation

Nanoparticles of poly(sebacic anhydride)-substituted phthalocyanine **3** were prepared by adding dropwise a dilute THF solution of **3** (0.25 mL, 2.5 $\times$ 10^{-3} g mL^{-1}) into a bulk aqueous solution (25 mL) containing sodium dodecyl sulfate (SDS) as the stabilizer. The SDS concentration was about twice its critical micelle concentration (CMC) (1.6×10^{-2} mol dm^{-3}). The mixture was then sonicated for 1 h.

Degradation

In a typical degradation experiment, a dust-free NaOH aqueous solution (0.14 g, 4.5 $\times$ 10^{-3} mol dm^{-3}) was added to a 2-mL dust-free suspension of **3** nanoparticles, both of which had been clarified by a 0.45 μm Millipore poly(tetrafluoroethylene) (PTFE) filter. The pH value was found to be 10.0 immediately after mixing. Both $R_{vv}(q)$ and $G^{(2)}(t,q)$ were simultaneously measured during the degradation. The release of phthalocyanine from the nanoparticles was monitored by fluorescence spectroscopy. It is worth noting that the addition of a trace amount of NaOH has little polyelectrolyte effect on the LLS measurements.

RESULTS AND DISCUSSION

Starting from the readily available silicon phthalocyanine dihydroxide (**2**),[8] melt condensation with oligo(sebacic anhydride) prepared by heating sebacic acid (**1**) in acetic anhydride[12] gave the phthalocyanine-containing poly(sebacic anhy-

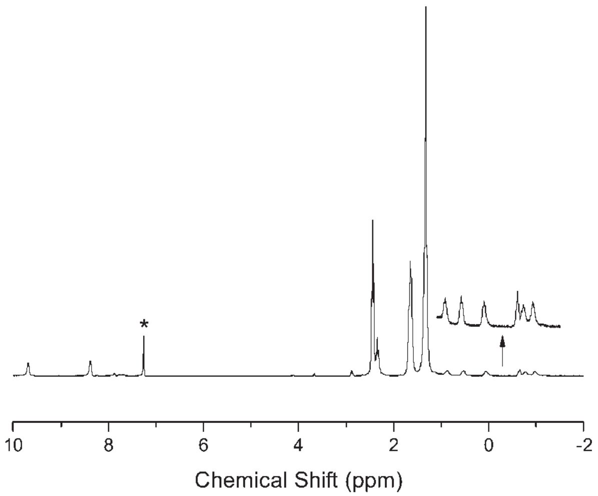


Figure 1. ^{1}H NMR spectrum of **3** in $CDCl_3$; * indicates residual $CHCl_3$.

dride) **3** in a 70% yield (Scheme 1). Figure 1 shows the ^{1}H NMR spectrum of **3** in $CDCl_3$, in which two aromatic signals at δ 9.7 and 8.4 are observed for the phthalocyanine α- and β-ring protons, respectively. Several upfield signals are also seen in the region of $\delta = 1$ to -1 that are attributed to the polymer methylene protons nearest to the ring center. The signals are shifted upfield because of the shielding effect due to the phthalocyanine ring current. The typical resonances for a poly(sebacic anhydride) backbone appear at δ = 2.3–2.5, 1.6–1.8, and 1.2–1.4, with a 1:1:2 ratio. The molecular weight (5400), as determined from the integrations of these signals, is comparable with those obtained by GPC measurement [number-average molecular weight (M_n) = 2800, weight-average molecular weight (M_w) = 3140]. On the basis of the molecular weight of the prepolymer (1900),[13] it is likely that **2** reacts with only two oligomeric chains at the axial positions during condensation, and further polycondensation with oligo(sebacic anhydride) does not occur. With a larger amount of the prepolymer, equivalent to 50 and 100 equiv. of **1** with respect to **2**, the resulting polymers had similar molecular weights (M_n = 3150, M_w = 4570 for the former and M_n = 3270, M_w = 4130 for the latter) as determined by GPC, but with a larger polydispersity (1.5 and 1.3, respectively vs 1.1 for **3a**). The ^{1}H NMR spectra of these two polymers showed very strong signals due to the poly(sebacic anhydride) backbone, whereas the signals due to the phthalocyanine ring protons and the shielded methylene protons were just discernible. These observations suggest that the excess prepolymer undergoes polycondensation, yielding free poly(sebacic anhydride) that contaminates the phthalocyanine-containing poly(sebacic anhydride) formed.

Polymer **3** exhibited electronic absorptions and emission that are typical for nonaggregated phthalocyanines. As shown in Figure 2, the absorption spectrum of **3** in $CHCl_3$ displays a strong and sharp Q band at 685 nm, together with a Soret (or B) band peaking at 360 nm and two weak vibronic bands at 616 and 654 nm. On excitation at 610 nm, the polymer shows a strong fluorescence emission at 693 nm, with a stoke shift of 8 nm. The fluorescence quantum yield (0.59) is higher than that of the unsubstituted ZnPc in 1-chloronaphthalene, which was used as the reference. To evaluate the photosensitizing efficiency of this polymeric phthalocyanine, the singlet oxygen quantum yield (Φ_Δ) was also determined by a steady-state method with 1,3-diphenylisobenzofuran as the scavenger. The concentration of the quencher was monitored spectroscopically at 411 nm along with time, from which the value of Φ_Δ could be determined.[10] It was found that polymer **3** is a singlet-oxygen generator and the value of Φ_Δ (0.24) is comparable with those of silicon(IV) phthalocyanines with two axial poly(ethylene glycol) chains (0.16–0.20).[14] The electronic absorption and photophysical properties of the other batches of polymers contaminated with free poly(sebacic anhydride) were also studied and found to be comparable with those of **3**. This indicates that the presence of the free polymer chains does not exert a significant influence on these properties of the polymeric phthalocyanines.

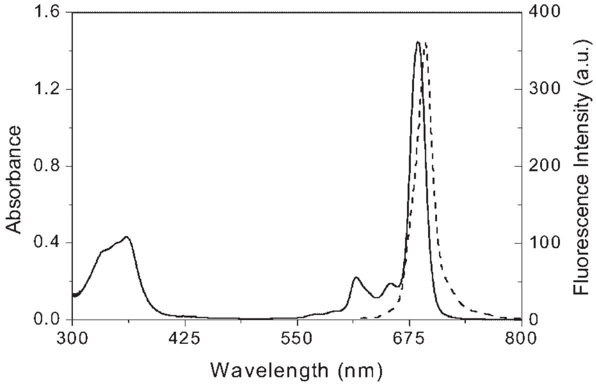


Figure 2. Electronic absorption (—) and normalized fluorescence (---) spectra of **3** in $CHCl_3$ with a concentration of 1.5×10^{-5} g mL^{-1}.

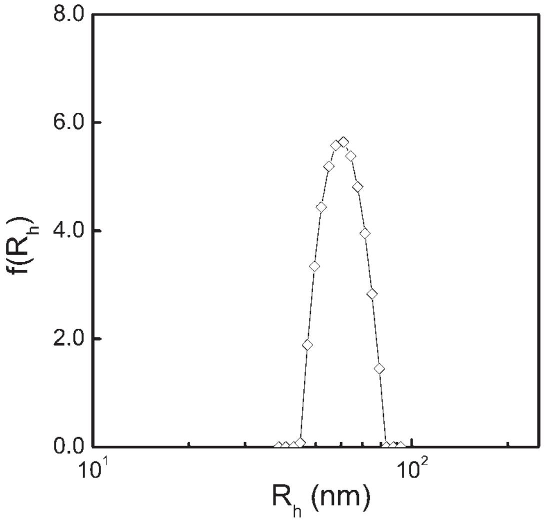


Figure 3. Intensity-weighted distribution of hydrodynamic radius for the nanoparticles of **3** in the presence of SDS (2 CMC) at 25 °C, where the concentration of **3** $= 2.5 \times 10^{-5}$ g mL^{-1} and the scattering angle is 15°.

By adding a dilute THF solution of **3** (2.5×10^{-3} g mL^{-1}) into a bulk aqueous solution (100 folds by volume) containing SDS at twice the CMC, nanoparticles of **3** were formed. Figure 3 shows that the resulting nanoparticles are narrowly distributed, with an intensity-average apparent hydrodynamic radius $\langle R_h \rangle$ of 65 ± 1 nm determined by dynamic laser light scattering at 15°. It is worth noting that at this scattering angle, the correction of $q \to 0$ is less than a few percent. Therefore, the extrapolation is not necessary. Also note that f(R_h)s measured at different scattering angles are monomodal. Because of axial substitution, the phthalocyanine rings are not significantly aggregated within the nanoparticles. This is reflected by the typical electronic absorptions for nonaggregated phthalocyanines at 356, 617, 654(sh), and 683 nm, and a relatively strong fluorescence emission at 685 nm ($\Phi = 0.32$, by using the refractive index of water in the calculation).

The measured $KC/R_{vv}(q)$ increases linearly with q^2 (not shown). According to eq 1, the extrapolation of $KC/R_{vv}(q)$ to $C \to 0$ and $q \to 0$ leads to the value of M_w (6.5×10^7 g mol^{-1}), whereas the slopes for the lines plotting $[KC/R_{vv}(q)]_{C\to 0}$ versus q^2 and $[KC/R_{vv}(q)]_{q\to 0}$ versus C give the values of $\langle R_g \rangle$ (50 ± 1 nm) and A_2 (≈ 0), respectively. From the values of M_w and $\langle R_h \rangle$, the average particle density $\langle \rho \rangle$ was found to be very small (0.09 g cm^{-3}). This suggests the formation of loose aggregates trapping about 90% of water in their hydrodynamic volume, assuming that the bulk polymer has a density of about 1 g cm^{-3}. The ratio of $\langle R_g \rangle / \langle R_h \rangle$ was found to be 0.77, which is virtually identical to the value of 0.774 predicted for a uniform sphere, indicating that the aggregates are spherical and uniform.[15]

Degradation of these nanoparticles in the presence of NaOH (pH 10.0) was performed *in situ* inside an LLS cuvette.[16] Figure 4 shows that $[R_{vv}(q)]_t/[R_{vv}(q)]_0$ decreases with time. Eq 1 shows that at both $C \to 0$ and $q \to 0$, the ratio $[R_{vv}(q)]_t /[R_{vv}(q)]_0$ is proportional to $[CM_w]_t/[CM_w]_0$, where the subscripts "t" and "0" denote the quantities at time t and the initial values, respectively. Therefore, a decrease in $[R_{vv}(q)]_t/[R_{vv}(q)]_0$ clearly indicates the degradation, but it could be related to a decrease in either M_w and/or C. Previous studies showed that the degradation of polymeric nanoparticles in the presence of enzymes or under a basic condition follows a one-by-one random process,[16,17] very similar to the hydrolysis reaction of small molecules in solution.

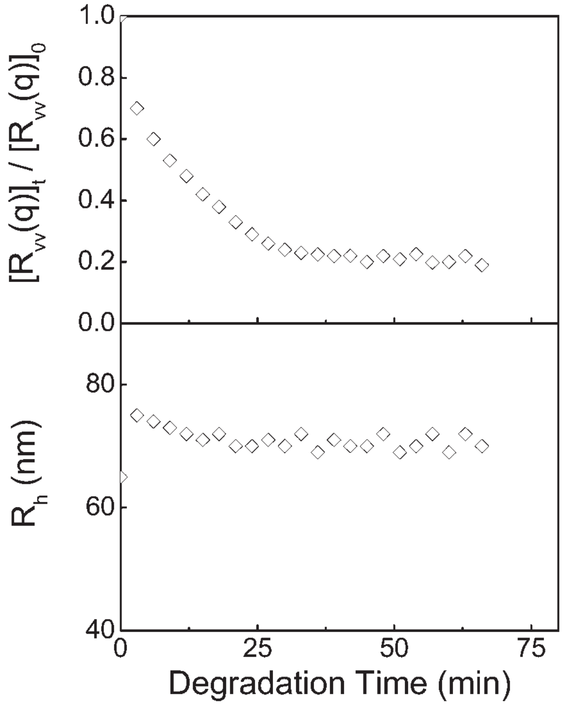


Figure 4. Degradation time dependence of the relative Rayleigh ratio $[R_{vv}(q)]_t/[R_{vv}(q)]_0$ and the average hydrodynamic radius $\langle R_h \rangle$ of the SDS-stabilized nanoparticles of **3** at 25 °C and pH = 10.0, where the initial concentration of **3** (C_o) $= 2.5 \times 10^{-5}$ g mL^{-1}.

Note that LLS can only detect those remaining nanoparticles, not small molecules (products) generated from the degradation because the scattering intensity is proportional to the square of mass. On the other hand, if the degradation of all the particles occurs at the same time and the degradation of each nanoparticle follows a step-by-step process, we would observe a gradual decrease in the particle size. However, Figure 4 shows that the average apparent hydrodynamic radius ($\langle R_h \rangle$) of those remaining nanoparticles is nearly a constant during the degradation. It should be stated that there was no noticeable change in the distribution width of f(R_h) during the degradation and also no change in the ratio of $\langle R_g \rangle / \langle R_h \rangle$ at the end of the degradation. Therefore, we can only attribute the decrease of $[R_{vv}(q)]_t / [R_{vv}(q)]_0$ to the decrease of the concentration, that is, $[R_{vv}(q)]_t/[R_{vv}(q)]_0$ is proportional to C_t/C_0 during the degradation. It is helpful to note that the particle concentration used here is so low that we do not need to consider a possible effect or correction of the second virial coefficient (A_2) term in eq 1. The slight increase of $\langle R_h \rangle$ could be attributed to the swelling of the nanoparticles caused by the small hydrophilic acids generated during the degradation. These molecules were initially trapped inside and it takes time for them to diffuse out.

Although the absorption spectrum did not change significantly upon degradation, the change in fluorescence spectrum was more noticeable. As shown in Figure 5, the fluorescence intensity increases significantly during the initial degradation period and reaches a steady value after about 1 h (inset of Figure 5). It is likely that during nanoparticle formation the polymer chains collapse, leading to a slight aggregation of the phthalocyanine rings. A partial scissoring of the polymer chains on degradation releases some phthalocyanine-containing fragments, in which the residual axial substituents together with the broken poly(sebacic anhydride) chains and the surfactant molecules reduce the aggregation of phthalocyanine, leading to a higher fluorescence intensity. Compared with the zinc phthalocya-

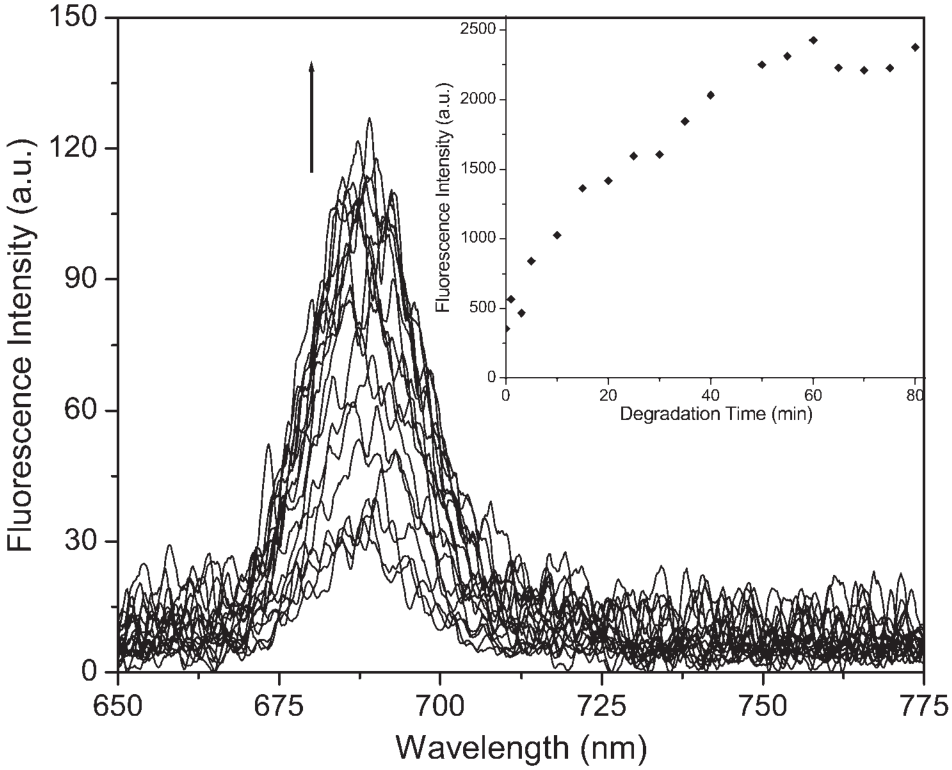


Figure 5. Change in fluorescence spectrum of SDS-stabilized nanoparticles of **3** during degradation at 25 °C and pH = 10.0, where the initial concentration of **3** (C_o) = 2.5 × 10^{-5} g mL^{-1}. The inset shows the degradation time dependence of the fluorescence intensity.

nine analogue reported by us previously,[7] in which poly(sebacic anhydride) chains are linked to the periphery of the phthalocyanine ring, the changes in absorption and fluorescence spectra for **3** were much smaller. This may be because of the axial substitution in **3**, which effectively prevents the aggregation of phthalocyanine, both during the nanoparticle formation and degradation.

In summary, we have prepared a silicon(IV) phthalocyanine conjugated axially with poly(sebacic anhydride) and micronized the polymer into nanoparticles via a microphase inversion method. The degradation in an alkaline medium was monitored by a combination of laser light scattering and fluorescence spectroscopy. Because of the axial substitution, the change in aggregation state during nanoparticle formation and degradation, as reflected by the fluorescence spectra, is relatively small. The results suggest that this novel polymer-based colloidal system is potentially useful for the delivery and release of photosensitizers in PDT.

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