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Water-Soluble Distyrylbenzene Fluorophore and Fluorescence Behavior in a Polymeric Vesicle

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Water-Soluble Distyrylbenzene Fluorophore and Fluorescence Behavior in a Polymeric Vesicle

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요 약. 수용액에서 vesicle을 형성하는 고분자, poly(sodium acrylamidoundecanoate)(**PSAU**)와 수용성 형광다이, **TPADSB-C**를 합성하고 흡수 및 형광 분광기를 이용하여 광학적 특성을 연구하였다. N-phenyl naphthylamine을 형광 probe로 사용하여 **PSAU**의 농도가 0.01 g/L 이상에서 고분자들의 응집에 의해 vesicle을 형성함을 확인하였다. 수용성 형광다이의 형광 특성을 vesicle의 존재유무에 따라 조사함으로써 형광다이 주위의 미세환경의 변화에 따른 광학적 특성의 변화를 측정하였다. 형광다이를 고분자 vesicle안에 침투시킬 경우 형광체 주변의 미세 환경(극성 등)의 변화에 따라 수용액 대비 발광 파장은 blue-shift하였고 형광 효율도 90%로 증가하였다. 본 연구는 형광다이를 함유하고 있는 고분자 vesicle이 바이오이메징 응용에 있어 효과적이고 안정적이면서 biocompatible한 레이블용 테그로 사용될 수 있음을 보여준다.

주제어: Vesicle, Fluorescence, Water-soluble Chromophore, Distyrylbenzene, Microenvironment

ABSTRACT. A vesicle forming polymer, poly(sodium acrylamidoundecanoate) (**PSAU**) and a water-soluble distyrylbenzene-based fluorophore, **TPADSB-C** were synthesized and characterized by using UV-vis and photoluminescence (PL) spectroscopy. An inter-chain vesicle formation of **PSAU** was observed at ~0.01 g/L from N-phenyl naphthylamine fluorescence measurement with changing **PSAU** concentration in water. Above critical aggregation concentration of **PSAU**, optical properties of **TPADSB-C** were investigated to study the microenvironment modulation through dye incorporation in the polymeric vesicle. The emission of **TPADSB-C** in the presence of **PSAU** vesicles was blue-shifted and the PL quantum efficiency was increased to 90% due to the microenvironment (e.g. polarity) change in aqueous solution. This study shows that the polymeric vesicle containing molecular fluorophores has a great potential as an efficient, stable and biocompatible labeling tag in biological cell imaging.

Keywords: Vesicle, Fluorescence, Water-soluble Chromophore, Distyrylbenzene, Microenvironment

INTRODUCTION

Among the various microstructures formed by the amphiphilic molecules, a vesicle is an especially interesting structure as it has two distinct domains, the lipophilic membrane and the interior aqueous cavity. It can entrap large quantities of chemicals (or molecules) either in the lipophilic membrane or in the aqueous cavity. Therefore, vesicles formed by synthetic surfactants have attracted

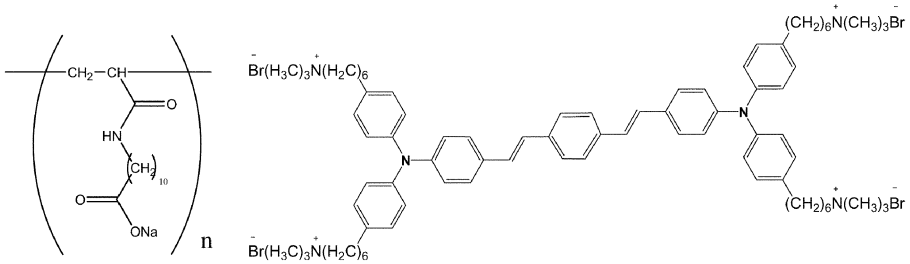
tremendous attention because of their potential uses as agents for encapsulation and eventual release of drugs, flavors, and fragrances, and also as microreactors for the synthesis of monodispersed nano-sized semiconductor particles.[1-6] The microstructures formed by the amphiphiles are in dynamic equilibrium with the surfactant monomers in solution. One way to obtain structural stabilization of the molecular self-assemblies is chemically tethering the surfactant monomers through polymerization. Polymerization prevents the surfactant reorganization necessary for the formation of reversed micelles and flip-flop. The vesicles formed by polysoaps are reported to be more stable and less permeable than respective monomeric ones.[7] Therefore, polymerized vesicles are expected to act as an excellent micro-container for chemicals such as cosmetic substances and pharmaceutical drugs. The polymerized vesicles are well known structures as a biomimetic system which is capable of carrying out cell-like functions.[8]

In this contribution, we report the synthesis and optical characterizations of a vesicle-forming amphiphilic polymer, poly(sodium acrylamidoundecanoate) (**PSAU**) and a water-soluble distyrylbenzene-based fluorophore, **TPADSB-C** (*Fig.* 1). Inter-chain vesicle formation of **PSAU**, the chromophore inclusion in the vesicle and its optical properties with/without the vesicles were studied by using UV-vis and photoluminescence (PL) spectroscopy. The microenvironment modulation around the fluorophores using the vesicles is discussed in terms of utilizing them as optical tags in biological cell imaging.

EXPERIMENTAL

All chemicals except acryloyl chloride (TCI) were purchased from Aldrich Chemical Co. and used without further purification. All solvents used were of reagent - grade and whenever necessary purified, dried, and distilled before use. ^{1}H NMR spectra were recorded on a JEOL (JNM-AL300) FT NMR system. UV-visible spectra were measured with a Jasco (model V-630) spectrophotometer. The steady-state fluorescence spectra were measured on a Jasco FP-6500 spectrofluorometer. A saturated solution of the probe, N-phenyl naphthylamine (NPN) was prepared by adding an excess compound to deionized water with stirring at 40 °C for 24 h. After equilibration at room temperature, the remaining undissolved molecules were removed by filtration through Millipore syringe filter (0.22 mm). A polymer stock solution (2 g/L) was prepared in the above probe solution. Appropriate volume of the saturated probe solution was used to make various dilutions of the polymer stock solution. All fluorescence spectra were measured at equilibration (after 24 hrs) after sample preparation.

Synthesis of PSAU. PSAU was obtained by free radical polymerization of sodium acrylamidoundecanoate (SAU) in water using $K_2S_2O_8$ as a radical initiator at 60 °C under a N_2 atmosphere according to the procedures reported elsewhere.[9] The polymer solution was dialyzed for 72 h using 12 kDa molecular weight cut off dialysis bag against alkaline water (pH~10) and lyophilized to get dry **PSAU**. ^{1}H-NMR



PSAU **TPADSB-C**

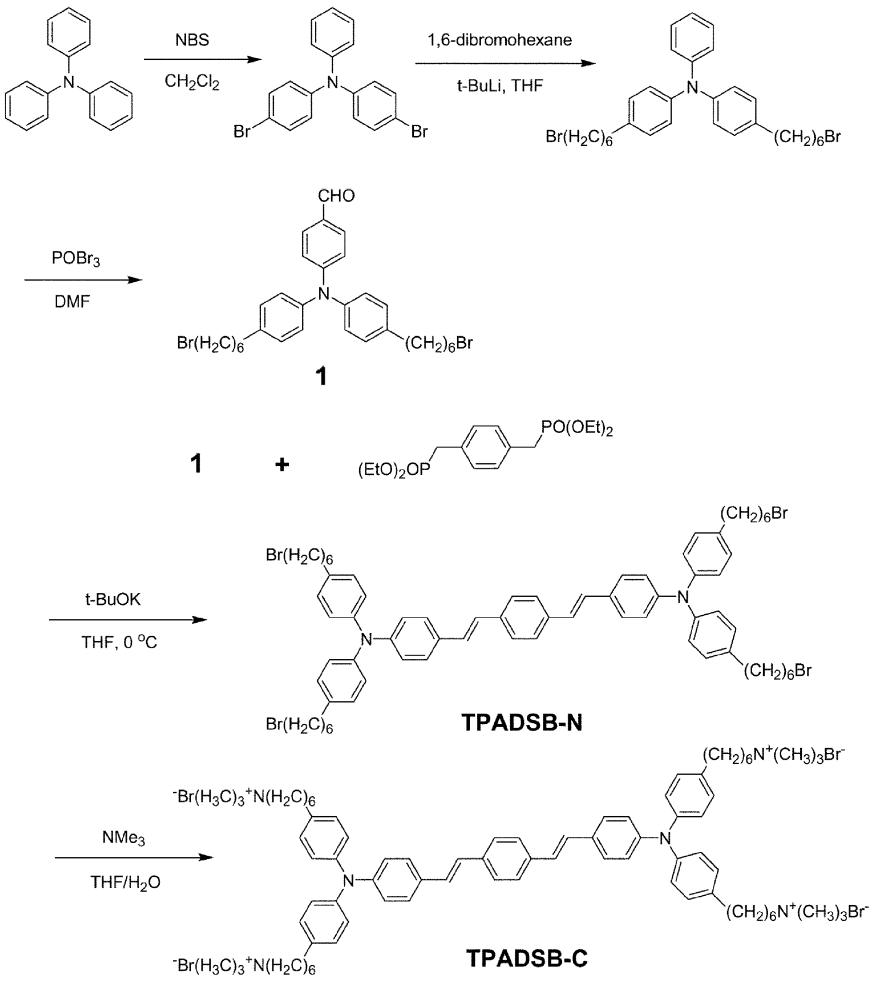
Fig. 1. Chemical structures of **PSAU** and **TPADSB-C**.

for SAU (300 MHz, $CDCl_3$): δ 6.29 (d, 1H), 6.06 (dd, 1H), 5.59 (d, 1H), 3.27 (qt, 2H), 2.3 (t, 2H), 1.5 (br, 4H), 1.21 (br, 12H). The polymerization was confirmed by the disappearance of the vinyl proton peaks at δ=5~7 ppm in the ^{1}H NMR spectrum of **PSAU** in D_2O.

N,N-Bis[4'-(6"-bromohexyl)phenyl]-4-aminobenzaldehyde (1). A 100 mL round-bottom flask containing 30 mL of dry DMF was cooled down to 0 °C. Phosphorous oxybromide (1.2 g, 4.2 mmol) was added maintaining the temperature below 5 °C. The reaction mixture was stirred for 30 min. A white solid was observed to be precipitated. Bis[4-(6'-bromohexyl)phenyl]phenylamine (0.8 g, 1.4 mmol) in 10 mL of dry DMF was added to the above solution and the temperature was slowly increased to 90 °C. After the solution was heated overnight, it was cooled down to room temperature and poured into cold water. The pH of the solution was adjusted to around 7 with aqueous NaOH solution. The product was extracted with dichloromethane, dried over magnesium sulfate, and purified by silica gel column chromatography (ethyl acetate/hexane (v/v) = 1/10). The product yield was 0.67 g (80%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 9.79 (s, 1H, -*CHO*), 7.66 (d, 2H, J = 8.8 Hz), 7.15 (d, 4H, J = 8.4 Hz), 7.09 (d, 4H, J = 8.4 Hz), 6.96 (d, 2H, J = 8.8 Hz), 3.42 (t, 4H, -CH_2Br, J = 6.8 Hz), 2.61 (t, 4H, ArCH_2-, J = 7.8 Hz), 1.88 (m, 4H), 1.66 (m, 4H), 1.50 (m, 4H), 1.40 (m, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 190.6, 153.8, 143.8, 139.9, 131.5, 129.8, 128.6, 126.6, 118.5, 35.5, 34.2, 32.9, 31.4, 28.6, 28.2. HRMS (EI): m/z = 597.1268 (M^+), Δ = 4.3 ppm.

1,4-Bis[N,N-bis(4"-(6'"-bromohexyl)phenyl)-4'-aminostyryl]benzene (TPADSB-N). A 50 mL two-necked flask charged with 1,4-bis(diethylphosphonatemethyl)benzene (0.14 g, 0.37 mmol) and 2.2 eq. of aldehyde **1** (0.5 g, 0.83 mmol) in 20 mL of dry THF was cooled down to 0 °C with an ice bath. To the above solution, 0.83 mL (2.2 eq.) of potassium tert-butoxide (in THF, 1 M) was added dropwise at 0 °C. The reaction mixture was stirred for 6 hrs at 0 °C and then quenched with water. The solvent was removed under reduced pressure and the resulting mixture was diluted with dichloromethane, washed with water and brine, and dried over magnesium sulfate. The crude compound was purified by silica gel column chromatography (dichloromethane/hexane = 1/5).The product yield was 0.38 g (80%). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.47 (s, 4H), 7.38 (d, 4H, J = 8.8 Hz), 7.09~7.01 (m, 22H), 6.98 (d, 2H, J = 16 Hz), 3.43 (t, 8H, -CH_2Br, J = 6.8 Hz), 2.59 (t, 8H, ArCH_2-, J = 7.6 Hz), 1.89 (m, 8H), 1.65 (m, 8H), 1.50 (m, 8H), 1.40 (m, 8H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 147.9, 145.5, 137.6, 136.9, 131.0, 129.4, 128.1, 127.4, 126.8, 126.4, 124.8, 122.8, 35.5, 34,3, 33.0, 31.5, 28.7, 28.3. MS (FAB): m/z = 1264 (M^+).

1, 4-Bis[N,N-bis(4"-(6'"-(N,N,N-trimethylammonium)hexyl)phenyl)-4'-aminostyryl]benzene tetrabromide (TPADSB-C). To a solution of **TPADSB-N** (0.15 g, 0.12 mmol) in THF (20 mL), was added an excess of condensed trimethylamine (2 mL) using a dry ice/acetone filled gas condenser at -78 °C. The reaction mixture was allowed to warm to room temperature slowly and stirred for 12 hrs. A small amount of water was added to the above solution to dissolve the precipitated compounds and the reaction solution was cooled down to -78 °C with a dry ice/acetone bath. An excess of condensed trimethylamine (2 mL) was added again and the resulting solution was allowed to reach room temperature and stirred for additional 12 hrs. After the reaction was completed, the remaining trimethylamine and solvent were removed under reduced pressure. A crude product was dissolved in a minimum amount of hot methanol and precipitated into diethyl ether several times. The yield was 0.15 g (85 %). ^{1}H-NMR (300 MHz, DMSO): δ 7.55 (s, 4H), 7.48 (d, 4H, J = 8.8 Hz), 7.20 (d, 2H, J = 16.4 Hz), 7.15 (d, 8H, J = 8.4 Hz), 7.08 (d, 2H, J = 16.4 Hz), 6.96 (d, 8H, J = 8.4 Hz), 6.88 (d, 4H, J = 8.8 Hz), 3. 28 (m, 8H), 3.05 (s, 36H), 2.55 (t, 8H, ArCH_2-, J = 7.6 Hz), 1.68 (m, 8H), 1.60 (m, 8H), 1.36 (m, 16H). MS (ESI): m/z = 671 $(M-2Br)^{2+}$, 421 $(M-3Br)^{3-}$, 296 $(M-4Br)^{4+}$.



Scheme 1. Synthetic routes to **TPADSB-N** and **TPADSB-C**.

RESULTS AND DISCUSSION

PSAU was obtained by free radical polymerization of sodium acrylamidoundecanoate in water using $K_2S_2O_8$ as a radical initiator.[9] The final polymer was purified by dialysis using 12 kDa molecular weight cut off bag against alkaline water (pH=10). For the synthesis of compound **1** (*Scheme* 1), triphenylamine was brominated with N-bromosuccinimide in dichloromethane and the successive lithiation/alkylation with t-BuLi and excess 1,6-dibromohexane yielded bis[4-(6'-bromohexyl)phenyl]phenylamine in 63% yield. To prevent intermolecular coupling reactions, a large excess (10 eq.) of 1,6-dibromohexane was added. Aldehyde functionality was easily introduced by Vilsmeier formylation to give the structure **1** in 78% yield. Horner-Emmons Wittig coupling between 1,4-bis(diethylphosphonatemethyl)benzene and 2 eq. of compound **1** using potassium tert-butoxide in THF at 0 °C gave **TPADSB-N**. The reaction temperature was kept around 0 °C in order to remove the possibility of halide elimination during the reaction. Formation of the final water-soluble **TPADSB-C** was easily achieved via quaternization with a large excess of trimethylamine in the mixed solvent of THF and water. After addition of trimethylamine, the insoluble solid forms gradually as the reaction proceeds, which means the ionic units are formed

via quaternization. To ensure that complete quaternization is achieved, it is recommended to re-dissolve the precipitated compounds by adding water and to add excess trimethylamine for a further reaction. Removal of unreacted trimethylamine does not impose any problem because of its low boiling point.S

The surfactant units in the polymer chain can assemble in a way to form unimolecular micelles in aqueous solution. This process is expected to be independent on the polymer concentration [**PSAU**]. On the other hand, the polymer chains can also undergo self-organization to form inter-chain aggregates at higher concentrations. In order to examine hydrophobic domain formation, we have performed fluorescence probe studies using NPN as a probe molecule. Though NPN is weakly fluorescent in aqueous solution, its fluorescence spectrum in the presence of **PSAU** shows 8 times intensity increase accompanied by a 52 nm blue-shift (*Fig.* 2). This suggests that the probe molecules are solubilized in nonpolar environments within the polymer aggregates.[10-11]

The change of relative fluorescence intensity (*I*/*I*o, where *I* and *I*o are the fluorescence intensities of NPN in the presence and absence of polymer, respectively) with increasing polymer concentration is shown in *Fig.* 3. Large enhancement of *I*/*I*o at higher polymer concentrations indicates the formation of inter-chain aggregates. The corresponding shift of the emission maximum of NPN ($\Delta\lambda = \lambda_{\text{in water}} - \lambda_{\text{in PSAU solution}}$) is also plotted as a function of

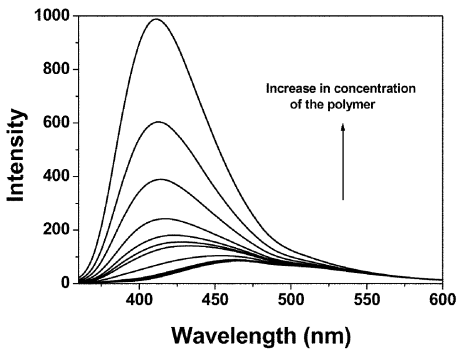


Fig. 2. Fluorescence spectra of NPN with increasing [**PSAU**].

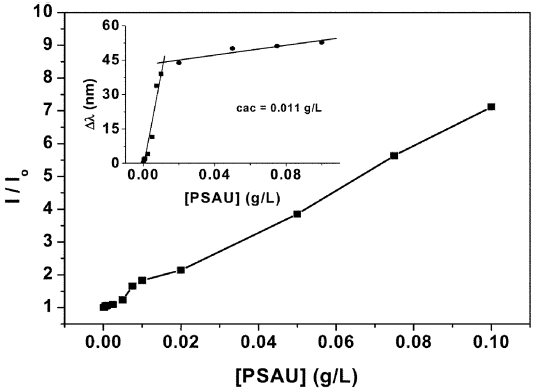


Fig. 3. Plot of relative PL intensity (*I*/*I*o) of NPN as a function of [**PSAU**]. Inset) plot of the shift of emission maximum of NPN as a function of [**PSAU**].

polymer concentration (Inset *Fig.* 3). Although spectral shift shows saturation after complete solubilization of the probe within the hydrophobic domains of polymer aggregates, the emission intensity keeps increasing. The plot shows that the onset of inter-chain aggregation occurs at polymer concentration of ~0.01 gL^{-1}. In analogy with the critical micelle concentration (cmc) of monomeric surfactants, the concentration corresponding to the inflection point can be referred as a critical aggregation concentration (cac). Thus, cac of **PSAU** obtained from the plot is about 0.01 gL^{-1}. The absence of any concentration-independent region at low [**PSAU**] (inset *Fig.* 3) suggests that single polymer chain has also hydrophobic domains that can bind the probes in dilute aqueous solutions.

Fig. 4 shows absorption and PL spectra of **TPADSB-N** in toluene and **TPADSB-C** in water. Both **TPADSB-N** and **TPADSB-C** have same electronic conjugation. The charged ionic units only adjust the solubility in different solvents due to break-up of π-conjugation with the main chromophore backbone. The emission spectrum in water is red-shifted (λ_{PL} = 520 nm), structureless and broad relative to that in toluene (λ_{PL} = 457 nm). The absorption spectra in toluene (λ_{abs} = 415 nm) and in water (λ_{abs} = 408 nm) show a negligible difference and a slight blue-shift in water is originated from conformational distortion due to poor solubility and/or hydrogen bond-

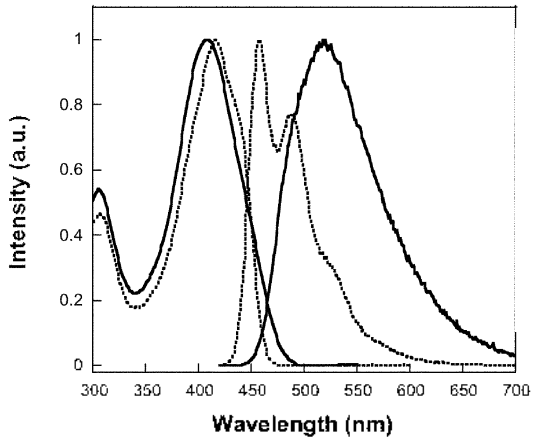


Fig. 4. UV-vis absorption and fluorescence spectra of **TPADSB-N** in toluene (dotted line) and **TPADSB-C** in water (solid line).

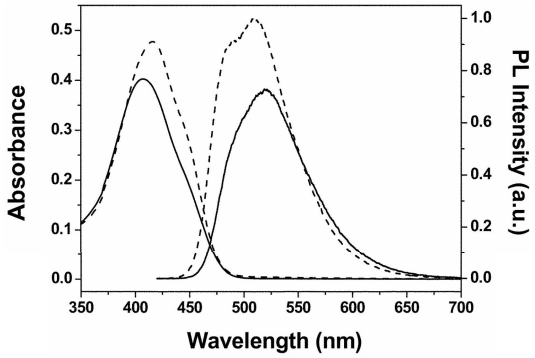


Fig. 5. Absorption and fluorescence spectra of **TPADSB-C** in water (solid line) and in **PSAU** vesicle solution (dotted line).

ing interaction with water.

The steady-state PL and UV-vis spectra of **TPADSB-C** ([**TPADSB-C**] – 1×10^{-6} M) in the presence and absence of **PSAU** in water are presented in *Fig.* 5. The **PSAU** concentration (3 g/L) was adjusted to exceed its cac (~0.01 g/L) for complete formation of inter-chain vesicles. The emission of **TPADSB-C** is blue-shifted (λ_{PL} ~520 nm → 510 nm) in **PSAU** solution due to incorporation of the chromophores inside of the vesicles. The PL quantum efficiency (Φ_{PL}) of the chromophore was measured relative to fluorescein in water (pH~10). The Φ_{PL} of **TPADSB-C** in water is 73% in the absence of **PSAU** and 90% in the presence of **PSAU** vesicle. The enhancement in the quantum efficiency is caused by the nonpolar microenvironment around the chromophores due to inclusion inside the **PSAU** vesicle. The inside of bilayers of the vesicle is hydrophobic and nonpolar relative to water, which is similar to organic solvents. **TPADSB-C** is water-soluble with charged ionic groups but the main conjugated backbone is still hydrophobic. The chromphores are expected to exist in the bilayers through hydrophobic interactions. Molar absorptivity of the chromophore is also increased with the **PSAU** vesicles (*Fig.* 5).

It is interesting to mention that the fluorescence spectrum with **PSAU** shows a blue-shifted emission with enhanced Φ_{PL} (90%) but it is different from the spectra in toluene and in water. However same UV-vis and PL spectra of **TPADSB-C** in a sodium dodecylsulfate (SDS) micelle were reported with regard to those of **TPADSB-N** in toluene.[12] It is expected that the microenvironment inside the SDS micelle is very similar to nonpolar organic solvents such as toluene, which is not the same case with the **PSAU** vesicle. There are two different micro domains inside the vesicle, the aqueous core and the hydrophobic bilayer. **TPADSB-C** can exist in both of the core and the bilayer. The measured absorption and PL spectra of **TPADSB-C** in **PSAU** vesicles are intermediate between those in toluene and water. The chromophores in two different micro domains probably contribute together to the resulting spectra. The polymeric vesicle is a very interesting microcontainer which modulates the microenvironment (such as polarity) around the molecular fluorophores in bulk aqueous solution. The encapsulated fluorophores inside the vesicle have a great potential as efficient molecular labels with biocompatibility in biological imaging using confocal microscopy and two-photon induced fluorescence microscopy.

In summary, an inter-chain vesicle formation of poly(sodium acrylamidoundecanoate) (**PSAU**) was studied using NPN fluorescence measurement with changing **PSAU** concentration in water. **PSAU** shows a critical aggregation concentration at ~0.01 g/L. Above cac, UV-vis and fluorescence spectroscopy of the water-soluble fluorophore (**TPADSB-C**) was investigated to study the microenvironment modulation through dye incorporation in the polymeric

vesicle. The emission of **TPADSB-C** in the presence of **PSAU** vesicles was blue-shifted and the PL quantum efficiency was increased to 90% due to the microenvironment (e.g. polarity) change in aqueous solution. This study shows that the polymeric vesicle containing molecular fluorophores has a great potential as efficient, stable and biocompatible labeling tags in biological cell imaging.

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