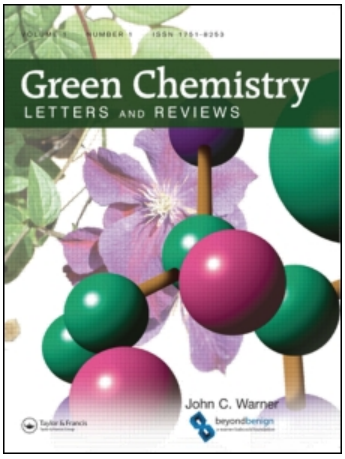

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Core-shell thymine containing polymeric micelle system: study of controlled release of riboflavin

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Core-shell thymine containing polymeric micelle system: study of controlled release of riboflavin

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A series of bioinspired poly(vinylbenzylthymine)-*b*-poly(vinylphenylsufonate) was synthesized and its core-shell thymine containing polymeric micelle system was prepared. The study of control release of riboflavin with micelles was examined by photometrical assay and demonstrated that the guest materials could be encapsulated by hydrogen bonding with the attached thymine in the core.

Keywords: self-assembly; block copolymer; core-shell polymers; thymine; bioinspiration

Introduction

Nature provides abundant and elegant examples of the synthesis of materials in terms of both atom economy and energy utilization. Bioinspiration, identifying naturally occurring mechanisms that can be extrapolated to synthetic systems, is one way to develop environmentally benign materials using the principles of green chemistry (*1*,*2*). Thymine, one of the nucleic bases in DNA, is not only well known to stabilize the double helix structure of DNA by strong hydrogen bonding against adenine, but is also known to photodimerize when exposed to UV light and thus disrupt the helical structure of DNA (*3–5*). Deriving inspiration from this biological mechanism, we have sought to create novel materials.

Thymine-functionalized polymers have been well studied because the thymine moiety can form complexes through hydrogen bonding (*6–17*). Rotello et al. found that diaminotriazine and thymine-functionalized polymers form self-assembled micro scale spheres or vesicles in non-polar media based on hydrogen bonding (*6–8*). Ward and Long et al. demonstrated specific and reversible adhesion of terminal thymine-functionalized polystyrene to adenine recognition sites on a silicon surface (*9*). Puskas et al. have used thymine-functionalized polymers for application in biotechnology such as affinity bioseparation (*10*,*11*). We have synthesized a thymine-functionalized styrene monomer, vinylbenzylthymine (VBT), and found that its copolymers with anionic and cationic vinyl monomers form highly sensitive photocrosslinkable polymers by the photodimerization of thymine as a result of irradiation with short wavelength UV light (*13–17*). When irradiated with UV light, the 2π electron system of two adjacent thymines form a cyclobutane crosslink. It is also known that this cyclobutane ring can be opened either by UV irradiation or by enzymatic action (*16*).

Self-assembling amphiphilic block copolymer nanoparticles obtained in aqueous media through a micellization process are of interest because of their potential for application as industrial surfactants, nanocapsules for biomedical usage, electronic pollution control devices, removal of organic contaminates in water, and in other nanofabricated materials (*18–24*). The self-assembly of an amphiphilic block copolymer can be explained by the core segregation created by the interaction between the hydrophilic and hydrophobic blocks with one another and with the surrounding medium. The hydrophilic block becomes a shell which is often called the corona, and the hydrophobic block becomes the core in a hydrophilic selective solvent. There are studies using hydrogen bonding in amphiphilic block copolymers as a driving force for micellization or as a template for small molecule recognation (*25*,*26*). In addition, hydrogen bonding can be used to form micelles from the self-assembly of a pair of homopolymers or in organic solvents such as 1,4-dioxane and ethanol (*27–31*).

In the previous paper, we have reported the stability enhanced nanopolymeric micelles aggregates system from amphiphilic block copolymers of VBT and vinylphenylsufonate (VPS) (*32*). The stability of the micelles could be controlled by the amount of core-photocrosslinking of the attached thymines. Furthermore, such thymine containing amphiphilic block copolymer micelles aggregates showed the ability of the hydrogen bonding of the attached thymines inside the micelles to effect the micelle properties. These results suggested that the micelles from VBT and VPS

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block copolymers have the potential to encapsulate guest materials by hydrogen bonding with the attached thymine in the core.

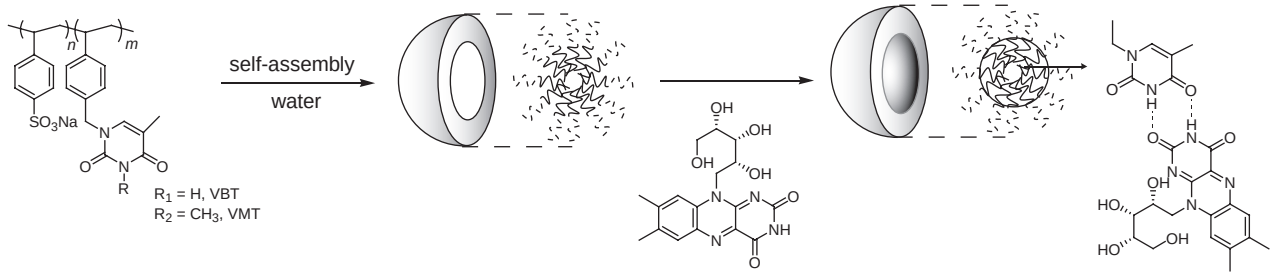
We report here a control release study of bioinspired core-shell thymine containing polymeric micelles system from poly(VBT)-*b*-poly(VPS). A series of amphiphilic block copolymers of VBT and VPS, and of 3-methyl-1-(4-vinylbenzyl)thymine (VMT) and VPS have been synthesized and a suitable comonomer molar ratio of amphiphilic block copolymer for a micelles aggregate system was determined (Scheme 1). A study of the controlled release of riboflavin was examined by photometrical assay.

Results and discussion

Poly(vinylbenzylthymine (VBT)-b-vinylphenylsufonate (VPS)) synthesis

The thymine-functionalized monomer VBT and VMT synthesis and the block copolymers synthesis was carried out according as previously reported (*32*,*33*). The polymerization results are summarized in Table 1. VBT was not totally soluble in the water/ethylene glycol mixture but the reaction proceeded satisfactorily in this heterogeneous system. VMT was obtained by methylation of VBT to block the possibility of hydrogen bonding.

A series of four amphiphilic block copolymers of VBT and VPS in different monomer ratio were prepared using TEMPO-mediated living radical polymerization: poly(VBT(1)-*b*-VPS(1)), poly(VBT(1)-*b*-VPS(2)), poly(VBT(1)-*b*-VPS(4)), and poly(VBT(1)-*b*-VPS(8)) (Scheme 2). The number in the parentheses is the molar ratio of the respective monomers. The homopolymer of VPS was prepared first and then different monomer ratios of VBT were added to the reaction solution to prepare the block copolymers. Polymer structures were characterized by ^{1}H-NMR, ^{13}C-NMR, and IR; e.g. Poly(VBT(1)-VPS(1))-H: ^{1}H-NMR: (500 MHz, DMSO-*d*6) δ 0.71–2.10 (br, 9H, –CH_2–CHR–, –CH_3), 4.63 (br, 2H, –CH_2–), 5.88–7.96 (br, 9H, aromatic C–H), 11.3 (br, 1H, N–H). IR: $\nu_{N-H} = 3195$, $\nu_{C=O} = 1672$. $M_n = 7.9 \times 10^4$, $M_w/M_n = 1.73$. The molecular weight of the formed block copolymers were measured by gel permeation chromatography (GPC) with poly(styrene sulfonic acid sodium salt) standards and the molar ratios of VBT:VPS in the block polymer were determined by

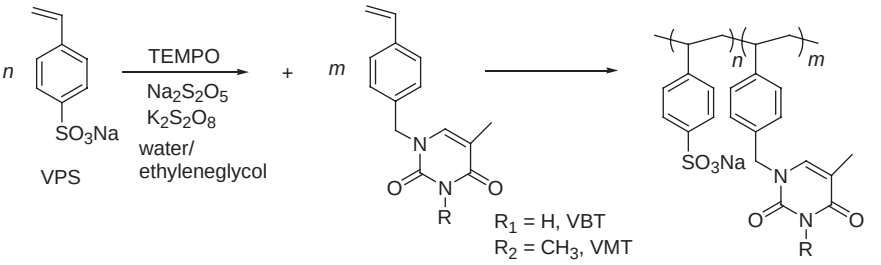


Scheme 1. Thymine containing polymeric micelle system.

Table 1. Blockcopolymerization of vinylbenzylthymine (VBT) and vinylphenylsufonate (VPS)[a].

Entry	Polymer code	VBT/VPS[b] Feed	VBT/VPS[b] Polymer[d]	Yield (%)	M_n (M_w/M_n)[c] ($\times 10^4$) (–)
1	Poly(VPS)[e]	0/1	0/1	80	3.3 (1.75)
2	Poly(VBT(1)-*b*-VPS(1))	1/1	1/1.04	100	8.1 (1.59)
3	Poly(VBT(1)-*b*-VPS(2))	1/2	1/2.17	98	5.7 (1.48)
4	Poly(VBT(1)-*b*-VPS(4))	1/4	1/3.50	91	5.2 (1.89)
5	Poly(VBT(1)-*b*-VPS(8))	1/8	1/9.11	76	3.5 (1.65)
6	Poly(VMT(1)-*b*-VPS(1))	1/1	1/1.23	75	3.5 (1.72)

[a]All of the polymerization was carried out in water/ethylene glycol = 1/1 (10 ml).
[b]Molar ratio of the respective monomers.
[c]Estimated by gel permeation chromatography with poly(styrene sulfonic acid sodium salt) standards in water/methanol = 70/30 buffer (sodium phosphate = 0.5M, sodium sulfate = 0.5M).
[d]Molar ratio measured by NMR integration.
[e]Homo poly(sulfonic acid sodium salt) polymer using TEMPO-mediated living radical polymerization.



Scheme 2. Poly(VBT)-*b*-(VPS) synthesis.

^{1}H-NMR integration. The amphiphilic block copolymer of VMT and VPS, poly(VMT(1)-*b*-VPS(1)), was synthesized to examine the effect of hydrogen bonding on the stability of micelles as previously reported (*32*). Polydispersity of the formed block copolymers became high compared to the other TEMPO-mediated living radical polymerizations (*34*). Polydispersity was low, however, when compared to previously reported VPS block copolymer synthesis in a heterogeneous aqueous solvent systems (*34*,*35*).

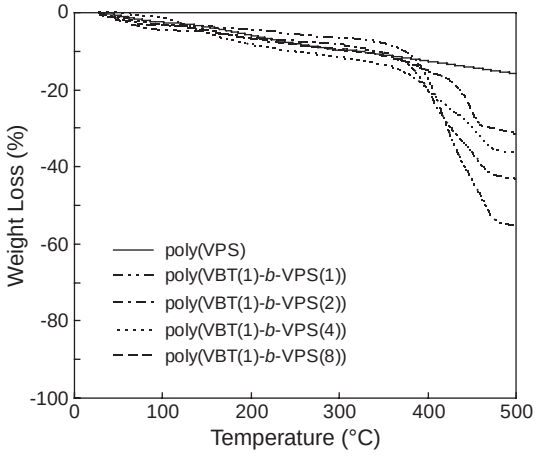


Figure 1. Thermogravimeteric analysis results for a series of poly(VBT(*n*)-*b*-VPS(*m*)).

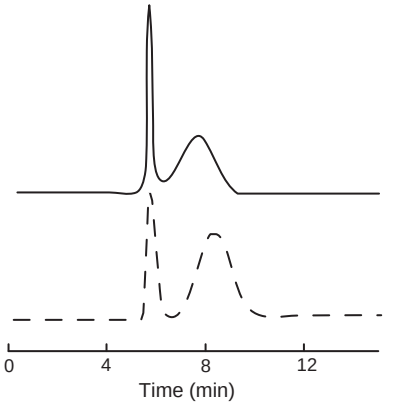


Figure 2. GPC trace of poly(VBT(1)-*b*-VPS(1)) and poly(VBT(1)-*b*-VPS(2)) in water/methanol = 70/30 buffer (sodium phosphate = 0.5M, sodium sulfate = 0.5M). Solid line: poly(VBT(1)-*b*-VPS(1))-H, broken line: poly(VBT(1)-*b*-VPS(2)).

The thermal properties of a series of amphiphilic block copolymers of VBT and VPS were analyzed by thermogravimeter in a nitrogen stream. The thermogravimetric curves of the block copolymers are compared in Figure 1. The initial weight loss below 100°C is a result of water evaporation. It should be noted that the VBT copolymers have a degradation stage at 375°C corresponding to VBT (*33*). VPS homopolymer did not show the degradation stage at 375°C and the degradation stage at 375°C of block copolymers of VBT and VPS increased as the ratio of VBT increases in the polymer system.

To determine the suitable comonomer molar ratio of amphiphilic block copolymer for a micelle system, obtained amphiphilic block copolymers before any micellization have dissolved in a water/methanol buffer and their GPC trace have been measured. Poly(VBT(1)-*b*-VPS(1)) and poly(VBT(1)-*b*-VPS(2)) showed the characteristically sharp micelle peak before the single chain polymer peak in GPC trace (Figure 2). Other poly(VBT(1)-*b*-VPS(4) and poly(VBT(1)-*b*-VPS(8)) did not show that sharp peak. These results obtained by dissolving the amphiphilic block copolymers in a buffer before any treatment for micellization suggest that the lengthening of the hydrophobic block chain facilitated micelle formation and that a 1:1 comonomer ratio as in poly(VBT(1)-*b*-VPS(1)) is the best polymer in our experiments for micellization and formed micelles in aqueous solution easily (*36*).

Controlled release study of riboflavin-loaded micelles aggregates

It is known that an amphiphilic block copolymer with a suitable hydrophilic/hydrophobic balance will form micelles in aqueous solution. Based on the GPC results, poly(VBT(1)-*b*-VPS(1)) was selected and dialysis method was used to form micelles and the size of the obtained block copolymer micelle aggregates (164 nm) was analyzed by dynamic light scattering

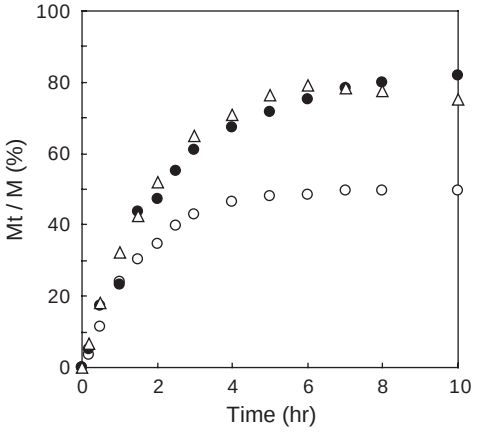


Figure 3. Release behavior of riboflavin. ● = riboflavin alone, ○ = riboflavin with the poly(VBT(1)-*b*-VPS(1) micelle aggregates, △ = riboflavin with the poly(VMT(1)-*b*-VPS(1)) micelle aggregates.

(DLS) as previously reported (*32*). A previous report suggested that hydrogen bonding of attached thymine in the core plays an important role in micelle formation and affects both the morphology and stability of the micelles (*32*). However, it was not possible to demonstrate the hydrogen bonding of thymine units in the micelle solution by IR. This was inconclusive because of the multiple broad peaks between 3500 and 3000 cm^{-1}. When a highly hydrophilic block and a highly hydrophobic block with a hydrogen bonding unit are used, the block copolymer has a strong tendency to form micelles in water and in this case, the effect of a hydrogen bonding between hydrogen units in the core on the micellization must be minimal. The hydrogen bonding in the core after the micellization, however, must be more important because of the hydrophobicity of the core and this hydrogen bonding sites from attached thymines in the core have the potential to encapsulate guest materials by hydrogen bonding (*26*). We chose riboflavin, which has a hydrogen bonding site in its structure and is known to bind with thymine (*37*), as the guest material and the controlled release of the loaded riboflavin from micelle aggregates was examined by photometrical assay to investigate the effect of hydrogen bonding inside block copolymers micelle of VBT and VPS. Riboflavin is a water soluble vitamin. It is well known that a hydrophobic chemical can be easily entrapped physically inside block copolymer micelles in aqueous solution by hydrophobic interaction because of its hydrophobicity of the micelles' core. When a hydrophilic chemical such as riboflavin is mixed with micelles, there is no interaction between the core and the hydrophilic chemical should distribute itself equally in the outer water phase and the micelles. However, if there are thymines inside the core, a hydrophilic chemical which has a hydrogen bonding site could hydrogen bond with the thymines and thus could be encapsulated inside the micelles.

To form the riboflavin-loaded micelles aggregates, an aqueous solution of riboflavin was simply mixed with a micelle aggregates solution and this mixture was dialyzed against water. The release behavior of riboflavin from a neat riboflavin solution, riboflavin with poly(VBT(1)-*b*-VPS(1)) micelle aggregates solution, and riboflavin with poly(VMT(1)-*b*-VPS(1)) micelle aggregates solution within a dialysis bag to outside water were examined. Data of riboflavin release behavior were summarized in Figure 3. After 10 hr of dialysis, ca. 80% of riboflavin was released from the neat riboflavin solution but only ca. 50% of the riboflavin was released from riboflavin with poly(VBT(1)-*b*-VPS(1)) micelle aggregates solution. The riboflavin release behavior of the riboflavin with poly(VMT(1)-*b*-VPS(1)) micelle aggregates in which the hydrogen bond donating ability was blocked by the methyl group demonstrated the same result of ca. 80% release as observed with the control neat riboflavin solution. In addition, ca. 30% of loaded riboflavin remained in micelle aggregates even after 48 hrs. These results suggest that when riboflavin was mixed with poly(VBT(1)-*b*-VPS(1)) micelle aggregates solution, some of the riboflavin was encapsulated in the micelle, suggesting that the block copolymers micelle of VBT and VPS have the hydrogen bonding site of the attached thymines in the core and can encapsulate guest materials by hydrogen bonding. This hydrogen bonding encapsulation has the potential to be used in the controlled release of materials from such micelles.

Experimental section

Materials and measurements

All reagents and materials were purchased from Sigma-Aldrich in the purest form available and used as received. NMR spectra were taken on a Bruker 500 MHz NMR spectrometer. IRs were recorded using a Thermo Electron Corp. class 1 laser product. Molecular weights were measured using an Agilent 1100 series GPC with Agilent GPC data analysis software equipped with RID using Tosoh G4000PW_{XL} columns. Calibration curves were obtained using poly(VPS) standards. The thermal stability of the block copolymers was studied using Q50

thermogravimetric analysis (TGA) from TA Instruments. To perform the TGA experiment, the polymer samples were carefully weighed, put into the furnace of the instrument and heated, under nitrogen, over a range of 30–500°C at 10°C/min.

Copolymer synthesis

The following is a typical procedure for polymerizations in Table 1 except entry 1 and 6. VPS (1.0 g, 4.85 mmol) was dissolved in 10 mL of 50% ethylene glycol/water and TEMPO (74 mg 0.47 mmol) was then added followed by Na_2SO_4 (34 mg, 0.18 mmol) and $K_2S_2O_8$ (64 mg, 0.24 mmol) and the solution was heated at 60°C for 1 h under nitrogen. The mixture became clear and was stirred at 125°C for 5 h under nitrogen to prepare homogenous VPS polymer. VBT (molar range from 4.85 to 0.61 mmol) was then added to the homogenous VPS polymer solution and stirring continued at 125°C under nitrogen for 5 h. The polymerized product was purified by adding the solution to 200 mL of acetone. The resulting solids were collected by filtration and dried under vacuum.

Micelle formation and its characterization

Micelle formation and its characterization were examined according as previously reported (*32*).

Controlled release study of riboflavin-loaded micelles

Ten mL of micelles solution (0.5 mg/ml) 10 mL was mixed with 10 mL of riboflavin aqueous solution (12.0×10^{-5} M) and sonicated for 30 min to form a riboflavin-loaded micelle solution. This solution was transferred into a pre-swollen semi-permeable membrane bag (Spectra/Por, SPEC-TRUM, molecular weight cutoff, 3500), and dialyzed against 200 mL of water at 36°C, stirring rate of 100 rpm. At intervals, the micelle solution in the dialysis bag was sampled and subjected to photometrical assay at 450 nm to determine the residual riboflavin content in the micelle (Scheme 2).

Conclusion

We have synthesized a series of VBT and VPS block copolymers by TEMPO-mediated living radical polymerization and formed their block copolymer micelle aggregates in aqueous solution. Control release behavior of riboflavin from riboflavin with micelles aggregates suggested that the block copolymers micelle aggregates of VBT and VPS have the hydrogen bonding site of the attached thymines in the core and could encapsulate guest materials by hydrogen bonding. This method provides a novel approach of designing controllable nanomicelles based on a bioinspired mechanism.

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