

Anitbacterial Coatings Based on Quaternized Poly(4-vinylpyridine) Block Copolymers

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INTRODUCTION

Marine biofouling involves colonization of under-water surfaces by marine organisms. Any surface immersed in sea-water rapidly acquires a bacterial biofilm. Bacterial biofilms can stimulate the settlement of invertebrate larvae and algal spores by producing chemical cues or triggers that are used by other organisms to colonize on the surface.¹⁻³ Quaternized polymers of 4-vinylpyridine have been shown to exhibit antibacterial property both, in aqueous solutions,⁴ and when tethered to surfaces⁵. In this work polystyrene-*block*-(N-hexyl-4-vinylpyridine) polymers were prepared and coated on glass microscope slides. A bilayer coating strategy was used wherein a base-layer of styrene-ethylene/butylene-styrene (SEBS) thermoplastic elastomer was coated with the pyridinium block copolymer. The polystyrene block of the pyridinium block copolymer is expected to anchor it to the SEBS layer by entrapment of the PS block in the phase segregated PS domains of the thermoplastic elastomer. The coatings were tested, and were found to show antibacterial activity against *Staphylococcus aureus*.

EXPERIMENTAL

Polymer Synthesis. Polystyrene-*block*-poly(4-vinylpyridine) (PS-*b*-P4VP) were prepared following the procedures in the literature.⁶ A low reaction temperature was used to avoid the side reactions of 4-vinylpyridine that can result in branching and crosslinking. The 4-vinylpyridine block was terminated by a (heptadecafluoro-1,1,2,2-tetrahydrodecyl) dimethylchlorosilane which is known to end-cap polymer chains during anionic polymerization.⁷ Block copolymers with different overall molecular weights, and relative weights of the two blocks were synthesized. Molecular weight of the styrene block was determined by gel permeation chromatography of the polymer in THF. The results indicated a narrow distribution, with the ratio of weight average molecular weight to the number average molecular weight between 1.05 and 1.1. The molecular weight of the 4-vinylpyridine block was estimated using the recipe used. Table 1 shows the molecular weight characteristics of the synthesized block copolymers.

Table 1. Molecular weight characteristics of polystyrene-*block*-poly(4-vinylpyridine) block copolymers

PS block (g/mol)	P4VP block (g/mol)	Total MW (g/mol)	wt % PS
11,000	21,000	32,000	34
62,000	66,000	128,000	48
76,000	48,000	124,000	61
14,000	8,000	22,000	64

The quaternization reactions carried out in dimethyl formamide using 1-bromohexane. For example, when 1.5 g of the 62k/66k block copolymer (7.3 mmol 4-VP) dissolved in 10 mL of DMF was reacted with 5 mL of 1-bromohexane (35.6 mmol) at 90 °C, the solution turned dark green in color within 2 hours. After ca. 12 hours, the green solution was cooled to room temperature, and its viscosity lowered by adding a small amount of chloroform. The polymer was precipitated by pouring the solution in diethyl ether cooled with ice-bath. The brown solid was

filtered, and dried under vacuum. Poly(4-vinylpyridine) homopolymers with weight average molecular weights of 60,000 g/mol and 160,000 g/mol, and poly(4-vinylpyridine-*co*-*n*-butylmethacrylate) copolymer with a molecular weight of 300,000 g/mol and 10 wt % BMA were also quaternized with 1-bromohexane. The block copolymers were characterized by IR spectroscopy using a KBr pellet. ¹H-NMR spectra were obtained using CDCl₃ as the solvent. Water contact angles were measured on the polymer surfaces using Ramé-Hart contact angle goniometer. NEXAFS spectra of the surfaces were obtained at the U7A NIST/Dow materials end-station at the National Synchrotron Light Source at Brookhaven National Laboratory.

Preparation of surfaces for antibacterial tests. The surfaces were prepared in two different ways. (1) A 10 % (w/v) solution of the SEBS thermoplastic elastomer (Kraton G1652M) was first spin coated on a glass microscope slide, and annealed in vacuum at 120 °C. A 5 % (w/v) solution of the quaternized PS-*b*-P4VP were then spin coated on top of the SEBS layer. The resulting bilayer coatings were further annealed in a vacuum oven at 150 °C for ca. 12 hours. (2) 5 % (w/v) solutions in methanol, of the quaternized homopolymers and quaternized 4-VP/BMA copolymer, were dried at ca. 25 °C on glass slides over a period of 24 hours. 5 % (w/v) solutions of the quaternized block copolymers in CHCl₃/CH₃OH mixture (1:1 v/v) were similarly dried after spreading over the SEBS base layer using a glass pipette. The surfaces were then dried under vacuum at ca. 25 °C to completely remove the solvents.

Antibacterial tests. Aqueous suspensions of *Staphylococcus aureus* with concentrations ranging from 10⁴ cells/mL to 10⁶ cells/mL were sprayed using a chromatography sprayer on the test surfaces. The sprayed surfaces were dried in air for ca. 2 min. Agar gel containing caesin peptone (17 g/L), soy meal peptone (3 g/L), glucose (2.5 g/L), NaCl (5 g/L) and dipotassiumhydrogenphosphate (2.5 g/L) as nutrients, was poured on the slides, allowed to solidify, and incubated at 37 °C overnight. The number of bacterial colonies over a 2 cm × 2 cm area on the slides was counted using a colony counter. Corning glass slides that were not coated with the quaternized polymers were used as controls.

RESULTS AND DISCUSSION

The low-surface energy semifluorinated end group is expected to bring the 4-vinylpyridine block to the surface which would otherwise be covered by the polystyrene block.⁸ Pyridinium polymers with higher molecular weights have been shown to exhibit stronger antibacterial activity in solution.⁹ A similar effect was observed when the polymer chains were attached to a surface.⁵ Hence block copolymers with different lengths of the P4VP block were synthesized. For preparing block copolymers with high molecular weight P4VP blocks, the molecular weight of the PS block was correspondingly increased to maintain the solubility of the block copolymer in the reaction medium.

The PS-*b*-P4VP polymers were soluble in dimethyl formamide, dimethyl acetamide, pyridine, a mixture of chloroform and methanol. They were insoluble (at room temperature) in acetone, toluene, nitromethane and dimethylsulfoxide. The polymers dissolved in chloroform, but the resulting solutions had a turbid appearance. Interestingly, the turbidity depended on total molecular weight of the block copolymer rather than the relative masses of the styrene and 4-VP blocks in the copolymer. The 62k/66k polymer with the higher molecular weight formed a clear solution, while the 14k/8k polymer with the lowest molecular weight formed the most turbid solution. Quaternization using chloroform or methanol as solvent requires a long time^{10,11} possibly due to the low temperatures that have to be employed. DMF is a good solvent for both PS and P4VP, and hence used as the reaction medium for quaternization. Almost complete quaternization, as indicated by shift of the IR peak at 1598 cm⁻¹ corresponding to the C=N stretching vibration of the pyridine ring, to 1639 cm⁻¹ after quaternization (cf. Figure 1) was obtained. The peak at 698 cm⁻¹ is characteristic of styrene and is absent in the 4-vinylpyridine homopolymers. The quaternized 4-VP homopolymers were insoluble in acetone, but soluble in water and highly soluble in methanol.

The surfaces were characterized by water contact angle measurements and NEXAFS spectroscopy. A 4k/11k PS-*b*-P4VP polymer (with a H end group instead of the fluorinated end group)

showed an advancing contact angle of 95° and a receding contact angle of 69° before quaternization, while a lower advancing contact angle of 66° and a receding contact angle of 27° after quaternization.

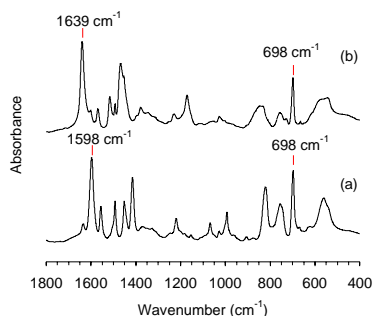


Figure 1. FTIR spectra of (a) PS-*b*-P4VP, and (b) PS-*b*-P4VP quaternized with 1-bromohexane.

Figure 2 shows the C edge and N edge NEXAFS spectra of the quaternized 4-vinylpyridine homopolymer, and the 11k/21k block copolymer. The peaks near the N-edge indicate the quaternized 4-vinylpyridine block within, approximately, the top 2 nm of the surface.

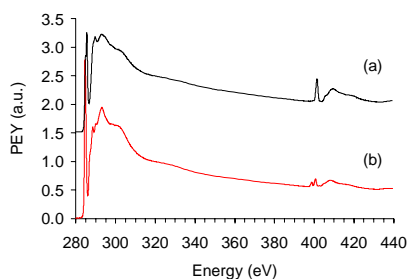


Figure 2. NEXAFS partial electron yield intensity vs. X-ray photon energy for (a) poly(N-hexyl-4-vinylpyridine) surface, and (b) polystyrene-*block*-poly((N-hexyl-4-vinylpyridine) surface for a 55° angle between the electric field vector of the polarized X-ray beam and the sample normal.

Antibacterial tests. The antibacterial-activity of the surfaces were quantified by the percentage reduction in the number of bacterial colonies on the test slides when compared to a plain glass slide that was used as control. The number of bacterial cells sprayed on the surface was controlled by adjusting the cell concentration in the suspension. Only the cells that remain viable after contact with the surface would be able to form colonies. The number of bacterial colonies that were formed on the control slides ranged from 50 cells/cm² to more than 500 cells/cm². The surfaces of the N-hexylated 160,000 g/mol P4VP homopolymer showed greater than 95 % reduction in the number of bacterial colonies. In the case of the 60,000 g/mol surface, the reduction in the number of bacterial colonies was (85±7) %. The quaternized poly(4-vinylpyridine-*co*-*n*-butylmethacrylate) surface also showed more than 90 % reduction. In the case of block-copolymer surfaces prepared by annealing the surfaces at 150 °C, the quaternized 11k/21k polymer did not show any antibacterial activity, but the quaternized 62k/66k polymer showed activity comparable to 160k homopolymer. Figure 3 shows the images of the microscope slides at the end of the antibacterial tests. All of the surfaces prepared using the CHCl₃/CH₃OH as the solvent exhibited antibacterial activity, irrespective of the length of the 4-vinylpyridine block. It is likely that the use of methanol, a good solvent for the pyridinium block but not for the styrene block, and a lower temperature for film formation resulted in a higher concentration of the relatively hydrophilic quaternized 4-vinylpyridine block at the surface.

CONCLUSIONS

Polystyrene-*block*-(N-hexyl-4-vinylpyridine) block copolymers exhibit antibacterial activity when coated on a surface, and show potential for use as antibacterial coatings for marine antifouling applications. The quaternized 4-vinylpyridine homopolymers also show strong antibacterial activity when coated on a surface. However, these surfaces cannot be used under water because of the water-solubility of the polymer. The bilayer coatings composed of SEBS the vinylpyridine block copolymers and expected to be more water-resistant, and therefore suitable for under-water applications. We have also prepared polystyrene-*block*-poly(N-hexyl-4-vinylpyridine-*co*-styrene) and expect it to show better performance with respect to durability under water. Quaternization using semifluorinated alkyl bromide results in a low-energy surface with a high degree of molecular order of the the fluoroalkyl side chains at the surface, as revealed by NEXAFS, and are expected to confer both, antibacterial and fouling-release properties to the coatings.

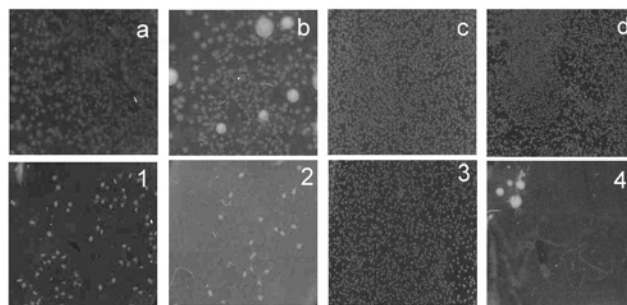


Figure 3. 2 cm x 2 cm regions of the microscope slides used for the antibacterial assays on quaternized 4-vinylpyridine surfaces: P4VP 60k (1); P(4VP-*co*-BMA), 300k (2); PS-*b*-P4VP, 11k/21k (3); PS-*b*-P4VP, 62k/66k (4); Images (a) to (d) are those of the corresponding plain glass control slides.

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