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Amphiphilic Random Copolymers Consisting of Styrene, EGMA, and HEMA for Anti-Biofouling Coatings

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*Amphiphilic random copolymers consisting of hydrophobic styrene and hydrophilic 2-hydroxyethyl methacrylate (*HEMA*)/(ethylene glycol) methacrylate (EGMA*) *were designed and synthesized for anti-*biofouling coating materials *in buffered aqueous solutions. Random copolymers such as PS-PEGMA, PS-PHEMA, and PS-PEGMA-PHEMA were synthesized, with different monomer ratios, by radical polymerization. The structures and molecular weights of the synthesized polymers were determined by ^{1}H-NMR and gel permeation chromatography, respectively. Protein adsorption experiments on the polymer surfaces were carried out using fluorescein isothiocyanate conjugate-labeled bovine serum albumin. Experimental results suggest that the surfaces of the amphiphilic random copolymers have a good anti-biofouling effect.*

Keywords biofouling; amphiphilic random copolymers; protein adsorption; TBT; BSA

Introduction

The marine biofouling of surfaces submerged in seawater is a worldwide problem caused by the accumulation of marine living organisms [1–7]. In water, the attachment of organisms follows a "successional" model [3–5]. First, organic molecules such as proteins adhere to the surface of a substrate. Next, the adsorption of bacteria and attachment of microalgae spores occur, leading to the formation of a biofilm or slime layer. Finally, large organisms settle and grow on the surface [1,3–5]. In the case of ships, marine biofouling decreases

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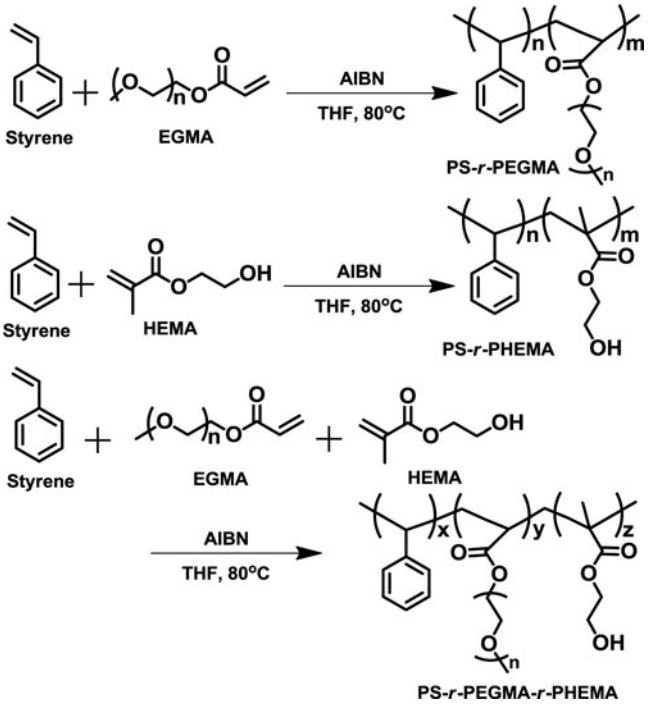
the fuel efficiency, cruising speed, and increases the frequency and cost of maintenance [8,9]. More than 4,000 marine biofouling species have been identified globally [10], and a variety of anti-biofouling methods have been investigated [7]. Tributyltin (TBT) has been widely used as a biocide in anti-fouling paints, commonly known as bottom paint, against a wide range of marine fouling organisms. However, they have been globally prohibited because they of their serious environmental effects such as induction of imposex in gastropods [10,11]. Subsequently, tin-free antifouling coatings such as copper-based booster biocides have been developed and used [12,13]. However, it is known that, like TBT, the use of copper-containing antifouling materials also causes environmental problems [12]. Therefore, research on environment-friendly anti-fouling coatings is urgently needed [14]. The use of homogeneous surfaces such as hydrophilic polyethylene glycol (PEG) and poly (2-hydroxyethyl methacrylate) (PHEMA) has received significant attention due to its exceptional resistance to protein adsorption and cell adhesion in aqueous media. Anti-biofouling effect of several kinds of amphiphilic block copolymers containing methacrylate (EGMA) and 2-hydroxyethyl methacrylate (HEMA) has been reported but amphiphilic random copolymers have not been reported in spite of their advantages in real application. In this study, amphiphilic random copolymers consisting of styrene, EGMA and HEMA were synthesized by changing the monomer ratios and investigated their anti-biofouling properties. To test the anti-biofouling effect, protein adsorption experiments on the polymer surfaces were carried out using fluorescein isothiocyanate conjugate-labeled bovine serum albumin (BSA). The synthetic routes and chemical structures of the random copolymers are shown in Scheme 1.



Scheme 1. Synthesis of amphiphilic random copolymers.

Experimental Details

Materials

Styrene (99%), 2-hydroxyethyl methacrylate (HEMA), (ethylene glycol) methacrylate (EGMA), phosphate buffered saline (PBS), fluorescein isothiocyanate conjugate-labeled bovine serum albumin (BSA), 2,2'-azobis(2-methylpropionitrile) (AIBN, 98%), and 1-methoxy-2-propanol were purchased from Aldrich. Tetrahydrofuran (THF) and toluene were purchased from Daejung. AIBN was recrystallized from methanol (300 mL) before use. Styrene, HEMA, and EGMA were passed through a basic alumina column to remove the contained inhibitors, 4-tert-butyl catechol and monomethyl ether hydroquinone.

Measurements

^{1}H NMR spectra were recorded using a Varian Mercury Plus 300 MHz spectrometer using deuterated chloroform as the solvent. The number- and weight-average molecular weights (M_n and M_w, respectively) and the polydispersity indices (PDIs) of the polymers were determined by gel permeation chromatography (GPC) relative to a polystyrene (PS) standard using a Waters high-pressure GPC assembly (model M590). Contact angle measurements were performed using a Photonx-300 Touch (Surface Electro Optics) at room temperature. The fluorescence imaging was performed using an Axioplan 2 fluorescence microscope.

Polymer Synthesis and Characterization

PS and the amphiphilic random copolymers were synthesized by radical polymerization. Styrene (0.038 mol) was dissolved in anhydrous THF (5 mL) and stirred at 80°C for 4 h. The reaction mixture was then concentrated and precipitated in a hexane (300 mL) solution. Finally, the precipitated polymer was recovered by filtration and vacuum dried at room temperature for 48 h to fully remove any residual solvent. ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.08 (br, 3H), 6.58 (br, 2H), 1.84 (br, 2H), 1.43 (br, 2H). Various monomer ratios were used for the synthesis of PS-PEGMA, PS-PHEMA, and PS-PEGMA-PHEMA via the polymerization process described above for PS. [Styrene]/[EGMA] = (9:1, 0.029 mol:0.003 mol), (8:2, 0.029 mol:0.007 mol), and (7:3, 0.029 mol:0.012 mol) amphiphilic random copolymers. ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.07∼6.57 (br, 3H, 2H), 4.00∼3.52 (br, PEG), 3.35 (s, 3H), 1.84∼1.40 (br, 1H, 2H, 3H). [Styrene]/[HEMA] = (9:1, 0.038 mol:0.004 mol), (8:2, 0.038 mol:0.009 mol), and (7:3, 0.038 mol:0.016 mol) amphiphilic random copolymers. ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.12∼6.68 (br, 3H, 2H), 4.00∼3.16 (br, 4H), 1.93∼1.73 (br, 1H, 3H), 1.48∼1.43 (br, 2H).

[Styrene]/[EGMA]/[HEMA] = (6:2:2, 0.035 mol:0.012 mol:0.012 mol), (6:3:1, 0.035 mol:0.018 mol:0.006 mol), and (6:1:3, 0.035 mol:0.006 mol:0.018 mol) amphiphilic random copolymers. ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.10∼6.67 (br, 3H, 2H), 4.00∼3.52 (br, PEG, 4H), 3.35 (s, 3H), 1.84∼1.49 (br, 1H, 2H, 3H).

Preparation of Surface and Protein Adsorption Experiments

Protein adsorption experiments were conducted to verify the anti-biofouling effect of the coatings. Prior to use, the silicon wafer substrate was cleaned using an isopropanol/acetone solvent, followed by treatment with a UV-ozone cleaner for 20 min. Samples for the protein

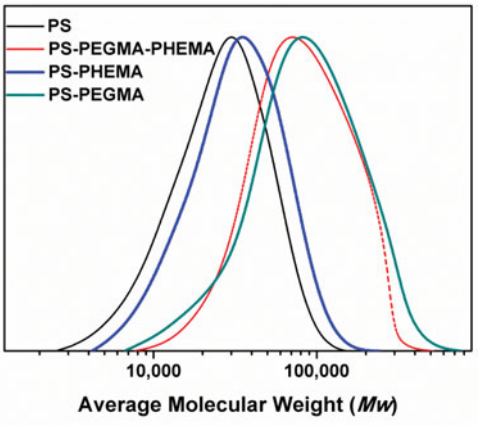


Figure 1. GPC curves for synthesized PS, PS-PEGMA, PS-PHEMA, and PS-PEGMA-PHEMA.

adsorption experiments were prepared by mixing the polymers (30 mg) in a toluene/1-methoxy-2-propanol (1:1, v/v, 1 mL) solution. The prepared solutions were first filtered through a 0.45 μm syringe filter and then coated on silicon wafers using a spin coater at 2000 rpm for 20 s. The coated films were annealed in an oven at 120°C for 12 h. The thin films, thus prepared, were used for the protein adsorption experiments. The protein solution was prepared by dissolving the BSA in the PBS solution (0.1 mg/mL). Subsequently, the polymer-coated silicon wafers were immersed in the protein solution for 2 h and then rinsed with deionized water to remove any unattached residual proteins. The samples were immediately analyzed under a fluorescence microscope, and the fluorescence intensity per unit area of the polymer surface was quantified using the Image J program.

Table 1. Molecular weights and polydispersities of the copolymers

Polymers (ratio, mol)	M_n (g/mol)	M_w (g/mol)	PDI
PS	19, 700	30, 900	1.56
PS-PEGMA (9:1)	104, 600	171, 400	1.63
PS-PEGMA (8:2)	83, 100	123, 400	1.48
PS-PEGMA (7:3)	62, 300	97, 400	1.56
PS-PHEMA (9:1)	24, 400	38, 600	1.58
PS-PHEMA (8:2)	34, 000	52, 400	1.54
PS-PHEMA (7:3)	39, 300	64, 400	1.63
PS-PEGMA-PHEMA (6:2:2)	61, 900	110, 200	1.78
PS-PEGMA-PHEMA (6:3:1)	63, 900	101, 900	1.47
PS-PEGMA-PHEMA (6:1:3)	65, 200	106, 700	1.63

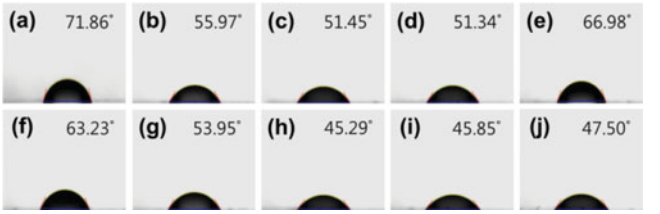


Figure 2. Contact-angle images of DI water drops on (a) PS (b) PS-PEGMA (9:1) (c) PS-PEGMA (8:2) (d) PS-PEGMA (7:3) (e) PS-PHEMA (9:1) (f) PS-PHEMA (8:2) (g) PS-PHEMA (7:3) (h) PS-PEGMA-PHEMA (6:2:2) (i) PS-PEGMA-PHEMA (6:3:1) (j) PS-PEGMA-PHEMA (6:1:3).

Results and Discussion

Characterization of the Polymers

We synthesized PS-PEGMA, PS-PHEMA, and PS-PEGMA-PHEMA with various monomer ratios and identified the polymer structure and molecular weights by ^{1}H-NMR and gel permeation chromatography (GPC), respectively. The ^{1}H-NMR spectra confirmed the synthesis of PS, PS-PEGMA, PS-PHEMA, and PS-PEGMA-PHEMA along with their compositions. The aromatic protons of the styrene peaks appeared at 7.12∼6.57 ppm, the protons of PEG and methylene groups appeared at 4.00∼3.16 ppm, and the proton peaks of alkyl groups in PS, PEGMA, and PHEMA appeared at 1.84∼1.40 ppm. Figure 1 shows the GPC curves of PS and the amphiphilic random copolymers. The GPC curves of PS and the amphiphilic random copolymers show a unimodal distribution. The weight-average molecular weights of PS-PEGMA, PS-PHEMA, and PS-PEGMA-PHEMA were 130,700, 51,800, and 106,200 g/mol, respectively. The measured molecular weights (M_w) and polydispersity indices (PDI) of the synthesized copolymers are summarized in Table 1.

Characterization of the Polymer Films

Contact angles of the polymer films are shown in Figure 2. The contact angles of PS-PEGMA, PS-PHEMA, and PS-PEGMA-PHEMA were smaller than that of the PS. On increasing the EGMA ratio of the copolymers, the contact angle decreased from 55.97° to 51.34° suggesting that the polymer films become more hydrophilic. Similarly, on increasing the HEMA ratio of the copolymers, the contact angles decreased from 66.98° to 53.95°. The average thicknesses of PS, PS-PEGMA, PS-PHEMA, and PS-PEGMA-PHEMA films were 198, 215, 195, and 228 nm, respectively.

Non-Specific Protein Adsorption Studies

For the protein adsorption experiment, the prepared polymer films coated on silicon wafers were treated with BSA. PS-PEGMA, PS-PHEMA, and PS-PEGMA-PHEMA were highly resistant to BSA adsorption, while PS showed relatively high protein adsorption.

Figure 3 shows the fluorescence images obtained after the adsorption of BSA on the polymer coated Si wafers. Figure 4 shows the fluorescence intensities of the polymer films after the BSA adsorption test. The fluorescence intensities of the BSA-adsorbed polymer films, obtained from the fluorescence microscope images, were quantified by an image processing program.

The measured fluorescence intensities observed for the PS, PS-PEGMA (9:1), PS-PEGMA (8:2), and PS-PEGMA (7:3) films were 127.75, 42.06, 37.14, and 36.77, respectively. As the result shows, copolymer films with higher PEGMA ratios showed lower fluorescent intensities. The fluorescence intensities of PS-PHEMA (9:1), PS-PHEMA (8:2), and PS-PHEMA (7:3) films were 94.49, 92.11, and 92.45, respectively.

The fluorescence intensities of PS-PEGMA-PHEMA (6:2:2), PS-PEGMA-PHEMA (6:3:1), and PS-PEGMA-PHEMA (6:1:3) were 41.99, 36.48, and 41.99, respectively. PS-PEGMA showed lower fluorescence intensities than PS-PEGMA-PHEMA and PS-PHEMA. From the protein adsorption experiments, it is evident that the hydrophilic EGMA and HEMA containing polymers showed a better anti-biofouling effect than the PS reference. Moreover, among the synthesized copolymers, PS-PEGMA was a more effective anti-foulant than PS-PHEMA or PS-PEGMA-PHEMA.

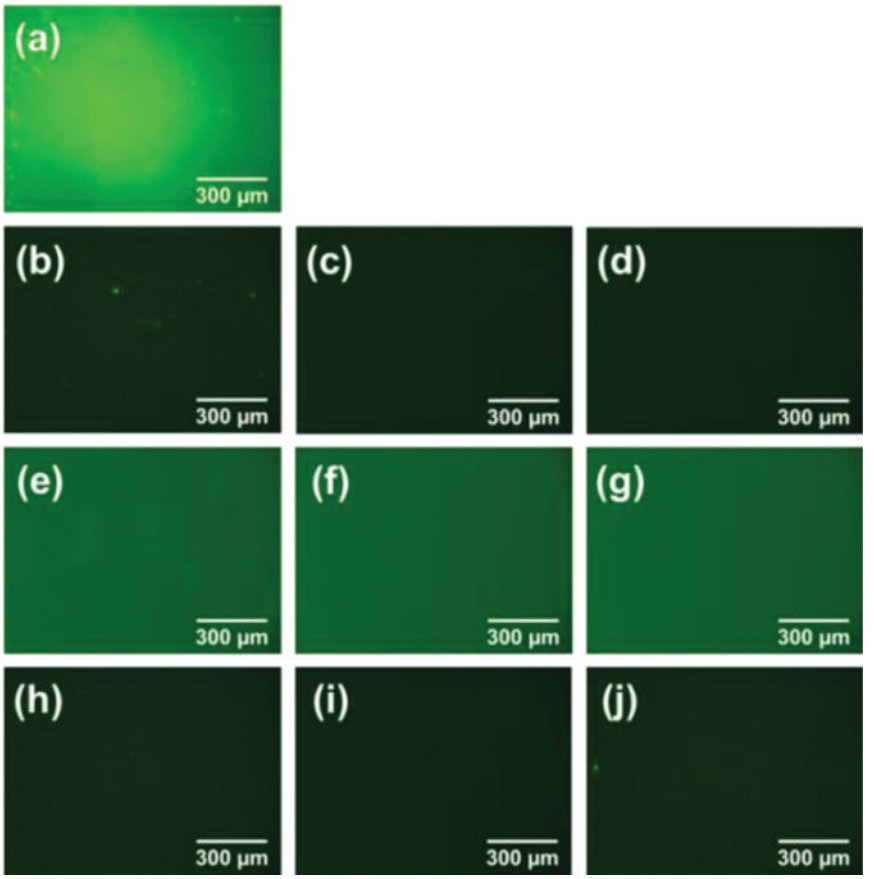


Figure 3. Images obtained after the adsorption of BSA on polymer coated Si wafers (a) PS (b) PS-PEGMA (9:1) (c) PS-PEGMA (8:2) (d) PS-PEGMA (7:3) (e) PS-PHEMA (9:1) (f) PS-PHEMA (8:2) (g) PS-PHEMA (7:3) (h) PS-PEGMA-PHEMA (6:2:2) (i) PS-PEGMA-PHEMA (6:3:1) (j) PS-PEGMA-PHEMA (6:1:3).

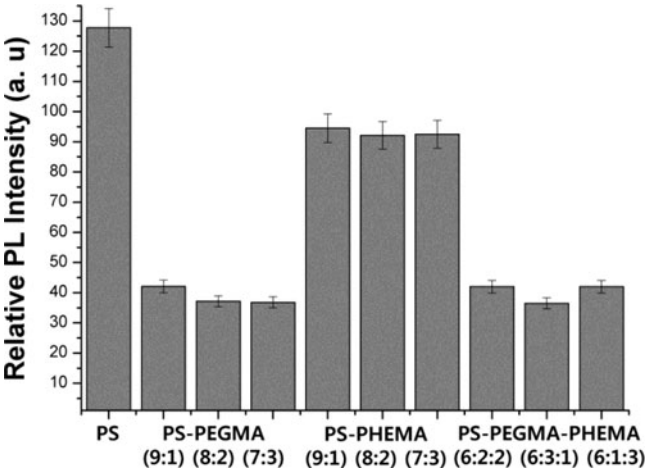


Figure 4. Fluorescence intensities of the adsorbed BSA on the polymer coated silicon wafers.

Conclusions

Amphiphilic random copolymers, PS-PEGMA, PS-PHEMA, and PS-PEGMA-PHEMA, were synthesized with different monomer ratios by radical polymerization. Protein adsorption experiments on the polymer surfaces were carried out using fluorescein isothiocyanate conjugate-labeled bovine serum albumin. The hydrophilic EGMA and HEMA containing polymers showed a much better anti-biofouling effect than the PS reference, and PS-PEGMA was a more effective anti-foulant than PS-PHEMA or PS-PEGMA-PHEMA.

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