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BIOTINYLATED POLY(3-HEXYLTHIOPHENE-*co*-3-METHANOLTHIOPHENE): A LANGMUIR MONOLAYER-FORMING COPOLYMER

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ABSTRACT

Copolymers of 3-substituted thiophenes have been synthesized by organosynthetic routes. The chemical synthesis of the copolymer was carried out by dehydrogenation of 3-hexylthiophene and 3-methanolthiophene. Attachment of biotin to the resulting copolymer, poly(3-hexylthiophene-*co*-3-methanolthiophene) [PMHT], is accomplished by room temperature esterification using *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine as catalyst. The resulting copolymers have well-defined chemical and electronic structures and molecular weights. The biotinylated copolymer forms a stable monolayer at the air–water interface due to the polar groups along the polymer backbone.

INTRODUCTION

In recent years, electrically conducting polymers have attracted much attention for the significant improvement of their processability and possible applications in electronics [1], sensors [2], and nonlinear optics [3, 4]. Several conducting polymers have been investigated in solutions [5, 6] or incorporated into macromolecular assemblies [7]. Polypyrroles and polythiophenes are in this class and are adaptable for such applications. A considerable amount of work has been done as they present a number of important advantages. They show high electrical conductivity together with excellent stability when exposed to ambient conditions, and they can be synthesized via both electrochemical [8, 9] and chemical oxidation [10, 11] polymerization techniques. Unfortunately, their insolubility and lack of processability prevented them from many potential applications. With the introduction of the poly(3-alkylthiophenes) improved processability of polythiophenes was achieved. Considerable effort has since been made in the design and synthesis of processable functional polythiophenes [12, 13]. Although the aromatic rings are responsible for stiffness and strong intra- and interchain interactions, solubility or fusibility is achieved by flexible alkyl chains on the backbone of these conducting polymers [14, 15]. A long-chain substituent at the 3-position of the thiophene has been shown to form a polymer soluble in common organic solvents [16, 17]. The synthesis of derivatives with substitution at the 3-position is generally easier to achieve with thiophene than with pyrrole, where ring nitrogen must be protected [18]. The orientation of conducting polymers has attracted interest because of a major breakthrough in the syntheses and processability of these materials [19–24].

The Langmuir–Blodgett (LB) technique has the ability to organize the molecules into highly ordered monolayer and to manipulate a multilayer film toward a desired architecture. Recently, this technique was used to prepare oriented and spatially organized protein molecular assemblies [25, 26]. The main aim of this line of investigation is to incorporate biological components into a conducting polymer to create a mono- and multilayer possessing interesting electronic and optical properties [27]. It was demonstrated earlier that one can use the LB trough cassette approach to create an ordered monolayer using biotinylated lipid which first binds to streptavidin. This streptavidin can then be made to bind to biotinylated phycoerythrin, a fluorescent antennae protein [28]. In general, this cassette approach could be utilized with any biotinylated species to get an ordered monolayer film with unusual optical and electronic properties for potential applications.

We have chosen to investigate a copolymer of 3-hexylthiophene and 3-methanolthiophene, with particular regard to the modification of the hydroxyl group of methanol by substituting with different functionalities to tune the polymer properties. We desire to extend the cassette approach to get an ordered biomolecular assembly with this conducting polymer template system. While the main objective of this paper is to report the design, synthesis, and monolayer formation characteristics of the copolymer, the eventual application is rather novel and interesting. There are reports in the literature regarding binding of labeled biomolecules to optic sensors [29, 30]. Our goal is to combine the conductivity properties of the copolymer with the fluorescent properties of phycobiliproteins in conjunction with building new types of biosensors.

A method for the synthesis of a copolymer of 3-hexylthiophene and 3-methanolthiophene (PMHT) is presented here. Further, attachment of a biotin molecule to the above copolymer is accomplished by room temperature esterification using *N,N*-dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine as catalyst to get biotinylated poly(3-hexylthiophene-*co*-3-methanolthiophene) (B-PMHT). Details of the syntheses and characterization of the copolymers PMHT and B-PMHT are presented. In addition, a series of pressure–area isotherms recorded for the monolayers of the copolymers is discussed.

EXPERIMENTAL

Instrumentation

^{1}H-NMR spectral data were obtained on a Bruker WP-270 NMR spectrometer. IR spectra were recorded on a Perkin-Elmer 1760X FTIR spectrophotometer. Ultraviolet-visible (UV-Vis) spectra were taken on a Perkin-Elmer Lambda 9 spectrophotometer. Number- and weight-average molecular weights (M_n and M_w) of the polymers were determined with a Waters 510 HPLC unit combined with a Waters 410 differential refractometer using chloroform as an eluent. The gel-permeation chromatography (GPC) calibration is based on polystyrene standards. The column set consisted of Waters styragyl 100, 500, 1000, and 10000 Å.

Materials

3-Hexylthiophene was purchased from TCI and used without further purification. 3-Methanolthiophene (Aldrich), ferric chloride (Aldrich), acetic acid (Aldrich), biotinic acid (Pierce), streptavidin (Biomeda), *N,N*-dicyclohexylcarbodiimide (DCC) (Aldrich), and 4-pyrrolidinopyridine (Aldrich) were also used as received. Chloroform (Aldrich), dichloromethane (DCM) (Aldrich), and methanol (Aldrich) were distilled under nitrogen before use.

Synthesis of Poly(3-Hexylthiophene-*co*-3-Methanolthiophene) (PMHT)

Synthetic grade anhydrous ferric chloride (14.59 g, 0.09 mol), was dried under vacuum at 100°C prior to use in a 250-mL three-necked round-bottomed flask. Nitrogen was introduced along with 100 mL dry chloroform into the reaction vessel. 3.36 g 3-hexylthiophene (0.02 mol) and 1.14 g 3-methanolthiophene (0.01 mol) in 10 mL chloroform was added dropwise under vigorous stirring. The reaction mixture was allowed to stir for 2 days until the reaction was complete. The reactant solution was precipitated into methanol (500 mL). The product was then treated with methanol in a Soxhlet extractor for 2 days. Total yield: 4.2 g (93%). M_n = 3057, M_w = 3159 (soluble fraction).

IR (KBr): 3400 (w, O—H) 2960 (s, C—H) 1465 (s, C—H bend) cm^{-1}. UV-Vis ($CHCl_3$): λ nm = 268, 406 (λ_{max}). ^{1}H NMR ($CDCl_3$): δ = 0.8 (t, 3H, CH_3), 1.27

(m, 2H, CH_2), 1.3 (s broad, 1H, OH), 1.43 (m, 2H, CH_2), 1.58 (m, 2H, CH_2), 2.2 (m, 2H, CH_2), 2.8 (m, 2H, CH_2), 4.1 (t, 2H, CH_2), 7.1 (s, 1H, arom.).

Synthesis of Biotinylated Poly(3-Hexylthiophene-*co*-3-Methanolthiophene) (B-PMHT)

A solution of 0.244 g biotinic acid (0.001 mol), 0.226 g *N,N*-dicyclohexylcarbodiimide (0.0011 mol), 0.165 g poly(3-hexylthiophene-*co*-3-methanolthiophene), and 0.014 g 4-pyrrolidinopyridine (0.0001 mol) in dichloromethane (50 mL) was stirred at room temperature until esterification was complete. The *N,N*-dicyclohexyl urea was filtered, and the filtrate washed with water (3 × 10 mL), 5% acetic acid solution (3 × 10 mL), again with water (3 × 10 mL), and dried ($MgSO_4$). The solvent was evaporated in a rotary evaporator under reduced pressure to produce the biotinylated copolymer. Yield: 0.190 g (93%). M_n = 3351, M_w = 3492.

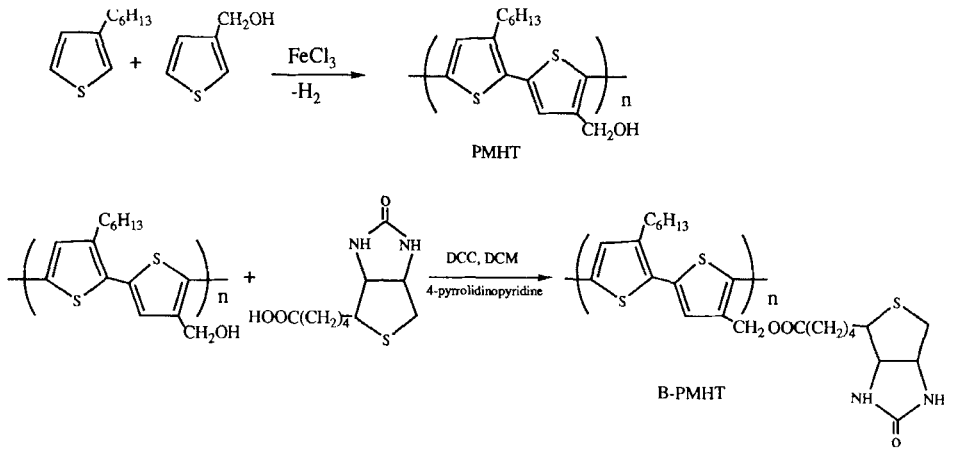
IR (KBr): 3324 (s, N—H), 1704 (s, C=O), 1640 (s, N—H bend) cm^{-1}. UV-Vis ($CHCl_3$): λ nm = 338, 410 (λ_{max}). ^1H NMR ($CDCl_3$): δ = 0.8 (s broad, 3H, CH_3), 1.08–1.3 (2m 7H, CH_2), 1.3–1.38 (m, 4H, CH_2), 1.59–1.62 (m, 8H, CH_2), 1.67–1.72 (m, 4H, CH), 1.9–1.95 (m, 2H, CH_2), 2.1 (t, 2H, CH_2), 2.22 (t, 2H, CH_2), 4.1 (d, 2H, CH_2), 5.3 (d, 2H, NH), 7.1 (s, 1H, arom.).

LB Monolayer Formation

All monolayer studies were carried out on a Lauda MGW Filmwaage trough with a surface area of approximately 930 cm^2. To obtain the pressure-area isotherms of PMHT and B-PMHT, 0.0005 mol chloroform solution was spread onto the purified MilliQ water subphase. For the measurement of the pressure-area isotherm of the B-PMHT monolayer upon streptavidin injection, the subphase was composed of an aqueous solution of 0.0001 mol NaH_2PO_4 and 0.1 mol NaCl at pH 6.8. Streptavidin (0.1 mg) in 5 mL of the buffered subphase was injected under the spread film and left to incubate for 2 hours at 30°C. Compression was then carried out at a speed of 2 mm^2/min until collapse of the film was observed.

RESULTS AND DISCUSSION

Synthesis of the biotinylated copolymer involves two steps (Scheme 1); the synthesis of the copolymer of 3-hexylthiophene and 3-methanolthiophene and then the attachment of biotin. In the first step, the copolymers were prepared using 3-hexylthiophene and 3-methanolthiophene as monomers by adopting a method [31] reported for the synthesis of polyalkylthiophene. During the synthesis, monomers were added to the ferric chloride solution, and the color of the solution gradually changed from yellow to dark brown, and finally to black. For the purification of the copolymer, ferric chloride was removed by Soxhlet extraction with methanol for 2 days. The copolymer was found to be partly soluble in chloroform. The insoluble fraction is the high molecular weight fraction and appears as a dark brown solid. The soluble part of the copolymer was separated and characterized. This component was used in the second step to attach biotinic acid by esterification [32]



SCHEME 1. The scheme for the synthesis of PMHT and B-PMHT.

of the hydroxyl group of the copolymer using DCC and 4-pyrrolidinopyridine as catalyst.

The infrared absorption spectra of PMHT and B-PMHT were carried out on KBr pressed disks and are shown in Figs. 1 and 2. B-PMHT exhibited new peaks at 1788 cm^{-1} due to carbonyl stretching of the ester group and a sharp peak at 3324

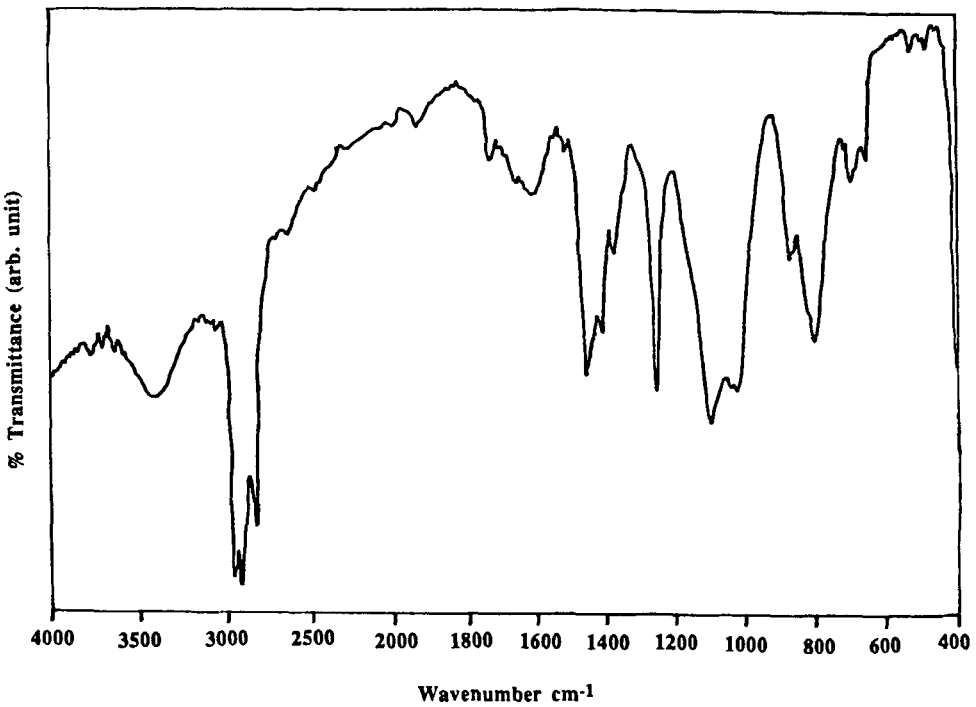


FIG. 1. FTIR of PMHT in KBr disk.

cm^{-1} for N—H stretching. Meanwhile, the broad O—H absorption peak at 3400 cm^{-1} shown in PMHT has disappeared in B-PMHT. Both PMHT and B-PMHT showed a principal absorption peak at 788 cm^{-1} due to the C—H out-of-plane vibration of the 2,5-disubstituted thiophene [33]. Further, both the copolymers exhibit a distinct peak around 820 cm^{-1} due to the C—H out-of-plane vibration of the 2,3,5-trisubstituted thiophene [34]. Ultraviolet-visible absorption measurements were performed in chloroform for PMHT and B-PMHT. Both the copolymers showed a λ_{max} around 410 nm with an absorption edge starting from 600 nm, indicating the presence of extended π-conjugation along the polymer backbone.

To evaluate the role of biotinylation of PMHT in monolayer formation and subsequently specific binding with streptavidin, a series of pressure-area isotherm measurements were performed. The isotherms of B-PMHT and streptavidin-injected B-PMHT monolayers are given in Fig. 3. The biotinylated copolymer (B-PMHT) exhibited significantly improved isotherms compared with the copolymer (PMHT). The significant improvement in the formation of B-PMHT monolayer indicates that biotinylation enhanced LB film formation by contributing a flexible spacer group and increased hydrophilicity to the copolymer molecule. A second advantage of B-PMHT includes increased stability of the monolayer during transfer and efficient deposition onto solid supports. It was found that a constant surface pressure of 15 mN was maintained over a period of 15 minutes with a transfer ratio of approximately 65%. The stability is also established by the extremely small hysteresis effect seen upon repeated compression and decompression cycles (Fig. 4).

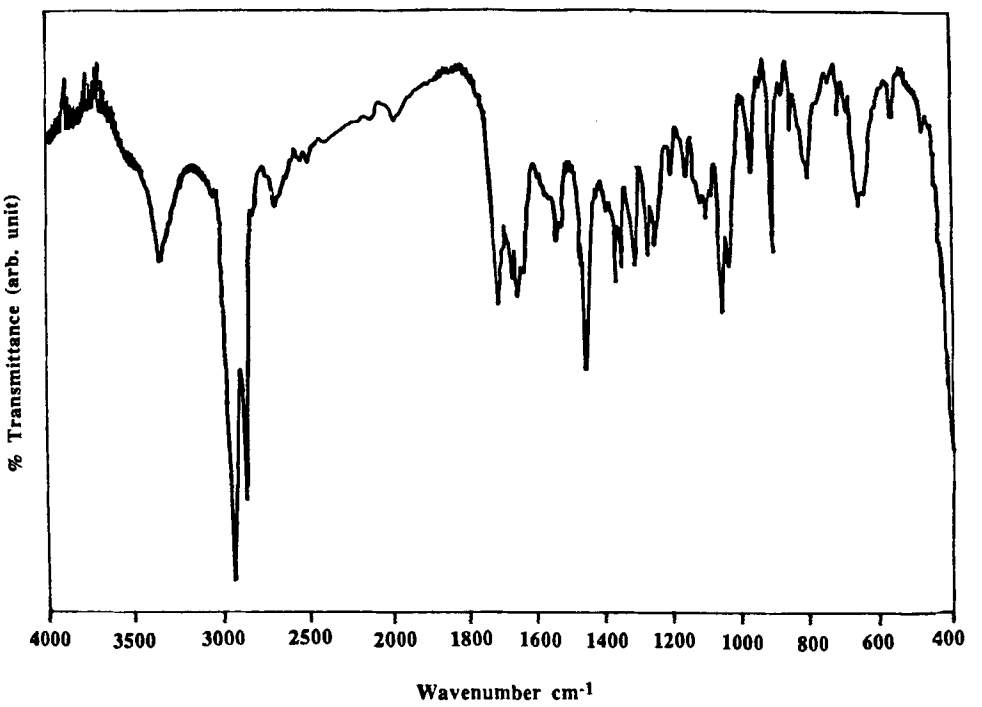


FIG. 2. FTIR of B-PMHT in KBr disk.

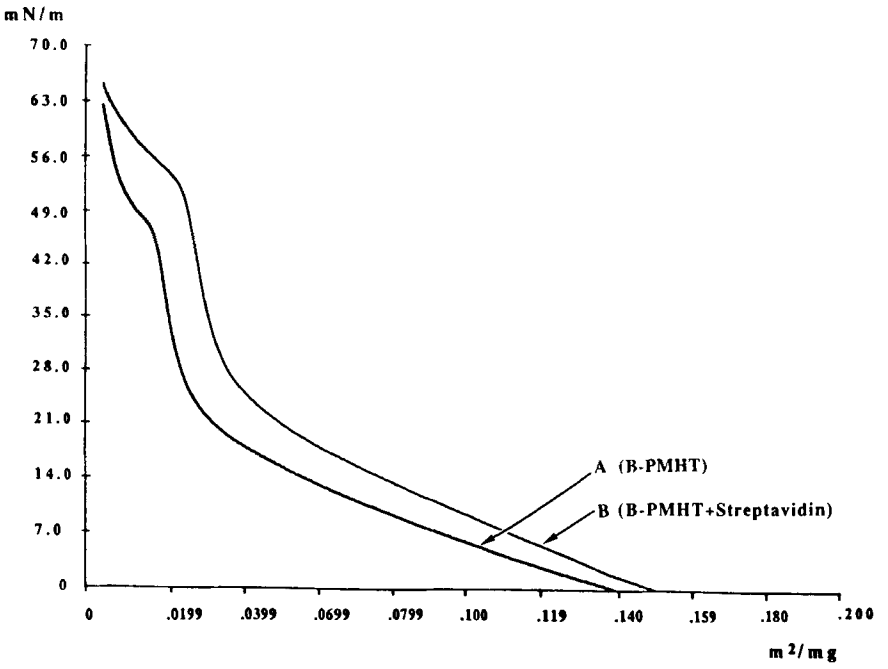


FIG. 3. Pressure-area isotherms of B-PMHT, streptavidin injected in B-PMHT.

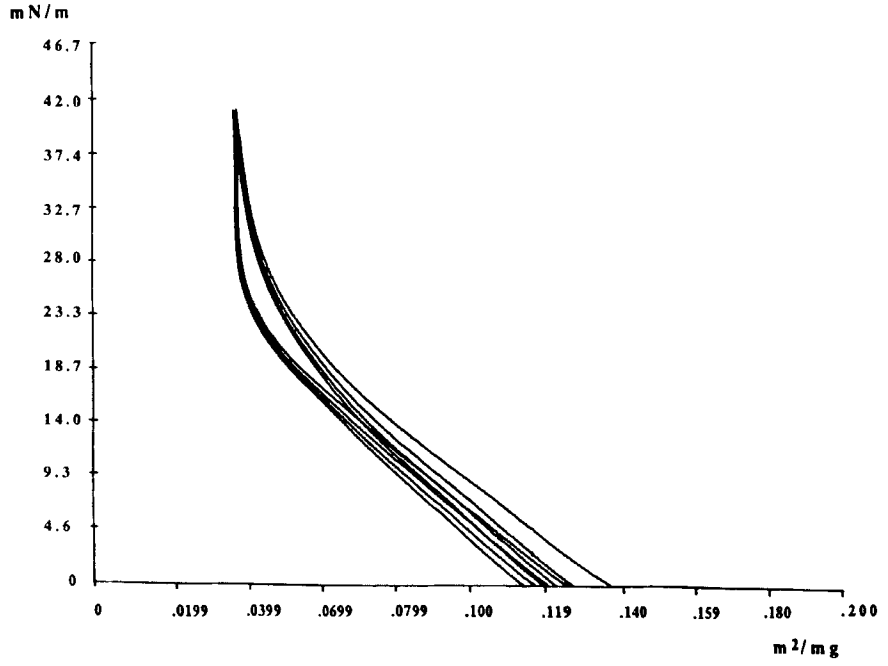


FIG. 4. Hysteresis effect upon repeated recompression and decompression cycles on B-PMHT.

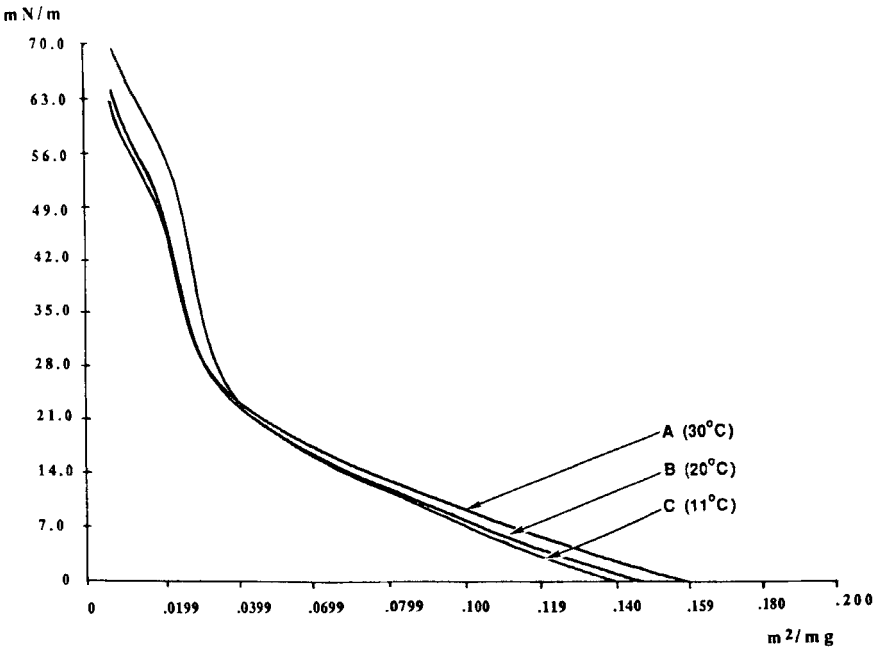


FIG. 5. Stability of B-PMHT at various temperatures.

B-PMHT was observed to possess fairly good mechanical properties as shown by the formation of an elastic-string-like fiber when the film was drawn up from the collapsed monolayer by using a tweezer with a Teflon-coated tip. It is further established that the B-PMHT monolayer is very stable at various temperatures (Fig. 5).

The isotherm in Fig. 3 showed a significant area expansion when the tetramer protein, streptavidin, was injected below the B-PMHT monolayer. This change supports the original goal of the biotinylation of this polymer, which was to employ the biotin-streptavidin complexation for subsequent immobilization of various proteins into conducting LB films. The expansion indicates the occurrence of effective binding between the biotin and streptavidin. Since there are four binding sites for biotin in the tetrameric protein streptavidin, there are still sites available for binding of additional biotinylated molecules. This strategy will be utilized for further attachment of other active components.

CONCLUSIONS

A Langmuir monolayer-forming copolymer containing a polythiophene backbone has been synthesized. The biotinylation of PMHT enabled better formation of Langmuir-Blodgett films at the air-water interface by contributing a flexible spacer group and enhancing the hydrophilicity of the copolymeric molecule. In addition, effective binding between this biotinylated polymer and streptavidin was

demonstrated. This bioconjugated copolymer is a potential material for further attachment of additional biotinylated molecules.

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