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Short communication

Electropolymerization molecularly imprinted polymer (E-MIP) SPR sensing of drug molecules: Pre-polymerization complexed terthiophene and carbazole electroactive monomers

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ABSTRACT

A novel chemosensitive ultrathin film with high selectivity was developed for the detection of naproxen, paracetamol, and theophylline using non-covalent electropolymerized molecular imprinted polymers (E-MIP). A series of monofunctional and bifunctional H-bonding terthiophene and carbazole monomers were compared for imprinting these drugs without the use of a separate cross-linker. A key step is the fast and efficient potentiostatic method of washing the template, which facilitated enhanced real-time sensing by surface plasmon resonance (SPR) spectroscopy. Various surface characterizations (contact angle, ellipsometry, XPS, AFM) of the E-MIP film verified the templating and release of the drug from the cross-linked conducting polymer film.

1. Introduction

Molecularly imprinted polymer (MIP) is now established as one of the most adaptable methods in fabricating tailor-made sensor films with an artificial receptor based on an imprinted cavity. This cavity retains the exact memory of the size, shape and chemical group orientation of the target analyte or template molecule (Mosbach and Ramstrom, 1996). Because of its low cost, simple preparation, high reliability, stability, and film formation on a wide range of transducers, MIP has been extensively applied in separation and isolation technologies having been developed to mimic biological receptors and enzymes (Wulff, 1995). Although MIP originated from the pioneering work of Polyakov in 1930s (Polyakov, 1931), many key enabling breakthroughs started only in the 1990s (Alexander et al., 2006).

Paracetamol and naproxen are efficient antipyretic and analgesic drugs that are potent ingredients in most pain-killer medicines. However, an overdose of paracetamol is a foremost cause of acute liver failure (Bosch et al., 2006; Hawton et al., 2001). In fact, paracetamol is reported as the most common drug used in self-poisoning (overdose) with a high rate of morbidity and mortality (Sheen et al., 2002). Also, a strict international regulation is imposed to pharmaceutical companies about the handling of naproxen in both raw material and final product because it is easily degraded under high temperatures (Adhoum et al., 2003). Theophylline is normally used as bronchodilators and respiratory stimulators for treatment of acute and chronic asthmatic conditions and is reported as the most frequent clinically monitored drug in the USA (Kawai and Kato, 2000; Rowe et al., 1988). The plasma level useful for effective bronchodilation action is within 20–100 μM concentration range. At higher concentration, it is lethal, leading to a permanent neurological damage (Kawai and Kato, 2000; Rowe et al., 1988). The safe and effective use of theophylline relies on careful dosage adjustments based on accurate measurements in blood serum. Therefore, fast and reliable detection of these drugs are of high importance.

In this communication, we report a novel approach of imprinting naproxen, paracetamol, and theophylline based on an electropolymerized MIP (E-MIP) of conducting polymers (CPs). A review of conducting polymers applied in chemical sensors and arrays has been published recently (Lange et al., 2008). In our work, the fabrication of the E-MIP was achieved by *in situ* electropolymerization of functional and cross-linking terthiophene and carbazole monomers that are non-covalently complexed with the template drug. These monomers have been investigated for their interesting electrochemical copolymerization behavior (Taranekar et al., 2005) and

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electrochromic properties (Witker and Reynolds, 2005). A quantitative electrochemical and electrochromic study of terthiophene and carbazole monomers to form conjugated polymer network (CPN) films have been done by our group using *in situ* measurements, electrochemical-surface plasmon resonance (EC-SPR) spectroscopy and electrochemical-quartz crystal microbalance (EC-QCM) (Taranekar et al., 2007a,b) methods.

The advantages of electropolymerization are: (1) thickness control of the polymer layer that is crucial to the sensing of the analyte, (2) ability to attach the sensor film to electrode surfaces of any shape and size, and (3) compatibility with combinatorial and high-throughput approaches critical for the commercial development of molecular imprinting (Lange et al., 2008; Malitesta et al., 1999). To the best of our knowledge, the imprinting of these three important drugs by poly(terthiophene) and poly(carbazole) has not been previously reported. Moreover, we demonstrate an efficient and fast protocol of removing the template drug, which improves the sensitivity of the analyte detection by SPR kinetic measurements. Our group has used this technique to study the formation of ultrathin CPN films (Jiang et al., 2007; Kato et al., 2003; Baba et al., 2002), interfaces (Baba et al., 2006), and kinetic processes at surfaces (Kaewtong et al., 2008; Sriwichai et al., 2008; Taranekar et al., 2007a,b). We have also demonstrated the use of SPR using an electropolymerized dendrimer-coated sensor chip for the detection of a nerve agent gas analog (Taranekar et al., 2006). A review of the SPR methods summarizes the potential for efficient optical/dielectric transduction with thin film sensing elements (Knoll, 1998).

2. Experimental procedures

2.1. Materials

All chemicals were used as received unless otherwise specified. The templates (naproxen, paracetamol, and theophylline), analogous analytes (1-napthalene sulfonic acid sodium salt, acetanilide, caffeine, theobromine, 3-aminophenol, and 4-aminobenzoic acid) used in selectivity studies, supporting electrolyte (tetrabutylammonium hexafluorophosphate or TBAH), tetrahydrofuran (THF), and acetonitrile (ACN) were purchased from Sigma–Aldrich. All the mono and bifunctional monomers used to fabricate the ultrathin films were synthesized in our laboratory, and the details of the synthesis can be found elsewhere (Taranekar et al., 2007a,b; Yassar et al., 1995). The E-MIP and non-imprinted (NIP) electrodeposition on Au-electrode and SPR substrate (Fig. 1) is described in detail in the Supporting Information.

2.2. Film fabrication and template washing

The E-MIP film deposition was done in an Autolab PGSTAT 12 potentiostat (Brinkmann Instruments now MetroOhm USA) coupled with an SPR instrument (Autolab ESPRIT from Eco Chemie). The SPR set-up is described in detail at the Supporting information as well as the electrochemical CV deposition procedure.

2.3. Film characterizations

A detailed description of the instrumentation specification and procedures for the experiments can be found at the Supporting Information document. In brief: ellipsometry was used to measure the thickness of the electropolymerized film using the Multiskop ellipsometer (Optrel GmbH, Germany) equipped with a 632.8 nm laser. Contact angle measurements were done on a CAM 200 optical contact angle meter (KSV Instruments Ltd.). Atomic force microscopy (AFM) measurements were done on a Pico-SCAN AFM from Agilent Technologies. X-ray photoelectron spectroscopy (XPS) measurement were done on a PHI 5700 X-ray photoelectron spectrometer.

2.4. Sensing

The sensing of the target analyte (imprinted drug) using the fabricated E-MIP film was performed on the Autolab SPR with a flow channel set-up. During sensing, the SPR was set to automated injection of the 0.1 M phosphate buffer saline (PBS) solution (baseline) for 120 s followed by sample injection (50 μL) for 900 s, and then rinsing of the MIP film with the background solution for 300 s. The SPR response due to the binding of the template and other analytes were compared and plotted after the abrupt change in angle, which is mainly due to the change in the refractive index of the bulk solution. All the SPR angular and kinetic curves were normalized to zero and plotted in OriginLab (version 7).

3. Results and discussion

3.1. Sensor fabrication

The formation of the E-MIP film was monitored *in situ* by EC-SPR. Among the synthesized monomers, the electropolymerization of the 3-carboxylic acid terthiophene (G0 3T-COOH) in the presence of the drug template (naproxen, paracetamol, and theophylline) was first investigated in a 2:1 monomer to template molar ratio. Terthiophene monomer was reported to have the advantage of forming a more ordered film and lower oxidation potential than mono and bithiophenes (Rasch and Vielstich, 1994; Roncali, 1992). The mechanism of the anodic electropolymerization of thiophene has been explained elsewhere (Roncali, 1992).

The electrodeposition of the E-MIP film showed a recurring oscillation of the SPR kinetic curves (Fig. 2a) for each CV cycles, which is due to changes in the dielectric property (Georgiadis et al., 2000) of the poly(terthiophene) film as it switches from oxidized to reduced states. This result is complemented by the change in the SPR angle as the potential is swept forward to 0.8 V (oxidation process) and reversed backward to 0 V (reduction process) (Fig. 2b). The profile achieved in the kinetic curve (Fig. 2a) agreed with our earlier results about the electropolymerization of poly(aniline) (Baba et al., 2004) and poly(carbazole) (Ravindranath et al., 2007) where the SPR response increases during oxidation and decreases upon reduction. The change in the dielectric property of the film has been explained as a result of the doping/dedoping of the conducting polymer. Upon oxidation, the doping process occurs when an anionic dopant (PF_6^- from the TBAH) from the bulk solution is incorporated into the film to compensate for the cationic charge carried by the polymer backbone (Hutchins and Bachas, 1995; Yamaura et al., 1988). The inserted dopant is then released back into the bulk solution upon reduction (dedoping process). At doped states, the electrical conductivity of a conducting polymer is highest (Georgiadis et al., 2000; Yamaura et al., 1988). The insertion/ejection of the counter ion from/to solution during the doping and dedoping of a conducting polymer has been observed previously using EC-QCM measurements (Deore et al., 1999).

The shifting of the minimum in the SPR angle (inset of Fig. 2a) after electropolymerization has been explained to be a combined effect of the change in dielectric constant and thickness as a result of the E-MIP film deposition on Au substrate (Ravindranath et al., 2007). The CV diagram depicts an increasing current in the oxidation regime (~0.6–0.8 V) from the 1st cycle to 20th cycle, indicating the deposition of the conducting polymer film (Fig. 2c). The same oxidation peak was observed with the electropolymerization of the monomer alone (NIP), which means that the template is electrochemically stable at the scanned potential window and

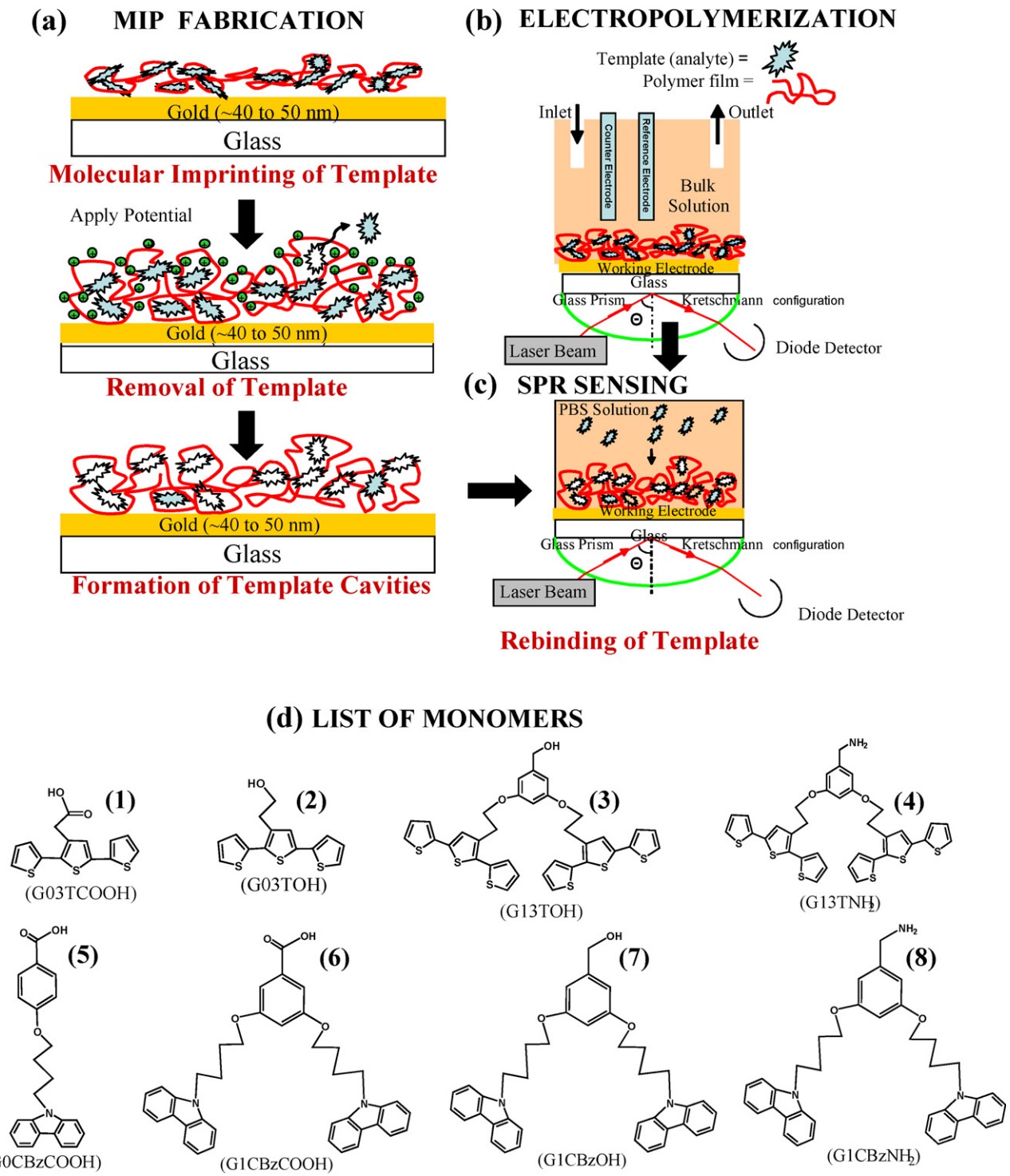

Fig. 1. (a) Sensor film fabrication by molecular imprinting and template removal by constant potential wash at 0.4 V (versus Ag/AgCl). (b) ESPR *in situ* set-up for electropolymerization and (c) SPR sensing of the imprinted guest molecule using (d) different monomers of poly(terthiophene), G0 3T-COOH (1), G0 3T-OH (2), G1 3T-OH (3), G1 3T-NH_2 (4) and of poly(carbazole), G0 CBz-COOH (5), G1 CBz-COOH (6), G1 CBz-OH (7), G1 CBz-NH_2 (8).

only the monomer undergoes electropolymerization (Supporting Information, Fig. 1a). A similar trend was observed in the SPR kinetic and CV diagram with imprinting of theophylline and paracetamol. To confirm the adsorption of the E-MIP film on gold substrate, a monomer free scan was performed on the same potential window (0–0.8 V) after electropolymerization. Prior to scanning the CV in the monomer free solution, the electropolymerized film was rinsed thoroughly in ACN to remove the weakly adsorbed molecules on gold substrate. A similar reduction–oxidation (redox) peak was seen in the CV diagram of the monomer free scan (inset of Fig. 2c), which corroborated the formation of the E-MIP film.

3.2. Surface characterizations

The surface analysis of the E-MIP films before and after the release of the guest molecules (naproxen, theophylline and paracetamol) are summarized in Table 1. After washing the films in ACN and simultaneously applying a constant potential (0.4 V), the

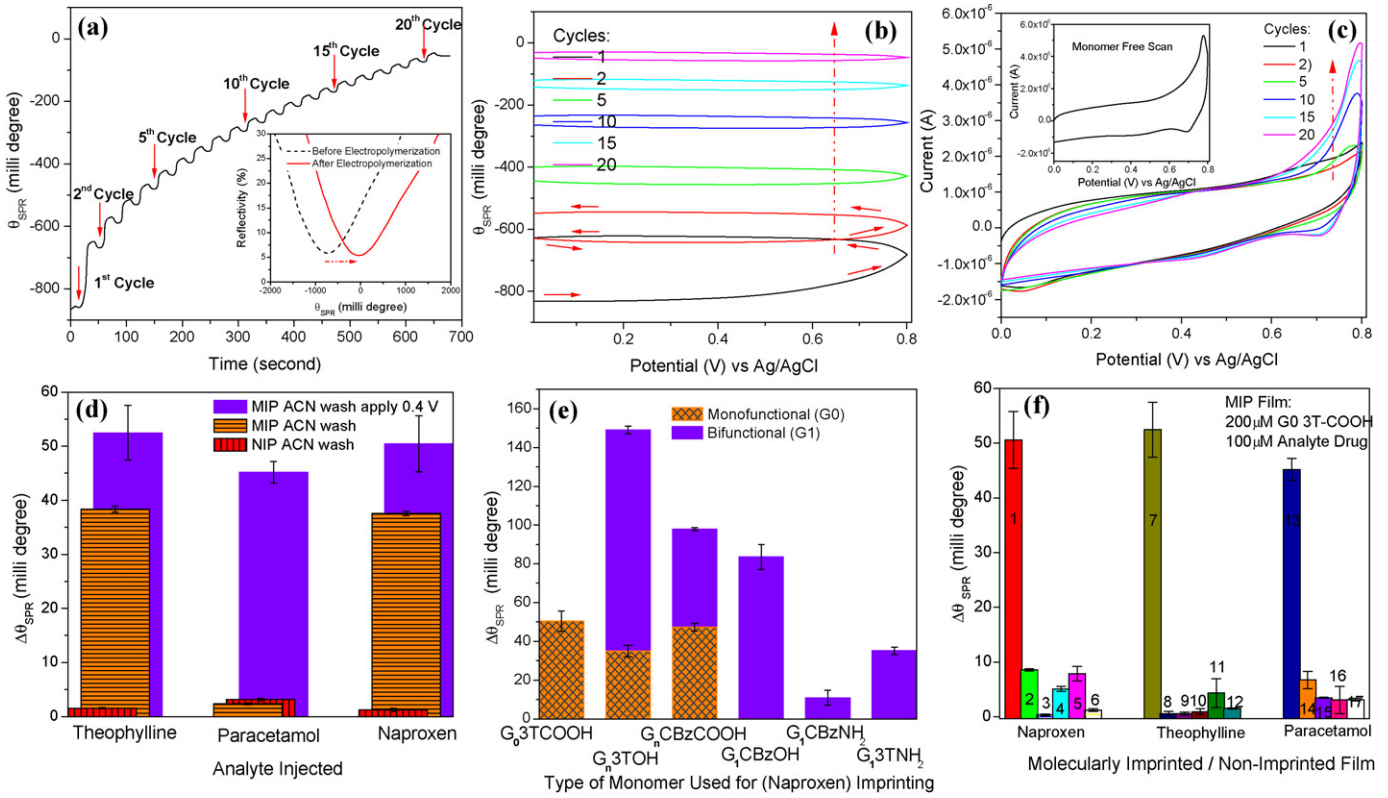

Fig. 2. *In situ* ESPR measurements of MIP film formation: (a) kinetic curve with (inset) SPR angular curve before (solid lines) and after electropolymerization of 200 μM G0 3T-COOH with 100 μM naproxen and (b) SPR scan and (c) current response versus scanning potential (representative cycles) with CV scan (inset) of the MIP film in monomer free solution. (d) SPR sensing (30 min analyte incubation) before and after constant potential (0.4 V vs Ag/AgCl) washing using different MIP films with the imprinted molecules of theophylline, paracetamol, and naproxen as compared to NIP. (e) SPR sensing of naproxen after constant potential washing using monofunctional (G0) and bifunctional (G1) monomers of carbazole and terthiophene. (f) Selectivity study of the different MIP films (naproxen, theophylline, paracetamol) and sensing response of the non-imprinted polymer film. The molecules used for this study are naproxen (1), (6), (10); 1 naphthalenesulfonic acid sodium salt (2); paracetamol (3), (11), (13), (17); theophylline (4), (7), (12); acetanilide (5), (14); caffeine (8); theobromine (9); 3-aminophenol (15); 4-aminobenzoic acid (16).

contact angle and thickness decreased, and the root-mean-square (rms) roughness increased. The observed trends are consistent with all three imprinted films. For this communication, only 0.4 V was applied during the washing. From the contact angle data, the films turned slightly hydrophilic after the release of the three organic guest molecules (templates), which are entrapped in the highly cross-linked poly(terthiophene) films. The release of the templates is evident from ellipsometry measurements which showed a decrease in film thickness. The theophylline E-MIP showed the highest thickness before the constant potential washing as compared to paracetamol and naproxen. This might be due to higher theophylline loading into the polymer film. The chemical structure of theophylline shows four possible H-bonding sites while the paracetamol and naproxen have only three possible H-bonding sites. Furthermore, the removal of the imprinted template molecule is confirmed by the increasing rms roughness as determined by AFM (Supporting Information, Fig. 2). As expected, the rms of the films had increased because of the many cavities formed in the polymer film. In order to quantify the amount of templates removed from the polymer film, high resolution XPS scan was performed with the theophylline- and paracetamol-imprinted films, which contain the N element not found in poly(terthiophene) film. After constant potential washing of the films, the N 1s peak area (~396 eV to 403 eV) had decreased by ~87% and ~81% for theophylline and paracetamol, respectively (Supporting Information, Fig. 3). These results confirmed that most of the imprinted guest molecules were removed from the polymer films. Earlier studies suggested that the N 1s peak located at this range were due to H-bonding interactions of tertiary amine and amide nitrogen atoms (Luo et al., 1998; Liu et al., 1999; Zhou et al., 1997, 1998). However, there is no

Table 1
Summary of the surface characterization measurements of the E-MIP film before and after template (drug molecule) removal by constant potential wash (at 0.4 V).

MIP film	Water contact angle (degree)	Ellipsometry thickness (nm)	AFM RMS (nm)	XPS N 1s (a.u.) peak area
A. MIP film with 200 μM G0 3T-COOH and 100 μM naproxen (NP)				
a. Before NP removal	54.50 ± 0.55	5.18 ± 0.03	1.26 ± 0.05	n.a.
b. After NP removal	53.31 ± 0.48	5.02 ± 0.10	1.67 ± 0.21	n.a.
B. MIP film with 200 μM G0 3T-COOH and 100 μM theophylline (Th)				
a. Before Th removal	56.20 ± 0.56	11.87 ± 0.26	1.11 ± 0.03	424
b. After Th removal	52.45 ± 1.22	10.73 ± 0.23	1.60 ± 0.02	55
C. MIP film with 200 μM G0 3T-COOH and 100 μM paracetamol (PCM)				
a. Before PCM removal	56.86 ± 0.52	2.06 ± 0.31	0.81 ± 0.06	732
b. After PCM removal	52.24 ± 0.98	1.78 ± 0.10	1.05 ± 0.16	138

Note: n.a; stands for not applicable.

N element with naproxen and thus, its percentage removal from the MIP film was not determined using XPS. Also, the S 2s peak (~225 eV to ~233 eV) of the films remained constant ~0.71%, which illustrates stability of the poly(terthiophene) film during washing. Furthermore, in a separate experiment, the thickness of the poly(terthiophene) was measured by ellipsometry after dipping the film to ACN for different time intervals until 17 h. The result showed no thickness change in the polymer film. This finding implies that the resulting polymer film, unlike the monomer and template, is not soluble in ACN. Therefore, ACN is a good solvent for the chosen polymer–drug E-MIP combination.

3.3. Sensing and selectivity studies

Unlike other monomers for MIP synthesis (O'Connor et al., 2007; Caballero et al., 2005; Blanco-Lopez et al., 2003; Peng et al., 2000; Weetall and Rogers, 2004; Sanbe et al., 2003; Chegel et al., 2009), the G0 3T-COOH and the rest listed (Fig. 1d) have the advantage of being both monomer and cross-linker to complex with the desired template in solution prior to the electropolymerization process (Batra and Shea, 2003). A similar concept for monomer orientation has been reported also (Yeh and Ho, 2005; Sibrian-Vazquez and Spivak, 2004). The functional group (–COOH, –OH, $–NH_2$) in the monomer is believed to be the binding site for the template via H-bonding (Mathieu and Trinquier, 2009; Khan and Sivagurunathan, 2008; Batra and Shea, 2003; Alexander et al., 2006) while the electroactive group (pendant carbazole or thiophene) is intended for cross-linking with another monomer to form a type of CPN. With the prior complexation of the monomer and the template in solution as suggested, a more robust cavity of the template is expected within the E-MIP film during imprinting. This can be generally termed as a "pre-polymerization complex" approach (Batra and Shea, 2003; Alexander et al., 2006).

The detection of the target analyte was accomplished by an SPR fixed angle or kinetic measurements. The sensing of naproxen, theophylline, and paracetamol using the three imprinted films of poly(terthiophene–COOH) is summarized in Fig. 2d (original kinetic binding curves in Supporting Information, Fig. 4). During the 30 min incubation with the templates after washing in ACN, the E-MIP films revealed an obviously higher angular change (delta) in the SPR than the NIP when exposed to the imprinted drug molecules except for the paracetamol-imprinted film. This result proves clearly that molecular imprinting has taken place (Batra and Shea, 2003; Alexander et al., 2006), where the sensing of the analyte drug by E-MIP is attributed to the presence of the complementary cavities that retained the exact size, shape, and orientation of the chemical functionalities of the template drug molecule. The diminutive SPR angular response of the NIP films upon exposure to a solution of drugs can be attributed to non-specific binding. Interestingly, the E-MIP films that were subjected to constant potential washing (at 0.4 V in ACN) demonstrated a considerable improvement on sensing compared to the earlier method of washing with solvent only. This outcome is more apparent with the paracetamol-imprinted film, where the earlier washing did not probably remove most of the imprinted drug. Among the solvents tried (dichloromethane, THF, PBS buffer), ACN has proven to be a good solvent for washing since the E-MIP film showed a higher sensing response during the rebinding studies. We suggest that the application of a constant potential during washing resulted to more swelling of the polymer film, which facilitates the release of more template from the polymer network. This hypothesis is currently being further investigated. To the best of our knowledge, the application of a constant potential during the washing of the template from an MIP film has not been previously emphasized.

Aside from using the same monomer (G0 3T-COOH) for the imprinting of the three drugs, several other terthiophene and carbazole monofunctional (G0) and bifunctional (G1) monomers (Fig. 1d) were also tested for molecular imprinting with naproxen as the model drug (Supporting Information, Fig. 5). The bifunctional monomers (G1) with –COOH and –OH functional groups have shown the best sensing response compared to the monofunctional monomers (G0) (Fig. 2e). This finding could be due to a greater amount of template–G1 monomer complexes deposited per unit volume on the film. The bifunctional NH_2 of both carbazole and terthiophene-based monomers exhibited lower template rebinding. This might be a result of the weaker H-bonding ability of the NH_2 than –COOH and –OH (Fiedler et al., 2006; Sibrian-Vazquez and Spivak, 2004; Jeffrey, 1997) when complexing with the analyte drug, and thus less number of templates were imprinted. However, the E-MIP films generated by the bifunctional G1 monomers required more time (>30 min) to reach the saturation point. A future work on determining the maximum point of the association curve is being done to understand the adsorption kinetics in detail. Preliminary sensitivity studies were made with the combination of G0 3T-COOH and theophylline (2:1 molar ratio). The injection of different concentrations (10–50 μM) of theophylline showed a linear increase in the SPR angle (Supporting Information, Fig. 6), which was not observed with the NIP film. Finally, the selectivity of the imprinted films was evaluated by exposing them to other analytes (Fig. 2f). The E-MIP films showed a greater binding response to the original imprinted molecule and only a limited response to the other analytes (Supporting Information, Fig. 7), even though some of them closely resemble the chemical structure of the template. This result is generally manifested in the three separate E-MIP films tested. Furthermore, the response of the NIP film to the target analyte represents any non-specific binding events which were found to be minimal (Supporting Information, Fig. 7).

4. Conclusion

We have demonstrated the feasibility of using a series of electropolymerizable terthiophene and carbazole monomers for the imprinting of drug molecules without the use of a separate cross-linker. The bifunctional (G1) monomers of –COOH and –OH functional groups were found to be most effective for imprinting than their monofunctional counterparts and the bifunctional NH_2, but results in a longer time to reach saturation. A possible compromise is the use of thinner films. We have also shown a novel and effective method of removing the template by potential-induced washing, which significantly improved the sensitivity of the E-MIP film. With the versatility and simplicity of the technique, the E-MIP sensor is a promising approach to the fabrication of different sensor films that can be easily attached *via* electropolymerization onto various, but limited to conducting, electrode transducers.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.10.027.

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