Pyrene-Imprinted Polythiophene Sensors for Detection of Polycyclic Aromatic Hydrocarbons

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ABSTRACT

A pyrene-imprinted polythiophene nanofilm is prepared by electrochemical deposition and used for highly sensitive detection of pyrene and its analogues. Using a molecular imprinting technique, pyrene-specific recognition sites are formed throughout the oligo/polythiophene film deposited onto a gold surface. These pyrene “imprints” possess complimentary geometric features to the analyte and are capable of forming π -donor and hydrogen bonding interactions with pyrene. Upon exposure to pyrene, the functionalized nanosurface produces specific fluorescence emission whose response is linearly proportional to its concentration range between 0.01 and 1.0 μM with good stability and reproducibility (relative standard deviation < 5.6%). The nanofilm sensor can also discriminate pyrene from its structural analogues in ultra-trace levels by shifting the fluorescence detection. Based on theoretical modeling studies, the strong polar hydrogen (Hp)- π interaction between the polythiophene film and pyrene, which generate fluorescent emission, was determined to be within -15 to -25 kJ/mol, a range close or even larger than common hydrogen bonds. The -SH groups of the thiophene moieties act as the Hp- π interaction donors while the aromatic ring of pyrene functions as an Hp- π acceptor. These interactions as combined with the size and shape of the pyrene binding sites lead to a highly sensitive and specific detection of pyrene. The nanofilm MIP sensor may have advantages in environmental monitoring of pyrene and other polycyclic aromatic hydrocarbons (PAHs) in aquatic, marine and air samples.

Keywords:

Conducting polymer; Electropolymerization; Nanosensor, Molecular Imprinting, Polycyclic aromatic hydrocarbons.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widely occurring environmental organic pollutants that pose a huge threat to human health and wildlife preservation due to their genotoxic, carcinogenic and teratogenic effects [1]. Composed of at least 2 fused aromatic rings in different configurations, these materials are typically formed from incomplete combustion of fossil fuels, tobacco, solid waste and other organic compounds [1–3]. Some important PAH sources include industrial stack emissions, fugitive emissions from manufacturing processes, municipal solid waste dumpsites, biomass burning and oil spills in marine ecosystems. Once released, PAH compounds can easily spread across the atmosphere and penetrate bodies of water and land after precipitation resulting to intercontinental transport. Traces of these compounds have also been detected in organisms and even food stuffs [4,5]. Due to their alarming abundance in the environment and adverse health effects, sensitive and selective analytical methodologies for the determination of PAHs in air, water and sediments should be pursued. Previous sensing protocols have employed powerful instrumental techniques such as fluorescence [6], electrochemical methods [7] and direct on-site monitoring [8] to detect these materials in environmental samples. In recent years, chemical sensors [9–11] are gaining attention in environmental analysis particularly in monitoring trace pollutants in ambient air, drinking water and sea food. In liquid media, sensors are common for the selective detection of ions but only a few applications are reported for monitoring organic pollutants in water [12,13].

Supramolecular recognition based on molecular cavities is a successful protocol in designing detection elements for sensor applications [14,15]. However, time-consuming syntheses and tailoring molecular sites to a special analyte are difficult to translate to real-world applications. In this regard, molecular imprinting is a promising technique for the preparation of sensitive coatings for chemical sensing as it offers a simpler, more rapid and robust approach. Currently, only a limited number of

studies have demonstrated molecular imprinting of PAHs [16–19]. One of the difficulties in imprinting PAHs is the lack of functionality in the chemical structure of these compounds, which prevents the formation of well-defined, functionalized and size-specific binding cavities in the polymer. Dickert *et al.* have reported the imprinting of PAHs onto molecularly imprinted polyurethanes that can detect different sizes of PAH molecules ranging from naphthalene (two rings) up to perylene (four rings) [18,20]. Kirsch *et al.* described the development of a screen-printed sensor for pyrene metabolites using styrene-co-divinylbenzene polymer [21] while Salinas-Castillo *et al.* reported a sensitive fluoranthene-imprinted sensor based on room-temperature phosphorescence optosensing [22]. Carlson *et al.* reported another MIP-based sensor using a proximal fluorescence technique [23]. A pyrene-imprinted self-assembled monolayer has been used by Luo *et al.* (2005) to prepare a selective voltammetric sensor [24]. Besides the development of sensing devices, molecularly imprinted solid-phase extraction (SPE) has been proposed by Lai *et al.* as a valuable technique for the extraction and clean-up of complex samples containing benzo[a]pyrene [25]. Typical configurations of MIP sensors are synthesized as bulk monoliths, however preparing thin MIP film sensors offers more sensitivity since the detection element is interfaced directly onto transducer surfaces. Electropolymerization has emerged as an attractive alternative in preparing thin-film formats of MIP with thickness between 50 and 900 nm. By changing the electropolymerization conditions including the applied current or voltage, deposition method, number of cyclic scans, among others, the thickness of the polymer film can easily be controlled and optimized based on the resulting sensor performance [26–29]. The Barrio group has pioneered the high performance development of electropolymerized MIP based microsensors [30]. Our earlier reports have used different functional monomer formulations to prepare MIPs as solid phase extraction (SPE) materials and sensor for detecting PAHs in environmental matrices [6,31,32]. The potential merits offered by molecular imprinting in sensing include the ability

to form binding sites with outstanding analyte specificity, robustness and chemical and physical stability. Unfortunately, limited selectivity was typically observed in the reported MIP sensors as a result of non-specific binding particularly for sensors applied in monitoring pollutants in water.

In this article, we report for the first time the preparation of pyrene-imprinted polythiophene thin film sensor using electropolymerization. Easily recognizable by its characteristic fluorescence emission, pyrene is selected as the model PAH compound to demonstrate the concept of combining electropolymerization and molecular imprinting as a capable technique for designing sensors for these toxic materials. The molecular recognition mechanism of the thin-film MIP was investigated using fluorescence and infrared spectroscopy and further studied by molecular modeling. The specific binding of pyrene onto the MIP was explained based on cross-selectivity studies with pyrene structural analogues. The developed sensory materials showed high sensitivity to pyrene in the presence of its structural analogues in low concentration range (0.1-100 μM) have great potential in *in-situ* environmental monitoring of these carcinogenic pollutants in aqueous solutions.

2. Experimental Section

2.1. Materials

The polymer precursor 2,2':5',2''-Terthiophene (CAS: 1081-34-1) was purchased from TCI America (Portland, OR). Pyrene, the main analyte for detection, and the analog molecules chrysene and benzo[a]pyrene were purchased from Sigma-Aldrich (St. Louis, MO). The solvents acetonitrile (ACN), dichloromethane (DCM) and ethanol of HPLC/Spectroscopic grade were obtained from Fisher Scientific (Pittsburgh, PA). All chemicals were used as received.

The gold surface of a QCM crystal was used as the working electrode in a standard three-electrode electrochemical cell setup. For the study, standard AT-Cut 5MHz gold-coated QCM crystals

(1 in. (25.4 mm), 25°C) with a chromium adhesion layer were sourced from INFICON, Inc. (East Syracuse, NY). Prior to electropolymerization, a Au QCM crystal was immersed in a piranha solution for 60 sec (1:3,v/v H₂O₂/H₂SO₄) and rinsed with Milli-Q water. After drying with nitrogen, the crystal was subjected to plasma treatment using the March Plasmod GCM 200 plasma cleaner (Concord, CA) at 10 W for 150 sec.

2.2. Electropolymerization of the pyrene-imprinted thin polythiophene film

A monomer-to-template ratio of 4:1 was used in the preparation of the MIP film. This optimum ratio was determined by plotting a Job's curve based on terthiophene-pyrene complexometric titrations. The fluorescence intensity (*FI*) of pyrene in the presence of terthiophene was monitored as a function of the pyrene:terthiophene ratio, which is denoted as $[Pyrene]/[Terthiophene]$. The combined concentration of the terthiophene monomer and pyrene was kept at constant molarity but the relative molar fractions of terthiophene and pyrene were varied in a compensatory manner to draw the Job's plot. Different values of *FI* counts were used to plot the complex concentration versus the molar fractions of pyrene at concentrations above the necessary threshold value to establish Job's plot. The lowest point of the plot is attributed to the prepolymer complex (PPC) concentration.

The molecularly-imprinted polymer (MIP) solution was prepared by mixing terthiophene (monomer, 4 mM concentration) with pyrene (template, 1 mM concentration) in acetonitrile (ACN) with 0.1 M tetrabutylammonium hexafluorophosphate (TBAH). The non-imprinted polymer (NIP) solution was prepared in the same manner without the addition of the template pyrene. The solutions were kept aside for about 10-12 hr before electropolymerization to allow the monomer-template complex to form. The MIP and NIP were directly coated on the surface of gold electrode by voltammetric cycling in acetonitrile in a standard 3-electrode cell set-up (Fig. 1A). The electrochemical polymerization via cyclic voltammetry (CV) was conducted by sweeping the potential

between 0 to 1.2 V for 20 consecutive cycles at a scan rate of 150 mV/sec. After electrodeposition, the film was washed with acetonitrile to remove excess and loosely-bound monomers from the polymer film. Finally, the template pyrene was extracted from the electropolymerized MIP film by immersing in acetonitrile, dichloromethane and ethanol solvents for 15 min each.

2.3. *Characterization of the Electropolymerized Pyrene-Imprinted Polyterthiophene Film*

As depicted in Fig. 1A, the electrochemical polymerization of the polyterthiophene MIP and NIP films via cyclic voltammetry (CV) was performed in a standard 3-electrode cell which employs a platinum counter electrode, Ag/AgCl reference electrode, and a gold QCM working electrode. The electrochemical cell was attached to and controlled by a potentiostat (Power Lab System, Milano, Italy). The experiment was conducted at ambient temperature conditions (20°C). Throughout the study, a 0.1 M TBAH solution in acetonitrile was used as the supporting electrolyte solution to characterize the electrochemical response of both MIP and NIP polymer films on the QCM gold electrodes.

The surface morphology of the MIP and NIP films was evaluated via tapping mode atomic force microscopy (AFM). These measurements were performed using a PicoScan 2500 AFM (Agilent Technologies) with a piezo scanner that was set to scan the films at 1-1.5 lines/sec. Commercially available tapping mode tips (TAP300-10, Silicon AFM probes, Tap 300, Ted Pella, Inc) with a resonant frequency in the range of 290-410 kHz were used as cantilevers. All AFM micrographs were filtered and analyzed using the Gwyddion Software (Ver. 2.19). Furthermore, a JEOL scanning electron microscope (JEOL-JSM-6510LV) was used to image wider areas of the films at a relatively lower magnification than the AFM. The SEM micrographs were recorded with an acceleration voltage 30 kV and working distance of 17 mm.

The MIP and NIP films were also characterized by attenuated total reflectance-infrared (ATR-IR) spectroscopy and X-ray photoelectron spectroscopy (XPS). ATR-IR spectra of the MIP and NIP film on Au were obtained using a Digilab FTS 7000 equipped with an HgCdTe detector from 4000 to 600 cm^{-1} with a spectral resolution of 4 cm^{-1} in absorbance mode. On the other hand, high resolution elemental analysis of the polymer thin films was performed using a PHI Versaprobe 5000 scanning X-ray photoelectron spectrometer, which has a monochromatic Al $K\alpha$ X-ray source ($h\nu = 1486.6 \text{ eV}$) with a 180° hemispherical energy analyzer. The resulting XPS spectra were processed using the Multipak V7.0.1 software.

The thickness of the electropolymerized MIP and NIP films are quantified using a Multiskop ellipsometer (Optrel Berlin) and the null ellipsometry technique. The instrument is built with a polarizer-compensator-sample-analyzer set-up and equipped with a He-Ne laser ($\lambda = 632.8 \text{ nm}$) with a 60° angle of incidence. Based on the experimentally determined ellipsometric angles, Δ and Ψ , the film thickness values were simulated by assuming a flat layered thin film model and setting the refractive indices to $n = 1.5$ for both MIP and NIP films.

Fluorescence measurements were performed on a Perkin-Elmer Model LS 55 fluorescence spectrometer (Waltham, MA, USA). The instrument consists of a 75-W xenon compact arc lamp (the light source), a model 101M monochromator and a model 814 detector. Using an excitation wavelength of 290 nm ($\lambda_{\text{excitation}}$), the fluorescence intensity of aqueous samples was recorded at the characteristic emission wavelength ($\lambda_{\text{emission}}$) of pyrene, which equals to 390 nm.

2.4. Binding Experiments

The binding properties of MIP and NIP films were evaluated by equilibrium binding analysis. Illustrated in Fig. 1B, the setup for binding experiments incorporates a flow cell system wherein the MIP or NIP-coated gold QCM crystal is mounted onto a holder with an axial flow cell adapter

purchased from Stanford Research Systems. The flow cell accessory has two ports: one port was used as an inlet while the other ejects the effluent (outlet). The pyrene sensing solution (1 μM concentration) was injected to the inlet port at a rate of 50 $\mu\text{L}/\text{min}$. The drain from the system was collected at regular intervals and the solutions were analyzed for pyrene concentration using a fluorescence spectrometer. The optimum contact time (i.e., 90 min) was determined by measuring residual pyrene concentration in solution at different flow times. The percentage binding of pyrene onto MIP or NIP was calculated from calibration curves obtained from external pyrene standards. Three repeated measurements were performed for every sample, and the mean of the concentration was recorded. The selectivity of the MIP for pyrene analogues such as chrysene and benzo[a]pyrene was evaluated by passing through the microsystem.

2.5. Molecular Modeling and Simulations

Quantum mechanical calculations were performed to investigate the molecular recognition of pyrene by the nano-film sensor *via* hydrogen bonds and other weak interactions. In practice, Møller–Plesset methods such as MP2 perturbation theory were reported to be very suitable for the study of organic molecule interactions on interfaces such as nanofilms. Concerning the basis set, the inclusion of polarization and diffuse functions is necessary to obtain reasonable results for expected weak interactions. Thus, 6-311G* is the minimum basis set that was selected for the study of hydrogen bonded systems. Furthermore, the basis set superposition error (BSSE) was used to consider the non-physical attraction between the two molecules. In particular, the BSSE counterpoise method developed by Boys and Bernardi was taken into account as a correction factor throughout this work.

Initially, MP2 calculations of terthiophene and pyrene were carried out using 6-311G* to determine the optimum basis set required for the study of the H-C $\cdots$ S-C hydrogen bond interaction in the complex system. However, MP2 calculations of thiophene layer on gold are very large and

complex systems that require extensive computations. In this regard, methods based on density-functional theory (DFT) are highly attractive alternatives. Therefore, the following DFT-based computational methods in conjunction to a B3LYP basis set were used to evaluate binding energy and distance computations for simulating CH $\cdots$ SC bonds. Consequently, the interaction energy E_{int} was further calculated using Eq. 1 and the single-point energy calculations of the terthiophene-pyrene complex ($E_{complex}$), terthiophene monomer ($E_{terthiophene}$), and pyrene (E_{pyrene}).

$$E_{int} = E_{complex} - (E_{terthiophene} + E_{pyrene}) \quad (1)$$

The binding enthalpies are given at 289 K (ΔH_{298K}). The distance d between S $\cdots$ H and the CH $\cdots$ N angle β were 1.89 Å and 127°- 173°, respectively. Similarly, the interaction between the gold surface and terthiophene were modeled using van der Waals density functional (vdWDF) with the Perdew-Burke-Ernzerhof (PBE) exchange functionality. The plane-wave cutoff has been fixed at 400 eV, which provided an accurate description of the ideal Au surface. The Brillouin sampling was performed using Monkhorst-Pack grids, determined such that the k-point spacing Δk is related to its corresponding lattice vector length via $k \geq 40$. This gives a k grid of $15 \times 15 \times 15$ for a single atom bulk unit cell. Except for convergence calculations, a unit cell has monolayer of Au atoms. A unit cell was constructed with 50 Au atoms and the optimized lattice constants for the two functional are for vdWDF/PBE and PBE respectively.

All calculations were performed using Gaussian09 program suite on Dell Precision T7500 workstation.

3. Results and Discussion

The thin film configuration is one of the most desirable MIP formats for sensing applications. In general, most MIPs are prepared as bulk monoliths via free radical polymerization and subjected to

mechanical grinding, which decreases the number of effective binding sites. This eventually results to poor sensitivity and lack of repeatable selectivity in MIPs [33,34]. The above mentioned constraints can be addressed by using the thin MIP film format as it improves the analyte mass transfer kinetics across the material. Earlier studies have reported that extracting the template from the MIP film is more difficult for thicker films. Moreover, template rebinding is poor because the cavities are deeply buried in the polymer film [35]. In this article, we report the preparation of an MIP thin film sensor using anodic electrodeposition and the self-assembly process for pyrene sensing. In addition, another advantage of the proposed fabrication method is the use of a single functional and cross-linking monomer without employing a separate cross-linking monomer and initiator. As illustrated in Scheme 1, the proposed fabrication method begins with the electrochemical polymerization and subsequent deposition of the terthiophene-pyrene (4:1) pre-polymerization complex onto a pre-cleaned Au QCM crystal. Pyrene was removed from the matrix by a series of solvent extraction steps to create the electropolymerized MIP film. The thin MIP film sensor was then exposed to varying concentrations of pyrene using the flow cell setup in Fig. 1B. Changes in fluorescence intensity of pyrene in the effluent were monitored and correlated to the sensing ability of the MIP detection system.

3.1. Electropolymerized pyrene MIP sensor

Prior to electropolymerization, the first step in the molecular imprinting process is the formation of a solution phase complex between the template and the functional monomer, which is referred to as the pre-polymerization complex (PPC). To establish the existence of the PPC, fluorescence spectrophotometric response from titration of the pyrene template with increasing concentrations of the monomer terthiophene was monitored. The fluorescence results showed a decrease in the peaks at 360 nm and 390 nm as the concentration of terthiophene increases. This indicates the formation of a complex between the terthiophene monomer and pyrene; and the complex

was further characterized by Job's plot analysis (Fig. 2A), which showed a 4:1 stoichiometry for the terthiophene monomer to pyrene.

After establishing the formation of the terthiophene-pyrene PPC, the pyrene-imprinted polyterthiophene film was electropolymerized onto the Au-coated QCM crystal via cyclic voltammetry using the standard 3-electrode cell in Fig. 1A. The resulting cyclic voltammograms (CV) for the deposition of the MIP and NIP films are presented in Fig. 2B and 2C. Based from Fig. 2B, the CV corresponding to the electropolymerization of the MIP film exhibits a typical redox peak of polythiophenes including an onset potential at 0.62 V [35]. In the first CV cycle, the anodic peak beyond 1.06 V has been reported to be due to the formation of the terthiophene radical cation, which is achieved by losing an electron in the neutral monomer species. Then, during the reverse scan (reduction), the radical cation is expected to couple with another charged specie in a 2.5 linkage to form a dimer. In the second CV cycle, a new oxidation peak appeared at a relatively lower potential (between 0.6 and 1.0 V), which is related to the formation of a more stable dication (bipolaron) with extended π -conjugation that is easily oxidized compared to the radical cation. Upon succeeding CV cycles until 20th scan, the current increases at this redox peak (between 0.6 and 1.0 V), which signifies stepwise growth of the polymer film as a result of continuous crosslinking between the terthiophene units. In addition, as the polymer loses electrons to form the polarons and assumes an overall positive charge, it adsorbs PF_6^- counterions from the TBAH supporting electrolyte. Then upon reduction (0 V), the polymer film returns to its neutral state as it ejects the counter ions to the bulk solution [36–38]. The overall electrodeposition of the polythiophene film onto the QCM crystal is evidenced by the net increase in the mass (0.29 ± 0.04 mg) and an average thickness of 400 ± 50 nm after 20 CV cycles. A similar electrodeposition behavior is observed with the NIP films (Fig. 2C). However, the NIP electropolymerization shows a greater current response as compared to the MIP film deposition. It is

possible that the analyte may have obstructed the inner chain electron transport during the MIP electropolymerization, and thus its current is relatively lower than the non-imprinted film.

Successful fabrication of the molecularly-imprinted polyterthiophene film is characterized using X-ray photoelectron spectroscopy (XPS). As shown in Fig. 3, the emergence of the singlet C 1s peak centered at 284.6 eV for the –C-C- and –C-H- moieties and the doublet S 2p peaks at 163.75 eV and 164.91 eV are the characteristic XPS peaks for polythiophene films. On the other hand, the morphologies of the MIP and NIP were imaged by atomic force microscopy (AFM). 2D and 3D topographical tapping-mode AFM images (Fig. 4A-D) show the existence of specific cavities in the MIP films while there are no defined cavities in NIP film. These micrographs provide an evidence of pyrene imprinting by clearly viewing the cavities in the MIP formed during electrodeposition of polythiophene and subsequent solvent washing. The overall rough surface morphology of the MIP film can also be observed through the scanning electron microscope as depicted in Fig. S1A and B.

3.2. Pyrene rebinding on MIP and NIP films

The recognition capability of the MIP film sensor towards pyrene was evaluated using the flow system in Fig. 1B. Driven by a syringe pump, a 1 μM pyrene solution in a 1:99 (v/v) acetonitrile:water solvent mixture was passed through the teflon chamber wherein the MIP film is mounted. After passing through the MIP-coated Au QCM surface, the drain was collected at regular intervals. The concentration of pyrene in drain samples were analyzed using fluorescence spectroscopy. Fig. 5A monitors the pyrene concentration (C) with respect to the initial concentration (C_0) as a function of time. The symmetrical S-shape of the plot signifies the breakthrough curve for pyrene which begins at non-equilibrium condition developed when the pyrene solution flows faster than the diffusion rate of pyrene onto the MIP film (Fig. 5A). At the breakthrough point, the curve rises quickly at a high slope which corresponds to the analyte rebinding. The pyrene breakthrough column started after 5 mL, when

the flow rate was 50 $\mu\text{L}/\text{min}$ and the concentration of pyrene in the effluent was below 0.03 μM (30 times less than the initial pyrene flow concentration, 1.0 μM). The course of the breakthrough curve indicated that pyrene were effectively trapped in the pyrene-imprinted sites of the MIP film. By passing 1.0 μM solution for about 8 min, the saturation was reached by the polymer thin film of mass 0.29 mg. The saturation capacity was attained by adsorbing 0.83 μM . When, the total mass of the film is 1 g, then the break-through point reached by adsorption 11.35 mg of pyrene from aqueous solution. The breakthrough capacity was found to be equal to 11.35 mg/g. The effect of flow rate on the adsorption of pyrene in the system was investigated by changing the flow rate between 20, 50 and 100 $\mu\text{L}/\text{min}$. The initial pyrene concentration was kept constant at 1.0 μM , the adsorption of pyrene decreased with increasing flow rate (Fig. 5B). At higher flow rates, the contact time of pyrene with the MIP film is shorter. It appears that mass transport of the pyrene binding to the binding sites may limit the effective capacity observed at these flow rates. These experimental results showed that the adsorption in this system is a rapid kinetic process. Such systems have potential application in the high throughput separation of structural analogues and enantiomers. The binding capacity of pyrene on the MIP and NIP films was measured by changing their concentration from 0.1 to 5.0 μM , and the obtained results were shown in Fig. 5C. It can be seen that the dynamic binding capacity of pyrene almost linearly increased when its concentration was less than 1.0 μM . The same binding capacity was observed when the pyrene concentration was higher than 1.0 μM . This result suggests that a non-linear adsorption isotherm occurred for adsorption of pyrene on the MIP films, which may be caused from the lack of binding sites on the MIP film for binding of pyrene. It is expected that this may result in peak tailing of template molecules on MIP films [39]. A rapid and high adsorption of pyrene onto the MIP films selectively at low concentration solutions is an important property for the real-time sensor development.

3.3. Molecular Recognition Mechanism

The recognition mechanism of the molecularly imprinted thin polythiophene film sensor for pyrene was investigated using fluorescence spectroscopy and computer-aided molecular modeling. At this point, the interaction between pyrene and terthiophene has not been well reported in literature probably since these two molecules do not have any functionalities. Based on the structures, hydrogen bonding and π - π interaction might form between the materials. The interaction of sulfur atoms toward the gold surface is highly spontaneous and stable which could result to the parallel orientation of sulfur-containing terthiophene moieties on the substrate after electropolymerization. Specifically, the potential types of chemical interactions between terthiophene and pyrene can be as follows: (i) gold-sulfur in the terthiophene ring (ii) gold- π electrons in the terthiophene ring and (iii) terthiophene-terthiophene interactions in the form of van der Waals and electrostatic interactions [40,41].

3.3.1 IR spectroscopy

The IR spectral data in Fig. 6A shows a distinct peak at 718 nm, which corresponds to the out-of-plane C-H vibrations of the terthiophene ring, suggests the fixing of sulfur onto the gold surface. This means that the transition moment vector of the vibration mode coincides with the electron field being normal to the gold surface. The stretching vibrations of both C-H and C=C of the terthiophene ring were not observable because the transition moment vectors of the terthiophene in plane vibration modes and the electric field vectors form right angles with each other. The constant peak at 718 nm implies that the intermolecular interactions are less prominent than the gold-terthiophene interactions. The IR spectra of MIP after pyrene binding showed clear peaks at 1417.41 and 1035.58 cm^{-1} and these peaks were not noticed for pyrene binding onto the NIP (Fig. 6A).

3.3.2 Fluorescence Spectroscopy:

Based on Fig. 6B, the fluorescence emission spectra of the MIP film after exposure to a pyrene solution showed clear peak at 408 nm that is not evident in the NIP film. The emergence of the peak between 350-450 nm is attributed to the monomeric fluorescence emission of pyrene [42]. Fig. 6C shows the FI response upon exposure of the MIP films to varying pyrene concentrations signifying specific interaction between MIP sensor and pyrene. Even weak interactions such as π - π and van der Waals are sufficient to induce specific orientations of the targeted template during polymerization thus enabling the design of sterically adapted sites suitable for reversible capture of the targeted analyte-pyrene. Additionally, the imprinted films exhibit a specific pattern of selectivity (Fig. 6C). The pyrene-imprinted film showed different sensor responses towards other PAH compounds used as interfering molecules – chrysene and benzo[a]pyrene. This clearly indicates that the binding site in the MIP film is specifically adapted to the size of a compound (pyrene) even if no covalent bonding between template and monomer is used. The sensor characteristic obtained by fluorescence measurement of pyrene is shown a linear response down to range of μM .

3.3.3 Molecular Modeling

The spectroscopic studies were further complimented using molecular modeling calculations. First, the most stable binding sites of MIP films were determined with PBE-D3 optimizations by bringing the pyrene molecule in contact with different imprinted sites and then computing the binding energies. It was found that the binding sites with energy higher than 15 kJ/mol represented relatively high mobility of pyrene on the MIP film surface. The orientation of the flat lying molecules with respect to the underlying surfaces has no significant influence to the binding and geometries. The model output is depicted in Fig. 7A. A stepwise rotation of the molecule around the z-axis changes the binding energy by < 1 kJ/mol in each case. Second, the polar hydrogen – π (Hp- π) interactions of terthiophene are point to π -plane interactions, possessing much more interaction conformations and a

broader energy range than that of the common hydrogen bond interactions. The Hp- π interactions would be the causative factor for the dynamic reversible binding between pyrene and terthiophene adhered on the gold surface. As illustrated in Fig. 7B, the sulfur atom of terthiophene strongly binds the gold atom like a pin-point. The most favorable orientation (perpendicular direction) for the Hp- π interactions is just the most unfavorable direction for common hydrogen bond interactions. In the present study, the Hp- π interaction donors are the atomic groups -SH while the Hp- π interaction acceptors are the aromatic compound moieties. The pyrene has π -bonds, possessing both polar hydrogen atoms and conjugate π -groups, playing the roles of both Hp- π interaction acceptors and donors. The Hp- π interactions are point to π -plane interactions, having many possible interaction conformations (Fig. 7C). The Hp- π interaction energies between P-T complex are in the range from -15 to -25 kJ/mol, close or even larger than the common hydrogen bonds. The bond length of Hp- π interactions is in the region from 2.00 to 3.00 Å at the perpendicular direction to the π -plane, which are much longer than common hydrogen bonds. The size and shape of pyrene binding sites generated on gold surface with polythiophene layer is capable of recognizing pyrene specifically and the sensing is primarily based on hydrogen bonding and π - π interactions between thiophene and pyrene.

4. Conclusions

The research work reported derives the following conclusions:

- i. A highly sensitive polythiophene sensor for detection of ultratrace levels of pyrene and other polycyclic aromatic hydrocarbons in the range of 0.01 – 1000 μ M has been developed. The proposed system promises to advance existing analytical methods in environmental monitoring.

- ii. As compared to other electrodeposited conducting polymer films, the polythiophene nanofilm do not have problems of tailing and surface fouling brought by weak adhesion on gold surface.
- iii. Resulting from the high mass transfer kinetics between polythiophene nanosurface and the pyrene solution, the rapid and high adsorption of pyrene onto the MIP films selectively at low concentration solutions is an important property for the real-time sensor development.
- iv. The thin-film nanosensor showed different sensor responses towards other PAHs as interfering molecules such as chrysene and benzo[a]pyrene. This behavior indicates that the binding sites in the MIP film is specifically adapted to the size of a compound (pyrene) even if no covalent bonding between template and monomer is used.
- v. Molecular modeling simulations and computations revealed that the size and shape of pyrene binding sites generated on gold surface senses the target analyte pyrene with hydrogen bonding and π - π interactions/stacking.

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Biographies

Dr. Brylee David B. Tiu is a research associate at Case Western Reserve University (CWRU) in Cleveland, OH. He has recently completed his Ph. D. in the Department of Macromolecular Science and Engineering in CWRU. His dissertation is primarily on electropolymerized conducting polymer

thin films as applied to molecularly imprinted polymer sensors and multi-component patterning with relevant inorganic and biological materials. Aside from continuing this work, he also researches on plant-virus nanoparticle arrays and corrosion-inhibiting materials for various industries.

Dr. Reddithota J. Krupadam is a Senior scientist at the National Environmental Engineering Research Institute in Nagpur, India and Faculty of Academy of Scientific & Innovative Research (AcSIR) in New Delhi, India. He received the National Award for Technology Innovation in the field of Polymer Science & Technology as bestowed by the Indian government for his work on molecularly imprinted polymer systems in 2014. His research interests include nanostructures, molecular imprinting and polymer nanocomposites useful in sensing and remediation of emerging pollutants such as polycyclic aromatic hydrocarbons, endocrine disrupting chemicals, viruses and pathogenic bacteria.

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Figures

Figure 1. Schematic diagrams of important systems used in the study: (A) Standard three-electrode cell for the electropolymerization of the terthiophene/pyrene pre-polymerization complex (PPC) onto a quartz crystal microbalance (QCM) electrode (B) Flow microsystem used for binding experiments: the system is connected to syringe pump that continuously pushes the pyrene solution to the MIP nanofilm mounted teflon chamber; the concentration of pyrene in outlet solution was measured.

Scheme 1. Stepwise fabrication diagram of pyrene-imprinted poly(terthiophene) thin film sensor beginning from the (1) electropolymerization of the terthiophene PPC onto a blank Au QCM crystal, (2) thorough solvent extraction of pyrene, and (3) rebinding of pyrene.

Figure 2. (A) Job's plot showing the optimum molar ratio between pyrene and thiophene. Cyclic voltammograms (CV) of (B) molecularly imprinted polymer (MIP) (C) non-imprinted polymer (NIP).

For Job's plot, the total molar concentration of pyrene and terthiophene are held constant ($1.0 \mu\text{mol}$), but their mole fractions varied. The interactions between pyrene and terthiophene was monitored by change in intensity of pyrene fluorescence emission spectral peak at 390 nm and the excited wavelength was 270 nm . The conditions maintained during electropolymerization are (i) pyrene and terthiophene

mixture solution of millimolar ratio 1:4, (ii) electrochemical cell parameters – Voltage, 0-1.2 V; Scan rate, 150 mV/s; Number of cycles – 20 and 0.1 M TBAH was used as an electrolyte.

Figure 3. High-resolution C 1s (A) and S 2p (B) X-ray photoelectron spectra of the pyrene-imprinted polyterthiophene film.

Figure 4. Topographical tapping-mode atomic force micrographs (AFM) of the (A) molecularly imprinted polymer (MIP) and (B) non-imprinted polymer (NIP) films; (C) and (D) are their respective

3D images.

Figure 5. Binding properties of MIP nanofilm (A) Break-through curve (B) effect of flow-rate on binding capacity and (C) binding isotherms.

Figure 6. Molecular recognition by nanofilm MIPs for pyrene (A) ATR-IR spectra of MIP after pyrene binding showing peaks at 1417.41 and 1035.58 cm^{-1} and these peaks are not prominent for NIP (B) fluorescence spectra showing difference between MIP and NIP after pyrene binding (C) MIP and NIP selectivity binding of pyrene and the FI response as a function of pyrene concentration (inset).

Figure 7. Molecular recognition by nanofilm MIPs for pyrene – A molecular modeling perspective (A) geometrically optimized gold and terthiophene monomer models (B) molecular view of arrangement of terthiophene molecules on gold surface in a pin-point manner (C) force-field model of pyrene and imprinted polythiophene film Perdew-Burke-Ernzerhof exchange functionality.

Biographies

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