Detection of aspartame via microsphere-patterned and molecularly imprinted polymer arrays

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HIGHLIGHTS
- The patterned polythiophene sensor exhibited linear sensitivity ranging from 12.5 μM to 200 μM.
- The sensor has high selectivity toward aspartame against other peptide analogs.
- H-bonding between monomer-template was crucial in successful aspartame imprinting.

ARTICLE INFO
Article history:
Received 5 October 2015
Received in revised form 14 January 2016
Accepted 21 January 2016
Available online 23 January 2016

Keywords:
Aspartame
Molecular imprinting
Conducting polymer
Electropolymerization
Colloidal lithography
Polythiophene
Quartz crystal microbalance
Electrochemical quartz crystal microbalance

ABSTRACT
A colloidal sphere-patterned polyterthiophene thin film sensor with high binding affinity and selectivity toward aspartame was fabricated using a technique combining molecular imprinting and colloidal sphere lithography. The successful imprinting of aspartame into electropolymerized molecularly imprinted polymer generated artificial recognition sites capable of rebinding aspartame into the microporous film, which was sensitively detected using quartz crystal microbalance measurements. The resulting sensor exhibited a good linear response after exposure to aspartame concentrations ranging from 12.5 μM to 200 μM and a detection limit of ~31 μM. It also demonstrated a high selectivity toward aspartame as compared to other peptide-based analogs including alanine–phenylalanine (Ala–Phe), alanine–glutamine (Ala–Gln), glycyglycine (Gly–Gly), and arginylglycylaspartic acid (RGD). The formation of the highly ordered and micropatterned surface was induced and monitored in situ by electrochemical quartz crystal microbalance and atomic force microscopy. Analyte imprinting and removal were characterized using X-ray photoelectron spectroscopy. Based on molecular modeling (semi-empirical AM1 quantum calculations), the formation of a stable pre-polymerization complex due to the strong hydrogen bonding interactions between the terthiophene monomer and aspartame played a key role in the effective aspartame imprinting and detection.

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1. Introduction

Despite widespread consumption, the controversy surrounding aspartame persists due to adverse health risk reports [1]. Concern
over this issue continues to heighten as it led PepsiCo Inc., one of the two biggest beverage companies in the world, to succumb under consumer pressure and completely exclude aspartame in its diet soda recipe beginning August 2015 [2]. Since its accidental discovery in 1969 [3], the non-carbohydrate-based aspartame gained massive attention because of its highly potent sweetness that is 200 times more than sucrose [4]. Consequently, fewer intakes are required to achieve the same effect, which is particularly attractive for weight control, body sugar management and even dental cavity prevention [5,6]. For most artificial sweeteners, the disparity in taste is inevitable but aspartame’s sweetness closely resembles that of sucrose and it also lasts longer, thus making aspartame a leading option [7]. However, upon ingestion, aspartame undergoes hydrolysis to form methanol after absorption in the intestinal lumen [1]; the remaining dipeptide is completely metabolized to form amino acid isolates L-aspartate and L-phenylalanine at the mucosal surface and absorbed by the body [8]. According to previous reports, the amount of produced methanol, which eventually converts to formaldehyde and then to formic acid for both human and rat experiments [9], is related to increased carcinogenicity of aspartame [4,10,11]. On the other hand, phenylalanine are reported to be neurotoxic and is capable of severely altering the concentrations of important inhibitory catecholamine neurotransmitters including norepinephrine, epinephrine, and dopamine within certain regions in the brain [1,12,13]. In addition, cases of memory loss, headaches, insomnia and even mental disorders have been macroscopically related to aspartame intake [12,14,15]. Hence, these findings provide the strong impetus in designing novel sensitive and selective aspartame detection systems for consumer protection.

Most widely-used detection protocols for aspartame employ capillary electrophoreses [16–18] and high performance liquid chromatography (HPLC) techniques [19]; however, these techniques require highly sophisticated equipment and time-consuming preparatory and pre-treatment steps [20]. Other reports involve the co-immobilization of enzymes such as alcohol oxidase (AOX) and carboxyl esterase (CaE) on screen printed electrodes using various crosslinking chemistries [20–22]. When the analyte reaches the functionalized electrode, the CaE hydrolyzes the methyl ester group to produce methanol leaving the dipeptide group. AOX oxidizes the methanol to produce hydrogen peroxide, which can be quantified electrochemically. Due to high cost and difficulty in maintaining the active ingredient, immobilizing enzymes may be effective but very limited and impractical especially for industrial applications [23]. In this regard, utilizing molecularly imprinted polymers offers a faster, longer-lasting, and more sensitive approach in detecting a wide range of analytes. Molecular imprinted polymers (MIPs) can be referred to as synthetic antibodies containing cavities with complementary shapes, sizes and strategically-situated recognition sites that posses a highly specific binding affinity toward the “imprinted” analyte [24]. The concept of molecular imprinting was first demonstrated by Polyakov from Kiev using silica particles that exhibited unusually specific adsorption toward the additives and solvents incorporated during its fabrication [25]. MIPs are typically prepared by co-polymerizing functional monomers and cross-linkers with the target analyte to form a composite wherein the analyte molecules are embedded in surrounding polymeric material [26]. Prior to co-polymerization, strong interactions either through covalent or non-covalent interactions should be induced to form the pre-polymerization monomer-template complex to hold the functional groups in the most thermodynamically stable position before the MIP is formed. Typically, cross-linkable monomers are added to help maintain the orientation of the pre-polymerization complex. Using solvent extraction and possible stimuli-triggered swelling techniques, the target analyte is removed from the matrix thus leaving “imprints” capable of re-capturing the analyte. Similarly, MIPs possess a lock-and-key mechanism; an artificial MIP recognition site is the lock while the analyte is the key. Due to this strong and selective binding affinity, MIPs have been employed for various chromatographic separation systems, binding assays and sensing devices [26]. So far, apart from the aspartame-imprinted zwitterionic polymer grafted onto a silica surface [1], the application of molecular imprinting in aspartame detection is very limited thus presents a huge opportunity area for development.

Synthetic schemes for producing MIPs are mostly based on bulk free radical polymerization of functional vinyl monomers [27]. However, extracting the imprinted analyte from these bulk MIP monoliths is a major concern that results to poor sensor performance. Based on recent reports, surface imprinting in thin film formats provide a more appealing strategy since the artificial recognition sites are easily accessible and more closely attached to transducer surfaces and the signal due to analyte rebinding is amplified [28,29]. In the past few years, our group has been using thin films of electrochemically polymerized conducting polymers in developing molecularly imprinted polymer sensors for various analytes including drug molecules [30] and several toxic chemicals [31,32]. Through this technique particularly with cyclic voltammetry, modifying electropolymerization variables such as the potential range, scan rate, and the number of scans provides direct control over the resulting thickness and the oxidation-reduction (redox) state of the polymeric film, which can potentially improve sensor performance [33]. In this study, we employed a terthiophene-based monomer with an acetic acid moiety (3-TAA) in synthesizing the MIP via anodic electrochemical polymerization. Aside from its chemical stability, 3-TAA has carboxylic acid units capable of forming hydrogen-bonding interactions with aspartame. Moreover, it does not require the use of a cross-linkable monomer to form a robust MIP film.

Meanwhile, recent reports have demonstrated the improved sensitivity of colloidally templated and microporous MIP sensors over planar formats by increasing the exposed surface area/volume ratio and providing more access to recognition sites that are obscured within the film [34,35]. Hence, in this investigation, we have fabricated a sensitive and highly specific aspartame-imprinted MIP sensor as synthesized via colloidal template-assisted electrochemical polymerization. As pioneered by Van duyne’s research group [36], colloidal sphere lithography employs hierarchically assembled colloidal spheres usually made of silica or polystyrene as sacrificial masks for subsequent deposition of other materials. The monolayer colloidal crystal (MCC), a hexagonally close-packed assembly that closely resembles a honeycomb structure, is the most common formation used in colloidal sphere lithography. Recently, our group has been widely employing the sacrificial polystyrene (PS) MCC template in co-patterning conducting polymers with various inorganic materials such as gold nanoparticles [37], carbon nanotubes [38], and even graphene [39]. Since the interstitial voids of the MCC still expose electrochemically accessible areas, the pre-polymerization complex composed of the terthiophene-acetic acid monomer and aspartame can be simultaneously electropolymerized and deposited within these tight spaces. An inverse opaline poly(3-TAA)/aspartame composite pattern is then revealed after dissolving the colloidal sphere templates. Moreover, the embedded aspartame molecules are extracted forming artificial recognition sites capable of rebinding the analyte to the MIP array. Mass adsorptions as monitored by quartz crystal microbalance (QCM) were mathematically correlated to the concentration of the analyte solution to which the MIP was exposed. The simple and robust protocol was able to produce a highly sensitive and aspartame specific detection system.
2. Materials and methods

2.1. Materials

Without prior purification, Polybead® polystyrene (PS) microspheres (500 nm in diameter, 2.59 wt% latex in water) from Polysciences, Inc. (Warrington, PA) have been employed as the sacrificial template for colloidal sphere lithography. The anionic surfactant sodium N-dodecyl sulfate (SDS) has been purchased from Alfa Aesar (Ward Hill, MA). The monomer (2-(2,5-di(thiophen-2-yl) thiophen-3-yl) acetic acid), an acetic acid functionalized-terthiophene molecule referred to as 3-TAA, has been synthesized in our group using a previously reported procedure [34]. Meanwhile, the sensing analyte aspartame or N-(L-α-Asparyl)-L-phenylalanine methyl ester (98%, 22839-47-0) was obtained from Acros Organics (Geel, Belgium). The supporting electrolyte tetraethylammonium hexafluorophosphate (TBAH) and phosphate buffer saline tablets were obtained from Sigma–Aldrich (St. Louis, MO). Most solvents including acetonitrile (ACN), tetrahydrofuran (THF), and methanol have been purchased from Fisher Scientific.

2.2. Formation of 2D PS monolayer colloidal crystals (MCC)

The sensor substrate selected is a standard AT-cut polished Au QCM crystal with a 5 MHz fundamental resonant frequency and 1 in (25.4 mm) diameter. The use of an electrically conductive substrate for the deposition is vital in nucleating the potential-induced oxidation coupling polymerization and the subsequent deposition of the newly formed polymer. Before any deposition, it was washed using Piranha solution (70% H2SO4 and 30% H2O2) then repeatedly rinsed with de-ionized H2O. Caution! Using the Piranha solution requires extreme care because it is highly reactive and corrosive particularly to organic materials. After drying with nitrogen, the crystal was subjected to plasma treatment in an oxygen plasma etcher (Plasmad, March) for 30 s. Prior to MIP synthesis, a sacrificial template made up of hexagonally packed array of PS monolayer colloidal crystals (MCC) has been assembled on the QCM crystal surface using the Langmuir–Blodgett (LB)-like deposition method [40]. In brief, a pre-cleaned Au QCM crystal was clipped in one end to achieve a vertical orientation and immersed in an aqueous dispersion containing 1 wt% PS microspheres and 34.7 mM SDS. The colloidal dispersion should be pre-sonicated for at least an hour to ensure uniform dispersion and minimize aggregation in the resulting array. Using a motorized dip coater, the substrate is slowly raised at a controlled rate of 0.1–0.3 mm/min from the colloidal dispersion. Finally, the substrate was dried in vacuum for at least an hour before use.

2.3. Molecular modeling for optimized monomer-template complex formation

Favorable formation of monomer-template pre-complex can be quantified as energy of formation (∆AEcomplex) and optimized by varying the concentration ratios of the components through molecular modeling studies. The modeling software Spartan ’08 Version 1.2.0 (Wavefunction, Inc.) was used to perform semi-empirical Austin Model 1 or AM1 calculations that considers the geometry, dipole moments and relevant interactions. These include hydrogen bonding, which is known to play a significant role in designing molecular imprinting sensors. ∆AEcomplex was calculated using Eq. (1). Based from Spartan ‘08 modeling results, a 2:1 ratio of 3-TAA and aspartame has been used to prepare the MIP solution. Specifically, an acetonitrile solution containing 400 μM 3-TAA, 200 μM aspartame and 0.1 M TBAH was used to fabricate the MIP film. It is important to note, however, that aspartame is not directly soluble in acetonitrile. Hence, a PBS solution with a 5 mM aspartame was initially prepared from which the required amount of analyte to achieve 200 μM in the final solution was taken and added to the mixture of acetonitrile and 3-TAA. The resulting solution was stored in refrigerated condition after sonication to allow better complexation between the monomer and template. On the other hand, the non-imprinted polymer (NIP) film was also synthesized using an ACN solution with 400 μM 3-TAA and 0.1 M TBAH.

2.4. Fabrication of patterned and molecularly imprinted poly(3-TAA) films

The MIP film has been electrochemically polymerized and simultaneously deposited by cyclic voltammetry method onto the PS-templated Au-coated QCM crystal. The set-up used was a standard three-electrode electrochemical cell that consists of an Ag/AgCl reference electrode, platinum counter electrode, and the Au-coated QCM crystal pre-patterned with PS MCCs as the working electrode. The electropolymerization was performed by gradually sweeping the potential between 0 V and 1.1 V at a scan rate of 100 mV/sec for 15 cycles. The resulting film was thoroughly rinsed with ACN to wash away the undeposited monomers and oligomers. A second cyclic voltammetry experiment was done using only an ACN solution with 0.1 M TBAH to confirm the stable formation of the polystyrene. Since the polystyrene particles are electrically insulating, only the areas that are not being covered by the colloidal sphere template are electrochemically accessible and capable of nucleating polymer formation. Consequently, immersing the PS-MIP film in THF for 30 min twice to selectively dissolve the PS sacrificial template unveils the resulting inverse opal pattern. Meanwhile, a similar procedure was done for the preparation of the NIP film (control) by electropolymerization of the same monomer but not including the analyte. The aspartate analyte was extracted from the MIP film matrix by thorough washing with methanol. The sample was dried in vacuum for at least an hour before sensing.

2.5. Quartz crystal microbalance for electrosynthesis of MIP film and aspartame detection

The RQCM—quartz crystal microbalance research system (Inficon, Inc.), which includes the main apparatus, probe and crystal, was used in monitoring mass changes during the electropolymerization of the MIP film and the rebinding process for aspartame. The apparatus consists of a built-in phase lock oscillator with a measurement resolution of <0.4 ng/cm² and frequency range of 3.8–6 MHz and is capable of crystal capacitance cancelation to remove unwanted capacitance effects of the crystal. QCM measurements were logged and graphically monitored using the RQCM data-log software. For the electrochemical polymerization, the Amel 2049 potentiostat and power lab system (Milano, Italy) were connected to the RQCM to perform an electrochemical-quartz crystal microbalance (EC-QCM) measurement, wherein any film depositions induced by electrochemistry is monitored gravimetrically. The voltage bias applied by the potentiostat is conducted through the QCM holder, POGO electrodes and to the main QCM surface, hence frequency changes and current measurements can be logged in real time while the electrolys isisynthesis is on-going. On the other hand, for the aspartame detection, changes to the resonant frequency after exposure to varying analyte concentrations are then used as the main transduction signal for the MIP sensor.

2.6. Characterization of colloidally-templated poly (3-TAA) films

An atomic force microscope (PicoScan 2500, Agilent Technologies) was used to study the surface morphology of the substrates in tapping mode. AFM is equipped with a piezo scanner that was set to run at 1–1.5 lines/s using commercially available tapping mode
tips (Tap 300-10, silicon AFM probes, Tap 300, Ted Pella Inc). The software Gwyddion 2.19 was used to analyze and enhance the AFM images.

X-ray photoelectron spectroscopy (XPS) for monitoring the presence of key elements were performed using an X-ray photoelectron spectrophotometer (PHI Model 5700), which was equipped with a monochromatic Al Kα X-Ray source (hν = 1486.7 eV). The X-ray source is positioned at 90° relative to the axis of a hemispherical energy analyzer. The spectrophotometer was operated both at high and low resolutions with pass energies of 23.5 and 187.85 eV, respectively, a photoelectron take off angle of 45° from the surface. All XPS measurements were performed at a base pressure of 1 × 10−8 Torr.

3. Results and discussion

Scheme 1 illustrates the step-by-step strategy in fabricating the molecularly imprinted polyterthiophene film for aspartame detection. First, a hexagonally close-packed formation of 500 nm PS latex microbeads, also referred to as a monolayer colloidal crystal (MCC) is assembled on a Au QCM substrate via the Langmuir–Blodgett-like deposition method. Employing the colloidal-templated substrate as the working electrode, the terthiophene-carboxylic acid/aspartame complex was electrochemically polymerized via cyclic voltammetry. The polyterthiophene film subsequently formed in the electrochemically accessible interstitial voids of the colloidal pattern. An inverse opal structure is then revealed after dissolving the latex PS spheres. Lastly, aspartame is removed from the matrix by thorough washing in methanol to expose molecular imprints capable of capturing the analyte.

3.1. Molecular modeling and simulation of monomer-template prepolymerization complex

In designing molecularly imprinted polymer sensors, inducing strong non-covalent interactions between the monomer and analyte molecules is vital in ensuring that the resulting film will have cavities with strategically placed chemical handles capable of capturing the analyte back to the film. Molecular modeling and simulating the interactions between the components is an effective approach to address this concern. Based from its chemical structure, 3-TAA has a carboxylic acid moiety that can potentially form hydrogen-bonding and electrostatic interactions with the amine and carboxyl groups of aspartame. The interaction energy of the resulting monomer-analyte pre-complex can be estimated using Eq. (1).

$$
\Delta E_{\text{complex}} = \left( \Delta H_f^{3-\text{TAA/Aspartame}} \right) - \left( \Delta H_f^{3-\text{TAA}} + \Delta H_f^{\text{Aspartame}} \right) \quad (1)
$$

It accounts for the individual enthalpies of formation of the complex, monomer and analyte, which have been determined using semi-empirical AM1 force field calculations as performed in Spartan '08 (V1.2.0; Wavefunction, Irvine, CA). By changing the composition ratios, the most thermodynamically stable complex formation can be achieved using 1:2 analyte-monomer ratio which accounted for an interaction energy of −25.1 kJ/mol. In comparison, a 1:1 system corresponds to 10.7 kJ/mol while a 3:1 monomer/analyte system is calculated to have 15.2 kJ/mol. The negative interaction energy value suggests a highly spontaneous complex formation, which is key in forming stable imprinting of the analyte within the polymeric system. Fig. 1 shows the theoretical model of the pre-polymerization complex, which also suggests a potential arrangement of 3-TAA and aspartame in the matrix. Space-filling and tube models are shown in Fig S1. Based on the molecular modeling simulation, the aspartame analyte can be found squeezed in the middle of two 3-TAA units. During polyterthiophene formation, interaction between the monomer and aspartame should be stable enough in such a way that a robust conducting polymer/analyte composite can form on the electrode surface.

3.2. Aspartame-imprinted poly(terthiophene-acetic acid) inverse opal film formation

The simulated optimum monomer:analyte ratio is then translated in preparing the electropolymerizing solution for synthesizing the MIP film. However, according to previous reports [34,35,41], incorporating nano/microstructures throughout the film improves the performance of the sensor by increasing the contacting surface area to analyte. Hence, a sacrificial micropatterning template composed of a hexagonally close-packed assembly of PS latex beads was first arranged on the QCM surface using a...
controlled vertical deposition technique. Covered areas within the insulating honeycomb structure also referred to as a mono-
layer colloidal crystal (MCC) will mask subsequent depositions,
however, the interstitial spaces between the spheres still expose
electrochemically-accessible areas capable of accepting electro-
chemically polymerized materials. Fig. 2A and B are 2D and 3D
topographical tapping mode AFM images that demonstrate suc-
cessful ordering of PS microspheres. According to the x-axis of
the cross-sectional profile in Fig. 2C, the average diameter of the
colloidal PS is 463.0 nm ± 4 nm, which is close to the actual 500 nm
PS diameter. Meanwhile, the y-axis of the cross-sectional profile
only accounts for 162.4 nm ± 8 nm for PS diameter since the tight
spaces between beads are difficult to reach and image accurately.
The highly ordered hexagonally close-packed surface was prepared
using the Langmuir–Blodgett-like deposition method, wherein the
QCM crystal is immersed in an aqueous dispersion of latex particles
and anionic surfactant and then slowly raised at a steady pace.
The anionic surfactant SDS assumes a vital role in inducing the well-
ordered arrangement of the colloidal particles as it helps meet the
required ionic strength to force the particles to the air–liquid inter-
face [40]. At this point, the colloidal materials are driven to a more
thermodynamically stable honeycomb orientation that is also capa-
ble of seeding wider range ordering. Moreover, the surfactant also
helps in inducing electrostatic repulsion within the particles, which
protects the pattern from colloidal aggregation. As the substrate is
slowly lifted, the close-packed formation is transferred onto and
maintained at the surface.

The fabricated PS MCC was then used as an electrochemical
mask for colloidal patterning the molecularly imprinted polye-
thiophene film. The PS-patterned Au-coated QCM crystal was
immersed in an acetonitrile solution containing 3-TAA (400 µM),
aspartame (200 µM), and tertbutylammonium hexafluoropho-
phate (0.1 M). Using a standard three-electrode cell with the
Au-coated QCM crystal as the working electrode, the potential
was swept back and forth between 0 and 1.1 V at 100 mV/s for
15 times. The ease in dissolving the sacrificial latex tem-
plate is one of the most attractive features of colloidal sphere
lithography but it also limits the range of solvents that can be
employed in depositing a material through it. Based on Fig. S2,
it can be seen that the AFM image is similar to Fig. 2A and that
the electropolymerization conditions did not disturb the highly
ordered system. After thoroughly washing the QCM crystal using
tetrahydrofuran to remove the PS microbeads and then extract-
ing aspartame via methanol dissolution, the successful fabrication
of the inverse opaline polymer film, as presented in Fig. 2D and
E, is unveiled. Fig. 2F shows the AFM cross-sectional profile of
the inverse honeycomb pattern. The macropore diameter or the
peak-to-peak distance of the macropore is determined to aver-
age at 473.6 nm ± 31 nm. Moreover, the center-to-center distance
equals 464.0 nm ± 12 nm. Both values closely resemble the geo-
metrical features of the original PS pattern. On the other hand, the
height of the cavities averages at 95.9 nm ± 7 nm. As summarized
by the chemical reaction in Fig. 3A, the real-time formation of
the poly[3-TAA]/aspartame complex was simultaneously facilitated
and monitored using electrochemical quartz crystal microbalance
(EC-QCM), a technique that measures current changes, resonant
quartz frequencies, and mass depositions. Cyclic voltammograms
of Fig. 3B and C, which correspond to the molecularly-imprinted
and non-imprinted polymer films, depict the current responses
due to the electropolymerization. The mechanism of polythiophene
electropolymerization has been previously reported [42]. Typical
to the oxidation–reduction behavior of polythiophenes, an onset
potential was observed at 0.4 V. Already evident in the first anodic cycle (forward scan from 0 V to 1.1 V), an oxidation peak emerged after 1.0 V that corresponds to the release of electrons from the monomer and subsequent formation of radical cation species also known as polarons. As the potential sweeps back from 1.1 V to 0 V, the polarons attach to each other through a 2.5–linkage and undergoes reduction as signified by the wide cathodic peak between 0.4 V and 1.1 V. For succeeding cycles, another anodic peak becomes evident at 0.6 V, which is attributed to the oxidation of the radical dications or bipolarons. The anodic potential is much lower than that of the polarons since the dications readily oxidize due to extended π-conjugation as compared to the radical cation species. Similar behavior is observed for the electropolymerization of both the aspartame-imprinted and non-imprinted polymer films. However, the total cyclic voltammogram area for the non-imprinted polymer film is much wider as compared to the imprinted film, which is intuitive because aspartame is insulating in nature and incorporating it in the matrix would result to decreased current peaks as compared to the electropolymerization of the monomer alone. On the other hand, the current responses observed in the cyclic voltammograms reflect corresponding frequency shifts ΔF in the QCM data as shown in Fig. 4A. For both MIP and NIP films, a decreasing staircase behavior is observed wherein the number of “stairs” corresponds to the number of electrochemical polymerization cycles. In a typical electrochemical oxidation–reduction cycle, a sudden decrease in ΔF is observed as the potential is swept from 0 V to 1.1 V (oxidation), which is followed by a slight increase in ΔF when the potential cycles back from 1.1 V to 0 V (reduction). In addition to the stepwise polymeric depositions per cycle, the oscillating behavior is also due to the cyclic capture and release of supporting electrolyte PF6– ions. During the anodic cycle, the monomer releases an electron forming the positively-charged polaron, capable of accepting negatively-charged supporting electrolyte PF6– ions. This behavior as combined with polymeric deposition is signified by a sudden decrease in ΔF. Meanwhile, during the reduction half-cycle, the cationic nature of the polymeric backbone neutralizes thus releasing the supporting electrolyte ions. Comparing the MIP and NIP EC-QCM data, it can be observed that the increased ΔF shifts due to reduction is much higher for the non-imprinted polymer film than the MIP. It is possible that during the polymerization of 3-TAA/analyte complex, aspartame hinders the diffusion of the supporting electrolyte ions in and out the resulting polymeric matrix, which is not the case for the electropolymerization of the pure monomer. After 15 cycles, the total ΔF shift amounted to −823 Hz for the 3-TAA/aspartame complex, which is much higher than that of the pure case which only achieved a total of −406 Hz. Based on the measured mass shifts presented in Fig. 4B, a total of 14.6 μg/cm² deposited due to the electropolymerization of 3-TAA/aspartame while for the 3-TAA only case, 7.3 μg/cm² of material was formed. These findings complement the claim made.
from the CV data that the interaction between the monomer and the analyte is strong enough to co-deposit the electrochemically inactive aspartame with the polyterthiophene on the QCM surface, which is vital in fabricating the analyte imprints of the MIP film.

The high resolution X-ray photoelectron spectroscopy (XPS) data presented in Fig. 5 and 6 confirm the electrodeposition of a robust poly(3-TAA)/aspartame composite array (after the removal of PS latex beads) on the Au QCM crystal. The emergence of the S 2s singlet peak at 228 eV and 2p doublet peaks at 163 eV and 166 eV, as shown in Fig. 5A and B, confirm the robust deposition of the patterned film [43]. Moreover, detected C 1s and O 1s intensities, shown in Fig. 5C and D respectively, are also characteristic peaks for polythiophene films. Two peaks can be observed from the C 1s spectra. The more intense C 1s peak centered at 284.6 eV is attributed to the —C—C— and —C—H—functional groups of the polymer; the less prominent peak at 288.9 eV correspond to the —O—C—O— moieties [43]. On the other hand, O 1s peak which begins at 529.01 eV and ends at 535.42 eV is due to the —OH and —O—C—O— units of poly(3-TAA) [43]. Aside from confirming the synthesis of polymeric film, XPS is also an important tool in determining the presence of aspartame in the system by monitoring the elemental marker N 1s. Based on Fig. 6, the electrodeposited poly(3-TAA)/aspartame indeed demonstrated an intense N 1s peak from 399 eV–402 eV and centered at 400 eV [43], which is, as expected, not present in the spectra for the non-imprinted film. In order to finally generate the imprinted cavities, the composite film was repeatedly washed in methanol for 1 h. As a result, the N 1s spectra significantly decreased suggesting complete removal of most amounts of analyte in the system.

3.3. Aspartame QCM detection using molecularly-imprinted and microporous poly(3-TAA) inverse opaline films

The capability of the aspartame-imprinted and colloidal-patterned poly(3-TAA) arrays to capture the analyte is measured using in situ quartz crystal microbalance adsorption studies. Fig. 7A
presents time-dependent $\Delta F$ measurements after the injection of 50 $\mu$M aspartame in PBS buffer solution (0.1 M, pH 7) onto MIP and NIP films. Adsorption onto the micropatterned MIP film had a much greater $\Delta F$ decrease equal to $-55$ Hz, which is more than twice as compared to the measured re-binding of $-21$ Hz on a non-imprinted film. Based from this concentration, the imprinting factor, which is defined as the ratio of the transduction response of the imprinted film over the NIP, is determined to be equal to 2.64. Fig. 7B compares $\Delta F$ signals due to aspartame adsorption on both MIP and NIP using various concentrations. Clearly, the adsorption data confirms the successful imprinting of aspartame within the MIP film and its ability to capture the analyte with higher sensitivity as compared to a non-imprinted polymer film. The sensing performance of the colloidal sphere-patterned, microporous MIP film was further evaluated by measuring the response over a range of concentrations from 12.5 $\mu$M to 200 $\mu$M aspartame. In situ QCM adsorption measurements are collated in Fig. 7C from which a relatively good linear calibration plot was constructed relating the final frequency shift as a function of aspartame concentration and presented in Fig. 7D. The determined Pearson’s coefficient $R^2$ is 0.9845 while the slope of the calibration plot is 1.2517 Hz/$\mu$M. Moreover, the limit of detection (LOD), which is the minimum analyte concentration that can be detected at a certain confidence level, was also determined based on Eq. (2), wherein $\sigma$ is the standard deviation of the y-intercept and $m$ is the slope of fitted line [31]. Assuming a confidence level of 95%, the LOD is calculated to be 31.75 $\mu$M (9.34 mg/L).

Limit of Detection (LOD) = \frac{3\sigma}{m} \tag{2}

On the other hand, the limit of quantification is calculated to be 105.84 $\mu$M (31.15 mg/L) according to Eq. (3) [44].

Limit of Quantification (LOQ) = \frac{10\sigma}{m} \tag{3}

In 2013, the European Food Safety Authority (EFSA) has stated that the acceptable safety limit for aspartame is 40 mg per kg of body weight while the US Food and Drug Administration (FDA) recommends 50 mg per kg of body weight [45,46]. Moreover, the maximum permitted level (MPL) for aspartame content in soft drinks is set to 600 mg/L by the EFSA [45]. Based on these values, the detection and quantification limits of the proposed MIP sensor are suitable for aspartame detection within the legally set safety limits.

Aside from the sensitivity of the detection mechanism, the selectivity of the micropatterned aspartame-imprinted poly(3-TAA) sensor was also investigated by measuring the $\Delta F$ response after exposure to aqueous solutions containing 200 $\mu$M of various analogs with chemical structures that closely resemble that of aspartame, the imprinted analyte. Fig. 8A shows the chemical structures of the selected analogs, which include peptides alanine–phenylalanine (Ala–Phe), alanine–glutamine (Ala–Gln), glycylglycine (Gly–Gly), and arginylglycylaspartic acid (RGD). As presented in Fig. 8B, the corresponding time-dependent QCM $\Delta F$ shifts reveal that aspartame, indeed, exhibited the highest amount of adsorption toward the aspartame-imprinted poly(3-TAA) array. This behavior can be attributed to the successful formation of cavities with same shape and size as aspartame within the microporous MIP film. The selectivity of the sensor can also be expressed in terms of the cross-reactivity ratio, which is defined the ratio of the $\Delta F$ shift due to the capture of the similarly structured analogs over the $\Delta F$ response when the micropatterned MIP film is exposed to the aspartame solution. Based on Fig. 8C, the cross-selectivity ratios arranged in a decreasing order are as follows: Ala–Gln (70.8%), Gly–Gly (59.2%), Ala–Phe (44.3%) and RGD (18.2%). The high interference ratio of Ala–Gln can be attributed to the three groups of amine moieties, which are capable of forming strong hydrogen bonding interactions to the $-\text{COOH}$ units of the polymeric film. On the other hand, it is possible that the smaller size of the
molecular structure of Gly–Gly caused the analog to fit into and be captured by the molecular imprinted cavities. Unlike the other molecules, the poor binding affinity of Ala–Phe and the RGD peptide might be attributed to the relatively larger sizes of the structures, which hindered the rebinding of the analogs onto the MIP sensor. Despite these issues, the aspartame-imprinted poly(3-TAA) film still exhibited high selectivity toward aspartame as compared to other structurally related analog molecules.

4. Conclusion

We have fabricated a micropatterned thin film sensor for aspartame based on a surface-bound molecular imprinting technology. Inspired by previous reports [34,41], colloidal sphere lithography was employed in order to introduce periodically-spaced macropores that exposed hidden artificial recognition sites and increased the surface area/volume ratio of the MIP sensor. Hexagonally close-packed PS MCC sacrificial masks were successfully prepared using the Langmuir–Blodgett-like deposition method. Prior to electropolymerization, the optimum 2:1 monomer/aspartame composition ratio was calculated from simulated semi-empirical AM1 calculations to maximize non-covalent interactions between the components. Using the PS-patterned gold substrate QCM crystal as a working electrode, the precopolymerization monomer/aspartame complex was simultaneously electropolymerized and deposited within the interstitial voids of the PS MCC template. Dissolving the PS mask revealed a highly ordered inverse opal pattern and array fidelity. Monitoring the N 1s peak obtained by XPS as well as the current responses and ΔF shifts from the electrochemical quartz crystal microbalance technique supported the claim the aspartame was embedded within the electropolymerized poly(3-TAA) due to the strong interactions between the components. By tracking the elemental N 1s XPS marker, aspartame was successfully extracted from the composite array by repeated washing in methanol. The micropatterned, aspartame-imprinted polyterthiophene array exhibited sensitivity toward analyte concentrations from 12.5 μM to 200 μM, as characterized by a Pearson’s coefficient R² of 0.9845. The nanostructured thin film sensor also showed specificity toward similarly structured analog molecules which includes peptides such as Ala–Gln. Gly–Gly, Ala–Phe and RGD, as arranged in a decreasing cross-selectivity ratio. Based on these results, it was confirmed that the molecular imprinting technology using a colloidal-templated and electrochemically polymerized conducting polyterthiophene film was able to build synthetic antibody-type recognition sites for rebinding aspartame which was tracked using the QCM technique. To our knowledge, this is the first account wherein an artificial nanostructured conducting polymer film is used to detect aspartame. Due to its promising sensitive and selective binding affinity toward aspartame, the technology can easily be translated to various separation systems. In particular, molecularly imprinted polyterthiophenes can be electrodeposited on commercially available mesh and be used to filter out the imprinted molecule from a given mixture, which can be manufactured on a larger scale. In terms of performance, other stimuli-responsive functional groups can be added to the polymer matrix to help induce swelling, which can be turned on and off upon trigger for developing smart functional detection systems.

Acknowledgments

We acknowledge funding from the National Science Foundation, NSF STC-0423914 and CMMI NM 1333651. Technical support from Biolin Scientific, Park Systems, and Agilent Technologies is also acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfa.2016.01.038.

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