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

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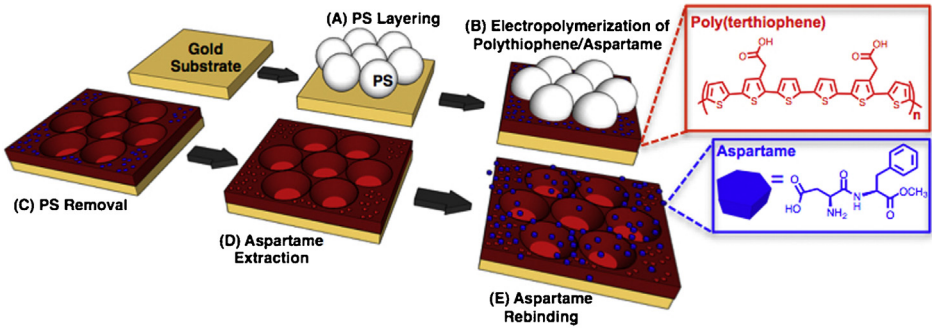
HIGHLIGHTS

- 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.

GRAPHICAL ABSTRACT



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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), glycylglycine (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.

1. Introduction

Despite widespread consumption, the controversy surrounding aspartame persists due to adverse health risk reports [1]. Concern

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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.