Molecular imprinted polymer functionalized carbon nanotube sensors for detection of saccharides

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In this work, we report the synthesis and fabrication of an enzyme-free sugar sensor based on molecularly imprinted polymer (MIP) on the surface of single walled carbon nanotubes (SWNTs). Electropolymerization of 3-aminophenylboronic acid (3-APBA) in the presence of 10 M D-fructose and fluoride at neutral pH conditions resulted in the formation of a self-doped, molecularly imprinted conducting polymer (MICP) via the formation of a stable anionic boronic ester complex between poly(aniline boronic acid) and D-fructose. Template removal generated binding sites on the polymer matrix that were complementary to D-fructose both in structure, i.e., shape, size, and positioning of functional groups, thus enabling sensing of D-fructose with enhanced affinity and specificity over non-MIP based sensors. Using carbon nanotubes along with MICPs helped to develop an efficient electrochemical sensor by enhancing analyte recognition and signal generation. These sensors could be regenerated and used multiple times unlike conventional affinity based biosensors which suffer from physical and chemical stability. © *2015 AIP Publishing LLC*.
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Molecular recognition forms the basis of developing highly selective tailor-made recognition systems that find widespread usage in medical, analytical, and biological applications. Molecularly imprinted polymers (MIPs) have been successfully demonstrated towards detecting biological entities such as receptors, enzymes, and antibodies.[1–4] It is based on the complexation between the target molecule and functional monomer(s) of the polymer that exhibits a high recognition affinity only towards the target molecule. The technique involves co-polymerization of functional monomers and cross linkers around template molecules[5] resulting in the formation of highly cross-linked polymers with chains in a fixed arrangement. Removal of the template thereafter renders the polymer matrix with well-defined binding sites that are complementary to the analyte both in shape and in positioning of functional groups, thus resulting in selective recognition of the target molecule.[6]

Conducting polymers (CPs) are a class of polymers which exhibit superior electrical and electronic properties of metals and semiconductors along with processing advantages of polymers.[7–9] Unique properties such as high electrical conductivity, tunable charge, transport properties, and good electrochemical reversibility have made them ideal components for use in developing electrochemical sensors.[10–12] CPs have also been suitably modified chemically with appropriate functional groups or functionalized with bioreceptors for specific recognition and detection of different biological and chemical target molecules.[13]

However, sensing ability of CPs has so far been confined to detection of large bioactive molecules such as DNA and peptides.[14,15] Detection of small molecular weight molecules still remains a challenge that plagues most of the biosensors systems developed so far, owing to the poor transduction mechanism that results from conversion of the binding event into a measurable signal, in particular, at a low concentration of the analyte. In addition, elimination of non-specific interactions also becomes a critical issue to address when detection of small molecules is concerned.

Molecular imprinting of conducting polymers (MICPs) is an approach which combines the enhanced selectivity arising from highly specific binding sites of molecular imprinting together with the properties of CPs, thus creating highly sensitive and selective platforms for analyte detection. Over the past few years, synthesis of MICPs and their applications for detecting a wide range of molecules have been reported. Examples include: over oxidized polypyrrole films for glutamic acid detection;[16,17] electrochemical synthesis of conjugated MICP, poly(3,4-ethylenedioxythiophene-*co*-thiophene-acetic acid), for detecting herbicide atrazine;[18] detection of bovine leukemia virus glycoproteins using polypyrrole based MICP;[19] and saccharide-imprinted poly(aniline boronic acid) for detecting D-fructose.[20]

In this work, we report the electropolymerization of D-fructose imprinted poly(aniline boronic acid) (PABA) on the surface of single walled carbon nanotubes (SWNTs). This approach takes into account the fact that polyaniline boronic acid–D-fructose film can be formed under neutral pH conditions in the presence of fluoride ion as catalyst and subsequently is used to prepare specific molecular recognition sites in polymer films after template removal for the detection of D-fructose based on the selectivity complexation reactions involving boronic acids. Each of the imprint's recognition cavities generated after extracting the template

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has a specific shape, size, and contain complementary functional groups spatially situated for subsequent recognition of the template through rebinding both with high affinity and specificity. One of the challenges which often limits the use of MIP matrices for electrochemical applications is poor electronic transduction that prevents electrical communication between remote binding sites and the electrodes, thus affecting the overall signal level. Carbon nanotubes (CNTs) have been extensively used as transducer active elements due to their ultra-small size, high specific area, and superior electronic and transport properties. Their reduced dimensionality and structure which comprises almost entirely of surface atoms results in high surface area, thus leading to enhanced sensitivity towards detecting an analyte. In this case, using single-walled carbon nanotubes along with MICPs resulted in the fabrication of efficient electrical sensor by enhancing analyte recognition and signal generation.

To overcome the inherent drawbacks of enzymatic sensors for detection of sugars (that mainly arise due to the instability of the enzymes), non-enzymatic approaches have been developed and demonstrated over the years. In this regard, functionalized polyaniline, particularly boronic acid and its derivatives,[21] have been studied to develop electrochemical platforms for detection of sugars. The mechanism involved is based on the complexation of saccharides (as well as alkyl and aromatic diols) with aromatic boronic acids, producing a stable boronate anion and a proton in the pH range of 6–10.[22,23] The complexation results in a stable ester where the binding constant is known to be dependent on the pH, electrolyte concentration, and p*K*a of the boronic acid involved.[24] For the complexation between PABA and saccharides, pH values above 8 are desirable.[25] However, owing to the conflicting pH requirements for the electrochemical synthesis of polyaniline (which is typically carried out near a pH value of 0), efforts were made to form a saccharide complex with PABA at near neutral pH conditions. Studies revealed that the equilibrium reaction of boronic acid with fluoride is known to produce a tetrahedral anionic complex[26] and this fluoride catalyzed reaction results in the self-doped structure of PABA under acidic conditions.[27] Phenylboronic acid based sensors are mostly used as soluble reagents in sugar sensing. To overcome this limitation and further expand the scope of application of these sensors, coupling of these sensing platforms with efficient transducer mechanisms has been studied. In a recent report, we demonstrated the formation of self-doped PABA polymer on the surface of SWNTs.[28] It involved sustained electropolymerization of PABA in the presence of 300 mM NaF in 0.1 M phosphate buffer saline solution (10× PBS) adjusted to pH 5.0 on aligned SWNTs surface. The PABA coated SWNT sensors could be used to detect D-fructose and D-glucose over a wide concentration range. However, the PABA coated SWNT sensors lacked the discriminating ability between saccharides as cis-diol groups of any saccharide could bind with the BA moieties to form tetrahedral anionic cyclic esters. This was a downside of a non-MIP based sugar sensors. Hence, it was necessary to develop a sensing platform that could exhibit high selectivity and still retain high sensitivity for a predetermined molecule. In the present work, SWNTs platform has been used for developing a highly sensitive and selective sensor based on molecular imprinting of the conducting polymer (MICP) PABA along with target molecule D-fructose.

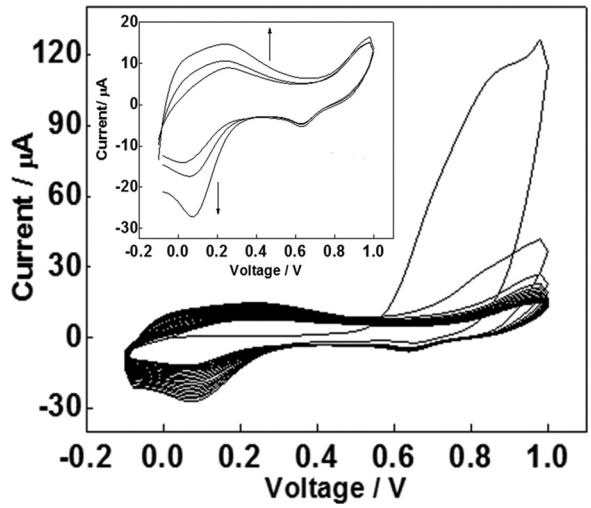

FIG. 1. Cyclic voltammogram of 40 mM 3-aminophenylboronic acid and 10 M D-fructose in 0.1 M phosphate buffer saline solution plus 40 mM NaF; pH 7.4. Scan rate: 100 mV/s. (Inset) CV under the same conditions of Fig. 1, showing continuous and sustained electropolymerization of molecularly imprinted polyaniline boronic acid.

Fig. 1 shows the cyclic voltammogram of 40 mM 3-aminophenylboronic acid in 10× PBS along with 10 M D-fructose in the presence of 40 mM NaF at pH 7.4 at a scan rate of 100 mV/s. Phenylboronic acid (PBA) and its derivatives are known to interact rapidly and reversibly with dicarboxylic acids, α-hydroxy carboxylic acids, and diols in aqueous media.[29] The most common interaction being with 1,2- and 1,3-diols to form five- and six-membered cyclic ester, respectively. In this case, electropolymerization of D-fructose and PABA in presence of fluoride resulted in the formation of a self-doped molecularly imprinted polyaniline. Based on the type of bonding between the template and functional monomers, two kinds of molecular imprinting strategies have been established, namely, covalent and non-covalent imprinting. In this work, the non-covalent approach has been followed because it avoids tedious synthesis steps, thereby ensuring simplicity and the ease in extraction of the template. In addition, the non-covalent approach is more versatile in terms of the vast number of compounds, including biological compounds, which are capable of non-covalent interactions with functional monomers.[30,31]

Significant and sustained polymerization of the saccharide complex with 3-aminophenylboronic acid in the presence of one molar equivalent of fluoride at neutral pH was observed as shown in the inset of Fig. 1. Both fluoride and D-fructose are involved in the process that resulted in the formation of a self-doped MICP as confirmed from literature.[20] Electrical characterization in terms of I-V characteristics was performed after electropolymerization of molecularly imprinted, self-doped PABA on the aligned SWNT surface across microfabricated electrodes (see supplementary material for details of SWNTs device fabrication, SWNTs functionalization with imprinted PABA, and sensing protocols).[32] As shown in Fig. 2, the devices exhibited higher resistance after

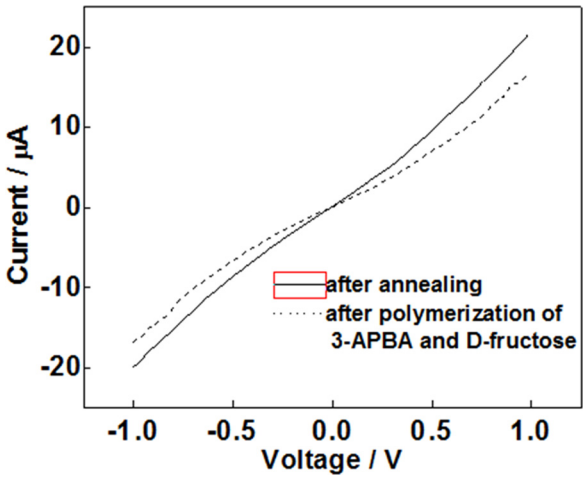

FIG. 2. Current versus voltage of dielectrophoretically aligned SWNTs network before and after electropolymerization of molecularly imprinted polyaniline boronic acid.

electropolymerization as compared to bare aligned SWNT devices. This is due to the fact that the formation of the fluoride D-fructose boronate complex leads to an increase in relative negative charge on the surface of the p-type SWNT channel, thereby resulting in an increase in the device resistance. Incubation of the imprinted SWNT based devices in buffer overnight resulted in the removal of D-fructose moieties. Thus, extraction of the template molecule led to the creation of binding sites on the polymer matrix that are complementary to D-fructose both in structure, i.e., shape, size, and positioning of functional groups. The imprinted polymer consisted of binding sites in a definite spatial arrangement that have a shape to match that of the removed template (in this case, D-fructose). The imprinted polymer coated devices were then used for detection of D-fructose and D-glucose separately. Changes in electronic properties accompanying molecular interactions between the sugar and the imprinted polymer matrix were monitored by measuring the normalized response $\Delta R\ (\%) = [(R - R_0)/R_0]$, where R is the resistance after exposure to sugar and R_0 is the initial sensor resistance of the sensor. The sensing results obtained are shown in the calibration plot (Fig. 3). Each D-glucose/D-fructose concentration was

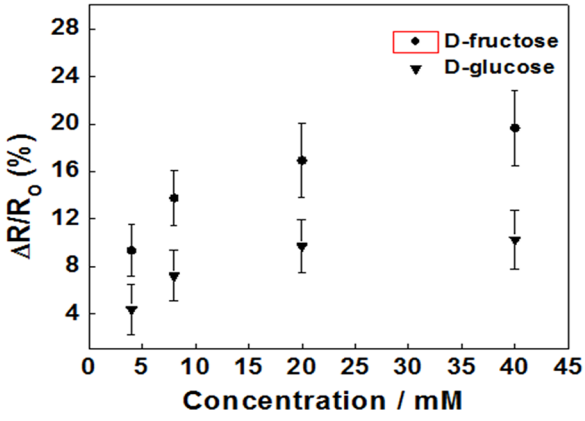

FIG. 3. Response of the D-fructose imprinted polyaniline boronic acid sensors towards D-fructose and D-glucose at pH of 7.4 in phosphate buffer saline solution.

measured on 8–9 devices to ensure reproducibility. The relative response of D-fructose and D-glucose to the imprinted film brings about a change in the net electrical charge of the SWNT based device based on predetermined selective molecular recognition and efficient electrical conductivity of the SWNTs, thereby resulting in a change of the overall conductance, which in turn is detected by the chemiresistive mode of sensor configuration.

The imprinted devices show an enhanced response towards D-fructose in comparison to D-glucose. This is due to the fact that imprinting followed by template extraction from the polymer matrix results in the creation of very specific molecular recognition sites for a given compound, its analogues, or for a single enantiomer. The three-dimensional cavities formed are complementary in both shape and chemical functionality arrangement to those of the template molecule. In this case, polymerization and subsequent template removal induces a permanent molecular memory to the imprinted polymer for the imprint species (in this case, D-fructose). This results in the selective detection and rebinding to the imprint D-fructose molecule with a high specificity.

The imprinted devices were tested against sugar concentrations from 4 mM to 40 mM. The response curves reach a plateau at concentrations beyond 40 mM and show no significant change with higher sugar concentrations. The estimated limit of detection (LOD) for fructose and glucose were found to be 2.04 mM and 3.16 mM, respectively. It was calculated based on 3SD/m equation, where m is the slope of the linear part of the calibration curve, and SD is the standard deviation of the blank measurement. It is noteworthy to mention here that for every given concentration of sugar, it was observed that the response of the imprinted devices towards D-fructose was almost double as that towards D-glucose. This enhanced response can be explained by the selective binding of D-fructose at the complementary binding sites through highly selective template molecule recognition during the sensing procedure, making it possible to detect D-fructose from a mixture of closely related compounds.

Polyaniline boronic acid–saccharide imprint, which was formed under neutral pH conditions in the presence of fluoride ion as catalyst, imparts higher selectivity towards D-fructose by creating recognition sites, which are formed by template directed formation of imprinted cavities that are of same chemical nature in terms of shape, size, and functionality as D-fructose. Thus, imprinting enables identification and classification of saccharides at the binding sites based on the selective complexation reaction between saccharides and boronic acid moieties prior to removal of template molecules formed in the MICP backbone. Apart from being a very straightforward and inexpensive technique, the MICPs developed were stable and could be used several times for repeated detection of D-fructose without significant loss of activity. The reduced dimensionality of SWNTs structures yields exceptionally high surface area and its excellent transport properties result in improved analyte recognition and signal generation. In addition to its excellent transducing properties, SWNTs lend high mechanical strength to the MICP, thus making it a very robust and durable system. Another significant benefit of using MIP based sugar sensors over non-MIP based sensing platforms is its enhanced

selectivity towards nonsimilar molecules that coexist along with D-fructose in blood and food samples. When physiological concentrations of such molecules, mainly ascorbic acid (AA), uric acid (UA), and citric acid (CA), were tested for their binding to the imprinted D-fructose devices, it yielded negligible response (results not shown). This confirms the excellent discriminating ability of the imprinted devices towards non similar molecules, which are not complementary to D-fructose in structure by offering highly specific binding sites. Thus, MIP based platforms can be used to address sensing issues where strong immunity against interfering species is desired.

In conclusion, single walled carbon nanotube based MICP devices were demonstrated towards detecting monosaccharides. This was achieved via electropolymerization that resulted in a self-doped polymer at neutral pH conditions involving the formation of a stable anionic boronic ester complex between PABA and D-fructose. These sensors exhibited selectivity in detecting D-fructose over D-glucose, since the binding sites on the polymer matrix left after template removal are complementary to D-fructose both in structure, i.e., shape and size, and positioning of functional groups. Using carbon nanotubes along with MICPs resulted in the fabrication of efficient electrochemical sensor by enhancing analyte recognition and signal generation. These sensors can be regenerated and used multiple times unlike conventional affinity based biosensors which suffer from physical and chemical stability. Thus, the versatile molecular recognition capabilities of MICPs coupled with the efficient transducing properties of SWNTs and CPs provide a strong platform for the detection of wide range of analytes such as biomolecules and drugs used in analytical technologies.

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[32]See supplementary material at http://dx.doi.org/10.1063/1.4930171 for details of SWNTs device fabrication, SWNTs functionalization with imprinted polyaniline boronic acid, and sensing protocols.