Talanta 128 (2014) 473–479

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Affinity chemiresistor sensor for sugars

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ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form
29 May 2014
Accepted 29 May 2014
Available online 6 June 2014

Keywords:
Single-walled carbon nanotubes
Non-enzymatic sensor
Sugar
Boronic acid

ABSTRACT

In this work, a non-enzymatic chemiresistive sugar sensor has been developed by combining a synthetic receptor with aligned single-walled carbon nanotubes (SWNTs) device. Briefly, boronic acid as a multivalent sugar receptor was immobilized on carbon nanotubes through amide bond formation. The interaction between three common sugars (D-glucose, D-fructose and sucrose) and boronic acid modified SWNTs device was studied. The effect of pH on the receptor–ligand binding was examined and highest response was observed at pH 9. The chemiresistive sensor exhibited specific and reproducible detection with sensitivity over the concentration range of 1–20 mM, 1–25 mM, and 1–30 mM for fructose, glucose, and sucrose, respectively. The sensor showed no interference from common electroactive compounds such as citric acid, uric acid, and ascorbic acid. Furthermore, the sensor retained 97.4% of the initial value after five regeneration cycles with an acidic buffer at pH 5, thus ensuring good reusability.

1. Introduction

Detection of sugars finds widespread applications in monitoring food quality and analysis [1] and also in clinical diagnostics governing the concentration of blood sugar [2,3]. Enzymatic sensors have been used extensively over the past few decades for detection of sugars. However, owing to the degradation of their activity with time and operational limitations such as pH, and oxygen dependency, these sensors often prove to be unsuitable for reliable and real time usage. Non-enzymatic sensors based on artificial receptor systems have been developed to address these issues.

Boronic acid compounds, such as phenylboronic acid (PBA), form one such field of interest which has been explored widely due to their sugar binding ability. The complexation of saccharides (as well as alkyl and aromatic diols) with boronic acid moieties produces reversible covalent complexes in nonaqueous or alkaline aqueous solution thus, leading to possibilities of numerous approaches to detect sugars using absorption spectroscopy [4–10], fluorescence spectroscopy [4,8,11–14], electrochemistry [4,15–20], quartz crystal microbalance/piezoresistive microcantilever [21,22], surface plasmon resonance [23], holography [24] and field-effect transistors [25–27].

Herein, 3-amino phenylboronic acid modified SWNTs device has been fabricated and its interaction with three common sugars namely D-glucose, D-fructose and sucrose has been investigated. It is well known that phenylboronic acid and its derivatives are usually used as soluble reagents for the detection of sugars which may reduce their applications in real time and high throughput measurements. In order to overcome this issue and to develop an alternative platform to the enzyme-based biosensors, the phenylboronic acid has been immobilized on the surface of the SWNTs which act as chemiresistor transducer.

2. Experimental

2.1. Materials

Single-walled carbon nanotubes with high carboxylated functionality, sold under the trade name of P3-SWNT, were purchased from Carbon Solutions, Inc. (Riverside, CA, USA). Dimethyl formamide (DMF), 3-aminophenylboronic acid hydrochloride salt 98% (APBA), mercaptohexanol (MCH), 1-pyrenebutanoic acid succinimidyl ester (PASE) were obtained from Sigma Aldrich (St. Louis, Mo, USA). Distilled water purified through a Milli-Q

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http://dx.doi.org/10.1016/j.talanta.2014.05.055
0039-9140/

plus (Millipore Inc.) ultrapure water system was used to prepare solutions.

2.2. Sensor fabrication

Gold microelectrodes were fabricated using standard photolithography and lift-off process in the Center for Nanoscale Science and Engineering, University of California, Riverside, cleanroom. In brief, 300 nm of SiO_2 was initially deposited on the highly doped p-type semiconductor Si wafer using chemical vapor deposition (CVD). Electrodes with 200 μm by 200 μm cross sectional area and 3 μm gap were defined by photolithography followed by e-beam evaporation of 20 nm thick Cr adhesion layer and 180 nm thick Au layer. As the final step, lift-off was performed to define the electrodes. The electrode pattern was cleaned with piranha solution (70 vol% H_2SO_4; 30 vol% H_2O_2) for 2 min, rinsed with water, dried under a stream of nitrogen and then annealed at 120 °C for at least 4 h to make the SiO_2 surface hydrophilic.

A uniform suspension of SWNTs (0.1 mg/ml) in DMF was prepared by ultrasonication (power level 9, Model 230D, Crest Ultrasonics, Trenton, NJ, USA) for 90 min followed by centrifugation (15,000 × g using a Beckman J2-HS centrifuge) for 90 min. The suspended SWNTs were aligned across a pair of gold microelectrodes using AC dielectrophoresis (DEP). A 0.1 μL drop of the dispersed SWNTs was dispensed in the gap between the gold microelectrodes pair and subjected to a 4 MHz (amplitude 3 V p–p) AC field across the electrodes for a few seconds using a function generator. The aligned SWNT devices were annealed at 300 °C for an hour under a continuous flow of nitrogen gas mixed with 5% hydrogen in order to reduce the contact resistance and remove any residues. Aligned SWNTs were incubated for 1 h at room temperature in a 6 mM solution of PASE, washed extensively with DMF to remove excess reagent followed by overnight incubation with a 100 mM of 3-aminophenyl boronic acid (APBA) solution in phosphate buffer (10 mM, pH 8) and rinsing with water and PB (pH 7.2, 10 mM phosphate buffer) to remove residual and physisorbed APBA. In order to improve the selectivity, the sensor device was incubated with 2 mM MCH in PB for 30 min followed by washing with PB.

2.3. Device characterization and sensing

Surface characterization to verify SWNT deposition and subsequent chemical functionalization of the sensor surface was performed using *I–V* and X-ray photoelectron spectroscopy (XPS) after each functionalization step. *I–V* characterization of the fabricated device was performed by measuring the source–drain current (I) as a function of source–drain voltage (V) from −0.5 V to +0.5 V using a HP 4155A (Agilent) semiconductor parameter analyzer. XPS characterization was performed using a Kratos AXIS ULTRA^{DLD} XPS system equipped with an Al Kα monochromated X-ray source. For the purpose of XPS characterization, samples were prepared on 1.0 cm × 1.5 cm Si/SiO_2 chips without the patterned electrodes. For these samples, SWNTs were deposited via drop-casting without A/C dielectrophoresis alignment; however, subsequent sample preparation followed the aforementioned sensor fabrication processes.

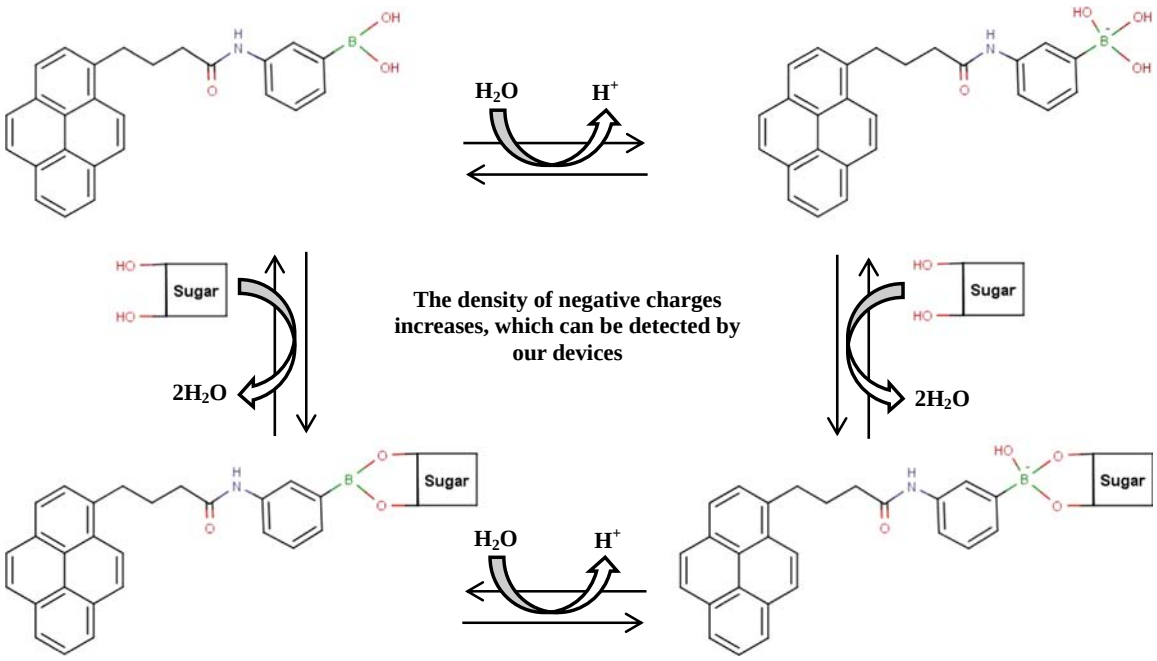
The sensing protocol consisted of monitoring the initial resistance (R_0) of the sensor fabricated by measuring the source–drain current (I) as a function of source–drain voltage (V) from −0.5 V to +0.5 V using a HP 4155A (Agilent) semiconductor parameter analyzer. The inverse of the slope of the *I–V* curve in the voltage window −0.1 V to +0.1 V was obtained which formed the base resistance of the device. The sensor was then incubated for 10 min at room temperature with different concentrations of sugar in buffer and the change in resistance was recorded.

3. Results and discussions

Carbon nanotubes (CNTs) are particularly interesting as an active material for sensors and biosensors due to their large surface areas and excellent electrical and physical properties. They have been successfully configured into field-effect transistor (FET)/chemiresistor devices, which can be used for detection of wide range of chemical and biological molecules [28–30]. The most common method for covalent biofunctionalization of SWNTs with biomolecules involves reactions with carboxylic acid residues. These carboxylic acid groups are usually introduced by oxidation using strong acids, and they occur predominantly at the more reactive ends or sidewalls of SWNTs. However, this technique may have effect on the electrical properties of the devices [31]. In this work, the non-covalent technique was employed as a method for the functionalization of the SWNTs surface. The principle is illustrated in Scheme 1. A bifunctional linker, PASE, was used to modify the SWNTs via π–π stacking interactions between the pyrene aromatic moiety and the CNTs sidewalls and then 3-aminophenyl boronic acid (APBA) was linked covalently by nucleophilic reaction between N-hydroxysuccinimide of PASE and the amine group of APBA.

XPS surface characterization results verified that SWNTs were deposited onto the Si/SiO_2 surface as shown by greater than 10-fold increase in the atomic concentration of carbon after SWNT deposition onto Si/SiO_2 (Table 1). Further deconvolution of the C 1s carbon peak (Fig. 1) showed the predominant contribution of sp^2 carbon peak at 284 eV compared to the much lower contribution from the sp^3 carbon peak (284.8 eV) from adventitious carbon, which is indicative of the presence of SWNTs which is mostly comprised of sp^2 carbons [32]. The lower intensity O–C=O peak at 288.5 eV corroborates with the specifications from the manufacturer of the SWNTs, which stated that the SWNTs contain 1–3% carboxylic groups [33]. Next, the addition of PASE onto SWNTs was verified by determining the atomic concentration of nitrogen which, at this step, is specific to PASE's chemical composition. Results showed that the atomic concentration of nitrogen increased by more than a 2-fold from 0.31% to 0.84% after the addition of PASE (Table 1) indicating the functionalization of PASE onto SWNTs. Subsequently, in order to verify that APBA was present at the sensor surface, the presence of boron from APBA was determined by comparing boron's atomic concentration before (undetectable) and after APBA functionalization (0.35%) as shown in Table 1 and Fig. 2-A and -B. Finally, to verify that APBA was covalently conjugated onto PASE by displacement of the succinate group, the peak shift of nitrogen, N 1s, peak was examined. Per the proposed structure of PASE conjugated with APBA (i.e. {3-[4-(pyren-1-yl)butanamido]phenyl}boronic acid) shown in Scheme 3, a decrease in binding energy of the N 1s core electrons was expected after conjugation due to the displacement of electron-withdrawing oxygen groups on nitrogen as the N-succinimidyl ester group on PASE is replaced by the amine from APBA. Thus, a shift in binding energy was observed for the N 1s peak from a higher energy at 399.84 eV to a lower energy at 399.42 eV (Fig. 3) for before and after APBA conjugation to PASE, respectively, indicating formation of the PASE–APBA conjugates.

Electrical characterization of the sugar sensors was performed by measuring the current across the source and the drain. Fig. 4 depicts *I–V* curves of the SWNTs devices after each modification step. Significant differences in the *I–V* curves were observed during stepwise modification of the SWNTs. The resistance for the bare SWNTs was estimated to be 35.3 kΩ and increased to 59.1 kΩ after PASE coating; this increase can be attributed to electron donation from pyrene moiety to SWNTs channel [34]. Subsequent immobilization of the APBA on the PASE-modified SWNTs devices generated more negative charge because at pH



Scheme 1. Schematic of the SWNTs-based chemiresistive sugar sensor.

Table 1
Relative atomic concentrations from XPS analysis.

Sample description	Atomic concentration (%)				
	Si	O	C	N	B
SiO_2 only	33.35	64.58	2.23	N/A	N/A
SiO_2 w/ SWNTs	24.56	51.75	23.38	0.31	N/A
SiO_2 w/ SWNTs/PASE	18.45	40.22	40.49	0.84	0
SiO_2 w/ SWNTs/PASE/APBA	14.87	31.54	51.38	1.86	0.35

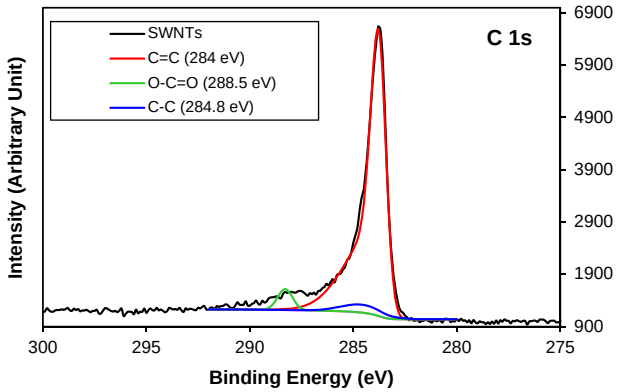


Fig. 1. Deconvoluted C 1s high resolution XPS spectra for biosensor surface after SWNT deposition.

7.4 the APBA (pKa 8.9) existed in equilibrium between uncharged and charged forms [35]. This was reflected by the increase of the SWNTs device channel resistance to 98.5 kΩ. A small increase in the resistance was observed after blocking the remaining NHS groups of the PASE with ethanolamine solution. The obtained data shows that the stepwise modification of the SWNTs devices was achieved successfully.

It has been reported that PBA and its derivatives interact rapidly and reversibly with dicarboxylic acids, α-hydroxy carboxylic acids, and diols in aqueous media [9]. The most common interaction is with 1, 2- and 1, 3-diols to form five- and six-membered cyclic ester, respectively.

As shown in Scheme 2, PBA exists in equilibrium between the trigonal neutral form with an sp^2 boron atom (electron-receptor) and the tetrahedral boronate anionic form with an sp^3 boron atom (electron-donor) forms in aqueous solution [16]. Both forms can react with cis-diol and the resulting esters exist in equilibrium between the neutral and anionic forms. The binding behavior of PBA with cis-diol-containing compounds is known to be highly pH-dependent. Therefore, it was necessary to investigate the effect of pH on the response of our proposed sugar sensors. The influence of pH on the response of the SWNTs devices was investigated in 10 mM PB at pH 7, 8, and 9 using D-fructose as a prototype of sugar because it is well know that phenylboronic acid has a good affinity towards D-fructose [4].

Fig. 5 shows the normalized response ΔR (%) [$=(R-R_0)/R_0$, where R is the resistance after exposure to sugar and R_0 is the initial sensor resistance] of the sensor at different pH in the presence of 30 mM D-fructose. As expected, the normalized response increased monotonically with increasing solution pH. For example, the resulting response of the SWNTs device at pH 7.2, 8, and 9 in the presence of 30 mM D-fructose was determined to be 12.8, 24.1, and 44.1%, respectively. In order to confirm that the observed increase in the normalized response was due to the binding between the PBA receptor and the D-fructose, an additional negative control (NC) using APBA-free SWNTs device (i.e., the procedure shown in the Scheme 1 was followed except that the immobilization of the APBA was omitted) was also carried out under the same conditions as reported previously. In this case, as depicted in Fig. 5, there was no significant increase in the normalized response ΔR (%) (Only 1.8%, 2.3%, and 3.1% increase were observed for pH 7.2, 8, and 9, respectively). This supports the fact that the changes were induced by the specific binding between the immobilized APBA receptors and the D-fructose in solution and not due to the nonspecific adsorption of D-fructose on the carbon nanotubes surfaces or/and the gold electrode (source and drain).

Based on the pKa value of PBA (8.9), at physiological pH conditions most of the PBA moieties exist in the trigonal neutral form,

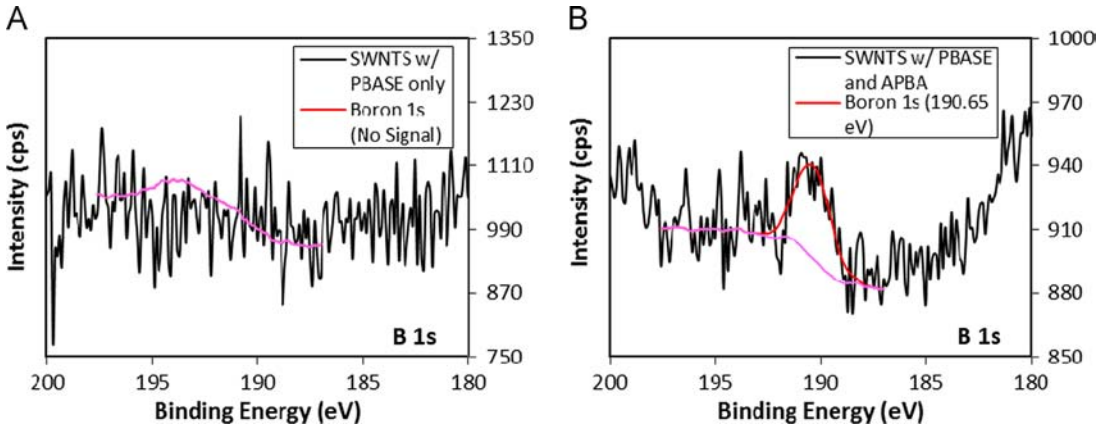
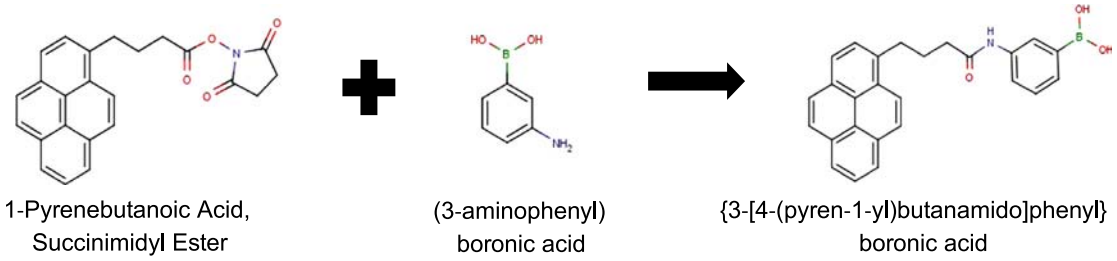
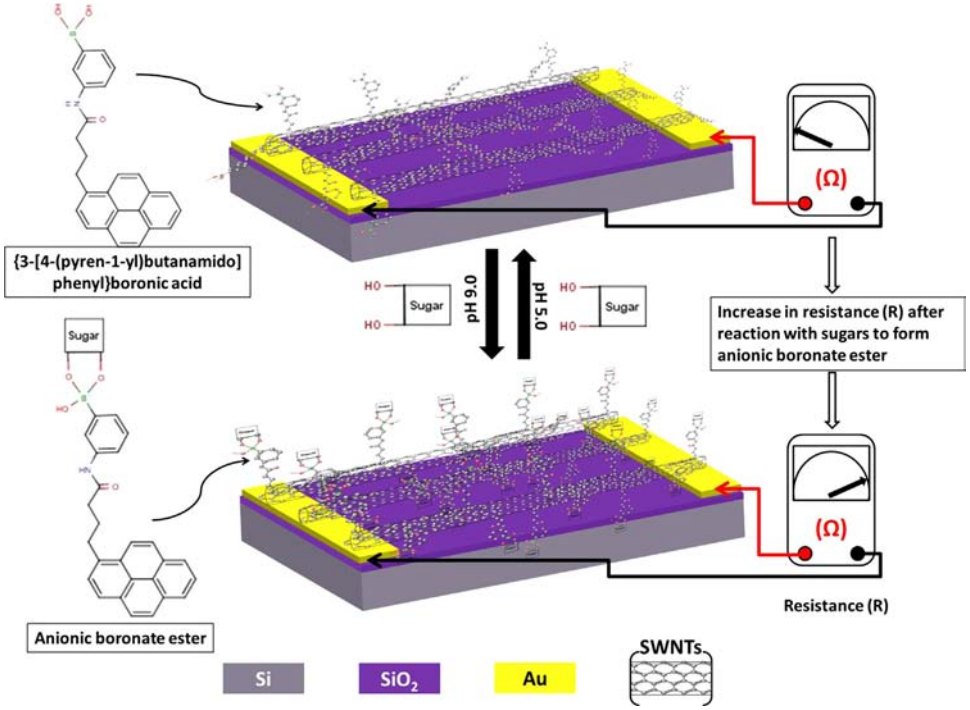


Fig. 2. B 1s XPS spectra for SWNTs with PASE before (A) and after (B) reaction with APBA.



Scheme 2. Schematic of the binding behavior of PBA moiety with cis-diol containing compounds.



Scheme 3. Schematic of the reaction of 1-pyrenebutanoic acid, succinimidyl ester (PASE) and (3-aminophenyl) boronic acid (APBA) to form the PASE–APBA complex ({3-[4-(pyren-1-yl)butanamido]phenyl} boronic acid).

which partially bind to diol groups of fructose due to their high susceptibility to hydrolysis as reported [36]. Therefore, the proposed SWNTs sensors had a poor fructose-sensitivity under physiological pH conditions. With increase in the solution pH, however, more PBA groups will be converted from the trigonal neutral to the tetrahedral anionic form, which facilitates the formation of a stable cyclic boronate ester. As a result, higher binding affinity was observed at pH 9, which can be used as an optimal pH for the subsequent work. This result is in good agreement with the previously reported data, where the authors have shown that for their PBA-fructose system, the optimal pH was 9 [21]. However, it was reported by other groups that the optimal pH for PBA-based sensors were in the range of 10–12 [10,19,23]. Their results are consistent with the theoretical

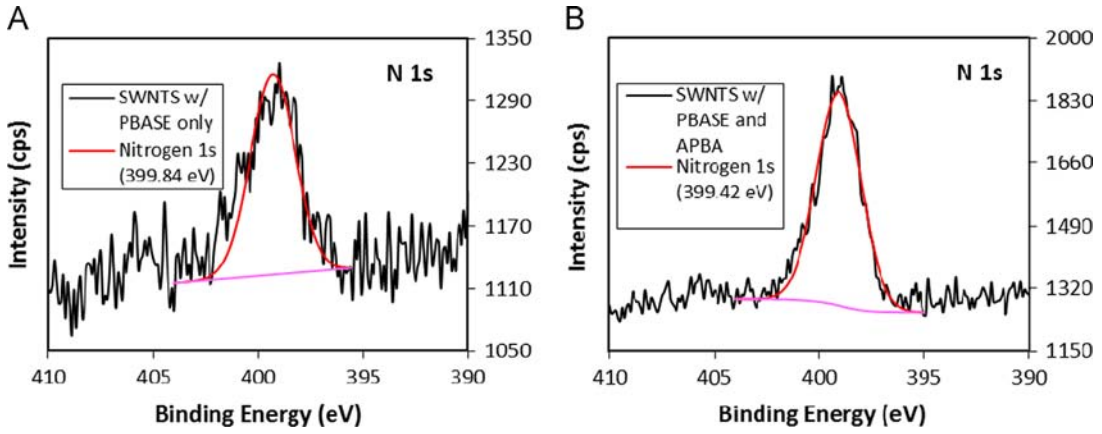


Fig. 3. N 1s XPS spectra for SWNTs with PASE before (A) and after (B) reaction with APBA.

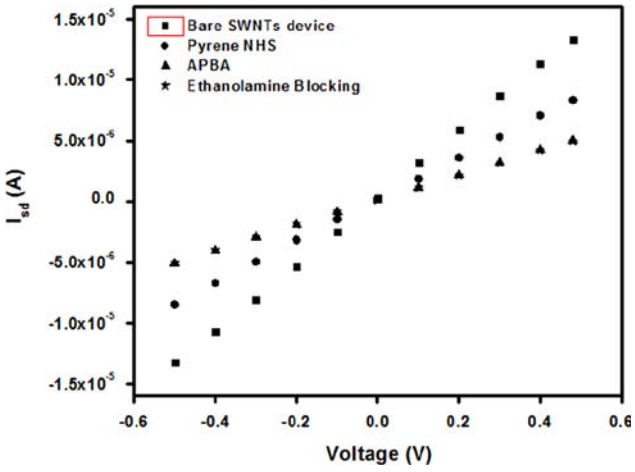


Fig. 4. (■) I_{DS} vs. V_{DS} for the bare SWNTs network, (●) after functionalization with PASE, (▲) immobilization of APBA and (∗) after blocking NHS groups of the pyrene with ethanolamine solution.

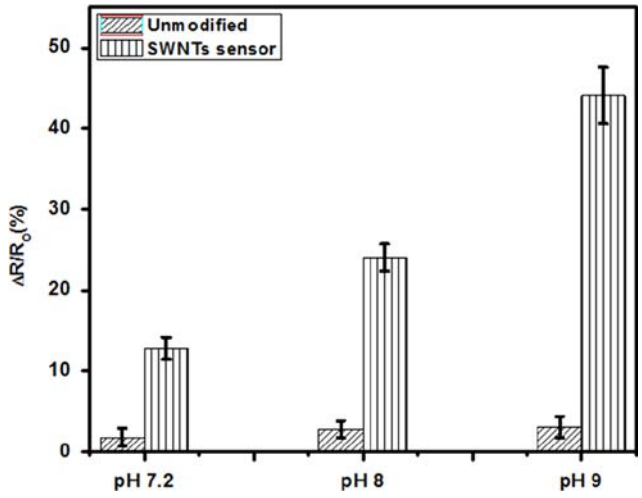


Fig. 5. Response of the APBA coated SWNT sensors and APBA-free SWNTs towards 30 mM of D-fructose at different pH.

expectation of the optimal pH to be between the pKa of the PBA (8.9) and the pKa of fructose (12.3) [37]. In our case, the solution pH above 9 was not tested since the high pH value affected the stability of the device itself (data not shown).

In order to demonstrate the practical utility of the proposed sensors, the PBA-modified SWNTs sensor was exposed to different concentrations of D-fructose (Fig. 6) in 10 mM PB buffer solution at pH 9. As expected, the normalized response increased in a concentration-dependent manner upon addition of D-fructose up to 30 mM and then tended to level off. It is well known that the oxygen–boron–oxygen (O–B–O) bond angle of boronic acid can be contracted upon association with a carbohydrate, resulting in an increase of the acidity of the boron center and consequently a decrease of the apparent pKa by 2–4 pH units [38]. This indicates that at a fixed pH, such as pH 9 in the current study, an increase of the fructose concentration causes a shift of the equilibrium towards a further stable charged phenylboronate ester form because of the decrease of the pKa value, thus giving rise to increasing the density of the anionic phenylboranate ester form which in turn results in an additional ΔR (%) increase.

In order to examine the general applicability of our sensor, it was exposed to aqueous solution containing various carbohydrates. These carbohydrates included glucose and sucrose, which are monosaccharide and disaccharide, respectively. The glucose was selected for this study since it is present in foods and biological fluid and is supposed to be the main target for medical diagnostics, while sucrose is a disaccharide composed of two monosaccharide glucose and fructose. As shown in Fig. 6, the sensitivity of the sensor was in the order of fructose > glucose > sucrose. This order of sensitivity is attributed to the structures of the sugars. Moreover, it is well known that the PBA has a strong preference for binding with the hydroxyl groups of sugar in their furanose forms. The high selectivity of the sensor towards fructose could be explained by its predominant furanose form (25%) compared to glucose (0.14%) [39]. The low selectivity for sucrose is attributed to the lack of the cis-diol moiety [40]. The change in the normalized response was linear for fructose concentration from 1 mM to 20 mM and had a regression equation of ΔR (%)=20 C+0.024, with a correlation coefficient of 0.998. The fructose response curve reached a plateau at a concentration above 20 mM. This behavior can be explained by the progressive saturation of the exposed binding site of the immobilized PBA. In the case of glucose, a linear relationship was found from 1 mM to 25 mM, covering the normal physiological range of blood glucose concentration in humans (healthy and diabetic persons). The linear regression was ΔR (%)=9.6 C+0.031, with a correlation coefficient of 0.993. Beyond 25 mM, the sensor response was saturated and showed no significant change with increasing glucose concentration. The SWNTs sensors exhibited a linear relationship between the normalized response and the sucrose concentration over the range of 130 mM and the linear regression equation was ΔR (%)=4.8 C+0.038, with a correlation coefficient of 0.991. The limit of detection (LOD) for fructose, glucose, and sucrose were estimated to be 0.6, 1.2, and 2.5 mM, respectively. The limit of detection (LOD) was calculated using the equation, LOD=3SD/m, where m is the slope of the linear

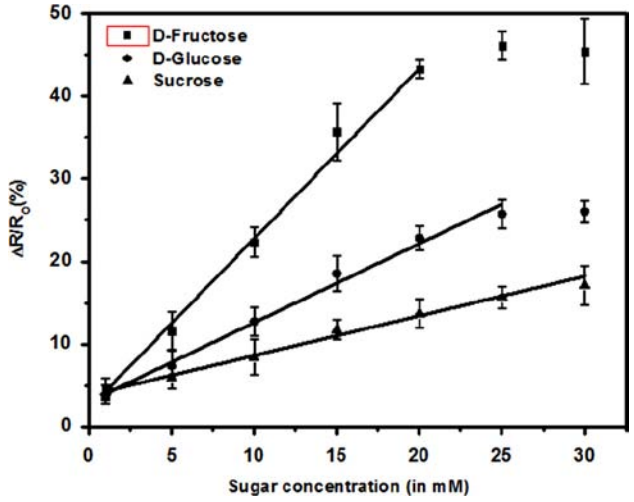


Fig. 6. Calibration curve showing the response of APBA coated SWNT sensors towards sucrose, D-glucose and D-fructose at pH 9.

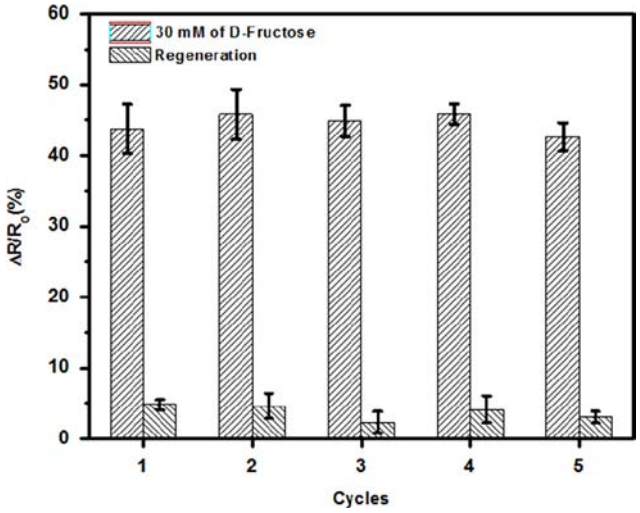


Fig. 7. Response of the APBA coated SWNT sensors towards 30 mM D-fructose at pH 9 and subsequent regeneration by washing with buffer at pH 5.

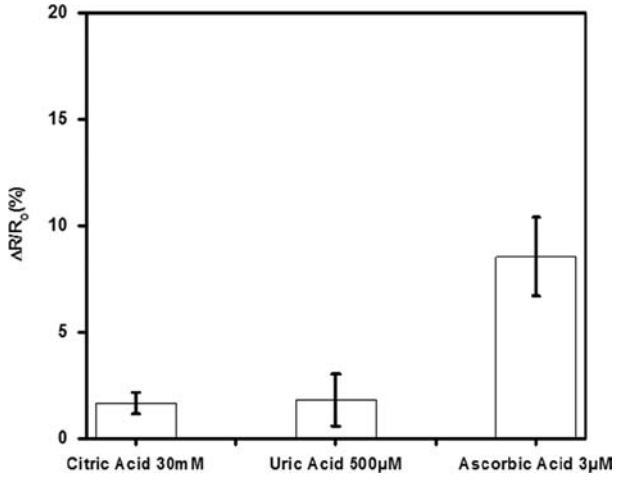


Fig. 8. Response of the APBA coated SWNT sensors towards 30 mM citric acid, 500 μM uric acid and 3 μM ascorbic acid after blocking with MCH.

part of the calibration curve, and SD is the standard deviation of the blank measurement. The selectivity of the APBA-functionalized SWNTs sensor was in good agreement with previous observations reported in the literature [24,41]. The two-fold higher sensitivity and lower limit of detection for fructose compared to glucose is not an issue for the sensor when used in clinical diagnosis for blood glucose measurement as the maximum fructose concentration in diabetic patients of 12.0 ± 3.8 μM [42] is well below the fructose LOD of the sensor (60 μM).

Regeneration and reusability are important aspects that govern the fabrication of sensing devices since simple regeneration procedure enhances the potential applicability and increases the cost-effectiveness of these sensors. It is well known that the capture/release process of cis-diol compounds with PBA can be manipulated easily through switching the pH solution [43]. This ensures enhanced sensor life-time and reusability without the need to functionalize the SWNTs surface and immobilize the PBA before each assay. In this context, after each assay the device was incubated in stripping acidic buffer (10 mM PB, pH 5) for 10 min to regenerate the sugar-free surfaces followed by three times washing with water and then dried with nitrogen. Fig. 7 highlights the changes in normalized response ΔR (%) as a function of the number of repeated cycles corresponding to sequential challenging the SWNTs sensors with 30 mM fructose and regenerating with acidic buffer. Regardless of the number of cycles, the normalized response ΔR (%) decreased significantly after treating the device with acidic buffer for 10 min, indicating that the D-fructose was dissociated from the immobilized PBA. This is due to the low stability of the PBA and its derivative in acidic medium. The as-renewed SWNTs sensor could retain 97.4% of the initial value after five assay runs, indicating that the proposed sensor can be used repeatedly for detecting fructose or other sugars. The relative standard deviation (RSD) values for the binding/release of D-fructose for 7 electrodes in parallel were found to be between 1.9% and 3.5%. These results suggest that repeated regeneration/reuse cycle can be carried out at a single electrode for multiple samples with high throughput.

For application in clinical diagnosis and food industry, the selectivity is a major concern. The challenge is to discriminate or to lower the interference signals and get a precise value of sugar level response in real samples. Electroactive species such as uric acid, ascorbic acid and citric acid are known to co-exist along with fructose in food samples. To investigate the effect of their interference, response of the APBA coated SWNTs sensors were measured in physiological concentration of uric acid (UA), ascorbic acid (AA) and citric acid (CA) under the same experimental conditions specified above. As can be observed in Fig. 8, none of the analyzed interfering compounds, except ascorbic acid, presented a significant normalized response ΔR (%) to APBA coated SWNTs sensors under the working conditions. The normalized response was 1.6% and 1.9% after adding 30 mM citric acid and 500 μM uric acid, respectively. This is due to the fact that no diol group exists in these two interfering species. However, ascorbic acid induced a significant increase in the normalized response (data not shown) which could be due to the binding ability of ascorbic acid with PBA through its planar diol and the fouling effect caused by the adsorption of the oxidized product of the ascorbic acidic (AA) on the gold electrode surface [44,45].In order to eliminate the interference effects of AA, the gold electrode was blocked with mercaptohexanol for 30 min in PB. As a result, the selectivity of the devices improved towards ascorbic acid and only 8.5% increase in the normalized response was observed towards 3 μM AA. L-lactic acid is another boronic acid binding α-hydroxy acid that is present along with glucose in blood. In a healthy adult, typical resting L-lactate concentrations are in the range of 0.36–0.75 mM while the average D-glucose concentration in the blood is ~5 mM. Hence, lactic acid at normal physiological concentrations is unlikely to overwhelm of the response of the sensor towards

D-glucose in blood. This has been confirmed from report published by Sartain et al. [46] wherein the response of boronic acid based holographic sensor to average concentration of D-glucose in blood sample was almost seven times more than its response to physiological concentration of L-lactate.

The obtained sensing results were compared with reports from literature which demonstrate boronic acid-modified carbon-based sensors for the detection of D-glucose [25,27]. While ours is a more detailed analysis in terms of (a) response to three main monosaccharides present in blood, (b) pH dependent sensing response thus establishing the fact that sensing response is maximum at a pH of 9, this approach is also advantageous in terms of (c) precise control over sensor construction as it employs dielectrophoresis for SWNTs alignment across electrodes. Furthermore, these developed sensors exhibit a wider linear range of detection for glucose from 1 mM to 25 mM, thus covering the normal physiological range of blood glucose concentration in humans, both healthy and diabetic patients.

4. Conclusions

In summary, we developed a simple chemiresistive sensing platform for the detection and quantification of sugars in alkaline solution using phenylboronic acid-modified carbon nanotubes. The SWNTs based sensor exhibited a strong preference for binding to fructose over other sugars, which is consistent with other relative systems. Moreover, it displayed a rapid response time, outstanding sensitivity, good selectivity, and cost-effective reusability. The ability to detect fructose and glucose in real/physiologically relevant samples is yet to be demonstrated, which can be done either after adjusting the sample pH to the working or operating the sensor at physiological pH.

Acknowledgments

Funding was provided by the National Institute of Food and Agriculture USDA award 2014-67021-21589 and the National Science Foundation awards 1307671 and Major Research Instrumentation Program, Grant DMR-095876.

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