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A saccharides sensor developed by symmetrical optical waveguide-based surface plasmon resonance

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We proposed a new saccharides sensor developed by symmetrical optical waveguide (SOW)-based surface plasmon resonance (SPR). This unique MgF_2/Au/MgF_2/Analyte film structure results in longer surface plasmon wave (SPW) propagation lengths and depths, leading to an increment of resolution. In this paper, we managed to decorate the dielectric interface (MgF_2 layer) by depositing a thin polydopamine film as surface-adherent that provides a platform for secondary reactions with the probe molecule. 3-Aminophenylboronic acid (3-PBA) is chosen to be the saccharides sense probe molecule in the present work. The aqueous humor of Diabetes and Cataract patient whose blood glucose level is normal are analyzed and the results demonstrated that this sensor shows great potential in monitoring the blood sugar and can be adapted in the field of biological monitoring in the future.

Keywords: Saccharides sensor; surface plasmon resonance; symmetrical optical waveguide; 3-Aminophenylboronic acid; dopamine.

1. Introduction

Saccharides are widespread in nature and play an essential role in nearly all the biological processes, such as cell-to-cell interactions, biological recognition,[1] and also in nutrition, metabolism, cell structure formation and immunological protection.[2–4] Therefore, the detection of sugar at high levels of sensitivity and selectivity is very important in medicine, the food industry and biochemical science.[5] In this respect, a number of detection methods such as capillary electrophoresis,[6] the enzymatic method,[7] chemiluminescence,[8] chromatography,[9–11] and the electrochemical method[12] have been proposed and all these methods have made a tremendous development in recent years. However, these methods are not sufficient for precise sugar detection.[5] There are some other sugar-sensitive systems based on optical methods such as UV-visible absorption, fluorescence,[13] and surface plasmon resonance (SPR).

Since the first demonstration of SPR for the study of processes at the surfaces of metals and sensing of gases in the early 1980s, SPR sensors have made vast advances in both development of the technology and its applications, and have become a central tool for characterizing and quantifying bio-molecular interactions.[14] As a label-free detection method, it has attracted increasing research interests due to its simple sample preparation, more cost effectiveness and well active conservation of biomedical samples in comparison with labeling strategies.[15] Even though SPR technology has made remarkable progress, further improvements in terms of the refractive index resolution SPR sensing performance are highly desired: Bardin *et al.* proposed an improved algorithm for SPR data processing[16]; Wang *et al.* used active electronic noise canceling to suppress intensity noise of the light source.[17] We had proposed a new symmetrical optical waveguide (SOW) based SPR-supporting bio-sensing system, which has a sandwich type 3-layer (MgF_2-Au-MgF_2) film architecture. A refractive index resolution of 8.1×10^{-8} RIU in fluid protocol and 3.5×10^{-7} RIU in atmosphere protocol is acquired by a simple SPR imaging system.[18]

Compared to other detection methods, SPR sensors have shown a great potential, and allowing real-time analysis of bio-specific interactions without the use of labeled molecules.[19] Consequently, it was essential to seek a particular material to interact with saccharides specifically. It has been reported that the phenylboronic acid (PBA) and its derivatives are excellent recognition elements for the construction of electrochemical and optical glucose sensors. PBA and its derivatives have a boronic acid group that has been known to form covalently bonded complexes with the 1, 2- or 1, 3-diol of sugars.[5] Their formation is fast and reversible in aqueous media,[20] and they are small and flexible molecules, can easily be incorporated as recognition motifs into larger structures, without changing the physical properties dramatically.[21] It makes the PBA derivatives represent useful compounds for developing an analytical method for saccharides.

In this manuscript, we proposed a saccharide sensor developed by SOW-based SPR. We chose 3-Aminophenylboronic acid (3-PBA) as our probe molecule. The probe must be modified on the surface which is difficult for the MgF_2 layer. So we managed to form a thin surface-adherent polydopamine film on the MgF_2 layer firstly, and then, we modified the 3-Aminophenylboronic acid molecule onto this polydopamine-coated film and form a 3-PBA film which interacted with saccharides in our work. This modification method does not take many steps or very long time to accomplish, which is much easier than any other methods which have been reported.

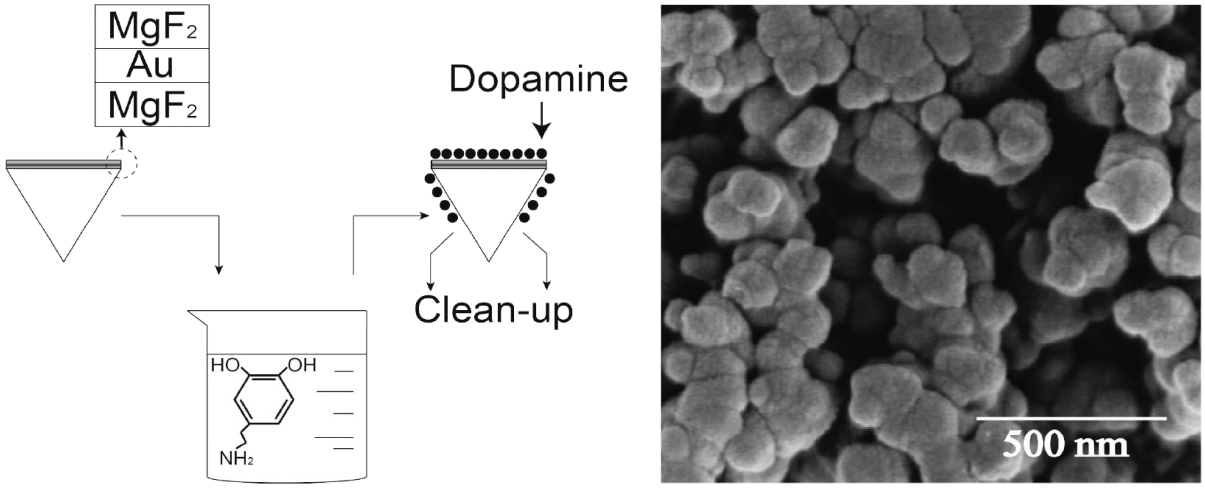
2. Materials and Methods

2.1. *Chemicals and reagents*

Dopamine Hydrochloride was obtained from Aladdin Chemistry Co. Ltd (PRC). 3-Aminophenylboronic acid Hemisulfate, 98+% was purchased from Alfa Aesar-Johnson Matthey (USA). Sucrose (GR) was bought from Guangfu fine chemical industry research institute in Tianjin (PRC). Deionized water was used for the preparation of the sample and buffer solution.

2.2. *Sensor chips*

Polished and cleaned SF4 glass equilateral prism was first deposited the MgF_2 (magnesium fluoride) layer about 505 nm by vacuum evaporation coating 39 nm of gold was deposited on the surface of MgF_2 layer by vacuum magnetron sputtering coating, and another MgF_2 layer about 645 nm was coated after that.[18] The thickness of two MgF_2 layers were measured by Ellipsometry (M-2000 UI, USA, J. A.



(a) (b)

Fig. 1. (a) Structure of our SPR sensor chip and process of dopamine modification. (b) The surface morphology of polydopamine coating.

woollam Co. Inc.), and the thicknesses of gold was measured with X-ray diffraction (XRD).

Dopamine is a small-molecule compound and has been identified to have strong surface-adherent capability by self-polymerization. It provides a versatile platform for secondary reactions through amine- or thiol-catechol adduct formation.[22,23] The coated prism was immersed into a dilute aqueous solution of dopamine, buffered to a pH typical of marine environments (2 mg of dopamine per milliliter of 10 mM tris, pH 8.5), resulting in a thin adherent polymer film by spontaneous deposition. The thickness of the dopamine layer reached nearly 15 nm after 3h immersion at a dark environment.[22] Figure 1(a) shows the demonstration of the SPR sensor layer structure and the process of dopamine modification. The surface morphology of polydopamine film is shown in Fig. 1(b), and the surfacing morphology was studied using a SEM (Hitachi S-4800). Afterward, we managed to modify 3-PBA molecules onto the dopamine polymer film by soaking into a 3-PBA dilute aqueous solution (2 mg of 3-PBA per milliliter of PBS buffer pH7.4). Four different sensor chips were tested to find the most appropriate time, which were separately soaked into the PBA solution for 2–8 h (interval 2 h). After the modification process, the optical surface of the prism must be cleaned with a solution consisting of acetone and ethanol in a 1:1 ratio, then rinsed with deionized water and dried with nitrogen.[18]

2.3. *SPR experimental setup*

In this work, our group had designed a miniature and portable SPR image system. This system was based on the traditional Kretschmann configuration,[24] the mechanical structure is shown in Fig. 2. In our system, light emerged from a light emitting diode (LED) (CWL = 632.8 nm, FWHM = 28 nm) with electric power of 3 W, traversed a 40× objective to guarantee the light well-distributed, and an aperture about $\Phi 300\,\mu$m to transform to a point light source. After that, the light passed through a collimating system which contained a spike filter (CWL = 632.8 nm, FWHM = 3 nm) and a Plano-convex lens ($f = 25$ mm) became parallel light, with a center wavelength of 633 nm. This light beam would be polarized after a polarizer, focused into a line by passing through a cylindrical lens ($f = 25$ mm), and incident on the surface of the prism. The emergent light was received by a charge coupled device (CCD, TUCSEN) camera, and saved the images for further data analysis. According to the distance between the prism and the surface of CCD detector, and the spacing between two pixels, we calculated that the linear relationship between the angular and pixel in this work is approximately 3.2×10^{-3} deg/pixel. A flow cell made of Plexiglas which is shown in Fig. 2, adhered on the surface of the prism. We used an infusion syringe pump (KDS 200, USA, KD Scientific Co. Inc.) to inject the samples.

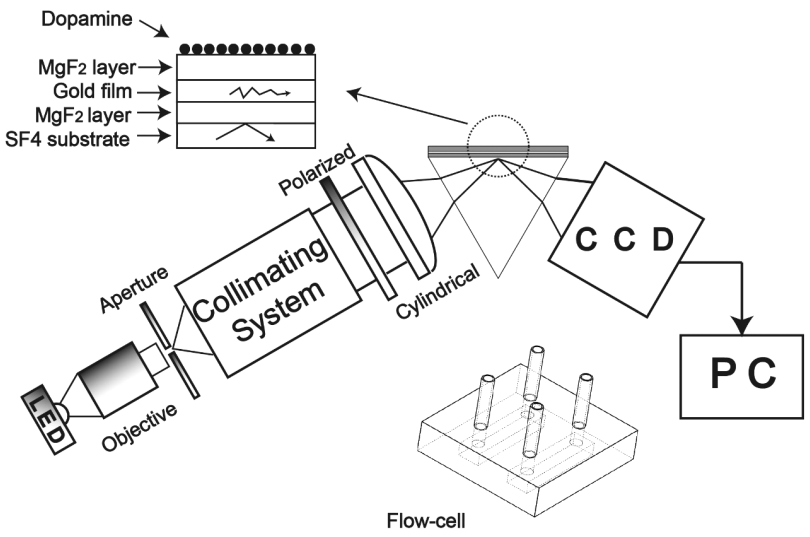


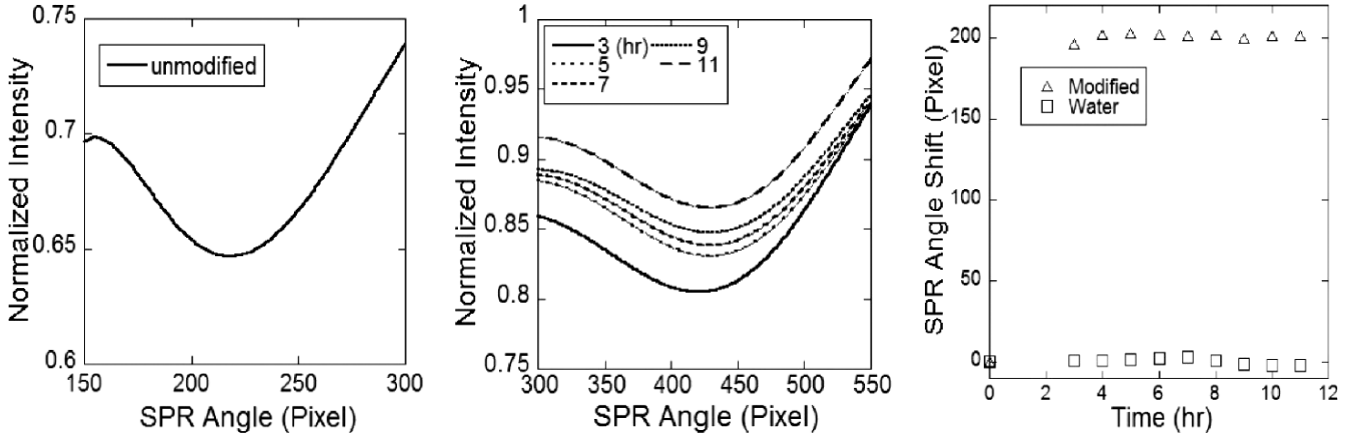
Fig. 2. Schematic of our SPR imaging system and flow cell.

3. Results and Discussions

In the surface-optoelectronic study of molecular interaction using SPR, the electron excitation phenomena on the metal surface induced by incident light can be measured as a SPR angle.[5] In this particular incident angle, the emergent light intensity would weaken because some of the incident light intensity would be absorbed by the excitation. This angle is due to the refractive index of the samples. Therefore, as the samples flowed upon the surface of sensor chips, this intensity change of the reflected light was then detected to demonstrate the refractive index change of analyte which directly correlated the effects of the biochemical reactions on the sensor surface.[18]

3.1. *The modification process*

The resonance angle shifts with the change of refractive index during the surface modification process. The SPR curve of water is first recorded as an initial reference. Figure 3 illustrated this SPR angle shift. As shown in Fig. 3(a), before the process began, the resonance location was at pixel No. 218, and the full width at half maximum (FWHM) was just about 0.256°. In Fig. 3(b), after dopamine molecule immobilized on the surface, it resulted in a significant change of SPR angle of 0.6239°, and the FWHM increased to 0.480°, while the PBA combining afterward did not bring significant angle shift which was just 0.6508° after 11 h. Meanwhile, the contrast ratio (the intensity ratio between the



(a) (b) (c)

Fig. 3. The influence of modification. (a) SPR curves and resonance angles of water before the modification. (b) SPR curves and resonance angles of water in different modification time. (c) SPR angle shift comparison of two prisms according to time.

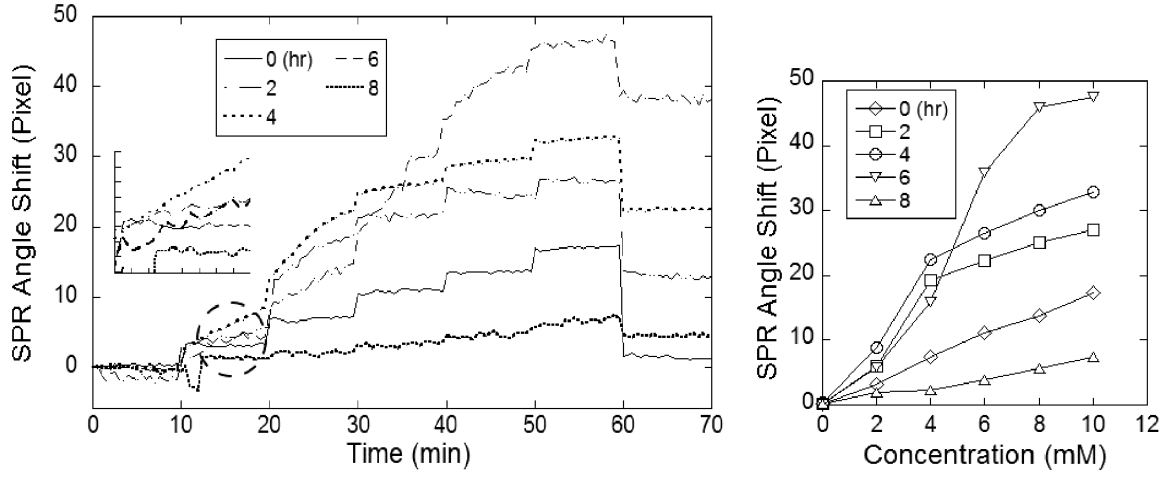
brightest and darkest area) decreased during both modification processes. In addition, another prism was detected which soaked merely in deionized water to monitor the influence of environmental change, and the result is shown in Fig. 3(c). The SPR angle fluctuated during this contrast test, and had a slight drop of about 0.006° after the end of all processes, which means the environmental change will not bring too much influence on the sensing process.

3.2. *Different PBA modification time*

The healthy concentration interval of d-glucose in blood is ideally 4–6 mM.[25] According to this, we have prepared five concentrations of sucrose for detection in this work, from 2 to 10 mM (interval 2 mM) dissolved in PBS buffer (pH 7.4), and 10 min for each concentration. We first injected the PBS buffer as a baseline, the flow velocity was 50 μL/min, and sucrose solution followed from low concentration to high concentration in the same velocity. During the process, every time before we shifted the concentration, we stopped the detection and injected the PBS buffer in high velocity (1000 μL/min) for 3 min to flush out the previous sugar solution. After all five concentrations had been detected, we injected the PBS buffer in high speed again to flush out the sugar molecule which did not bind with PBA closely enough. Then, we lowered the velocity (50 μL/min) for a contrast detection. This part was used as compared to the baseline. The difference between the ordinate values of last part and baseline is the total combined quantity in the experiment.

We first used an unmodified chip as the control experiment. This experiment indicated the SPR angle shifted nearly linear correlated to the concentration of the sugar solution. The value of ordinate of final section was nearly equaled to the baseline which means no combination happened on the surface.

Theoretically, the combined quantity of sucrose is in direct proportion to the PBA molecule incorporated with dopamine on the surface of sensor chip, which related to the soaking time. As it shown in Fig. 4(a), after 2 h of soaking in the PBA solution, we can see a clear binding process. When the sugar solution came in 20 min later, the curve was nearly flat which means this process was approaching completion. The difference of 2 PBS buffer's SPR angle stands for the combined quantity which shifted at about 0.045°. On the other hand, after 4 h, this binding process lasted for more than 30 min, and the combined quantity was nearly doubled, whereas after 6 h of modification, it lasted around 40 min, and the combined quantity was more than triple, which signified more PBA molecules on the surface as the soaking time increased. There was one more thing to be noticed — as we can see from the Fig. 4 (a), after the binding process ended, the sensitivity of refractive index was reduced. The reason must be plenty of sugar had combined with PBA molecules on the surface, they occupied most of the depth that the surface plasmon wave can penetrate.



(a) (b)

Fig. 4. Comparison of different modification time. (a) Detecting curve of sucrose using five sensor chips according to time. (b) Angle shift of different concentration by five sensor chips.

Nevertheless, as we have already known, the FWHM of SPR curve would increase and the Contrast Ratio of signal would decrease as the modification time grows. That means the signal quality was reducing as the soaking time grew. After 8 h, the quality of the signal we obtained was considered meaningless because it was easily disturbed by noise and other spurious signals. In conclusion, we chose 4 h as the most appropriate soaking time.

3.3. *Clinical sample experiment*

Generally, the blood or serum is used in blood sugar monitoring. Nonetheless, the refractive index of blood or serum was beyond the measuring range of our detection system. The aqueous humor contained glucose, vitamin C, lactic acid, Na, K, Cl and protein (0.2 mg/mL) etc., and was related to the venous circulation. Variations in blood glucose levels are reflected in corresponding variations in the concentration of the aqueous humor[26] What is more exciting, the refractive index of aqueous humor was approached with water, which was more suitable for our work than blood or serum.

We prepared two aqueous humor samples which were taken from diabetes patients, and a sample from a cataract person whose blood sugar concentration was normal. These samples were acquired from the Department of Ophthalmology of Xinhua Hospital in Shanghai. We follow the ethical protocols in using human samples in our experiment. Since the quantity of these samples we had obtained was few, the previous experimental methods could not be adopted in this detection. We just took 20 μL each time, and dropped it on the surface. All these experiments were done in similar circumstances, and the nonspecific sites were blocked by 0.5% bovine serum albumin (BSA) for 30 min. Yet in this way, the influence of evaporation existed during our experiment. We determined 15 min[27,28] was appropriate to this detection, which guaranteed the reaction time, and minimized the influence simultaneously. As shown in Fig. 5, the angle shifts of two diabetes samples was 0.065° and 0.068°, which is nearly three times more than the combined quantity of cataract patient, and this result clearly showed the difference in blood sugar levels between the diabetes patients and normal people, and proved theoretical analyses perfectly. It also demonstrated that our sensor can be used in medical monitoring without any doubt.

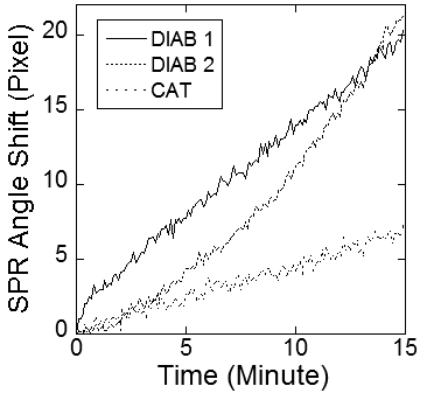


Fig. 5. Comparison of SPR angle shifts of the aqueous humor samples from diabetes patient and cataract person.

4. Conclusion

In our work, we developed a saccharides sensor developed by SOW-based SPR, which used a 3-layer (MgF_2–Au–MgF_2) film architecture, and a new probe molecule modification method was adopted. We managed a dopamine thin film on the MgF_2 film first, which provided a second reaction platform to combine with PBA molecule afterward. We conducted a series of experiments to find the most appropriate probe modification time under current condition. We can clearly see that the combination process between sugar and our probe molecules happened on the surface of our sensor chips when the sample solution flowed through, which means this sensor showed great efficiency on saccharides detection. It demonstrated that this sensor has a great potential and a bright future in blood glucose monitoring.

Acknowledgments

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