

Mesoporous nanospheres functionalized optical microfiber biosensor for low concentration neurotransmitter detection

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Abstract: A label-free and ultrasensitive microfiber interferometer biosensor has been demonstrated for detection of neurotransmitter molecule (5-HT). The surface morphology of the silicon dioxide nanospheres acting as molecule sieve provides an effective mean of gathering 5-HT molecules by designed mesoporous structure. The slight concentration change of 5-HT molecules is translated into a dramatic wavelength shift of the interferometric fringe pattern. The experimental results show that the biosensor has a linear response in concentration range from 100 fM to 1 μ M and a detection limit as low as 84 fM.

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1. Introduction

The neurotransmitter serotonin (5-HT), primarily known as a small monoamine molecule found in all bilateral animals, modulates neural activity and a wide array of neuropsychological processes [1]. Its concentration variations are implicated in the pathology of a number of increasingly prevalent psychiatric states and neurodegenerative diseases including cognition, mood, aggression, mating, feeding and sleep, qualitative and quantitative detection for low levels of 5-HT is thus of considerable value [2]. Conventional assays for the determination of 5-HT in vitro have been widely developed using high performance liquid chromatography (HPLC)-electrochemical detection [3,4], and HPLC-mass spectrometry (MS) [5, 6], capillary electrophoresis-laser induced fluorescence [7], immunoassay [8], and more recently, other electroanalytical methods [9,10]. Most of these techniques presently in use, however, generally suffer from some disadvantages with regard to large number of samples, complex sample preparation procedures, and even requiring analyte labeling.

Nowadays, there is considerable interest in developing label-free optical methods for biomolecules measurement [11–13]. These sensing technologies show great superiorities because of their flexibility to analyze biomolecular interactions without using fluorescence, absorptive, or radio-labels [14]. This simplifies the assay and allows time-resolved study of the kinetics of biomolecular interactions. Microfiber sensor devices used as biosensors exhibited remarkable optical and mechanical sensing properties including large evanescent fields, strong optical confinement, flexibility, configurability [15,16]. Such desirable characteristics have gained much attention in recent years and made microfibers an excellent platform for developing label-free optical biosensors. In particular, tapered microfiber interferometers show advantages of high sensitivity to surrounding refractive index (RI), ease of implementation, simplicity and robustness in structure [17], and have been applied to

detect various biomolecules, such as proteins, bacteria, DNA, by modifying its surface with specific biorecognition probes [18–22].

In this letter, we present a high sensitive biosensor to detect 5-HT molecules based on a nonadiabatic tapered microfiber interferometer coated by an array of mesoporous Ag@mSiO₂ nanospheres. Herein, the designed mesoporous silica-coated nanostructures serve as molecular sieve and cage to absorb specific volume of 5-HT molecules forming the aggregation of 5-HT molecules on the surface of microfiber sensing region. The 5-HT concentration fluctuations can be perceived by monitoring the optical wavelength shift of interferometric fringe as a result of localized surrounding environment variations of the fiber. The linear response range of the proposed biosensor ranges from 100 fM to 1 μ M. The detection limit is as low as 84 fM.

2. Experiment

2.1 Device fabrication and principle

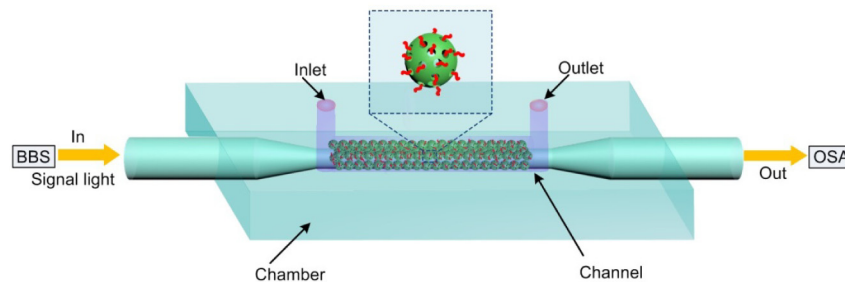


Fig. 1. Experimental setup: the proposed fiber-optic interferometric biosensor for low levels of 5-HT molecules detection. BBS: broadband light source; OSA: optical spectrum analyzer.

The optical set-up for ultrasensitive detection of 5-HT is shown in Fig. 1. The taper-based modal interferometer was fabricated as previously described in [23]. Simply, a nonadiabatic microfiber taper was obtained by locally heating and stretching a section of a highly Ge-doped fiber with assistance of a flame-brush technique. The fiber was slowly stretched with two linear stages when a 5 mm width flame scanned across it. The fiber-optic geometric parameters were mainly determined by the moving speeds of the flame and stages. A taper with a uniform region whose diameter and length were about 6.4 μ m and 1.5 cm, respectively, was fabricated. The transition region was 0.4 cm in length. When the fundamental core mode of the untapered fiber enters the downtaper part in transition region, it excites a higher order mode in the taper region because of the abrupt change in diameter leading to the broken adiabaticity. This allows the coupling and recombination of modes in the microfiber, thus achieving an interferometer. Although more than two modes may be excited, mode beating is mainly between the HE₁₁ and HE₁₂ modes because they have the similar azimuthal symmetry and the smallest phase mismatch [23]. Notice that any change of the external RI around the tapered region may have different influences on the fundamental mode and higher order mode. The higher order mode has larger RI sensitivity because some of its mode energy penetrates into the surrounding medium. As a result, the intermodal interference fringe pattern shifts with surrounding RI. The microfiber surface is modified with accessible mesoporous nanospheres which capture 5-HT molecules. The capture of 5-HT molecules onto the microfiber surface changes the localized surrounding environment. By monitoring the interference fringe shift, the concentration change of 5-HT molecules can be detected.

2.2 Material and reagents

All chemicals and solvents supplied by Aladdin were of analytical grade and were used without further purification. The monodispersed silver nanoparticle with amionacid groups which was beneficial for the hydrolysis of tetraethyl orthosilicate (TEOS) and deposited of silica through physical interaction was obtained from BaseLine Co. (Tianjin, China). The mesoporous silica-coated silver nanocage core-shell particles used in our work was synthesized via the developed Stöber method in propanol solution which was adapted according to the reference [24]. The procedure of creating $\text{Ag@SiO}_2\text{@mSiO}_2$ nanostructures is illustrated in Figs. 2(a) – 2(c). Briefly, 1 mL silver colloid nanoparticles with the diameter of 10 nm were dispersed in ethanol (10 mL) under sonication. Next, 25 mL of H_2O , 4 mL of ammonia aqueous solution (30 wt%) and 150 mL of isopropyl alcohol were added into the above dispersion. After 30 min, 30 μL of TEOS was dripped into the reaction mixture and stirred for 2 h. The Ag@SiO_2 particles were separated by centrifugation and washed with ethanol and water for three times. These particles were dried in the drying oven for further use. Then the preformed Ag@SiO_2 nanospheres were further deposited with mesoporous silica shell via a surfactant-templating sol-gel approach by using hexadecyl trimethyl ammonium bromide (CTAB) surfactant as template. Firstly, Ag@SiO_2 particles were dispersed in the solution containing 10 mL of ethanol, 15 mL of H_2O , 500 μL of ammonia aqueous solution and 0.01 g of CTAB with ultrasonic for 30 min. Then 50 μL of TEOS was added and the mixture was continuous stirred for 6 h. And 100 mL of isopropyl alcohol was added into above system and agitated for another 2 h to remove CTAB templating agent. The $\text{Ag@SiO}_2\text{@mSiO}_2$ core-shell particles were harvested after centrifugation and washed with ethanol and deionized water for three times. The fabrication of ordered, perpendicular, and open mesopore channels are highly dependent on the ratio of ethanol to water, the concentration of CTAB surfactant, the dispersibility and surface property of Ag@SiO_2 core nanoparticles, and reaction temperature. These nanoparticles were dried in vacuum drying oven under room temperature, and then were dispersed in ethanol solution with volume of 5 mL for further using. By drying a drop of the half diluted solution of the nanoparticles on platinum sheet, the sample was characterized by a field emission scanning electron microscopy (SEM) (ULTRA55, ZEISS, BRUKER). As shown in Figs. 2(h) and 2(i), the dispersed Ag@SiO_2 particles and $\text{Ag@SiO}_2\text{@mSiO}_2$ particles were uniform with 30 nm and 100 nm in diameter respectively, which both featured perfectly round shapes with high orderliness.

2.3 Modification of optical fiber sensor surface with mesoporous silica-coated nanostructures membrane

The presynthesized mesoporous silica-coated silver nanocage core-shell were subsequently decorated onto the tapered fiber surface using silane couple agent functionalized and the following procedure shown in Figs. 2(d) - 2(g). After rinsed with ethanol for three times, and cleaned for 1 h in a bath with a piranha solution (H_2O_2 : H_2SO_4 in a 1:3 ratio by volume) the freshly prepared tapered fiber probe with abundant hydroxyl groups hung on the silica surface was vertically dipped in a ethanol solution of (3-aminopropyl)triethoxysilane (APTES) (5 $\mu\text{L}/\text{mL}$) in anhydrous ethanol. For another 1 h, the probe with positive charges was taken out, rinsed with deionized water for three times to remove silane excess, dipped into reversely charged suspension of mesoporous silica nanocage core-shell for 1 h to realize a saturated adsorption of the nanostructures, washed with deionized water for three times, and dried in vacuum drying oven under 45 °C more than 6 h for future packaging in microchannel chip.

2.4 Sensor testing and characterization

Before testing, the morphology of the mesopore material coating microfiber region was characterized. And the SEM image of functionalized microfiber cross section was shown in Fig. 2(j).

During the experiments, the stability of sensor and biotesting was protected by designing and fabricating a microchannel chip, and the constant experimental temperature (25 °C) was controlled by water bath. The 5-HT solutions and other interfering solutions were injected into the micro-fluidic chip via an electronic-controlled pump. The light emitted from a broadband source (BBS) was launched into the microfiber taper interferometer. And the transmitted interference fringe was monitored with an optical spectrum analyzer (OSA).

Before the detection of 5-HT in phosphate buffer saline (PBS, 4 mL, 100 μ L/mL, PH 7.4), deionized water was used to remove any physically adsorbed or other interfering substances on the surface of the functionalized microfiber biosensor. Initially, the original standard sample of 5-HT solution with concentration of 1 mol/L was prepared and other standard samples were diluted by PBS buffer proportionally under dilution ratio (1:10) of original sample, thus a wide range from 100 aM to 1 μ M. Then the as-prepared biosensor was applied into the detection of 5-HT at different concentration levels. In each concentration assay, the 5-HT sample solution was injected for more than 30 min until the wavelength shift of the transmission spectrum in OSA varies little, to ensure thoroughly binding with as-prepared sensor. Herein, PBS buffer was also employed to rinse the 5-HT molecules adsorbed in the mesopore channels on the surface of nanospheres for three times before next concentration measurement.

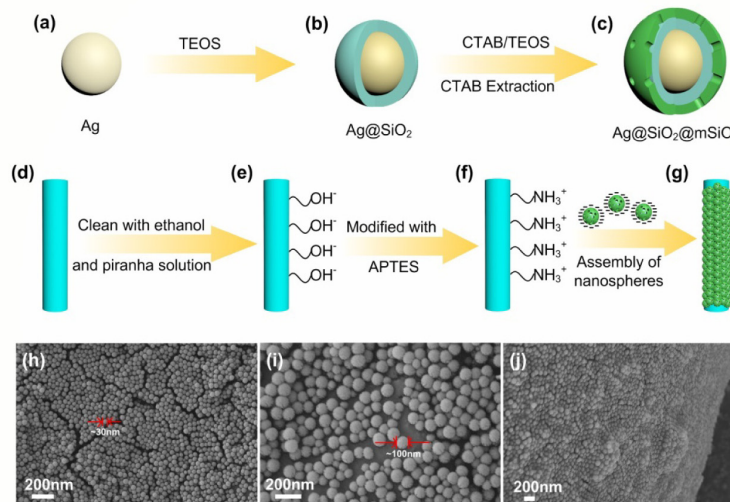


Fig. 2. A schematic process flow of the synthesis of $\text{Ag@SiO}_2\text{@mSiO}_2$ nanocarrier: (a) Monodispersed silver nanoparticles. (b) Depositing thin SiO_2 layer (~ 10 nm in thickness) on the surface of Ag nanoparticles using TEOS as silicon source. (c) Coating mesoporous SiO_2 layer (~ 35 nm in thickness) on the basis of Ag@SiO_2 nanospheres by introducing CTAB as template and followed by CTAB removal. A schematic diagram of the fabrication procedure for modified microfiber sensor with mesoporous nanostructures: (d) As-prepared microfiber device by tapering technique. (e) Cleaning microfiber with ethanol and piranha solution. (f) Amino groups with positive charges bonding to abundant hydroxyl groups on the surface of fresh microfiber with APTES. (g) Implementing the functionalized fiber device under the circumstances of nanospheres with negative charges. Top-view SEM image of dispersed Ag@SiO_2 nanospheres and $\text{Ag@SiO}_2\text{@mSiO}_2$ with higher amplification factor are shown in panels (h) and (i), respectively. (j) Side-view SEM image of such $\text{Ag@SiO}_2\text{@mSiO}_2$ nanospheres functionalized fiber sensing device cross section.

3. Results and discussion

Figure 3(a) shows the transmission notches around 1570 nm generated by the microfiber interferometer sensor. In the 5-HT solution with concentration from 100 aM to 100 fM, the notch wavelength varies little compared to that of PBS buffer (background noise). However, in concentration above 100 fM, the notch showed red shift obviously with sample concentration increasing. The curve above in Fig. 3(b) depicts the linear relationship between the transmission notch wavelength shift and the 5-HT concentrations. It can be seen that samples exhibited good linear optical response to the change of 5-HT concentration. The errors bars represent the standard deviations of three independently repeated measurement results at each 5-HT sample concentration and confirm the relatively good repeatability of the sensor. The linear regression equation obtained is $y = 1.04x + 14.71$, where y refers to the wavelength shift and x is the 5-HT concentration. From the fitting results, the sensitivity is 1.04 nm/LogM, and the concentration range is from 100 fM to 1 μ M. The limit of detection (LOD) was calculated as the ratio of three times the standard deviation of replicate runs of PBS spiked with each concentration and linear coefficient of the fitting curve, based on the equation: $\text{LOD} = 3\text{SD}/S$ [25]. It was estimated to be 84 fM in the linear concentration range. The above results show that in comparison with previous methods [3–10] and the same naked microfiber sensor which show weak sensitivity under very low concentration molecules detection, as shown in Fig. 3(b) below, the fiber-optics sensor exhibited much higher response activity for 5-HT with wider linear range and lower detection limit under similar circumstances. The high sensitivity of the sensor is attributed to both the fact that tapered microfiber possesses high RI sensitivity and the mesoporous nanoparticle arrays provide it with enhanced capability of biomolecule detecting response.

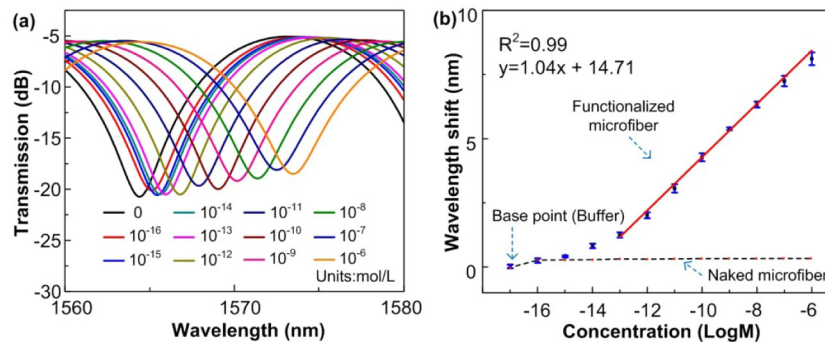


Fig. 3. (a) Measured transmission spectra with 5-HT concentrations of 0 to 1 μ M for a silicon dioxide nanospheres coated microfiber interferometer. (b) Above: a correlation curve of the data which corresponding relative wavelength shift of as-prepared biosensor in the presence of different concentrations of 5-HT. And the error bars represent the standard deviations of three independent measurements with a single interferometer. Below: corresponding relative wavelength shift of the same naked microfiber biosensor in the presence of same range of 5-HT concentrations variation.

To further verify whether our designed biosensor is specific for 5-HT, the detection reactivity to different potentially size-based interfering substances were chosen as control experiment. Smaller size molecules like K^+ , carbamide and larger size molecule like bovine serum albumin were examined to determine the selectivity of the sensor at the same concentration of 10 pM, as illustrated in Fig. 4. The results were analysed by placing the obtained mean value into the above calibration curve shown in Fig. 3(b), the calculated value that was equivalent to the concentration of 5-HT (e5-HT) was obtained. The cross-reactivity rate is expressed as $(\text{e5-HT})/10^{-11} \times 100\%$ [26]. The results indicated that the biosensor has reactivity $10^{-1.41}\%$, $10^{-0.56}\%$, and $10^{-1.14}\%$ with K^+ , carbamide, and bovine serum albumin, respectively. For examined interfering molecules, the cross-reactivity rate with 5-HT were

less than 0.1% than out of the detection range of the sensor. Moreover, the competing substances were added simultaneously to assess the prepared biosensor response. In solutions containing all 4 compounds above, the assayed response signal differed by only 0.9% from that associated with a pure 5-HT solution at equivalent concentration. It obviously found that only 5-HT induced a dramatic increase in optical signal response of the sensor when other experimental conditions were controlled to be the same. Whereas just a little changes compared to 5-HT detection limit were observed in the presence of the interference substances, indicating that the existing interferences did not largely affect the results under this detection circumstance. As mentioned above, the forming process of pore volume outer SiO_2 spheres is determined by several factors, mainly CTAB molecule chain which has 16 carbons. Thus, the open pores formed would be more suitable for the molecule with slightly smaller volume. Herein, 5-HT is monoamine molecule which has 10 carbons. In fluid circumstance, the unfolded 5-HT molecule chains make it easy to enter the open pores without exiting. However, the smaller size molecules easily flow in and out of the channels on the microspheres surface until reached to a balance, and the larger size molecules become it difficult to enter into the channels [27]. Thus, both of them demonstrate non-significant signal response.

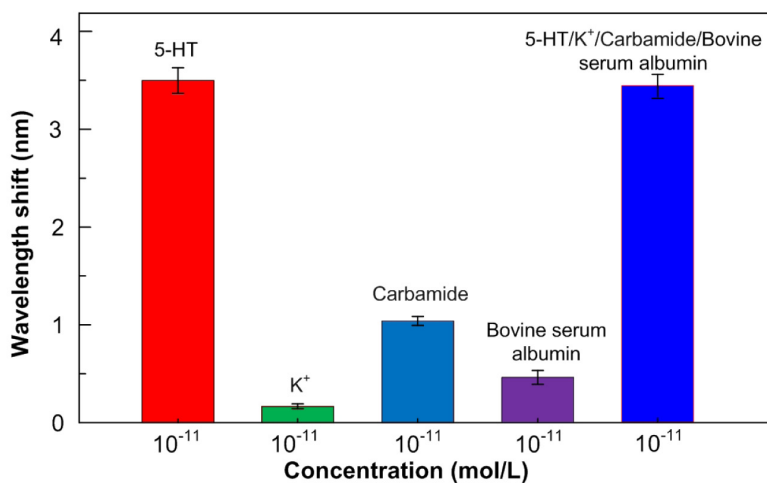


Fig. 4. Comparison of optical response of fiber-optic sensor to 10 pM 5-HT and other potential interferents: 10 pM K^+ , 10 pM carbamide, 10 pM bovine serum albumin, and a mixture of 10 pM 5-HT, 10 pM K^+ , 10 pM carbamide and 10 pM bovine serum albumin under the same experimental condition.

The high repeatability and stability are also very critical for the scientific and practical applications of fiber-optic biosensors. In order to examine repeatability of the neurotransmitter sensor, the experiment was repeated for each concentration and furthermore the repetitive process quantificationally was performed under low concentration of 10 pM for three times, as shown in Fig. 5. When the 5-HT molecule flows into channel of microspheres, the localized surrounding environment of the sensor varies, then leading to the transmission dip red shift sharply relative to baseline (PBS). And it progressed about 20 min until reached to its utmost. With the suitable pore size, the 5-HT molecule stayed in the pores on the microspheres without exiting, which endowed the detecting with good spectral reproducibility. In order to be able to show that biosensor regeneration was achievable with high fidelity, the unloading experiments were performed with buffer (PBS). The curled 5-HT molecule stretched and rushed out under flowing PBS circumstances. Obviously, the transmission wavelength dip turned blue shift with sample concentrations decreased, back to baseline about 30 min later. The loading experiments were converted from the relative shifts

of each loading/unloading cycle and offset so that the next loading/unloading cycle starts where the last one ended. Consequently, through a mild buffer exchange, the biosensor can be restored to allow repeated detection cycles. There is gradual decrease of maximum sensor intensity (approximately 7.5% per cycle) upon repeated reuse of the same sensor. The degradation of the sensor surface occurs when the functionalized microsphere is taken out from the surface during the rinsing procedure each time. This degradation due to bonding can be reduced or avoided in future assays which treatment process could be optimized.

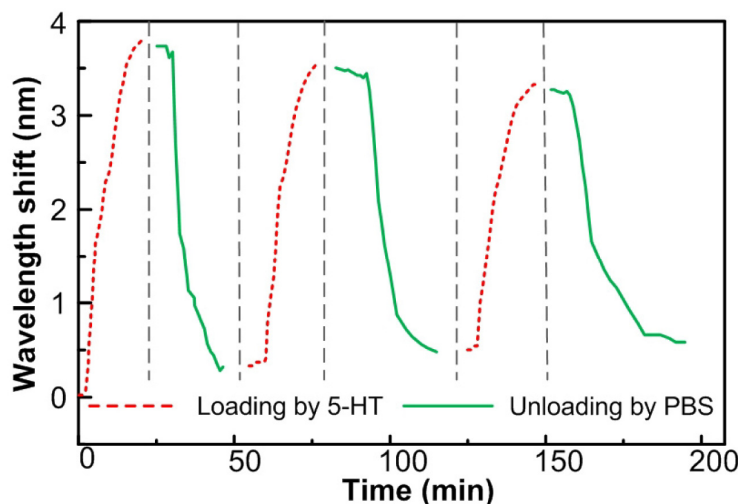


Fig. 5. Cyclical response of the as-prepared fiber-optic biosensor to detect 5-HT (at 10 pM) during 200 min. Loading experiments and unloading experiments were performed with 10 pM 5-HT and PBS, respectively.

4. Conclusion

A fiber-optic biosensor for 5-HT molecule detection with ultrahigh sensitivity has been developed by electrostatically assisted APTES-functionalized surface-assembly of Ag@mSiO₂ with controllable morphology. The results suggested that the sensor shows excellent reproducibility for 5-HT detection in a label free and reagentless manner with high sensitivity and relatively good selectivity. The LOD can reach up to 84 fM which is very critical for that the determination of low concentration molecules in body is difficult but significant in clinical. We hope that this proposed strategy may offer potential approach which will be beneficial for a wide range of applications including clinical diagnostics, biomedical engineering, and nanotechnology as well.

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