Hybrid Graphene/Conducting Polymer Strip Sensors for Sensitive and Selective Electrochemical Detection of Serotonin

Wed Al-Graiti  
*University of Wollongong*, wamag430@uowmail.edu.au

Javad Foroughi  
*University of Wollongong*, foroughi@uow.edu.au

Yuqing Liu  
*University of Wollongong*, yuqing@uow.edu.au

Jun Chen  
*University of Wollongong*, junc@uow.edu.au

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Abstract

© 2019 American Chemical Society. There is an urgent need for electrochemical sensor materials that exhibit electrochemically compliant properties while also retaining high durability under physiological conditions. Herein, we demonstrate a novel strip-style electrochemical sensor using reduced graphene oxide (rGO) and poly(ethylene dioxythiophene)/poly(styrene sulfonic acid) (PEDOT/PSS) nanocomposite films. The fabricated rGO-PEDOT/PSS sensor with and without nafion has shown an effective electrochemical response for both selectivity and sensitivity of the serotonin (5-hydroxytryptamine, 5-HT) neurotransmitter. The developed high-performance hybrid graphene/conducting polymer strip sensors are likely to find applications as chip electrochemical sensor devices for patients diagnosed with Alzheimer's disease.

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Hybrid Graphene/Conducting Polymer Strip Sensors for Sensitive and Selective Electrochemical Detection of Serotonin

Wed Al-Graiti,†,* Javad Foroughi,‡,*‡ Yuqing Liu,† and Jun Chen*,‡†

1Intelligent Polymer Research Institute, and 2School of Electrical, Computer and Telecommunications Engineering, Faculty of Engineering and Information Sciences, University of Wollongong, Wollongong, New South Wales 2522, Australia
3Chemistry Department, Science College, Thi-Qar University, Thi-Qar, Iraq

ABSTRACT: There is an urgent need for electrochemical sensor materials that exhibit electrochemically compliant properties while also retaining high durability under physiological conditions. Herein, we demonstrate a novel strip-style electrochemical sensor using reduced graphene oxide (rGO) and poly(ethylene dioxythiophene)/poly(styrene sulfonic acid) (PEDOT/PSS) nanocomposite films. The fabricated rGO–PEDOT/PSS sensor with and without nafion has shown an effective electrochemical response for both selectivity and sensitivity of the serotonin (5-hydroxytryptamine, 5-HT) neurotransmitter. The developed high-performance hybrid graphene/conducting polymer strip sensors are likely to find applications as chip electrochemical sensor devices for patients diagnosed with Alzheimer’s disease.

INTRODUCTION

Alzheimer’s disease (AD) is a long-term mental illness affecting the brain system and thus the memory and awareness of senior people. It can be clinically diagnosed by an extreme loss of serotonergic neurons when a severe drop in the level of the 5-HT neurotransmitter happens.1 Serotonin (5-HT, 5-hydroxytryptamine) is a double-functioning biomolecule present inside and outside the brain tissues acting as a neurotransmitter and a hormone, respectively.2,3 Once the 5-HT level is depleted (or raised), irregular biological and physiological disorders can occur among individuals. In addition, AD could lead to addiction to alcohol, infantile autism, sleep disturbances, depression, liver abnormality, and difficulty to adapt when 5-HT is dysfunctional.4,5 Therefore, early and direct detection of 5-HT is a pivotal subject for medical diagnostic clinics and biological researchers nowadays.6,7

Traditional determination methods for example, high-performance liquid chromatography (HPLC) and spectrophotometry, have been used for biosensing of 5-HT; however, they are not cost-effective and require prolonged procedures for sample preparation. Interestingly, due to the electroactive nature of 5-HT, electrochemical techniques (low cost, affordable for lab application, and highly selective and sensitive for biomolecules) have been introduced as suitable determination methods for fast detection of this neurotransmitter. Numerous materials are used to obtain satisfactory performance of electrochemical electrodes; however, 5-HT detection is still a challenge using unmodified sensors. 5-HT coexists with numerous species in biological samples; dopamine (DA) is one of them. 5-HT and DA are electroactive and have been found to have very close oxidation potentials when tested by bare electrodes, in turn resulting in signal overlapping of both chemicals.8 Therefore, most of the reported sensors lack good selectivity and high sensitivity when simultaneously detecting these species. Another issue is that most of the traditional electrochemical sensors are fabricated in large sizes, which are not ideal for implantation purposes because they cause large tissue damage.5,9 Furthermore, detection of 5-HT has not been widely reported so far.5,10 However, it is suggested that, for optimum detection of 5-HT, many aspects of the prepared sensor should be considered such as material chemistry, electrode design, electrochemical properties, ability to be activated, and surface properties.

The promising electrochemical performance of graphene (G) has created a new platform that is used for a number of applications including biosensing technology.11,12 Graphene has been suggested as an excellent candidate for sensor devices because it has fascinating electrical, thermal, and electrochemical properties. Biocompatibility, great conductivity, high electron mobility, ability to be modified, and high surface-to-volume ratio all highlight the versatility of graphene and its composite-based electrodes.13,14 Hence, graphene is currently applied in multiple electrochemical experiments and has led to significant achievements.10,15 Therefore, scientific researchers have focused on applying this unique material for biodetection of 5-HT in pristine and/or composite structures as well as as a modifier for conventional electrodes.2,16 However, the actual capability of reduced graphene oxide (rGO) for use as an electrochemical sensor can reduce as a result of accumulation that develops among the graphene tiers. This phenomenon is
attributed to electrostatic and π−π interactions between carbon molecules within the layers. Therefore, scientists have directed their attention to overcome the accumulation drawback and use rGO effectively and efficiently.

Coating graphene electrodes with conductive polymers (CPs) has been introduced as a perfect solution for eliminating accumulation and increasing the electrocatalytic properties of the electrode at the same time. In a typical chemical reaction at the electrode’s surface, conductive polymers work as a connector to facilitate the movement of electrons from various oxidized analytes to the electrode. Many studies are being carried out on conductive polymers in the field of electrochemistry, particularly, neurotransmitter sensing. Therefore, such polymers having super electronic and electrocatalytic properties have been in demand in the construction of electrochemical sensors, for example, graphene-based electrodes modified with poly(ethylene dioxythiophene)/poly(styrene sulfonic acid) (PEDOT/PSS) because of fascinating conductivity, the flexibility of electron transfer with reasonable oxidation potentials, and water solubility to obtain the interactive effect of all components when performing toward aimed analytes.17,18 Also, PEDOT/PSS is a very promising conductive polymer to decrease the restacking problem of graphene layers. The polymeric backbone of PEDOT can also be easily functionalized to increase its conductivity, biocompatibility, and stability through the incorporation of various dopants, counter ions, and biological moieties. The most frequently used PEDOT derivative is PEDOT doped with poly(styrene-sulfonate) (PSS), PEDOT:PSS, with an electrical conductivity that can go as high as 4600 S cm−1.19,20

Nafion as a perfluorinated ion-exchange and negatively charged polymer is also extensively used due to its good biocompatibility and excellent selectivity to cations rather than...
anions. Modification of electrochemical electrodes with nafion has shown improved selective adsorption. This approach is quite useful to increase absorption of 5-HT cations and for recording better measurements.

In this paper, we demonstrate the fabrication of an rGO–PEDOT/PSS sensor in strip style for qualified voltammetric determination of the 5-HT neurotransmitter. The applied electrochemical techniques displayed sensitivity and selectivity toward 5-HT compared to co-existent DA molecules. In addition, the sensor’s performance has been enhanced by adding nafion in rGO–PEDOT/PSS dispersion and the effect of nafion on selective detection of 5-HT when simultaneously tested with DA (the interference) has been investigated. The physical and chemical characterization of the developed electrochemical sensors has been carried out.

RESULTS AND DISCUSSION

Physical Characterization of rGO–PEDOT/PSS Strip.

Scanning electron microscopy (SEM) images of the as-prepared rGO–PEDOT/PSS film are shown in Figure 1a. As can be seen from Figure 1a, as-prepared films show a smooth and flat surface, which suggests the tight interaction between PEDOT/PSS and graphene sheets in addition to the role of PEDOT/PSS in decreasing the restacking problem within graphene layers. The obtained SEM image at higher resolution in Figure 1a (inset) showed minor curves on the rGO–PEDOT/PSS film due to minimal restacking of rGO. The results show that obvious changes occurred in rGO–PEDOT/PSS films once mixed with the nafion polymer. The SEM image in Figure 1b revealed the wrinkled surface of the rGO–PEDOT/PSS–nafion composite film, which could be attributed to the solvent evaporation process. The rGO–PEDOT/PSS–nafion film showed random dark spots distributed on its surface in Figure 1b (inset: magnified image). These dark spots were not seen on the rGO–PEDOT/PSS film with no nafion (Figure 1a magnified image). It could be due to distortion in the polymer structure when mixed with graphene that causes polymer rearrangement.

Figure 1c represents the Raman spectra of nafion, PEDOT/PSS, rGO, rGO–PEDOT/PSS, and rGO–PEDOT/PSS–nafion samples. D and G bands are two predominant bands highlighted in Raman spectra. The D band is related to any disorders in the structure of the film’s components caused by defects, functional groups, and surface faults. The G band is generated from heterogeneous structures and carbon bond vibrations within the graphitic layer. As shown in Figure 1c, sharp and clear D and G peaks appear at 1340 and 1580 cm$^{-1}$, respectively, in Raman spectra of rGO, rGO–PEDOT/PSS, and rGO–PEDOT/PSS–nafion samples. PEDOT/PSS showed relatively weak peaks at 1530, 1430, 1260, 1000, and 850 cm$^{-1}$, which could not be seen in rGO–PEDOT/PSS or rGO–PEDOT/PSS–nafion due to low intensity. The increased ID/IG ratio recorded for composite films from 1.15 (the rGO–PEDOT/PSS) to 1.27 (rGO–PEDOT/PSS–nafion) suggests the role of nafion in generating a high proportion of defects and fractions on the rGO–PEDOT/PSS film surface. The rGO sample displayed an even higher ID/IG ratio of 1.43 than rGO–PEDOT/PSS and rGO–PEDOT/PSS–nafion samples. PEDOT/PSS–nafion and revealed highly defective surface resulting from the reduction process of GO to rGO.

FTIR spectroscopy is a complementary technique to Raman analyses for sample surfaces to monitor any changes when modified (Figure 2d). PEDOT/PSS vibrations at 1580 and 1508 cm$^{-1}$ belong to the thiophene ring. S–O and S–phenyl groups were also detected at 1166, 1125, and 1028 cm$^{-1}$. However, PEDOT/PSS peaks decreased after mixing with rGO to prepare an rGO–PEDOT/PSS composite film. Notably, the peaks increased significantly after the addition of nafion to prepare an rGO–PEDOT/PSS–nafion composite film.

**Figure 1**. Results and discussion: (a) 100 μL of the composite, (b) 200 μL of the composite, (c) 300 μL of the composite, and (d) 400 μL of the composite. The area of a single film was 2 cm$^2$. The testing system includes a constant level of DA (5 μM) with (0.1–10 μM) 5-HT.

**Figure 2**. DPV results of the as-prepared strip films (rGO–PEDOT/PSS–nafion (0.5 wt %)) with different compositions (thicknesses): (a) 100 μL of the composite, (b) 200 μL of the composite, (c) 300 μL of the composite, and (d) 400 μL of the composite. The area of a single film was 2 cm$^2$. The testing system includes a constant level of DA (5 μM) with (0.1–10 μM) 5-HT.
Nafion role in creating oxygen functional groups on electrodes surface showing more curvature structure.

The sensing ability of rGO electrodes toward 5-HT concentrations was also investigated using DPV as highly sensitive and precise electrochemical technology. Figure 1e shows DPV curves of rGO−PEDOT/PSS with and without nafion strip films in a phosphate-buffer saline (PBS) solution containing 10 μM 5-HT. Using the rGO−PEDOT/PSS strip (red line), inadequate current signal response was recorded, at less than 0.5 μA. However, the rGO−PEDOT/PSS−nafion (5 wt %) strip (orange line) gave a well-distinguished oxidation signal of 5-HT at around 20 μA current signal. In a comparison between concentric nafion (5%) and diluted nafion (0.5%) mixed with rGO−PEDOT/PSS, a vast improvement in the 5-HT current signal was noticed in the diluted nafion−rGO−PEDOT/PSS strip (purple line, Figure 1e). For 10 μM 5-HT, the diluted nafion electrode recorded an outstanding oxidation signal current response at around 40 μA, which is double of that shown at the concentric nafion-modified electrode.

Similarly, Figure 1f shows DPV curves of rGO−PEDOT/PSS with and without nafion strips in a PBS solution containing 10 μM DA. The current response of the DA oxidation signal increased and became more prominent after rGO−PEDOT/PSS modification with concentric nafion (orange curve). Furthermore, the diluted nafion (0.5%)-modified rGO−PEDOT/PSS electrode showed significant current response of DA (50 μA), which is two times higher than that recorded by the concentric nafion-modified electrode (which was around 25 μA only). The presence of diluted nafion enhances the current intensity dramatically about 100 times and 2 times compared to untreated rGO−PEDOT/PSS or concentric nafion-modified rGO−PEDOT/PSS strips, respectively. This can be explained by the remarkable selective adsorption of nafion, especially when it has lower thickness,
which probably controls the transferring process of ions in the solution to the surface of the electrode.\textsuperscript{32}

Hence, the rGO–PEDOT/PSS–naion (0.5\%) film has been selected to investigate the targeted biomolecule 5-HT based on the already proven electrochemical performance in Figure 1e–f. However, the drop-casted amount of film dispersion needs to be further investigated as well due to the direct effect on the strip performance subsequently. Therefore, DPV experiments were carried out using rGO–PEDOT/PSS and diluted naion for prepared films at different thicknesses. In particular, 0.1, 0.2, 0.3, and 0.4 mL of the (diluted naion–rGO–PEDOT/PSS) composite were separately drop-casted on the same dimensional gold mylar substrates (area \(= 2 \text{ cm}^2\)). The recorded thickness of each film was varied and corresponded to the amount of deposited composite (as in Figure 2). The testing system included a PBS solution containing 5 \(\mu\)M DA with consecutive additions of 5-HT (0.1–10 \(\mu\)M).

As-prepared films with 100 \(\mu\)L of the composite and low concentrations of 5-HT were less defined at 0.36 V (showing a minor oxidation signal as shown in Figure 2a). Moreover, higher concentrations of 5-HT revealed a clear oxidation signal with excellent sensitivity. Besides, current response of a DA oxidation signal descended and did not appear after a few additions of 5-HT.

On the other hand, for the as-prepared film with 200 \(\mu\)L of the composite, highly separated signals belonging to 5-HT, at 0.4 V, and DA, at 0.16 V, were noticed with high sensitivity (Figure 2b). Although DA was found at a high level, these results confirmed the enhanced sensitivity of 5-HT, about 2.5 times that of the first film. Furthermore, the oxidation signal of 5-HT slightly shifted to a positive potential, which indicated increased electrocatalytic activity of this film. A dramatic decrease in DA response was observed in the same DPV shown in Figure 1b.

Moreover, the as-prepared film with 300 \(\mu\)L of the composite demonstrated extremely low current response of 5-HT and DA oxidation signals in Figure 2c. The reason is that the extra amount of diluted naion in the composite possibly obstructed some active sites on the strip surface, which indicates low permeability and weakened catalytic activity. Likewise, DPV findings in Figure 2d for the as-prepared film with 400 \(\mu\)L of the composite were complementary to DPV outcomes in Figure 2c. Figure 2c,d explains the claimed concept of the thickness effect on the voltammetric performance of the electrodes.

It can be concluded that the thickness of deposited rGO–PEDOT/PSS with a diluted naion composite film plays an important role in obtaining improved selective adsorption of serotonin. Therefore, according to the obtained results for developed strip films in Figure 2, the strip film with 200 \(\mu\)L of the composite can be considered as the best-performing film among others due to improved sensitivity and selectivity toward 5-HT in the presence of DA interference. The separated oxidation signals of 5-HT and DA facilitated the study of the oxidation process of 5-HT and demonstrated the optimum amount of naion–rGO–PEDOT/PSS composite used for electrode fabrication in Figure 2b. The capability of the diluted naion–rGO–PEDOT/PSS strip in detecting 5-HT and DA simultaneously has been investigated. PBS solution containing 5 \(\mu\)M DA with a range of 5-HT concentrations (0.1–10 \(\mu\)M) was used for DPV. Figure 2a exhibits the oxidation signal of 5-HT, which was very stable at 0.4 V with no shifts. Moreover, a well-defined and gradually increasing current response was observed for the sequential additions of 5-HT. It is worth mentioning that DA, which was present at a high and constant level, did not interrupt the oxidation signal of 5-HT. Furthermore, DA showed a clear oxidation signal at 0.16 V with a decent current response. However, the DA signal rapidly depleted and disappeared after a few consecutive additions of 5-HT. This result showed that both 5-HT and DA have competitive adsorption at the electrodes surface when simultaneously tested.

**Electrochemical Performance of the rGO–PEDOT/PSS–naion Strip. Separate Detection of 5-HT and DA.** A modified rGO–PEDOT/PSS electrode with the diluted naion (0.5\%) strip film has been proven to be promising for highly sensitive and selective adsorption of 5-HT or DA. The obtained results of DPV are shown in Figure 3a,b for the individual targeted oxidation of 5-HT and DA from 1 to 10 \(\mu\)M, respectively. DPV curves showed increased current responses with increased 5-HT concentration at 0.4 V in Figure 3a using an rGO–PEDOT/PSS–naion (0.5\%) strip sensor. The 5-HT signal was notable even at a low concentration of 1 \(\mu\)M. In addition, a high DA oxidation signal was observed at 0.16 V when the above strip sensor was used as shown in Figure 3b. The oxidation signal of DA gradually increased with an increase in its concentration. Moreover, 1 \(\mu\)M 5-HT elucidated satisfactory current intensity higher than that recorded for 1 \(\mu\)M DA. With continued additions of 5-HT and DA, 5-HT showed a slightly lower current response due to secondary products such as 5-HIAA from the oxidation process of 5-HT.

**Simultaneous Detection of 5-HT and DA.** An rGO–PEDOT/PSS–naion (0.5\%) strip film was used for simultaneous detection of 5-HT and DA in a PBS solution. The determination of 5-HT and DA was accomplished inside a cabinet to minimize the effect of the environment on running experiments. The solutions were daily and freshly prepared in the laboratory using precise scales and an isolated hood for nanoscale powders. The applied experimental protocol for measuring 5-HT did not interrupt by the later addition of DA.

The electrochemical performance of an rGO–PEDOT/PSS–naion (0.5\%) strip was recently evaluated toward simultaneous detection of 5-HT and DA (at 10 \(\mu\)M of each) through a CV experiment. At 20 mV/s scan rate, 5-HT and DA showed well-separated and sharp oxidation signals at 0.4 and 0.16 V (vs Ag/AgCl), respectively (Figure 2c). Increasing the scan rate from 20 to 200 mV/s for the same system as shown in Figure 3d, a symmetrical CV shape was observed with a proportional and an identical redox couple of 5-HT and DA. Figure 3c,d shows the enhanced sensitivity recorded by the rGO–PEDOT/PSS–naion (0.5\%) strip film toward 5-HT in the presence of DA. As can be seen from Figure 3c,d, the oxidation mechanism of 5-HT and DA is a diffusion-controlled process.

The capability of an rGO–PEDOT/PSS–naion (0.5\%) strip in detecting 5-HT and DA simultaneously has been demonstrated using the DPV method. Figure 3e exhibits DPV of 5-HT from 0.1 to 10 \(\mu\)M in a PBS solution containing DA (5 \(\mu\)M). The oxidation signal of 5-HT was very stable at 0.4 V, and no shifts were observed. The current response was well defined and typically increased with the sequential additions of 5-HT without interruption of DA that already existed at a high and constant level.\textsuperscript{24} Furthermore, DA showed a clear oxidation signal at 0.16 V with a decent current response.
However, the current response of greater DA concentrations μM injections of DA from 0.1 to 0.8 PSS be observed that the 5-HT signal was still well recognized in competitive adsorption when simultaneously tested. It can also minimized concurrent with the continuous additions of DA HT. The current response of the 5-HT signal slightly clear signal noticed at 0.4 V could be attributed to oxidized 5-corresponding to the DA oxidation process. The sharp and each DA addition, a linearly increased signal appeared curve of corresponding 5-HT concentrations vs current response.

Table 1. Composition of the As-Prepared rGO–PEDOT/PSS Films With and Without Nafion

<table>
<thead>
<tr>
<th>RGO–PEDOT/PSS</th>
<th>nafion</th>
<th>volume of final dispersion drop-casted on gold mylar substrate</th>
<th>dimensions of each film (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>film</td>
<td>volume (mL)</td>
<td>concentration (mg/mL)</td>
<td>mass (g)</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>1.9</td>
<td>0.380</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>1.52</td>
<td>0.304</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1.52</td>
<td>0.304</td>
</tr>
</tbody>
</table>

Amperometric Detection of 5-HT and DA. Figure 4a illustrates the amperometric test to investigate the effect of dopamine interference on the 5-HT signal at 0.4 V by increasing the 5-HT concentration from 0.1 to 10 μM under moderate and constant stirring. The current signal of the rGO–PEDOT/PSS–nafion (0.5%) electrode increased rapidly after each addition of 5-HT and then reached a stable-state current within 2 s, showing the fast oxidation behavior of 5-HT. The amperometric current–time curve showed that the pre-existing DA did not interfere with the oxidation of 5-HT. This could be ascribed to the significant electrocatalytic activity of the fabricated strip electrode and remarkable selectivity of the nafion polymer to 5-HT oxidation at that potential.24 The current of 5-HT oxidation signals was calibrated in Figure 4b, revealing a linear relationship between 5-HT concentrations and current responses as per indicated equations of the anodic current Ipa = 0.9213 C5-HT (R² = 0.94) for low concentrations and Ipa = 0.122 C5-HT + 0.9 (R² = 0.9466) for higher concentrations of 5-HT.

CONCLUSIONS

In summary, we have demonstrated a novel strip sensor based on nanostructured hybrid graphene and conducting polymer films for separate and simultaneous detection of 5-HT with dopamine. The as-developed rGO–PEDOT/PSS–nafion strip sensor can be manufactured through simple drop-casting and is capable of rapid electrochemical detection of 5-HT in the presence of DA. The as-prepared strip sensor enabled detection of lower concentrations of 5-HT than previously reported for electrochemical sensors. Indicative anodic peaks of 5-HT and DA were noticed at 0.34 and 1.6 V, respectively, showing excellent selectivity to 5-HT. Moreover, a detection limit of 5-HT as low as 0.1 μM was indicated. The as-prepared strip sensor is reusable and demonstrated excellent sensitivity and selectivity of 5-HT detection when presented with
were purged with nitrogen (N2) to obtain oxygen-free environment for every single electrochemical testing. The blank PBS solutions (pH 7.4, phosphate-buffered saline (PBS) buffer) containing 4000 μL of rGO–PEDOT/PSS dispersion and 100 μL of concentrated nafion (5%) were prepared. The concentration of targeted analytes, the mass of rGO–PEDOT/PSS, as well as the ratio of sodium chloride (NaCl) and ascorbic acid (AA, C6H8O6) were bought from Sigma-Aldrich. 5-HT, DA, and AA solutions were instantly prepared with phosphate-buffered saline (PBS) buffer before every single electrochemical testing. The blank PBS solutions were purged with nitrogen (N2) to obtain oxygen-free electrolytes. Nafion (5%) was supplied by ion Power, Inc. PEDOT/PSS pellets were obtained from Agfa, and graphite flake were purchased from Sigma-Aldrich.12

Table 2. Thickness of the As-Prepared Sensor Films as a Function of the Volume of rGO–PEDOT/PSS–Nafion (0.5 wt %) Solutions for Developed 2 cm² Casting Films using a Profilometer

<table>
<thead>
<tr>
<th>film</th>
<th>volume of the solutions (μL) @ 2 cm²</th>
<th>thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>0.52</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>1.13</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>2.20</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>3.80</td>
</tr>
</tbody>
</table>

Preparation of sensor components including the concentration of each component, the mass of rGO–PEDOT/PSS, and the ratio of nafion in the composites, and films’ dimensions are all described in Table 1. To prepare rGO–PEDOT/PSS–nafion (0.5 wt %) composite films, the following amounts of the composite were dropped onto gold mylar substrates: 100, 200, 300, and 400 μL. The thickness of each type of rGO–PEDOT/PSS–nafion (0.5 wt %) film was measured using a profilometer and shown in Table 2. As can be seen in Table 2, the thickness of as-prepared films increased from 0.52 to 3.8 μm for 100 to 400 μL samples, respectively. The measured thickness is corresponding to the dropped amounts of the composite as the size of the substrate was constant. These results indicated that the preparation method of the films was precise and well controlled (figure 5).

Preparation of rGO–PEDOT/PSS–nafion strips is illustrated in Figure 1. Preparation of sensor components including the concentration of each component, the mass of rGO–PEDOT/PSS, the ratio of nafion in the composites, and films’ dimensions are all described in Table 1. To prepare rGO–PEDOT/PSS–nafion (0.5 wt %) composite films, the following amounts of the composite were dropped onto gold mylar substrates: 100, 200, 300, and 400 μL. The thickness of each type of rGO–PEDOT/PSS–nafion (0.5 wt %) film was measured using a profilometer and shown in Table 2. As can be seen in Table 2, the thickness of as-prepared films increased from 0.52 to 3.8 μm for 100 to 400 μL samples, respectively. The measured thickness is corresponding to the dropped amounts of the composite as the size of the substrate was constant. These results indicated that the preparation method of the films was precise and well controlled (figure 5).

The electrochemical performance of the four films was investigated toward 5-HT with DA simultaneously. The obtained voltammetric results indicated the contribution of film thickness to strip performance when the testing conditions are the same during all of the experiments such as the concentration of targeted analytes, film size, and input parameters. The obtained results suggested that as-prepared dopamine simultaneously. It can be concluded that the as-prepared rGO–PEDOT/PSS–nafion strip sensor may be a promising device in 5-HT detection with potential for use in medical clinical application.
strip sensors with 200 μL of the rGO–PEDOT/PSS–nafion (0.5 wt %) film showed higher sensitivity and selectivity for 5-HT among other prepared sensors.

**Characterization of the As-Prepared rGO–PEDOT/PSS Strip.** The morphology of (rGO–PEDOT/PSS with and without nafion) strips was determined using field emission SEM (FESEM), JEOL7500FÀ (JEOL Ltd., Tokyo, Japan). Raman and FTIR spectroscopies were carried out using a Jobin Yvon Horiba 800 (Horiba Jobin Yvon, Edison, NJ) and the Shimadzu A1M8000 FTIR Spectrometers (Shimadzu Corporation, Kyoto, Japan).22

**Electrochemical Characterization of the rGO–PEDOT/PSS Strip.** Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were essential voltammetric techniques conducted using a CH Instruments electrochemical workstation (CH Instruments, Inc., model 660D, made in the U.S.A.). A three-electrode setup system was used where an rGO–PEDOT/PSS film was used as a working electrode (WE), Pt mesh was used as the counter electrode (CE), and Ag/AgCl (in 3M NaCl) was used as the reference electrode. Phosphate-buffer saline (PBS) (at pH = 7.4) was the basic electrolyte in all experiments. The high and low potentials (E) were set at +0.6 and −0.1 V, respectively, in CV testing with applied scan rates ranging from 0.01 to 0.2 (V/s). For DPV detection, the parameters were as follows: Incr. E = 0.004, amplitude = 0.05, pulse width = 0.05, and pulse period = 0.2.

**AUTHOR INFORMATION**

**Corresponding Authors**

*E-mail: Foroughi@uow.edu.au. (J.F.).
E-mail: junc@uow.edu.au. (J.C.).

**ORCID**

Javad Foroughi: 0000-0003-1979-5213
Jun Chen: 0000-0003-2218-7382

**Present Address**

School of Electrical, Computer and Telecommunications Engineering, Faculty of Engineering and Information Sciences, University of Wollongong, New South Wales 2522, Australia (J.F.)

**Notes**

The authors declare no competing financial interest.

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