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A new molecular imprinted PEDOT glassy carbon electrode for carbamazepine detection

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ABSTRACT

An electrochemical sensor for the detection of carbamazepine was fabricated by the electropolymerization of PEDOT on glassy carbon electrodes. Molecular imprinted polymer sites were synthesized by cyclic voltammetry on the electrodes' surfaces providing high selectivity and sensitivity towards carbamazepine molecules. Scanning electron microscopy validated the formation of the polymer. Extraction of carbamazepine from the polymer was performed by immersion in acetonitrile and validated by ultraviolet–visible spectroscopy along with cyclic voltammetry experiments comparing pre- and post-template extraction data. Further cyclic voltammetry and square-wave voltammetry tests aided in characterizing the electrodes' response to carbamazepine concentration in PBS solution with $[Fe(CN)_6]^{3-/4-}$ as a redox pair/mediator. The limits of detection and quantification were found to be 0.98 x 10^{-3} M and 2.97 x 10^{-3} M respectively. The biosensor was highly sensitive to carbamazepine molecules in comparison to non-imprinted electrodes, simple to construct and easy to operate.

1. Introduction

Carbamazepine (CBZ) 5H-Dibenzo[b,f]azepine-5-carboxamide is amongst the most used antiepileptic drugs (AEDs) for the treatment of epilepsy. In the routine management of epileptic patients, physicians frequently sample CBZ blood concentration for various reasons such as to a) ensure sufficient dosage in the patient; b) assess treatment adherence or compliance; c) avoid overshoot of dosing and development of overt clinical toxicity. Blood levels are particularly important in the context of patients that are elderly, pregnant, have co-morbid conditions such as liver disease, or take several medications that interact with each other.

As such, the development of a potentially implantable miniature biosensor for continuous monitoring of CBZ would give clinicians a valuable window into patients' health and their response to therapeutics by correlating serum CBZ concentrations with their clinical condition.

Biosensors are nowadays ubiquitous in biomedical diagnosis as well as a wide range of other areas such as point-of-care monitoring of disease treatment or progression (Mehrotra, 2016), environmental monitoring (Adams et al., 2019), food quality control (Sun et al., 2017), drug discovery (Myszka and Rich, 2000), forensics and personalized treatment (Mehrotra, 2016; Nikhil et al., 2016). They essentially involve the quantitative analysis of various substances by converting their biological properties into measurable signals. The performance of a biosensor is mostly dependent on the specificity and sensitivity of the biological reaction, which is highly determined by the sensor's biosensing and transducing elements.

To date, only a handful of biosensors have been reported for AED detection. Furthermore, CBZ biosensors often lack selectivity, require bulky equipment and special treatment rendering in-vivo detection illusory.

Reported studies of CBZ detection include techniques such as high-performance liquid chromatography (HPLC) (Mohamed Shah et al., 2013; de Almeida et al., 2013; Queiroz et al., 2008), spectrophotometry (Rezaei et al., 2005; Deepakumari and Revanasiddappa, 2014), cyclic voltammetry (CV) (Pruneanu et al., 2011; Lavanya et al., 2016), differential pulse voltammetry (DPV) (Pan et al., 2014; Lin et al., 2012; Wang et al., 2011), and amperometry (Unnikrishnan et al., 2012; Balasubramanian et al., 2018). Amongst the aforementioned techniques, those based on electrochemical detection (such as CV, DPV or amperometry) are utilized in a wide range of applications in medical, biological and environmental analyses due to their excellent speed, simplicity,

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low-cost and the potential of being integrated within implantable devices.

Despite their excellent characteristics, electrochemical based detection systems often lack sensitivity and selectivity. Consequently, sensitivity has been improved by altering the electrode's surface prior to template detection using different material such as graphene-gold nanoparticles (Pruneanu et al., 2011; Lavanya et al., 2016), iron doped tin dioxide nanoparticles, and graphene oxide-g- C_3N_4 composite films to increase surface conductivity and thus enhancing sensitivity (Balasubramanian et al., 2018). On the other hand, to permit the selective detection of CBZ, molecular imprinted polymers (MIPs) have been utilized (Khalilian and Ahmadian, 2016; Beltran et al., 2009; Mohiuddin et al., 2019). MIPs have proven to be robust, slightly affected by pH variations, reproducible, simple to fabricate, and most importantly, cheap. They are molecules designed to mimic the behaviour of antibodies.

Once the MIP is polymerized across the template, the template is then removed to leave a gap which resembles its structure. This gap is similar to the target template and has an affinity to bind to a molecule with a similar structure.

Chemically synthesized MIPs have outstanding characteristics: they have high sensitivity, are robust, are easy to prepare, and can be produced in bulk. However, they are difficult to immobilize on the electrode's surface never mind control their thickness. An alternative method involves preparing MIPs through electropolymerization which permits the formation of a MIP layer directly on the electrode's surface while controlling its thickness through the regulation of the electrochemical conditions (e.g. applied voltage, monomer concentration, etc). Electrochemical polymerization requires less time, less expertise, and costs less than additional polymerization. The most reported electropolymerized polymer for drug or bio-particle detection has been pyrrole (Schweiger et al., 2015; Nezhadali and Shadmehri, 2013; Hélder da Silva et al., 2014; Zhong et al., 2015). On the other hand, poly(3, 4-ethylenedioxythiophene) (PEDOT) has been reported to have superior conductivity, increased stability, lower density and operating voltages (Wang, 2009). In its doped state, PEDOT's conductivity is one order of magnitude higher than that of polypyrrole (PPy). Furthermore, its highly stable oxidized state maintains its conductivity for several months even at high temperatures (GueyeAlexandre et al., 2016; TemmerAli et al., 2013).

In the present study, a selective electrochemical sensor for the detection of CBZ was developed based on molecular imprinting of PEDOT and PPy. PEDOT was found to have superior electrical qualities than that of PPy. As provided in Fig. 1, in phase one of our experiment, molecular imprinted PEDOT (MIPEDOT) was prepared on GCE. Furthermore, scanning electron microscopy (SEM) images were taken to observe the sensor's morphology. Additionally, CBZ extraction validation was conducted through ultraviolet–visible (UV–vis) spectroscopy along with CV. In phase 2, CV measurements along with SWV were conducted to study the effect of CBZ concentration on the electrochemical response of the molecular imprinted PEDOT modified glassy carbon electrode (MIPEDOT-GCE). All characterization measurements were conducted in a phosphate buffered saline (PBS) solution. To assess the effect of CBZ on the current response of the sensors, a redox couple of $[Fe(CN)_6]^{3-/4-}$ was needed since CBZ is not electroactive within the potential window of PBS.

To our knowledge this is the first report of CBZ detection by molecular imprinted PEDOT GCEs.

2. Materials and methods

2.1. Reagents

CBZ powder was purchased from Sigma-Aldrich (Steinheim, Germany). All other commercially available reagents were purchased of analytical grade. Pyrrole along with 3,4-Ethylenedioxythiophene (EDOT) were purchased from Sigma-Aldrich, purified by distillation, purged by nitrogen, and kept in darkness at 4 °C. PBS was used as the electrolyte. Stock solutions of CBZ were prepared in methanol/water mixture and stored at 4 °C when not in use.

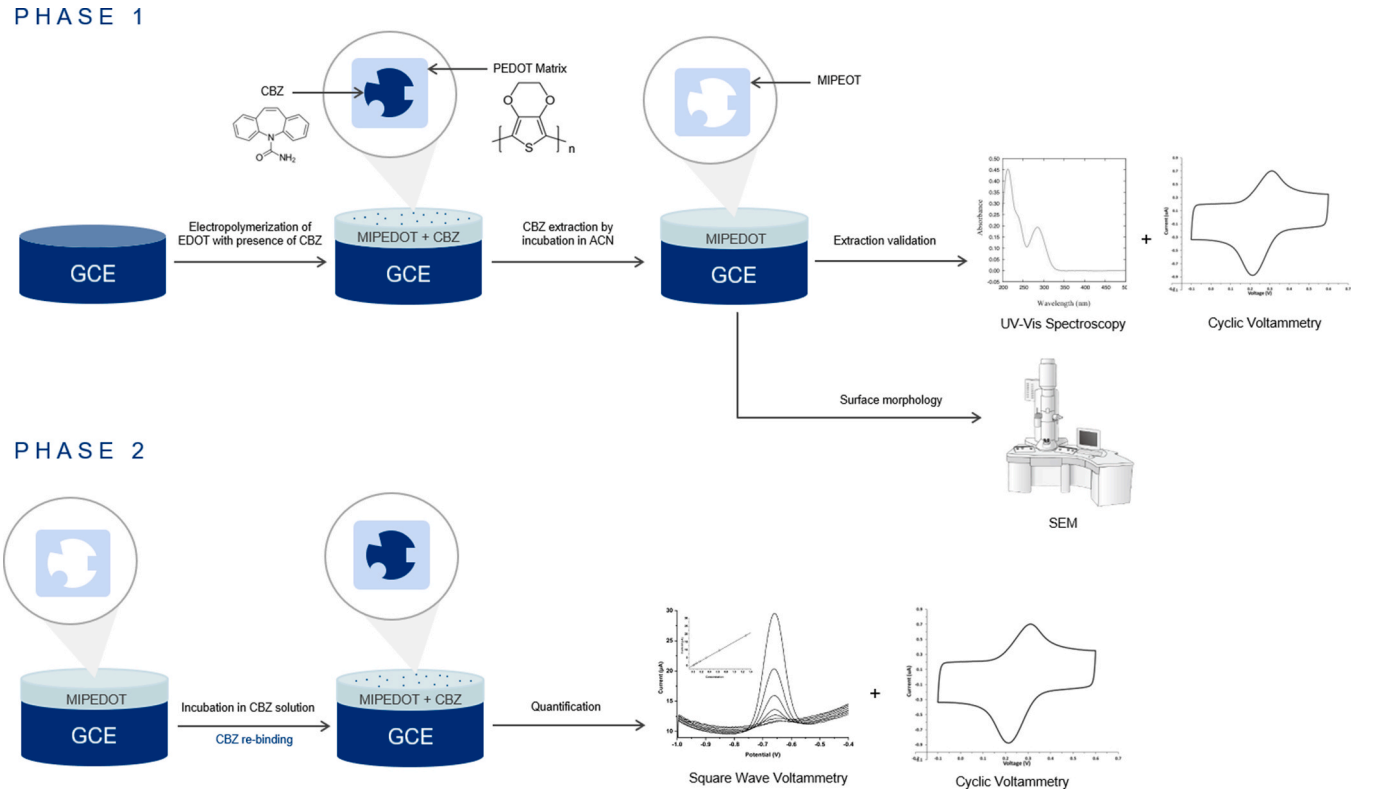

Fig. 1. Schematic illustration of experimental protocol.

2.2. Apparatus

A three-electrode setup was used for all measurements: a MIP-modified GCE (2 mm diameter) as the working electrode; a Pt wire electrode as the counter electrode; and a potassium chloride (KCl)-saturated Ag/AgCl electrode as the reference electrode. CV and SWV electrochemical studies were conducted using a Bio-Logic VMP-300 Multi Potentiostat equipped with EC-LAB software. SEM images were conducted at an electron acceleration voltage of 10 kV.

2.3. Preparation of PPy and PEDOT films

Bare GCEs were polished using 0.3 um and 0.05 um alumina slurry on micro-cloth pads followed by sonication in distilled water for 5 min. The electrodes were then sonicated in pure ethanol and again in distilled water for 5 min each. Followed by activation in sulfuric acid 0.5 M by CV at 100 mV/s between -0.2 V and +1.6 V for 20 cycles. The response of the bare GCEs was then recorded by CV scans in PBS solution containing $[Fe(CN)_6]^{3-/4-}$. The voltage was cycled from -0.2 V to +0.6 V at 100 mV/s until stable voltammograms were acquired. Finally, the electrodes were rinsed with distilled water followed by acetonitrile and left to dry at room temperature.

PPy was electropolymerized by immersing the GCEs in a solution of ACN containing 0.05 M $LiClO_4$ and 0.05 M pyrrole, followed by applying CV in the potential range of -0.0 V to +1.0 V for 10 cycles at a scan rate of 100 mV/s. PEDOT was prepared by dissolving 0.2 M Bu_4NBF_4 and 0.2 M EDOT in ACN and performing CV in the potential range of -0.0 V to +1.4 V for 10 cycles at a scan rate of 100 mV/s. Both PEDOT and PPy modified electrodes were slightly rinsed with ACN and left to dry at room temperature before characterization in PBS solution.

2.4. Preparation of molecular imprinted PEDOT-modified GCE

Similar to the preparation of PEDOT films, the modified MIPEDOT-GCE was prepared by washing the GCEs then immersing them in a solution of ACN containing 0.2 M Bu_4NBF_4 and 0.2 M EDOT with the addition of 0.015 M of CBZ. CV was then conducted in the potential range of -0.0 V to +1.4 V for 10 cycles at a scan rate of 100 mV/s. The imprinted template molecule was then extracted by immersing the electrode in ACN solution and constantly stirring for 30 min. The control (non-imprinted polymer electrode, NIP) was prepared similarly but without the addition of CBZ as a template molecule during the electropolymerization.

2.5. Electrochemical characterizations

CV measurements were conducted to study the oxidation and reduction potential of CBZ by immersing a bare GCE in a solution of CBZ dissolved in ACN and cycling its potential from -0.4 V to 1.0 V versus Ag/AgCl for 10 cycles at a scan rate of 100 mV/s.

CV measurements to compare the conductivity of PEDOT-GCE and PPy-GCE were conducted. Furthermore, the characterization of both the MIPEDOT-GCE and the NIPEDOT-GCE was carried out by CV and SWV measurements in a solution of PBS (PH = 7.4) containing 0.01 M $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl.

All SWV measurements were conducted by scanning the electrodes from -0.0 V to +0.7 V versus Ag/AgCl with a pulse height of 10.0 mV, pulse width of 50.0 ms and step height of 10.0 mV.

All other CV measurements conducted in PBS (PH = 7.4) with 0.01 M $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl were cycled from the potentials of -0.1 V to +0.6 V versus Ag/AgCl at 100 mV/s for 20 cycles.

Detection of CBZ was performed as follows: incubating the MIPEDOT-GCE and NIPEDOT-GCE in CBZ solutions for 15 min; measuring the electrode's response by SWV and CV; extracting entrapped CBZ molecules by immersing electrodes in ACN and stirring for 30 min; the electrodes were then utilized for successive detections. All measurements were undertaken at room temperatures.

3. Results and discussion

3.1. CBZ redox potentials

In order to determine the concentration of a template, its respective oxidation peak is measured. The template's concentration would have a direct effect on the electrode's response in solution. For such a relation to exist, the template must exhibit redox currents within the potential window of the solvent utilized. Initially, CV was conducted on a bare GCE in a solution of ACN with 0.1 M CBZ and 0.05 M $LiCLO_4$ and the potential of the GCE was cycled from -0.4 V to +1.0 V versus Ag/AgCl. No apparent peaks were found. This implies that no oxidation nor reduction current of CBZ are present in the redox potential window of PBS congruent with reported oxidation and reduction peaks between -2.3 V and -2.2 V versus Ag/AgCl (Atkins et al., 2010). Hence, a redox mediator such as $[Fe(CN)_6]^{3-/4}$ is needed to electrochemically probe the presence of CBZ at the electrode's surface.

We hypothesized that CBZ molecules present inside the polymer matrix on the electrode's surface could have an effect on the conductivity and the electroactive surface of the electrode. These changes in the conducting polymer properties would translate in a current variation due to the redox activity of $[Fe(CN)_6]^{3-/4}$.

3.2. Conductivity of PEDOT versus PPy

Conductivity of the modified electrode relies on several factors including its surface area, its composition, and its morphology. Typically, an electrode with higher conductivity is preferred in biosensors to increase sensitivity via an enhanced signal to noise ratio. Both PPy and PEDOT were electropolymerized on the GCE's surface and characterized by CV in a solution of PBS (PH = 7.4) with 0.01 M $[Fe(CN)_6]^{3-/4}$ and 0.1 M KCl. Fig. 2 shows the polymerization graphs: PEDOT offered larger redox currents, therefore PEDOT was chosen to be the imprinted polymer rather than PPy.

3.3. Characterization of modified GCEs

SEM micrographs are presented in Fig. 3. Porous structures are observed with the NIPEDOT Fig. 3(a) yielding a different morphology than that of MIPEDOT polymer Fig. 3(b). On the other hand, CBZ extraction Fig. 3(c) resulted in a further change in polymer morphology which could be related to CBZ molecules being separated from the polymer matrix. A more elaborate study of the effect of CBZ imprinting and extraction was conducted through performing CV in a solution of

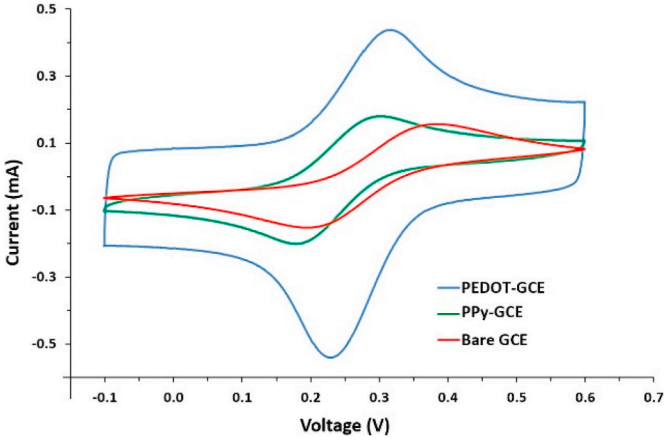

Fig. 2. Cyclic Voltammograms of PBS solution of 0.01 M $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl onto: Bare GCE, PPy-GCE, and PEDOT-GCE.

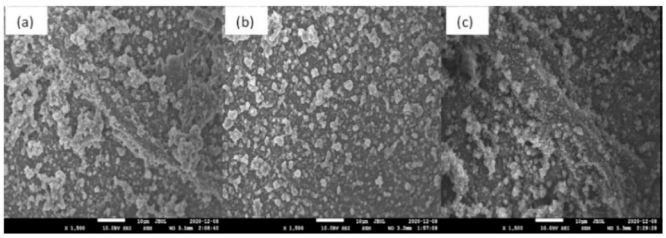

Fig. 3. Surface morphology using SEM; (a) NIPEDOT, (b) MIPEDOT pre-extraction of CBZ, (c) MIPEDOT post-extraction of CBZ.

PBS (PH = 7.4) with 0.01 M $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl.

Fig. 4 illustrates the response of the modified electrodes post-polymerization, and post-template extraction by incubation in ACN solution for 30 min with stirring. For NIPEDOT-GCE which was polymerized in the absence of CBZ molecules, there is no noticeable change in current after extraction of CBZ. On the other hand, there is a significant increase in redox currents post extraction of template from the MIPEDOT-GCE. This change in current magnitude for the MIPEDOT-GCE electrodes post extraction could be attributed to an increase of conductivity and electroactive area due to the absence of CBZ from the polymer matrix. However, more in-depth characterizations are needed to confirm this hypothesis.

3.4. Template extraction

One of the main challenges in electropolymerized-MIP-based sensors is the extraction of the template molecules from the polymer matrix. Exposing the electrode's surface to harsh extraction methods may risk delamination of the polymer. A few papers have reported extraction of the template through over-oxidation of the polymer (Hélder da Silva et al., 2014; Sun et al., 2013; Hrichi et al., 2019); however, over-oxidization of conducting polymers leads to loss of polymer activity. Alternatively, since CBZ is readily soluble in ACN, the extraction from the imprinted polymer was carried out by incubating the electrode in ACN for 30 min with constant stirring.

Extraction validation was implemented by conducting UV–vis spectroscopy on the ACN solution after incubation. A peak appeared at a wavelength of 230 nm reflecting successful extraction of CBZ from MIPEDOT-GCE. On the other hand, the ACN solution incubating NIPEDOT-GCE did not show any presence of CBZ.

3.5. MIPEDOT-GCE calibration curve

In order to establish the calibration curve of MIPEDOT-GCE to CBZ, both NIPEDOT-GCE and MIPEDOT-GCE were incubated in stock solutions of CBZ in methanol/water mixture (20:80). Electrodes were then removed, left to dry and then transferred into a solution of PBS (PH = 7.4) with 0.01 M $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl for SWV experiments. All measurements were performed in triplicates in order to achieve a standard deviation ensuring results are valid. SWV was conducted on a blank MIPEDOT-GCE to measure the response current. Following that, the electrode was incubated for 15 min in the prepared CBZ stock solutions from a range of 1.0×10^{-4} M to 2.0×10^{-3} M. SWV was conducted again to measure the variation in peak current due to the presence of CBZ molecules within the polymer matrix. The same electrode was then washed and incubated in another stock solution with different concentrations in order to finalize the experiments.

According to the SWV results there was no variation in oxidation current before and after NIPEDOT-GCE incubation; however the MIPEDOT-GCE showed a linear current response from $[Fe(CN)_6]^{3-/4-}$ redox activity with CBZ concentrations as presented in Fig. 5. Consequently, MIPEDOT- GCE can be used for quantitative analysis of CBZ.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 0.98×10^{-3} M and 2.97×10^{-3} M respectively according to the linear regression method (Shrivastava Guptaet al., 2011). The sensitivity of the polymer is dependent on the template extraction procedure, the conductivity of the polymer and the electrode's surface area. Through further optimization of the extraction protocol, a wider number of molecular imprinted gaps would be freed hence, enhancing detection. Furthermore, increasing both surface area and conductivity may be realized through introducing conductive graphene along with the polymer.

3.6. Future work and limitations

Table 1 summarizes the main advantages of the proposed sensor over existing sensors; molecular imprinted PEDOT sensors are selective, rapid, robust, easy to fabricate, and cost-effective. Future work involves implementing the modified electrodes on a complete system on chip enabling continuous detection and quantification of CBZ levels. Furthermore, the sensor is not limited to CBZ detection. Applications of molecular imprinted polymers can extend to encompass a wider range of AEDs. As such, electrodes could be designed containing multiple imprinted sites selective to a combination of AEDs.

Sensitivity of the proposed sensor is inferior to that of time-

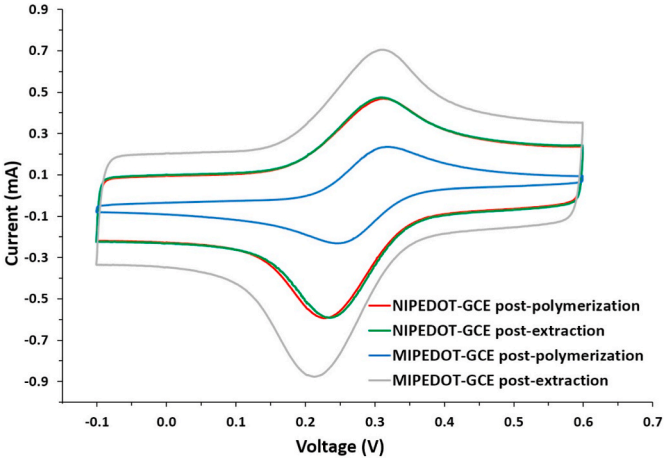

Fig. 4. Cyclic Voltammograms of NIPEDOT-GCE post-polymerization, NIPEDOT-GCE post-extraction, MIPEDOT-GCE post-polymerization, and MIPEDOT-GCE post-extraction.

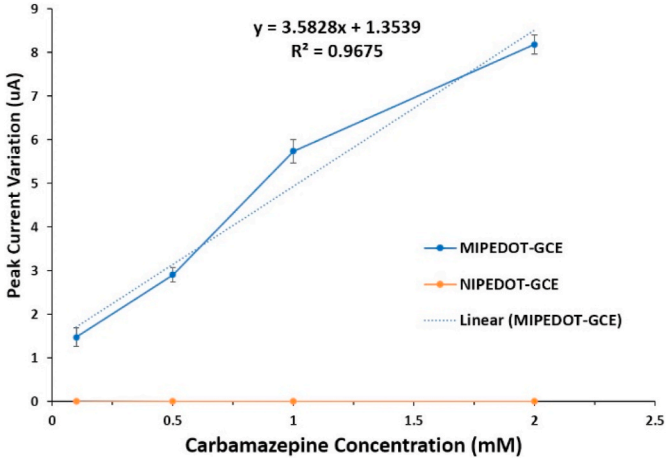

Fig. 5. Square wave voltammogram data of MIPEDOT- GCE's and NIPEDOT-GCE's peak current variation with respect to prepared CBZ stock solutions with different concentrations.

Table 1
Table of comparison.

Technique	Electrode	Selectivity	Complexity	Speed	Reference
SWV, CV	MIPEDOT-GCE	Yes	Simple	Fast	This work
CV	Au-Gr-AuNPs[a]	No	Simple	Fast	Pruneanu et al. (2011)
FPIA[b]	N/A	No	Moderate	Slow	Lin et al. (2012)
DLLME[c], RP-LC[d]	N/A	Yes	Complex	Slow	Mashayekhi et al. (2010)
LC-MS/MS[e]	N/A	Yes	Simple	Slow	Shokry et al. (2015)

[a] Graphene-gold nanoparticle composite deposited on gold electrode.
[b] Fluorescence polarization.
[c] Liquid chromatography-tandem mass spectroscopy.
[d] Reverse-phase liquid chromatography.
[e] Dispersive liquid-liquid microextraction method.

consuming, and complex chromatography-based methods. However, as previously mentioned, this limitation could potentially be overcome by adding graphene to the polymer. Graphene has excellent conductive properties and could vastly increase the sensitivity of the sensor. Another limitation to the proposed sensor is the template's extraction. Since CBZ molecules are well fused within the polymer matrix, removing them with rigorous methods deteriorates the sensor's performance through the delamination of the polymer. The utilized method through ACN incubation might not fully liberate the imprinted sites. Therefore, advanced extraction methods must be researched in order to provide complete template removal.

4. Conclusion

Both PPy and PEDOT were polymerized and their conductive properties have been investigated through CV measurements. The latter provided superior conductivity and robustness. As such, a novel electrochemical sensor for CBZ detection was reported using the molecular imprinting of PEDOT on GCE. A mediator $[Fe(CN)_6]^{3-/4-}$ was used to study the effect of CBZ levels on the sensor's response since CBZ provided no redox peaks within the redox potential of PBS. Polymerization of PEDOT was conducted via CV and the modified electrodes were characterized using both CV and SWV. Molecular imprinted PEDOT films were immobilized on the electrodes' surface through electropolymerization. SWV experiments were conducted to validate CBZ detection followed by SEM imaging and UV–vis spectroscopy to characterize surface morphology and the extraction protocol. Afterwards, subsequent to incubation in stock carbamazepine solutions, the sensor's LOD and LOQ were found to be 0.98×10^{-3} M and 2.97×10^{-3} M respectively. The proposed biosensor achieved great sensitivity and selectivity towards CBZ molecules. Although not as sensitive as chromatography or other complex techniques, this biosensor is fast, cheap, easy to fabricate and could be implemented on a system on chip. Furthermore, the biosensor's sensitivity could potentially be improved by introducing graphene to within the polymer matrix and enhancing the extraction protocol. This would increase conductivity at the electrode's surface and free up more polymer sites for successive detection increasing the signal to noise ratio.

CRediT authorship contribution statement

A. Hammoud: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft. **D. Chhin:** Writing – review & editing, Investigation, Resources. **D.K. Nguyen:** Supervision, Project administration, Writing – review & editing. **M. Sawan:** Supervision, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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