Asian Journal of Chemistry; Vol. 34, No. 6 (2022), 1526-1530

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2022.23705

Fabrication of Pyromellitic Acid Polymer Modified Electrochemical Sensor for the Determination of Tryptophan

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Received: 31 December 2021; *Accepted*: 16 March 2022; *Published online*: 18 May 2022; AJC-20817

Herein reporting a sensitive electrochemical strategy for the quantification of tryptophan on indium tin oxide electrode with an electropolymerized pyromellitic acid. In all perspectives, the characteristic features of the developed electrode were consistent with the requirements of a sensor. The morphological and functional identification of the modified electrode were done by scanning electron microscopy and infrared spectroscopy. The proposed electrode material has long-term stability and is easy to handle for real-life sample analysis. The fabricated electrode has a linear range of 10 to 300 µM with a limit of detection of 1.15 µM. The calculated sensitivity of the electrode was 1.3 µM/µA/cm^2.

Keywords: Tryptophan, Pyromellitic acid, Electropolymerization, Chemical sensors, Indium tin oxide.

INTRODUCTION

Electropolymerized chemical sensors can be explored as a feasible method for biomolecule quantification by improving the sensitivity and selectivity towards analytes. Electroconducting polymers (ECPs) are a significant type of conducting polymers with electronic properties like those of metals. In this way, these materials are referred to as manufactured metals then, they offer properties of regular organic polymers. ECPs are commonly used for the fabrication of chemical and biosensors due to their particular electrochemical properties [1-5]. Examples of ECPs include, polyacetylene, polyphenylene, polypyrrole, polythiophene and polyaniline [6,7]. The non-conductive polymeric (NCP) film on the electrode develops a thick film enough to be overly resistant to the transfer of electron between the electrode surface and the monomer molecules or radicals in solution [8].

Electropolymerization of a particular concentration of a monomer in the presence of an electrolyte of an acid or a base of a salt can yield conducting and non-conducting polymers [9]. The most widely recognized electrochemical technique for the synthesis of ECPs and NCPs is anodic oxidation of appropriate electroactive functional monomers [10]. In the anodic oxidation process, the growth of a polymer film and doping of counter ions takes place because of oxidation happen at the same time and the potential of monomer oxidation prompting polymerization is higher than that of charging of oligomeric intermediates or the subsequent polymer [5].

This work describes the quantification of tryptophan in real-life samples on an indium tin oxide (ITO) electrode by employing the electro polymerization of pyromellitic acid. Tryptophan serves as an essential amino acid and also a precursor to the biomolecule serotonin, the hormone melatonin and vitamin B$_3$ [11-13]. Reduced (15 µM) or increased (> 450 µM) concentrations of tryptophan in human body may influence the biological transformation of tryptophan to serotonin and kynurenine, resulting in a variety of physiological and neurological issues in the human body [14]. Hypertryptophanemia and Hartnup disease are caused by elevated tryptophan levels in human serum [15]. Emotional lability, hyperaggressive behaviour and myopia are symptoms of these physiological diseases [16,17]. Alzheimer's disease and pellagra [18] are the neurodegenerative disorders arises due to tryptophan deficiency.

To detect and determine the quantity of tryptophan in real-life samples, many methods have been reported, including, electrochemical sensors [19-27], HPLC, spectrophotometry and capillary electrophoresis [28,29]. Pyromellitic acid (PMA) was chosen for the development of electropolymerized ITO electrode, where PMA acts as a ligand [30] for molecular framework, supramolecular hydrogels [31] and coordination polymers [32] due to the presence of four carboxylic acid substituted on the aromatic ring. In this study, we used the electropolymerized pyromellitic acid (PMA) on indium tin oxide (ITO) to selectively quantify tryptophan in real-life samples.

EXPERIMENTAL

All the solvents and reagents used for the investigations were of analytical grade, while Millipore water (18.2 MΩ cm) was used for the preparation of solutions throughout the studies. Serotonin was purchased from Sigma-Aldrich, USA. Sodium dihydrogen orthophosphate (NaH_2PO_4), disodium hydrogen orthophosphate (Na_2HPO_4) and H_2SO_4 were purchased from Merck, USA. All other analytes and common reagents were purchased from Loba Chemie, India and used as received. A glass plate with one side coated with indium tin oxide (ITO) (1.1 mm thickness) and a resistivity of 35-40 Ωcm was acquired from Macwin, India.

Instrumentation: Electrochemical studies were performed with a CHI610E electrochemical analyzer (Bioanalytical system, USA) and an electrochemical workstation from CH Instruments, Austin, USA. The working electrode was a one-sided ITO coated glass plate electrode, the reference electrode was an Ag/AgCl (1M KCl) and the counter-electrode was a platinum wire. At room temperature (25 ºC), electrochemical measurements were performed. The pH measurement was performed using a digital Lab line pH meter. Scanning electron microscope (SEM) images were recorded using JoeL 6390 EO. Ultrasonic cleaning of electrodes was carried out in an ultrasonicator (Lab Equipment, Pvt. Ltd., India). Attenuated total reflection infrared spectroscopy was conducted on a Perkin-Elmer Fourier transform infrared spectrometer. Perkin-Elmer spectrometer was used for recording Ultraviolet-visible spectra.

Fabrication of electrode: Prior to modification, the ITO electrode (area of 10 mm^2) was sequentially sonicated in acetone and water for 10 min to remove any adsorbed substances on the electrode surface and then air dried. Before polymerization, a 10 mL solution of 2 mM PMA in 1 M H_2SO_4 was sonicated for 15 minutes. Cyclic voltammetric technique was employed for the synthesis of ITO/*p*-PMA by electropolymerization of a sonicated PMA solution on an indium tin oxide electrode. The polymerization window was from -0.2 V to +2.0 V at a scan rate of 100 mV/s for 10 cycles (Fig. 1a). The modified ITO electrode was then cleaned with Millipore water to remove the adsorbed PMA molecules on the ITO surface and allowed to dry.

Electrochemical measurements: A desired volume of tryptophan from 10 mM stock solution was taken to an electrochemical cell and diluted to a total volume of 10 mL with 0.1 M PBS (pH 5.0) as the supporting electrolyte. Using the electrochemical workstation, cyclic and differential pulse voltammograms were recorded within the potential window 0 V and 1 V at 0.1 V s^{-1}.

RESULTS AND DISCUSSION

Morphology studies: Scanning electron micrographs were used to compare the surface morphology of unmodified and PMA-modified ITO electrode surfaces. The SEM image of the ITO/*p*-PMA electrode surface (Fig. 1c) was found to be polymer-covered when compared to the SEM images of bare ITO (Fig. 1b).

FTIR studies: Fig. 2a depicts the FTIR spectra of an ITO and an ITO/*p*-PMA electrode over the 1500-400 cm^{-1} range. The absorption of In-O-In are represented by a strong band at 525 cm^{-1} for bare ITO and the absorption of Sn-O-Sn are represented by a band at 762 cm^{-1} [33]. The Si-O-Si stretching vibration in glass corresponds to the shoulder at 900 cm^{-1} [34]. The absence of corresponding In-O-In and Sn-O-Sn bands in the ITO/*p*-PMA spectra indicates the formation of polymer film on the ITO surface. The absence of PMA monomer's characteristic peaks such as a broad -OH stretching at 3069 cm^{-1}, a sharp -C=O signal at 1713 cm^{-1}, a -C-O stretching at 1153 cm^{-1} and a -OH bending at 803 cm^{-1} [35] confirmed the formation of polymer layer.

Electrochemical characterization: Fig. 2b depicts the electrochemical response of 5 mM $[Fe(CN)_6]^{3-}$ at a bare ITO (2.29 µA) and the modified ITO in 0.1 M KCl. The reddish line depicts the response of the NCP film of PMA on the bare ITO with $[Fe(CN)_6]^{3-}$ and a low current (1.1 µA) response indicating a poor electron transfer reaction than the bare ITO.

Electrochemical parameters: The differential pulse voltammetry (DPV) was used to optimize the monomer concentration, which was changed from 1 mM to 10 mM. Fig. 3a depicts the results of plotting the measured peak potential and peak current against concentration of monomer from 1 mM to 10 mM. The maximum current with the lowest potential for Trp's DPV response was obtained from 2 mM PMA in 10 mL solution of 1 M H_2SO_4. As a result, the optimal monomer concentration was determined to be 2 mM. The kinetics of the electron transfer

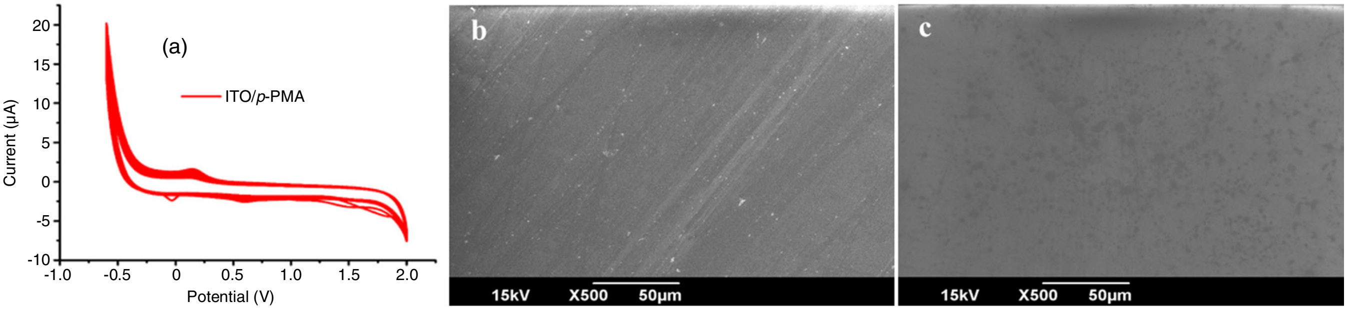

Fig. 1. (a) CV of electro polymerization of PMA on ITO, (b) SEM image of bare ITO and (c) SEM image of ITO/*p*-PMA

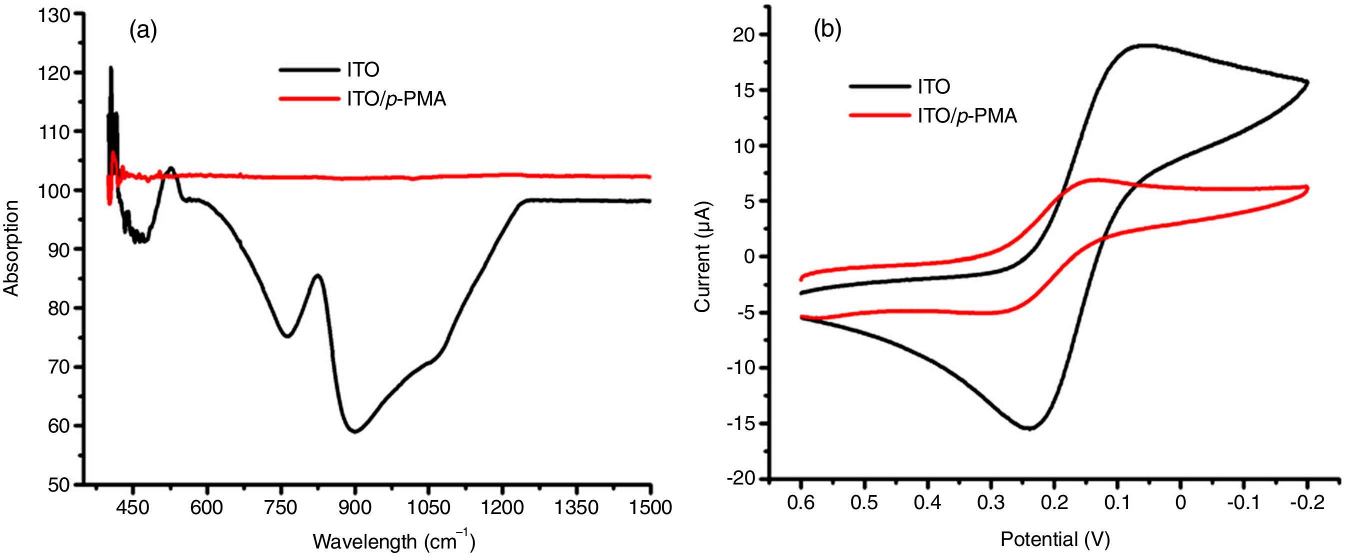

Fig. 2. (a) IR spectrum of the bare and the modified ITO, (b) CV response of bare and modified electrode in 5 mM $K_3[Fe(CN)_6]$ and 0.1 M KCl

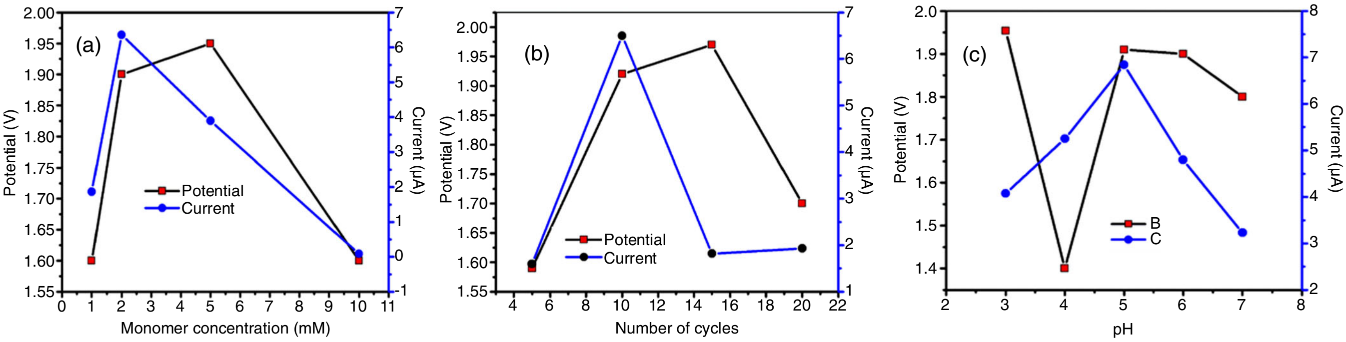

Fig. 3. (a) Monomer concentration *versus* peak potential and current, (b) number of polymerization cycles *versus* peak potential and current, (c) pH of 0.1 M PBS *versus* peak potential and current

process are influenced by the polymer thickness. The electropolymerization cycles were changed from 5 to 20 to optimize the film thickness of the polymer and cyclic voltammograms were recorded. Fig. 3b depicts a plot of the corresponding maximum current response over ten cycles. An increase in peak current was observed up to 10 cycles and further increasing the number of cycles resulted in a decrease in peak current. Because of the mass transfer resistance, the interaction of Trp with the polymer decreases as the film thickness increases. The effect of different electrolytes such as 0.1 M HCl, 0.1 M H_2SO_4, 0.1 M acetate buffer, 0.1 M NaOH and 0.1 M PBS on the oxidation current of tryptophan was investigated, with the 0.1 M phosphate buffer providing the best analytical response. The modified electrode was electrochemically tested against 100 µM tryptophan in 0.1M PBS with a pH range of 4 to 7, and the oxidation current to the pH of 0.1M PBS is plotted in Fig. 3c. Further analysis was carried out with 0.1 M PBS of pH 5 as the maximum peak current with the lowest potential was observed for pH 5. In acidic pH, the amine group in tryptophan molecule is protonated, resulting in a weak intermolecular interaction with the PMA film, whereas in alkaline pH, the acid group is not accessible for the interaction with the polymeric film [36].

Evaluation of sensor: The technique of DPV was investigated for the analysis of tryptophan on the ITO/*p*-PMA electrode in phosphate buffer medium with pH 5 (Fig. 4). A wide DPV signal of + 1.20 V was observed in bare ITO. This

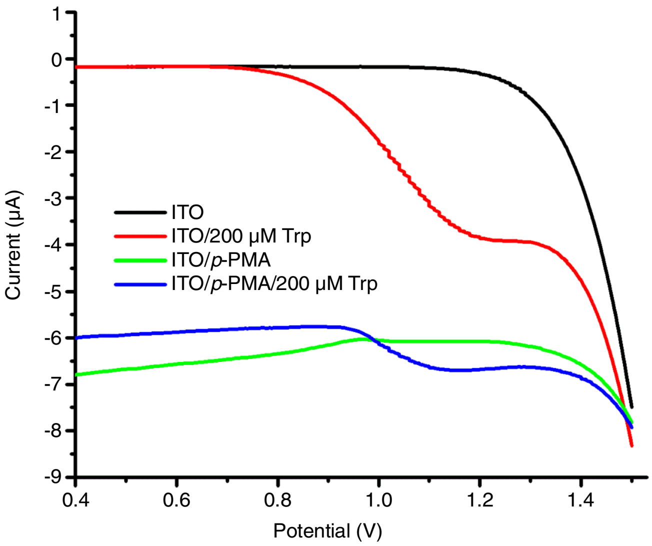

Fig. 4. DPV signals of 200 µM tryptophan on ITO and ITO/*p*-PMA

is equivalent to oxidizing 200 µM tryptophan with a current of 3.5 µA. ITO/*p*-PMA shows a wide signal at +1.21 V with a current of 0.63 µA, suggesting that the PMA polymer is electrically active with respect to tryptophan. Despite the fact that the peak current of tryptophan at ITO/*p*-PMA (0.63 µA) is lower than that of bare ITO (3.5 µA), it has been shown that the linear range and selectivity of other interfering molecules are large.

Fig. 5a shows the determination of tryptophan concentration from 10 to 300 µM using the fabricated ITO/*p*-PMA electrode. As shown in Fig. 5d, the anodic peak current of tryptophan from ITO/*p*-PMA linearly increased with the concentration. The first calibration segment ranged from 10 to 80 µM and had a regression coefficient (R) of 0.99 (N = 3), while the second ranged from 80 to 300 µM and had an R-value of 0.99 (N = 5) (Fig. 5b). These findings support the proposed electrode's practical applicability. Table-1 summarizes a comparative literature study on electrochemical methods using ITO/*p*-PMA to quantify tryptophan. The sensitivity of the electrode to tryptophan was 1.3 µA/µM/cm^2, the quantification limit was 10 µM, and the lower limit of detection was 1.15 µM.

TABLE-1
SUMMARY OF A COMPARATIVE LITERATURE STUDY ON ELECTROCHEMICAL METHODS USING ITO/*p*-PMA TO QUANTIFY Trp

Electrode	Method	Linearity (µM)	LOD (µM)	Ref.
MCPE/MWCNTs	Amperometry	0.6-100	0.03	[11]
GCE/AgNPs/MIL	DPV	1-150	0.14	[13]
ITO/AuNP-MWNT	SWV	0.5-90	0.025	[14]
GCE/ PARS/AuNPs	DPV	0.02-20.0	0.006	[22]
CPE/LCPFs	Amperometry	0.05-5	0.01	[23]
ABPE/GR	LSV	0.1-100	0.06	[26]
CILE/GNP	DPV	5-900	4	[27]
ITO/APTES/GO/ HAS	CV	100-1000	-	[28]
ITO/*p*-PMA	DPV	10-300	1.15	This work

Selectivity, reproducibility, repeatability and stability of the sensor: Interference of biomolecules and macro ions in the human body has been studied for the use of electrodes in actual sample analysis. Selectivity studies conclude that sensors are selective for other biomolecules in real samples except serotonin, tyrosine, dopamine and ascorbic acid. As a result, the ITO/*p*-PMA electrode demonstrated the selectivity of tryptophan from other biomolecules in the real sample. The proposed ITO/*p*-PMA electrode exhibit high reproducibility with repeatability and stable for 25 days.

Real-life sample analysis: Normal tryptophan levels in human serum have been reported to be 15-65 µM and the developed electrodes were used to quantify tryptophan in human blood at 10-300 µM. Serum was separated from blood samples and the response of ITO/*p*-PMA electrode was analyzed. There was no response of the developing electrode to tryptophan in the blood sample, so the spike analysis was conducted to quantify it.

Conclusion

Herein, reported the use of pyromellitic acid as a monomer for electropolymerization on an indium tin oxide (ITO) electrode for the real-life sample analysis of tryptophan ranging from 10 to 300 µM. The proposed sensor's linear range and selectivity were comparable to other reported electroanalytical techniques for quantifying tryptophan. The percentage of recovery of the spiked quantity of tryptophan from the sample of serum improved the analytical utility of the developed sensor.

ACKNOWLEDGEMENTS

The authors thank Amrita Vishwa Vidyapeetham, Amritapuri campus, for providing internal support to carry out the research work, as well as Mahatma Gandhi University, Kottayam, for

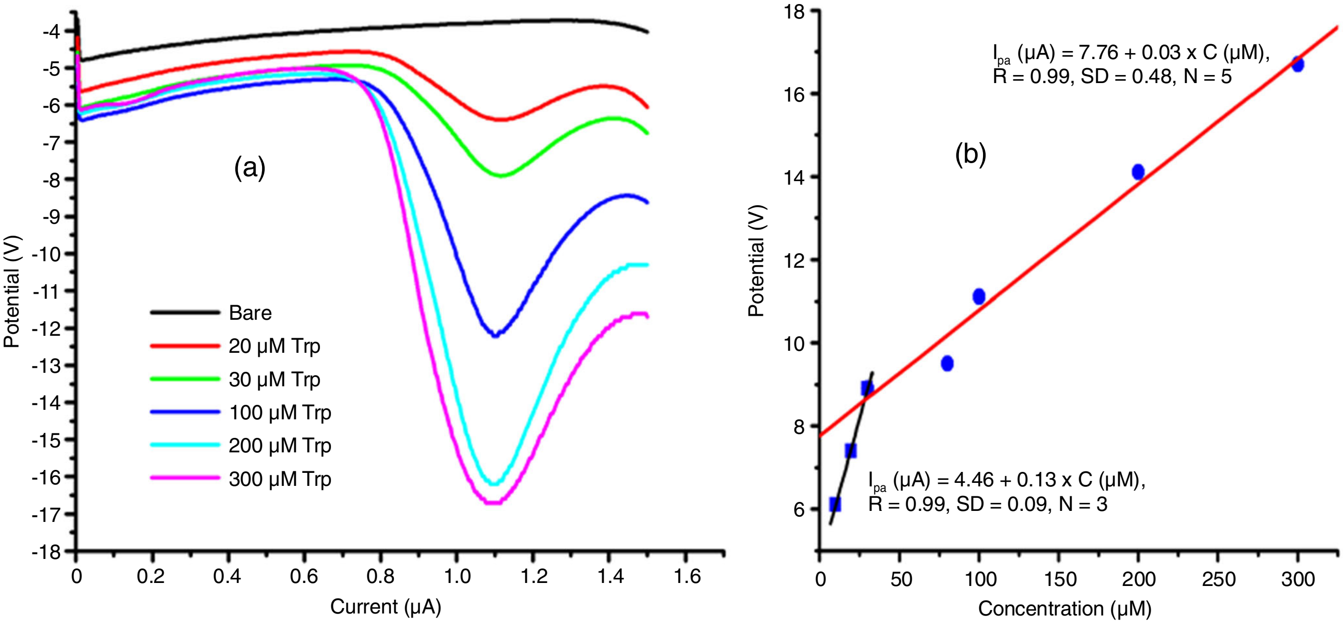

Fig. 5. (a) Concentration range of tryptophan and (b) the calibration graph

conducting the scanning electron and infrared spectroscopic analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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