Electronic Supplementary Material (ESI) for New Journal of Chemistry.

A facile and economic electrochemical sensor for Methyl malonic acid: A potential biomarker for Vitamin B_{12} deficiency

Supplementary material file

SI. Results and discussion

SI 1. Electrochemical preparation of PEDOT/PGE and Ag-PEDOT/PGE electrodes

The uniformity and homogeneity of the PEDOT layering the bare PGE surface can be controlled by the successive potential cycles. The electro-deposition of PEDOT was carried out by dipping the bare PGE in an electrolyte containing 0.1M EDOT, 0.01M surfactant, and 0.1M H_2SO_4 in an aqueous medium, for 25 successive cycles in potential range -1.9 V to 1.9 V at a scan rate of 0.05Vs^{-1} (Fig. S1).

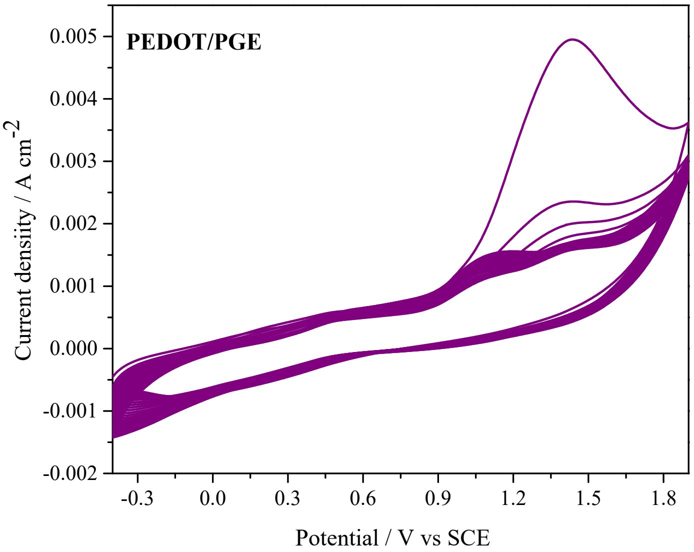


Figure S1 Electrodeposition of PEDOT on PGE by 25 scanning potential cycles at a scan rate of 0.05 V/s in an electrolyte medium of (0.01 M) EDOT, (0.01 M) SDS, and H_2SO_4 (0.1 M) H_2SO_4 in an aqueous medium.

The mass of PEDOT deposited on the PGE was estimated using Faraday's law. The quantity of charge utilized for deposition of PEDOT was employed to estimate the quantity of PEDOT deposited which was revealed to be 56.72 μg/cm^2. The electropolymerization reaction of EDOT to PEDOT is represented in Scheme 2.

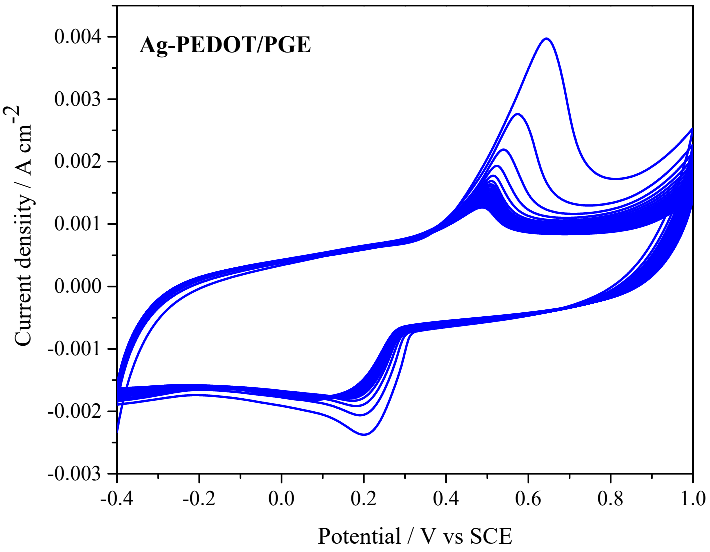


Figure S2 Electrofunctionalisation of Ag nanoparticles on PEDOT/PGE by 25 scanning potential cycles at a scan rate of 0.05 V/s in an electrolyte medium of (0.01 M) EDOT, (0.01 M) SDS, and H_2SO_4 (0.1 M) H_2SO_4 in an aqueous medium.

the redox peaks for the electrofunctionalisation of Ag nanoparticles over the PEDOT modified PGE were found to be at 0.2 and 0.6 volts. It can be said that the reduction of Ag+ ions is diffusion-controlled and PEDOT serves a simple conducting film [26] (Fig. S2).

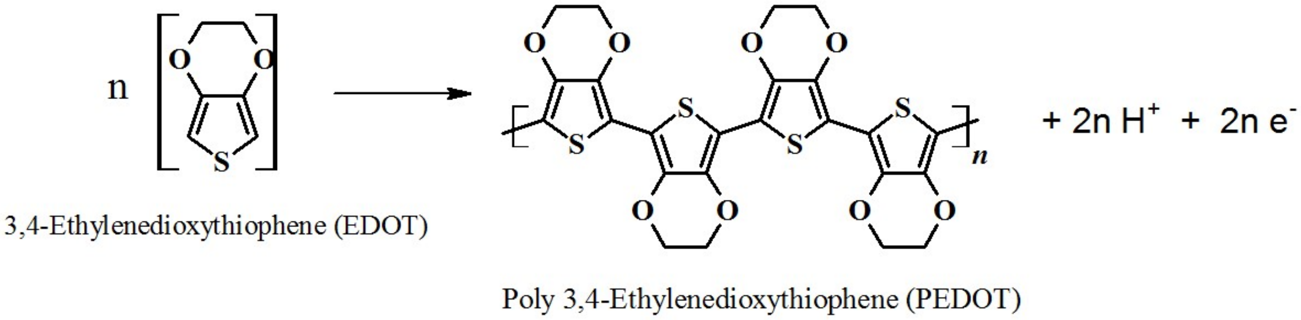
SI 2. Determination of electrochemically active surface area using potassium ferrocyanide/ferricyanide at bare PGE, PEDOT/PGE, and Ag-PEDOT/PGE electrodes

The voltammetric behaviour of all the electrodes (bare PGE, PEDOT/PGE, Ag-PEDOT/PGE) towards $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ were studied at a scan rate of 0.05 V/s (Fig. S3). The effective surface area of the electrode was determined using the data obtained from the redox couple of cyclic voltammograms. Respective slopes were obtained by drawing graphical plots between anodic peak currents and the square root of scan rates. The values of the slope, concentration, and diffusion coefficient (5.6×10^{-4} cm^2 s^{-1}) were substituted in the Randles-Sevcik equation (Eq. S1) to calculate the electrochemically active surface areas of the electrodes (bare PGE, PEDOT/PGE, and Ag-PEDOT/PGE).

The Randles–Sevcik equation (equation S1)[30]

$$i_p = 2.69 \times 105 A D_0^{1/2} n^{3/2} v^{1/2} C \qquad \text{(S1)}$$

Where n represents the number of electrons participating in the redox reaction, A indicates the electroactive surface area in cm^2, D_0 is the diffusion coefficient in cm^2/s, C is the concentration of $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ in the bulk solution in mol/cm^3 and $\nu^{1/2}$ is the scan rate in V/s. The value of electroactive surface area calculated for bare PGE, PEDOT/PGE, and Ag–PEDOT/PGE electrodes were, respectively 1.26 cm^2, 3.52 cm^2, and 8.72 cm^2. The results depict that the surface area for the bare PGE electrode is low in comparison to the surface areas calculated for the electrochemically modified electrodes (PEDOT/PGE and Ag–PEDOT/PGE). The increase in the surface area of the modified electrodes can be attributed to the deposition of thin layers of PEDOT over the bare electrodes thereby increasing the surface area as a whole. It also suggests the highly porous morphology of PEDOT which enhances the uniform dispersion of Ag nanoparticles over the electro-polymerized PEDOT pertaining to further increase in the active surface area. All of this can be justified by the enhanced current density value obtained for the modified electrodes when compared to bare PGE (Fig. S3).



Scheme S1 The schematic representation of electropolymerization of EDOT to PEDOT.

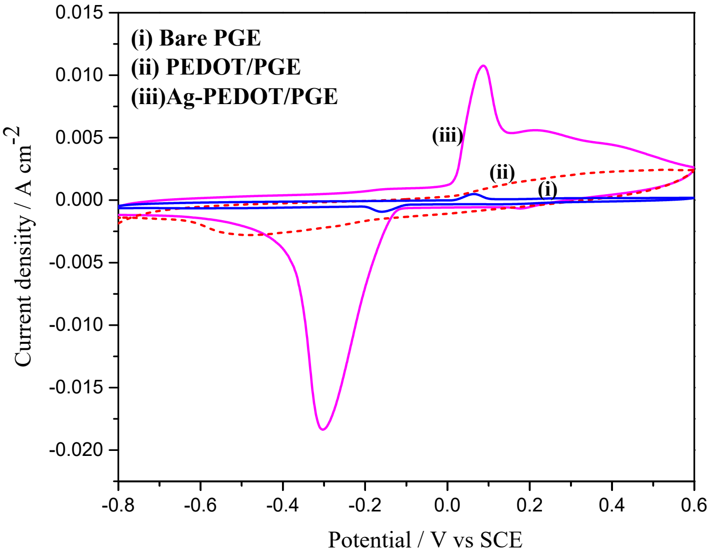


Figure S3 Cyclic voltammograms of 1 mM potassium ferrocyanide/ferricyanide at bare PGE (curve i), PEDOT/PGE (curve ii), and Ag-PEDOT/PGE (curve iii) in 1 M KCl.

SI 3. Determination of conducting behaviour of electrodes using Electrochemical impedance spectroscopy (EIS).

Electrochemical Impedance Spectroscopy (EIS), being an effective technique for determining the interfacial properties of an electrode surface, was employed for studying the characteristics of charge transfer processes at the electrode interface. The EIS measurements displaying the charge transfer behaviour of the electrodes are compared and represented as Nyquist plots. The Nyquist plots of bare PGE (curve a), PEDOT/ PGE (curve b), and Ag-PEDOT/PGE (curve c) electrodes in 1 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ using 1 M KCl as the electrolyte are shown in the (Fig S4).

The EIS measurements data were fitted to a suitable Randles equivalent electrical circuit model which comprises parameters like Warburg coefficient (W), double-layer capacitance (C_{dl}), electrolyte solution resistance (R_s), and resistance to charge transfer resistance at electrode surface (R_{ct}). The parameter R_{ct} can control the electron transfer kinetics at the electrode surface for redox processes of species $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$. This parameter varies with the adsorption of modifiers at the electrode surface and can be stated as inversely proportional to the electrical conductivity on the electrode surface i.e., lesser is the R_{ct} value, higher or enhanced is the conducting property at the electrode surface and vice versa. Charge transfer resistance (R_{ct}) at the electrode surface can be calculated from the semicircular diameter of the

Nyquist plots. Favourable diffusion activity of $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ towards the developed Ag-PEDOT/PGE electrode is indicated by the increase in the slope of the linear portion (Warburg impedance) in the plot. The double-layer capacitance seemed to increase on consecutive modifications of bare PGE to PEDOT/PGE and Ag-PEDOT/PGE. The R_{ct} value obtained from the Nyquist plot (curve i) of bare PGE was significantly high with a value of 127.3 Ω, showing that there is high charge transfer resistance at the electrode surface pertaining to poor conductivity and low sensitivity. This R_{ct} value is significantly reduced to 74.8 Ω when bare PGE was modified with PEDOT polymer. The moderately low value of charge transfer resistance is indicative of higher conductivity at the electrode surface and also implies that PEDOT is a conducting polymer. The electrodeposition of Ag on PEDOT/PGE to form the final modified electrode reduced the R_{ct} value immensely to 47.5 Ω. This further dip in the R_{ct} value confirms a better conducting nature of the final electrode sensor (Ag-PEDOT/PGE) in comparison to the PEDOT modified PGE. Thus, the low R_{ct} value infers that modification of the PEDOT/PGE with Ag nanoparticles facilitates enhanced conductivity towards the determination of $[Fe(CN)_6]^{3-/4-}$ and confirms faster electron transfer kinetics. The above results when summed up, concludes that the final modified electrode can act as an excellent electrochemical sensor and serves as an efficient platform for analyte detection.

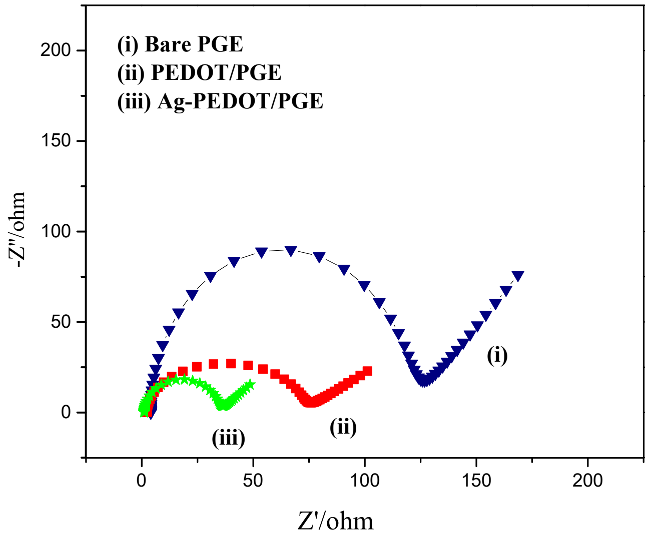


Figure S4 Nyquist plots of bare PGE (curve i), PEDOT modified PGE (curve ii), and Ag-PEDOT/PGE (curve iii) electrodes in 5 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ solutions containing 0.1 M KCl.

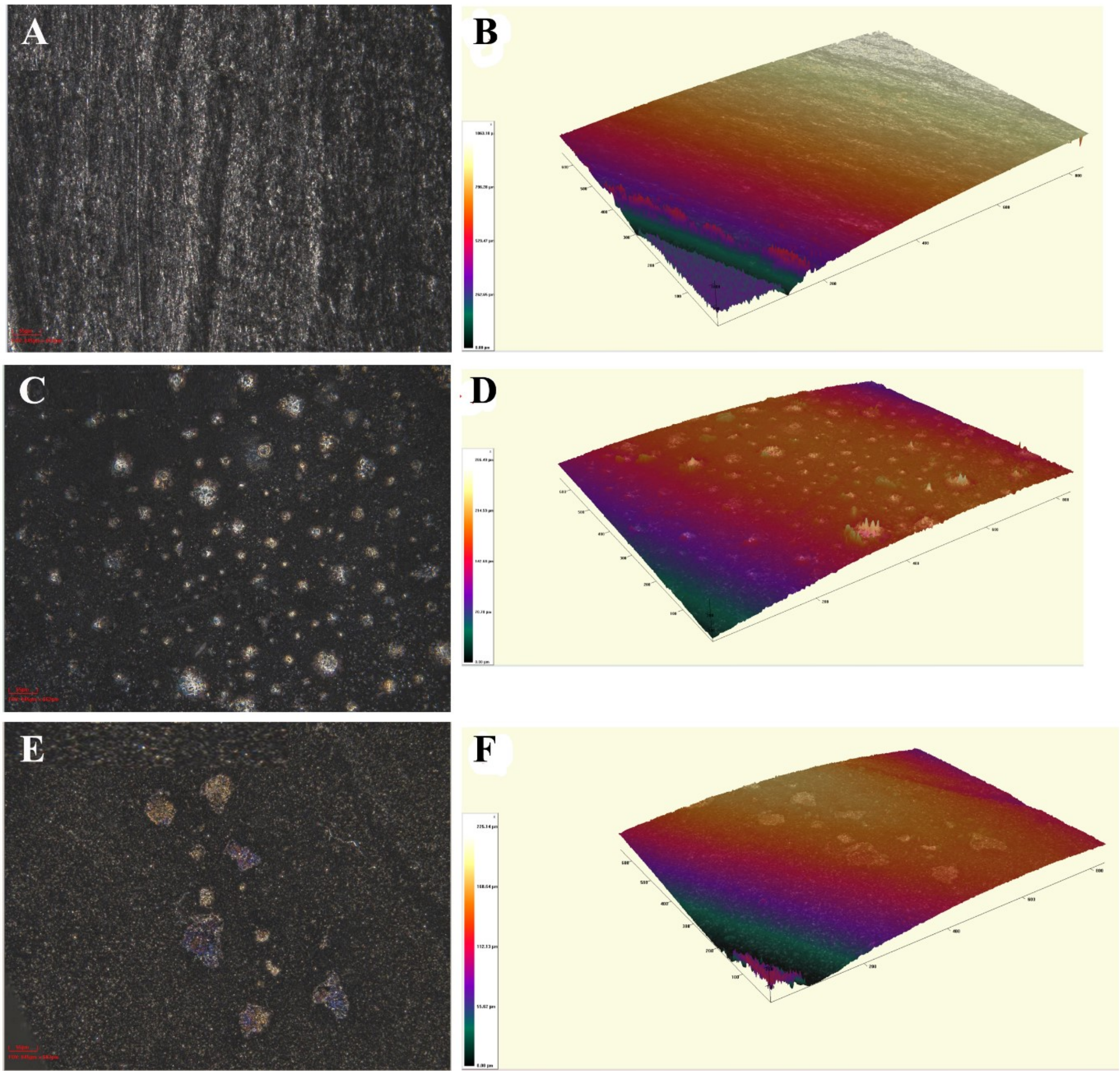


Figure S5 Optical profilometer images at magnification 20x: Two dimensional (2-D) and three dimensional (3-D) images of bare PGE (a and b), PEDOT/PGE (c and d), and Ag-PEDOT/PGE (e and f) electrodes.

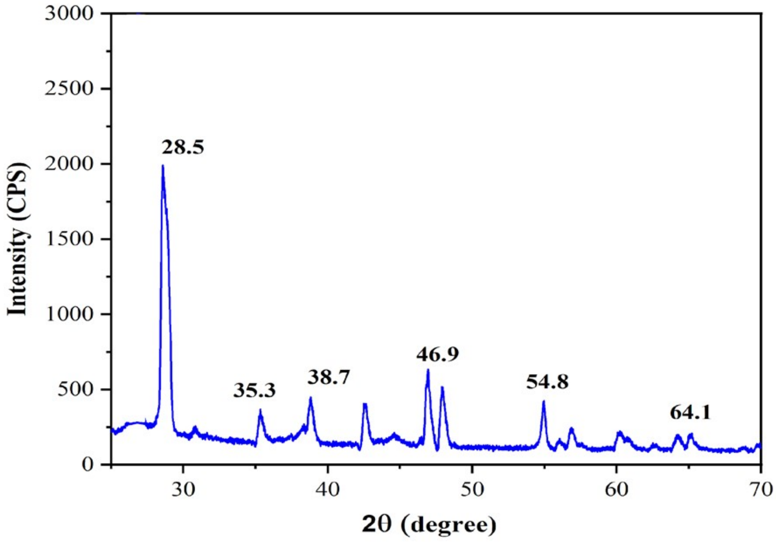


Figure S6 XRD pattern of Ag-PEDOT nanocomposite deposited on PGE.

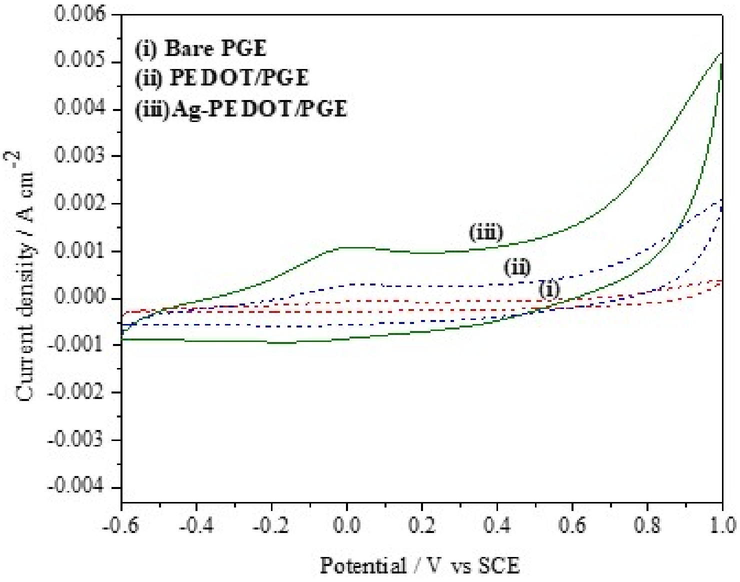


Figure S7 CVs of MMA (30 nM) in PBS (pH 7.0) at a scan rate of 0.05 V/s (i) bare PGE (ii) PEDOT/PGE (iii) Ag-PEDOT/PGE.

Figure S8 Cyclic voltammograms of 30 nM MMA at pH 2, 4, 6, 7, 8, 10 and 12.

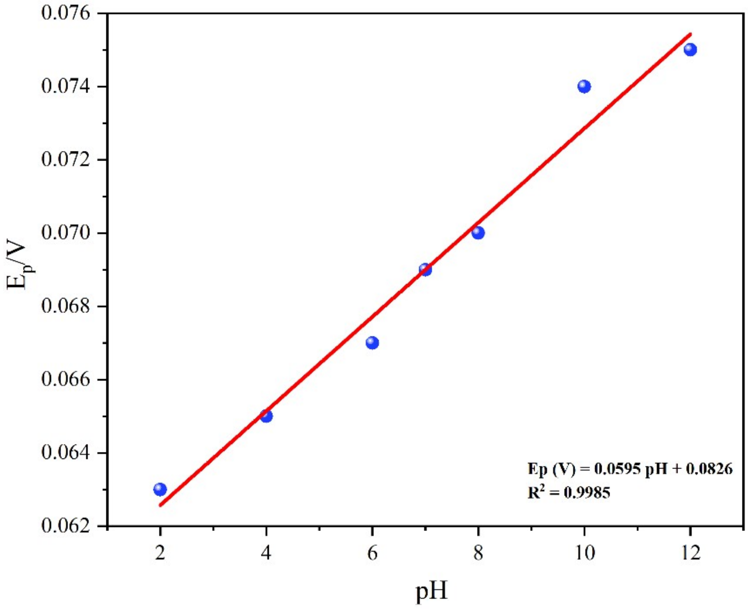


Figure S9 Dependence of cathodic peak potentials on pH at PEDOT/PGE.

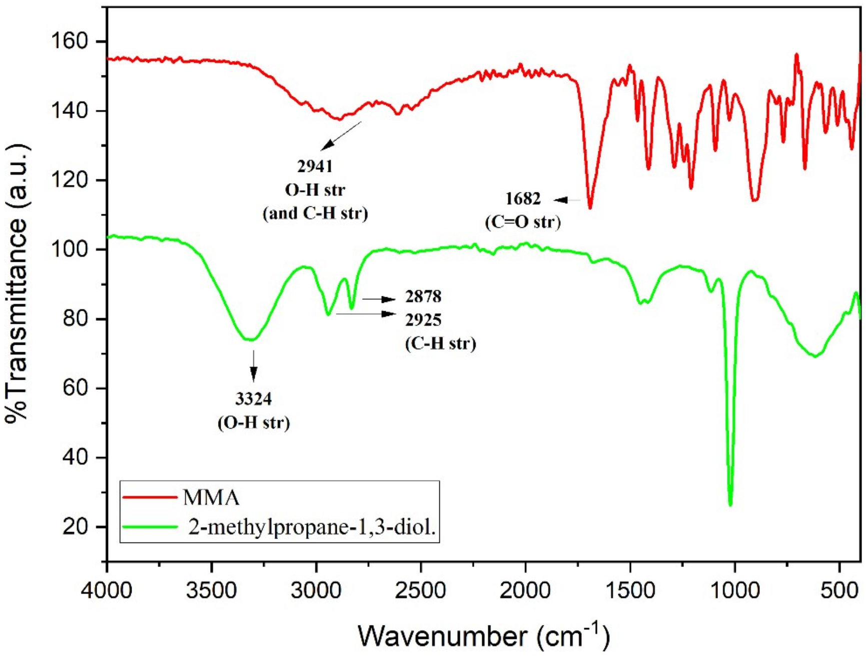


Figure S10 FTIR spectra of (i) MMA and (ii) 2-methylpropane-1,3-diol.

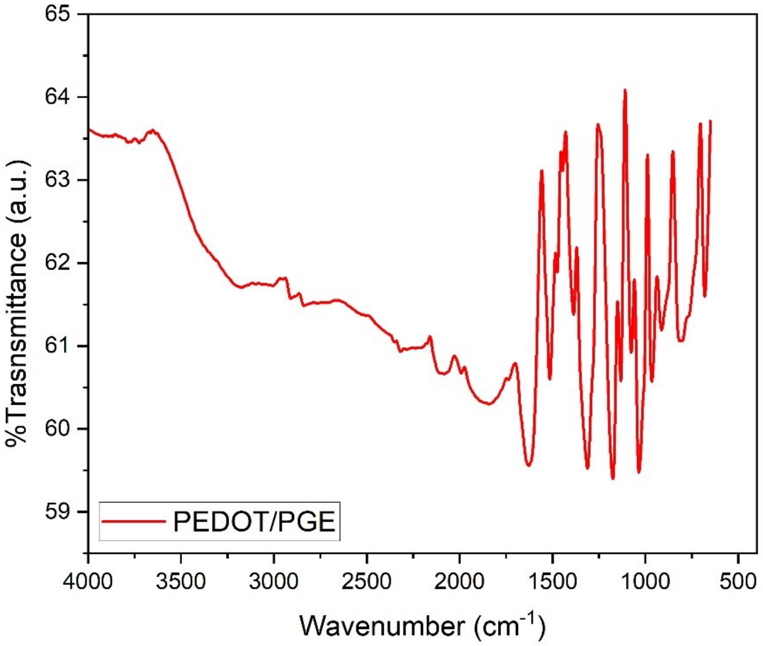


Figure S11 FTIR spectra of PEDOT/PGE.

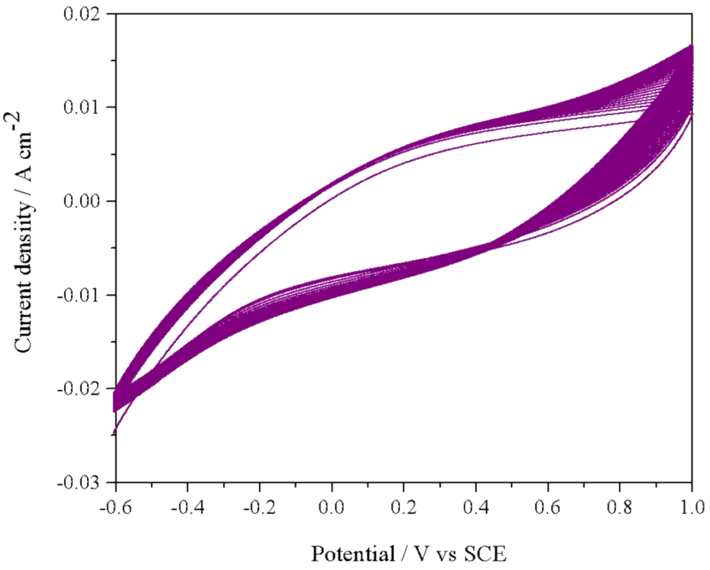


Figure S12 CV of 40 continuous cycles for Ag-PEDOT/PGE in PBS (pH 7.0) without MMA.

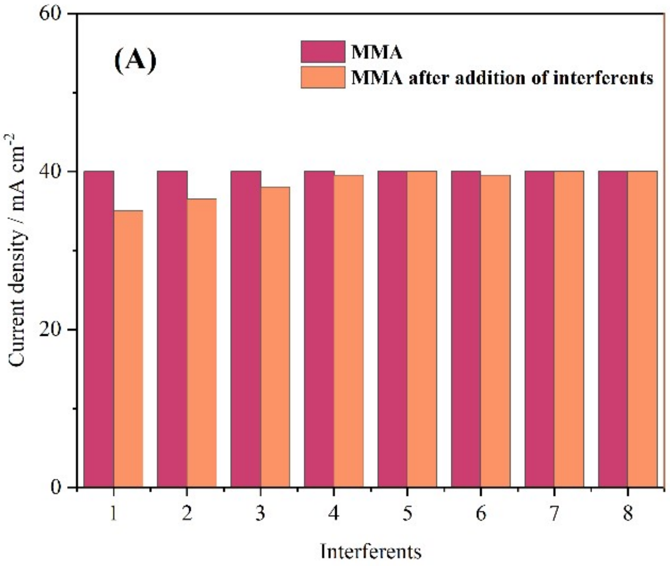


Figure S13 Influence of various interferents on the DPV current density values for quantification of MMA at Ag-PEDOT/PGE in PBS (pH 7.0); X-axis (Interferents): 1 - Homocysteine, 2 - Succinic acid, 3 – Ascorbic acid, 4 – Malic acid, 5 - Glucose, 6 – Uric acid and 7 – Urea, 8 – Glycine.

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Table S1 Roughness values were acquired for electrode surfaces utilizing an optical profilometer.

Electrode	**Root Mean Square Roughness (µm)**	**Standard Deviation**
Bare PGE	2.3186	0.621
PEDOT/PGE	5.9216	0.725
Ag-PEDOT/PGE	9.1046	0.835

Table S2 Comparison of efficiency of the developed electrochemical sensor (Ag-PEDOT/PGE) with other reported methods.

S.No	Technique used	Detection limit	Comments	Reference
1.	Isotope-dilution gas chromatography–Mass spectrometry (GC-MS) method	0.03 µM/L	The reported GC–MS analysis was found to be having high selectivity, good sensitivity, and heightened precision.	48
2.	HPLC combined with liquid chromatography electrospray ionization tandem mass spectrometry	0.1 µM/L	Facile and dependable high efficiency technique for MMA quantification that is applicable for automation.	49
3.	Liquid chromatography (LC)-MS/MS system using TurboIon Spray ionization and quantified by the multiple reaction monitoring mode of MS/MS	0.05 µM/L	The LC–MS/MS based MMA analysis requires minimal chromatographic separation which takes in < 60s per sample.	50

4.	Electrochemical sensing	0.2095 μM/L	The proposed electrochemical sensing method has a LOD and LOQ of 0.2095 μM and 0.2935 μM, respectively. In addition, the fabricated electrode required no pre-treatment of biological samples, was stable and reproducible.	23
5.	Electrochemical sensing	1.32 pM	The developed sensor exhibits a broad linear dynamic range (4.01 pM - 52.5 nM) with a very low detection limit (1.32 pM).	35
6.	Electrochemical sensing	0.16 pM	The reported electrochemical sensing methodology showcased a wide linear dynamic range (0.50 pM - 55 nM) and ensures high selectivity along with rapid detection.	Present work

Table S3 Repeatability study of Ag-PEDOT/PGE

Working Electrode Ag-PEDOT/PGE	**Potential (V)**	**Peak current value (A)**	**RSD (%)[a]**
1	**-0.135 V**	**0.0011**	**0.96**
2	**-0.134 V**	**0.0011**	**0.89**
3	**-0.135 V**	**0.0012**	**0.98**
4	**-0.136 V**	**0.0015**	**0.95**
5	**-0.133 V**	**0.0014**	**0.93**

- [a] Mean value of five determination
- Concentration of MMA taken is 30 nM