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Innovative electrochemical sensor for the precise determination of the new antiviral COVID-19 treatment Favipiravir in the presence of coadministered drugs

Mona A. Mohamed^{1,2§}, Ghada M. G. Eldin^{§1}, Sani M. Ismail², Nadia Zine², Abdelhamid Elaissari², Nicole Jaffrezic-Renault^{*2}, and Abdelhamid Errachid^{*2}

¹*Pharmaceutical Chemistry Department, National Organization for Drug Control and Research, Egyptian Drug Authority, Giza, Egypt*

²*Institut de Sciences Analytiques (ISA)-UMR 5280, Université Claude Bernard Lyon 1, 5 rue de la doua, 69100 Lyon, France*

§Equal first author contribution

Abstract

Due the current pandemic of COVID-19, an urgent need is required for serious medical treatments of a huge number of patients. The world health organization (WHO) approved Favipiravir (FAV) as a medication for patients infected with corona virus. In the current study, we report the first simple electrochemical, greatly sensitive sensor using MnO₂-rGO nanocomposite for the accurate determination of Favipiravir (FAV). The developed sensor showed a high improvement in the electrochemical oxidation of FAV comparing to the unmodified screen-printed electrode (SPE). The suggested platform constituents and the electrochemical measurements parameters were studied. Under optimal experimental parameters, a current response to the concentration change of FAV was found to be in the linear range of 1.0×10^{-8} – 5.5×10^{-5} M at pH 7.0 with a limit of detection 0.11 μ M and a quantification limit of 0.33 μ M. The developed platform was confirmed by the precise analysis of FAV in real samples including dosage form and plasma. The developed platform can be applied in different fields of industry quality control and clinical analysis laboratories for the FAV determination.

Keywords: COVID 19, Favipiravir, Graphene derivatives, real sample analysis

*To whom correspondence should be addressed:

nicole.jaffrezic@univ-lyon1.fr and abdelhamid.errachid-el-salhi@univ-lyon1.fr

1. Introduction

During the year 2020, the world faced the worst public-health crisis in past hundred years, COVID-19 pandemic. Coronaviruses are large genome viruses of the *Nidovirales* order, with a positive-strand RNA [1]. European and almost all countries have applied non-pharmaceutical involvements, like school closure and national lockdown that lead to huge economic crises [2]. Regardless of the major efforts to stop its spread, COVID-19 has affected considerable health and financial burden, stressing the urgent need for antiviral treatments[1]. Great efforts have been devoted to the development of an antiviral agent for the halt of the progression of this ailment. During the pandemic influenza in Japan 2014, Favipiravir (FAV) was approved for the treatment of that ailment which showed high potential for the *in vitro* activity against the acute respiratory syndrome coronavirus-2 [3]. FAV was first applied as a medication against COVID-19 in China-Wuhan, at the red zone of the pandemic origin. After that as the pandemic been in Europe, FAV got approval for emergency use in Italy, and currently has been in use in Japan, Russia, and many other countries around the globe including Egypt [4]. The main advantages of using FAV to treat a new indication, a process called “drug repurposing,” are that FAV is available in high doses and the safety measurements have previously been conducted on a high patients number [5, 6]. Accordingly, the approved medical agent FAV has the potential to be rapidly used on a large scale and be used as a first line of protection to administer to suspect or contact cases during a pathogen lockdown [5].

Based on the above mentioned facts, there is urgent need to develop a sensitive, reliable, fast, low expensive analytical method to trace and determine FAV individuality and in the presence of co-administered drugs. By reviewing the previously published works regarding the determination of FAV, only few methods have been reported for the determination of FAV including HPLC method [7], and spectrofluorometric method [8]. One electrochemical method has been reported for the single determination of FAV[9].

The MnO₂ nanomaterials have been used in different applications due to their high capacitance and low cost preparation; they are ecofriendly nanomaterials with an extraordinary stability in alkaline solutions[10]. By the incorporation of carbon with a high electrical conductivity, the conductivity of MnO₂ nanomaterials were further enhanced, reduce the equivalent resistance and charge-transfer resistance [10-12]. Graphene nanostructured materials have received great

attention in the past recent years as these materials offer high conductivity, excellent mechanical characteristics, high chemical/thermal stability, with tremendous surface area [13-16].

So, due to the urgent need to develop a fast, precise and sensitive strategy for the sensitive determination of FAV, we have fabricated the present electrochemical protocol. In this work, hydrothermal one-pot strategy was developed to prepare directly MnO₂-rGO nanocomposite using graphene oxide (GO). To the best of our knowledge, there were no literature reporting the use of MnO₂-rGO nanocomposite for the modification of screen-printing electrodes (SPE) for the electrochemical determination of FAV.

2. Experimental

The whole data and description of the used chemicals, apparatus, and methods are described in the electronic supplementary information (ESI) section S2.

3. Results and Discussion

3.1 Characterization of MnO₂-rGO nanocomposite

The structural morphology of the prepared MnO₂-rGO nanomaterial is displayed in Figure 1A. TEM photo is used to examine the structural morphology of MnO₂-rGO. TEM displays the structure of graphene with transparent layers and the manganese oxide distribution on its surface, (Fig. 1A). The MnO₂-rGO provided high surface area over the common graphite. The bulky particles of the graphite was exfoliated to layers with higher surface area. While the existence of MnO₂ nanoparticles with particle' diameter in the range of 30 to 85 nm offer much higher surface area that lead to improving the adsorptive affinity towards the FAV throughout the MnO₂-rGO/SPE surface. Also, the EDX spectrum was utilized to examine the elemental configuration of the prepared material. Figure 1B shows the EDX of nanocomposite, indicating the presence of Mn, O, and C.

The non-destructive method XRD is a beneficial tool in the description of MnO₂. Inset in Figure 1B shows different peaks at 2 θ 18.10°, 29.0°, 31.14°, 37.50°, 44.54°, 50.68°, 59.98° and 64.70° [11]. The sharp peaks are confirming the stability of MnO₂ crystalline nanoparticles. The peaks are correlated to 200, 310, 110, 002, 210, 512, 521 and 541 planes of crystalline MnO₂ [17-19], Fig. 1C.

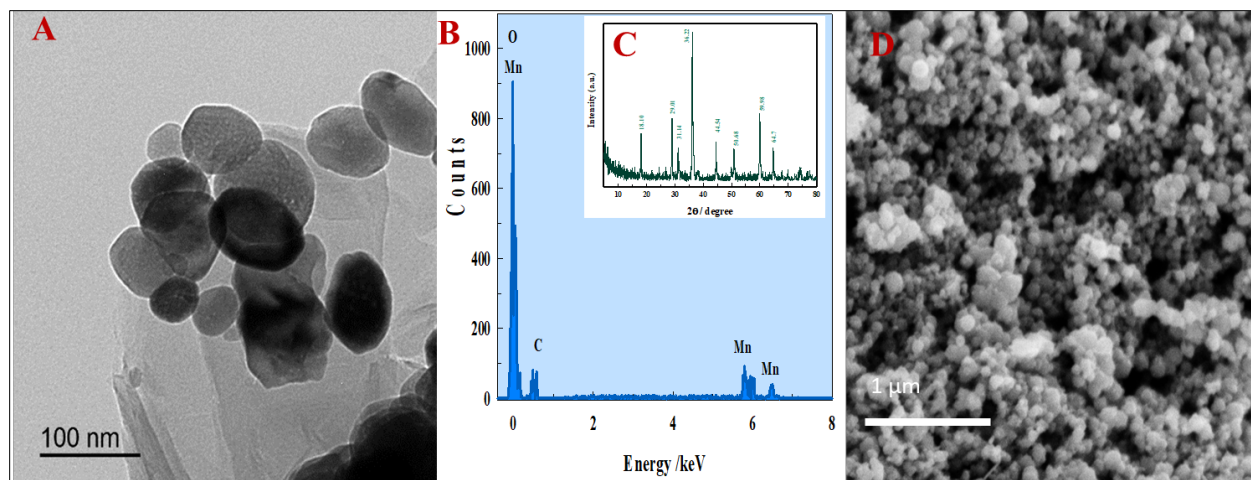


Fig. 1. A) TEM, B) EDX spectra, C) XRD) D) FE-SEM of MnO₂-rGO.

Field Emission Scanning Electron Microscopy (FE-SEM) Figure (1D) displays the hydrothermal synthesized MnO₂ nanoparticles of a spherical shape that covered the rGO surface. Fig S1 depicts the FTIR of graphene oxide and MnO₂-rGO nanomaterial. By inspection the FTIR spectra of GO, the C=C stretching and C-O-C epoxy stretching peaks appeared at 1642 and 1225 cm⁻¹, while the bands at 1725 and 1054 and 3224-3350 cm⁻¹ correspond to C=O stretching, C-O in alkoxy group and OH for the adsorbed H₂O. These FTIR indicate the graphite oxidation [20-23]. While for MnO₂-rGO nanocomposite, epoxy, alkoxy and carbonyl peaks were decreased because of the reduction of GO. Moreover, the clear events at 582 and 468 cm⁻¹ are recognized for the MnO₂ nanoparticles that completely cover the reduced graphene sheets [24].

3.2 Electrochemical characterization of the SPE modified with MnO₂-rGO nanocomposite

Next, the electrochemical characteristics of the developed platforms were depicted in 5.0 mM potassium ferricyanide (K₃Fe(CN)₆) in 0.1 M KCl. MnO₂-rGO/SPE (1:1) performance was compared to the bare SPE, and GO/SPE, Figure 2A. The redox peaks for MnO₂-rGO/SPE were 3.33, and 1.62 times higher than those of SPE, and GO/SPE, respectively, which could be attributed to the increase in the surface area of MnO₂-Gr compared to GO. The impedance spectroscopy (EIS) is a potent method for proper specification of the different parameters like mass transport and kinetic as well as the coefficient of charge transfer.

Figure 2B displays the electrochemical impedance spectroscopy (EIS) plot of bare SPE, GO/SPE, and MnO₂-Gr/SPE platforms, which were detected in 5.0 mM potassium ferricyanide (K₃Fe(CN)₆) in 0.1 M KCl. A simple corresponding circuit model was applied to adequate the obtained data.

R_s, C and R_{CT} symbolize solution resistance, a capacitance for the double-layer and electron transfer resistance, respectively. Z_W is a Warburg -type impedance coupled to R_{CT}, which represents the bulk diffusion. A semicircle with a Warburg-type line was shown in the spectra. The reduction in the semicircle diameter was indicated in the Nyquist plot for the electrochemical platforms GO/SPE and MnO₂-rGO/SPE, indicating an enhancement of charge transfer kinetics for those sensors comparing to the unmodified bare SPE.

The capacitance (C) was in parallel with the R_{CT} and W impedance. The resistance of charge transfer R_{CT} was valued to be 5970.0 Ω for the unmodified SPE, while it diminished to 4880.0, and 1600.0 Ω for GO/SPE, and MnO₂-rGO/SPE, respectively, indicating the significantly lower R_{CT} value at the MnO₂-rGO/SPE. The obtained cyclic voltammograms data were in a good agreement with the previous data gained from the electrochemical impedance measurements, showing that MnO₂-rGO offers high area along with good electron conduction.

The value of resistance of charge transfer R_{CT} can be applied to estimate the rate constant of the electron transfer (*k*^o) through the MnO₂-rGO/SPE. Using the following equation[25]:

$$k^o = \frac{RT}{F^2 R_{CT} AC}$$

where *R* is defined as the universal gas constant, *T* is the thermodynamic temperature (298.15 K), *F* is the Faraday's constant (96485 C mol⁻¹), *A* is the sensor area and *C* is the electroactive species concentration in the electrolyte medium. Heterogeneous electron transfer rate constant (*k*^o) were estimated and the calculated values were: 1.08×10⁻⁷, 1.10×10⁻⁷, and 1.75×10⁻⁷cm s⁻¹ for the bare SPE, GO/SPE, and MnO₂-rGO/SPE, respectively.

By comparing the *k*^o constant values, the highest value of *k*^o that is related to MnO₂-rGO/SPE indicates the effect of MnO₂-rGO nanomaterial for the enhancement of electron transfer kinetics throughout the electrode surface.

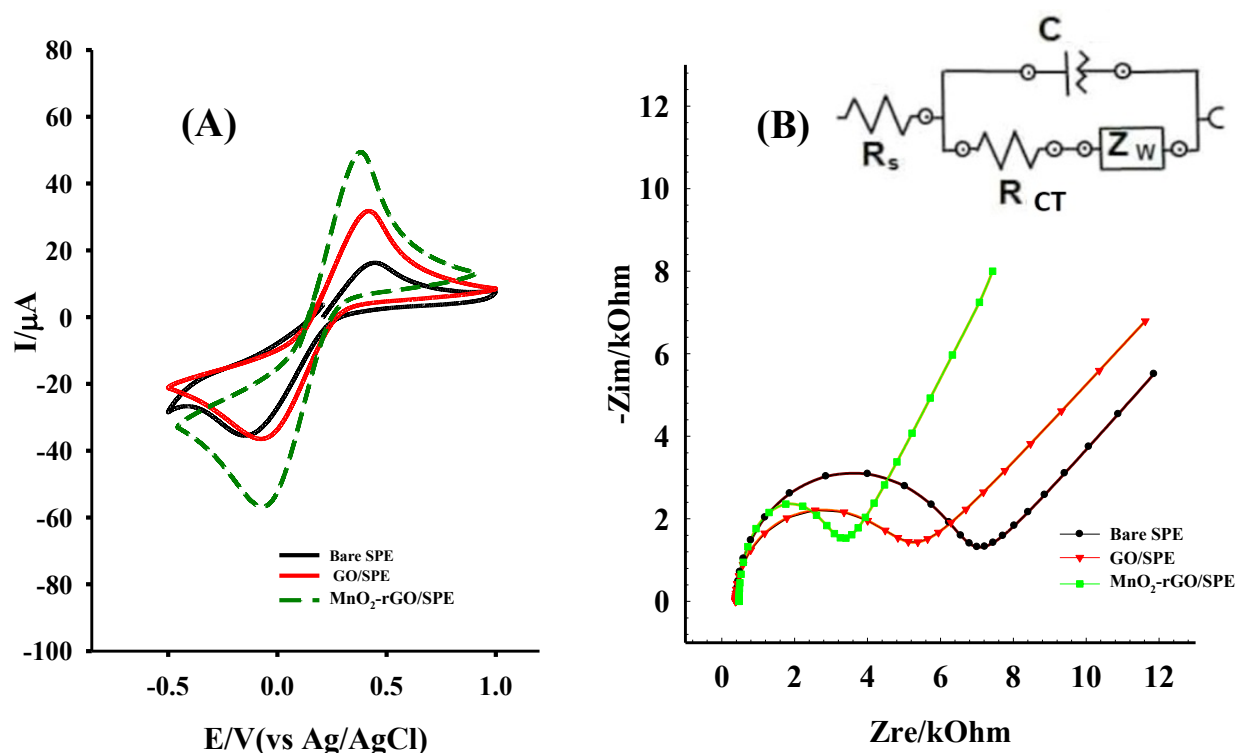


Figure 2. (A) The recorded CV curves at scan rate (v) of 0.1 V s^{-1} in $5.0 \text{ mM K}_3\text{Fe(CN)}_6$ (1:1) in 0.1 M KCl solution at SPE, GO/SPE, and $\text{MnO}_2\text{-rGO/SPE}$ electrodes, (B) the electrochemical impedance of each electrode.

3.3 Electrochemical detection of Favipiravir with the SPE modified with $\text{MnO}_2\text{-rGO}$ nanocomposite

Figure 3A displays the characteristic SWV of $5.5 \times 10^{-4} \text{ M FAV}$ in pH 7.0 B.R. buffer at unmodified SPE, GO/SPE, and $\text{MnO}_2\text{-rGO/SPE}$. Upon adding of 1.0 mM FAV , the unmodified SPE showed an anodic peak current of $14.70 \mu\text{A}$ at 1.27 V , which related to the oxidation of FAV. As can be seen in Figure 3A, the value of the current of the electrode rises due to the addition of GO, where the anodic peak appears at 1.25 V with a current value of $18.10 \mu\text{A}$. After modification of the sensor with $\text{MnO}_2\text{-rGO}$, the peak current increased to $25.14 \mu\text{A}$ at 1.14 V . Consequently, the successful application of $\text{MnO}_2\text{-rGO}$ for the determination of FAV enhanced the analytical signal, due to a higher electron transfer with high electrical conductivity.

One of the important factors that affect the electrochemical process is the solution pH. Different pH values were tested using $5.5 \times 10^{-4} \text{ M FAV}$, Figure 3B. Upon varying the pH from 2.0 to 9.0,

the FAV oxidation peak was shifted towards lower potentials values, i.e. more negative values, which might be explained as reduction–deprotonation of FAV[26]. The relation between the peak potential (E_p) and pH values of the buffer solution was linear as: E_p (V) = 1.558 V – 0.046 V pH⁻¹, ($R^2 = 0.9845$). The slope value follows Nernst behavior where the included number of electrons and protons are equal during the electrochemical oxidation of FAV. Furthermore, the physiological pH 7.0 was selected as the optimum working one.

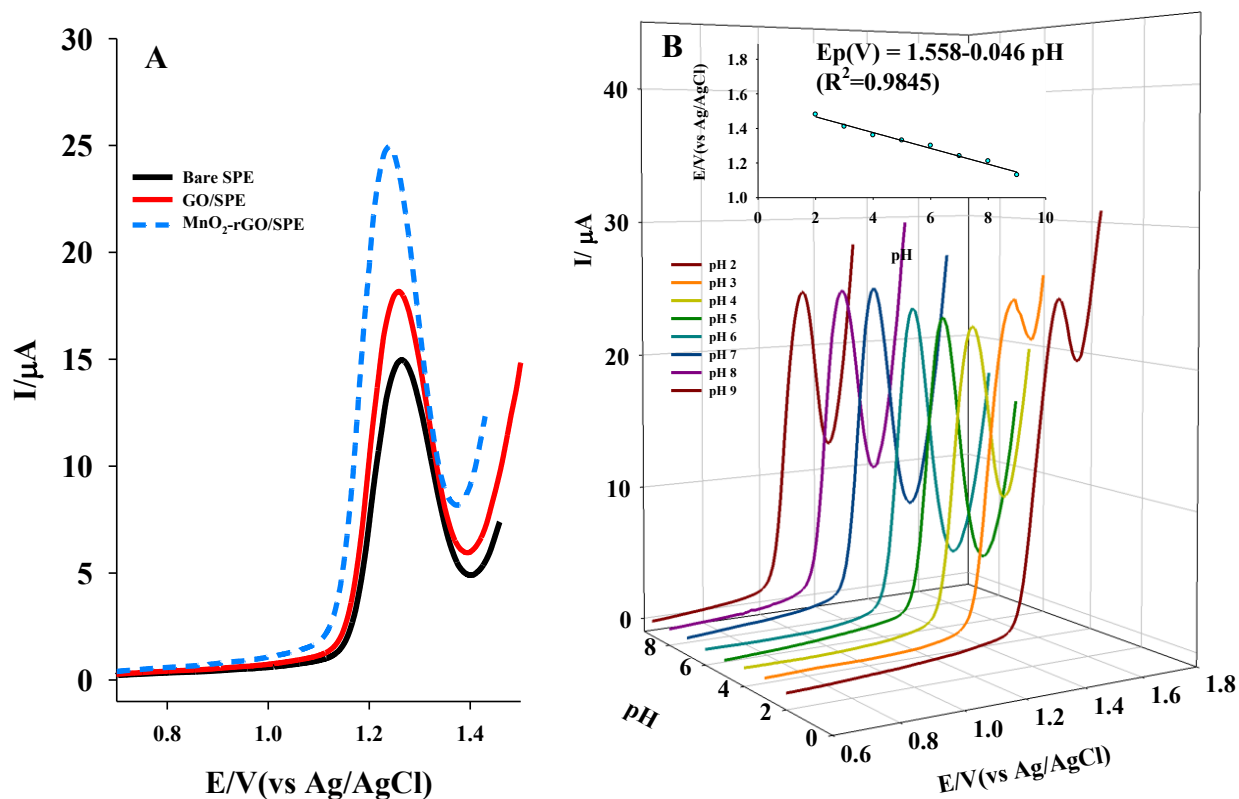


Figure 3. (A) SWV of 5.5×10^{-4} M FAV in B.R. buffer solution pH 7.0 at a SR of 0.1 V s^{-1} at SPE, GO/SPE, and MnO_2 -rGO/SPE electrodes, (B) SWV of 5.5×10^{-4} M FAV at different pH values using MnO_2 -rGO/SPE at $\nu=0.1 \text{ V s}^{-1}$, $P_w=0.50 \text{ V}$, $P_H= 0.25 \text{ V}$, $S_H=0.02 \text{ V}$. The inset: the plot of I_p versus pH.

Chronoamperometric measurements (CA) at 1.23 V for 20 s were performed for the determination of diffusion coefficient (D) applying Cottrell's law [27], Figure 4. The calculated D value was found to be $4.67 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ for FAV at MnO_2 -rGO/SPE in 0.04 M B.R. buffer (pH 7.0).

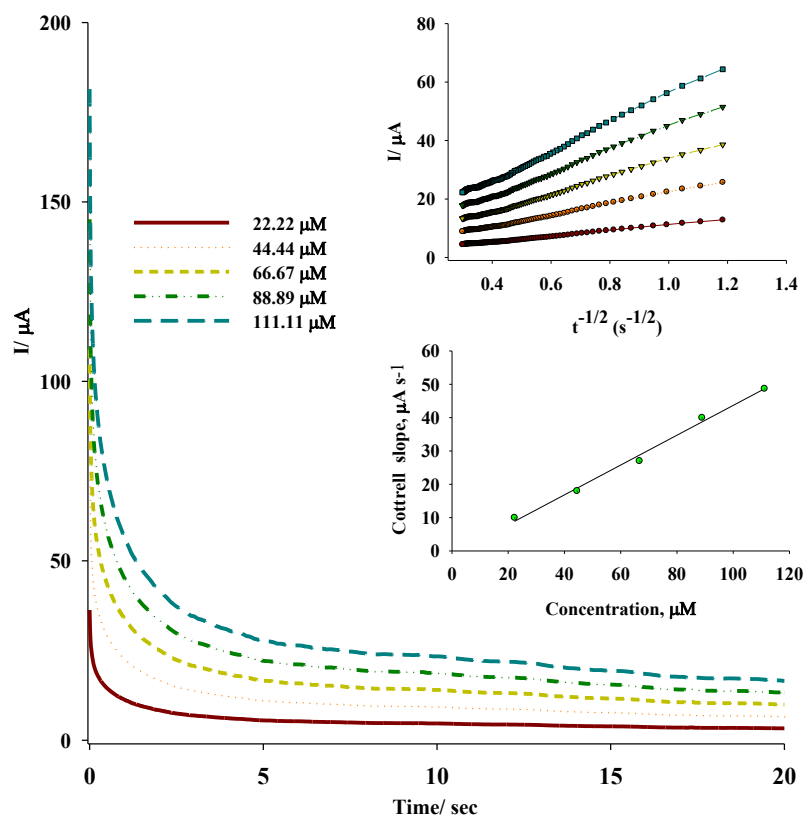


Fig. 4. CA measurements for the oxidation of FAV concentrations using MnO₂-rGO/SPE in B.R buffer pH 7.0, with a P_s = 500 mV. Inset displays the variant of CA currents at t=20 s vs. [FAV].

3.4 Analytical performance of the SPE modified with MnO₂-rGO nanocomposite

For the determination of FAV, SWV experiments were done using MnO₂-rGO/SPE in solutions of varying FAV concentrations using pH 7.0 B.R. buffer. As depicted in Figure 5A, the calibration curve is drawn as peak current I_p vs. FAV concentration. For the range $1.0 \times 10^{-8} - 5.5 \times 10^{-5}$ M, the regression equation was $I(\mu\text{A}) = 0.048C - 1.10$; ($R^2 = 0.9990$). The calculated values for the limit of detection (LOD) and limit of quantification (LOQ) were found to be 9.0×10^{-9} and 2.9×10^{-8} M, respectively.

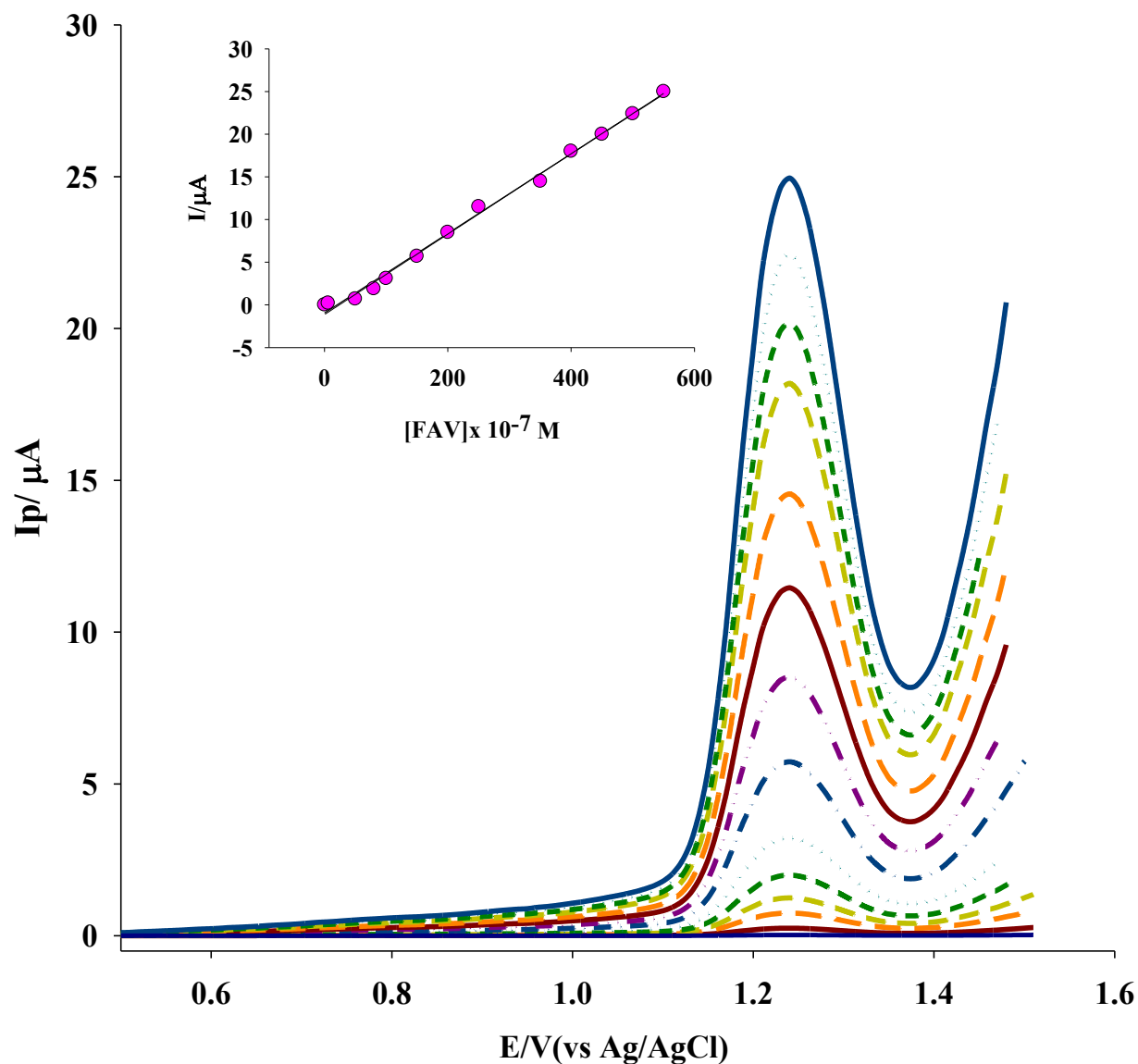


Figure 5. A) SWVs measurements at $\text{MnO}_2\text{-rGO/SPE}$ in a pH 7.0 B.R. buffer for the concentration range $1.0 \times 10^{-8} - 5.5 \times 10^{-5} \text{ M}$ FAV at $\nu = 0.1 \text{ V s}^{-1}$, $P_w = 0.50 \text{ V}$, $P_H = 0.25 \text{ V}$, $S_H = 0.02 \text{ V}$. The inset displays the plot of the I_p and FAV concentration in the concentration range 1.0×10^{-8} to $5.5 \times 10^{-4} \text{ M}$.

To check the specificity of $\text{MnO}_2\text{-rGO/SPE}$, its electro-analytical response was examined for the determination of FAV in the existence of co-administered COVID-19 drugs including fingolimod, remdesivir, and hydroxychloroquine (HCQ). Fingolimod and remdesivir did not show any electrochemical activity using $\text{MnO}_2\text{-rGO/SPE}$. Paracetamol (PAR) also is prescribed for COVID 19 patients for fever control. Fig 6A shows voltammetric response of FAV in presence of PAR as

the peak at 0.43 V is corresponding to PAR, while the other at 1.23 V is due to FAV oxidation. These findings are indicating that the simultaneous determination of the co-administered drugs of FAV is practicable using the $\text{MnO}_2\text{-Gr/SPE}$ platform. While for testing hydroxychloroquine (HCQ), two separated peaks appeared at 1.01 and 0.81 V at pH 7. Upon adding 5.5×10^{-4} M FAV, FAV well-separated peak appeared at 1.23 V, Fig 5B. Comparing to the published electrochemical work [9], our sensor offers wide range of linearity and sensitive determination of FAV alone and the presence of different coadministered drugs were demonstrated for the first time.

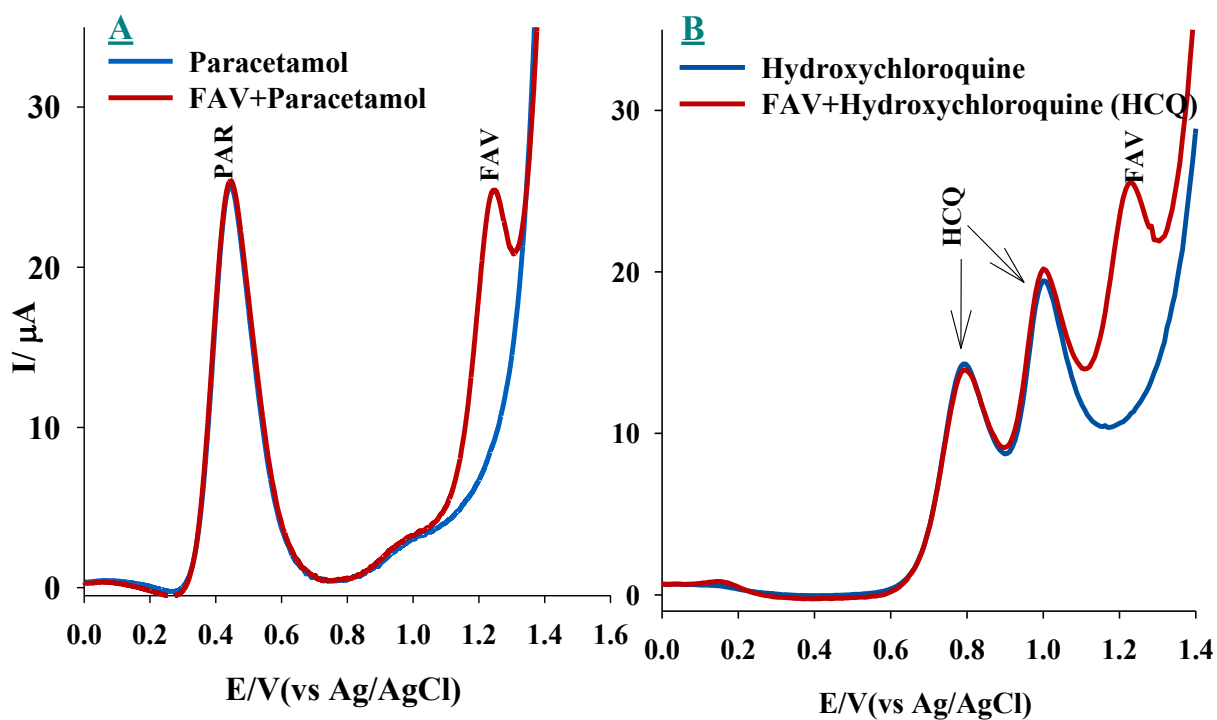


Figure 6. SWVs of A) HCQ, and FAV B) PAR, and FAV at $\nu=0.1 \text{ V s}^{-1}$, $P_w=0.50 \text{ V}$, $P_H= 0.25 \text{ V}$, $S_H=0.02$ using pH 7.0 B.R. buffer.

Repeatability, reproducibility, and storage stability of the $\text{MnO}_2\text{-rGO/SPE}$ electrochemical sensor

The $\text{MnO}_2\text{-rGO/SPE}$ sensor showed high repeatability and reproducibility for FAV analysis. Five determinations of 1.0×10^{-5} M FAV were performed for a single $\text{MnO}_2\text{-rGO/SPE}$ (intra-day repeatability). The relative standard deviation (RSD) for five determinations of FAV was 1.37%. Furthermore, the series of five different $\text{MnO}_2\text{-rGO/SPE}$ sensors made-up in the same procedure

(inter-day reproducibility) have showed the response with an RSD of 1.53% during the electroanalytical determination of 1.0×10^{-5} M FAV (Figure S2).

After the preliminary determination of FAV, the MnO_2 -rGO/SPE sensor was kept in the buffer (pH= 7.0) at 25 °C. The FAV electrochemical response was checked regularly over 4 weeks (Fig. S2). So, four sensors prepared in the same manner were tested in parallel (n=3). After four weeks, the final current decreased by 12% of the initial current value that proposes the sensor was rationally stable through the checking period. The high stability of the sensor can be gained because of the hydrophobic property of the graphene materials[28-30], which prevents the formation of a water film on the sensor surface.

Interference studies

Croscarmellose sodium, sodium lauryl sulphate, titanium dioxide, sodium dihydrogen orthophosphate ($\text{H}_6\text{NaO}_6\text{P}$), talc, castor oil, mannitol, cellulose, magnesium oxide, and povidone, were examined in order to check any change in the FAV electrochemical signal. Using 1.0×10^{-5} M FAV in a pH 7.0 B.R. buffer (0.04 M) solution, each was added, and the magnitude of the anodic peak current was monitored. From the chosen potential interfering substances, none were found to interfere with the electroanalytical sensing of FAV. The tolerance limit was less than $\pm 5.0\%$ for each interfering substance. Correspondingly, it was found that the use of 40-fold of inorganic ions (e.g. Mg^{2+} , K^+ , Zn^{2+} , Na^+ , Fe^{3+} , Ca^{2+} , Cl^- , NO_3^- and SO_4^{2-}) did not affect the electrochemical response, as shown in Figure S3.

In addition, the important commonly interfering materials in the biological fluids ascorbic acid (vitamin C, AA) and uric acid (UA) have been tested in the presence of 1.0×10^{-5} M FAV. Also, the precursor of dopamine, levodopa (LEVO) has been checked. The high peak potentials separation between the oxidation peaks of these compounds and FAV indicates the promising determination of FAV in biological fluids without interference, Figure 7.

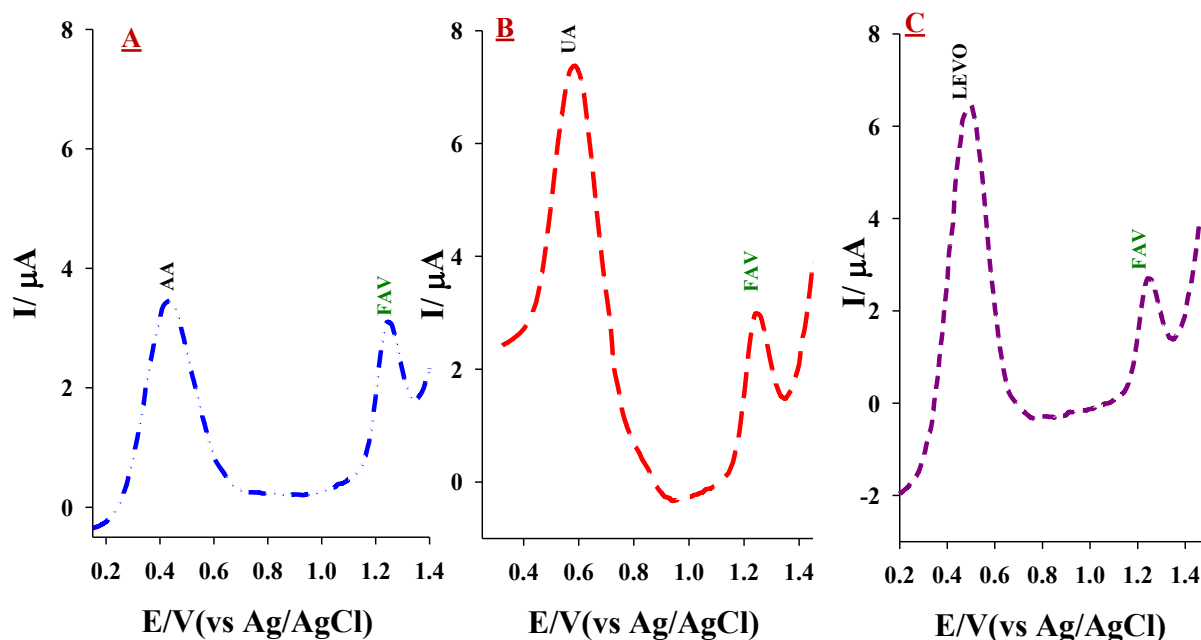


Figure 7. SWVs using a MnO₂-rGO/SPE in a pH 7.0 B-R buffer containing 5.0×10^{-5} M FAV, 5.0×10^{-3} M AA, UA and LEVO, respectively, for each at scan rate: 0.1 V s^{-1} , $P_H = 0.25 \text{ V}$, $P_W = 0.50 \text{ V}$, $S_H = 0.02 \text{ V}$.

Analysis of FAV in real samples

The practicability of MnO₂-rGO/SPE for the determination of FAV in real samples were tested using SWV technique. The standard addition method was used to evaluate the analytical performance of the sensor for FAV determination. The recovery experiment was presented in Table 1, which confirm that, the MnO₂-rGO/SPE is very sensible for the determination of low and high concentrations of FAV in different real samples.

Table 1. Determination of FAV in human plasma and synthetic tablets using MnO₂-rGO/SPE.

Sample	Added (μ M)	Found (μ M)	Recovery%
Plasma	12.00	11.82	98.50
	55.00	54.03	98.23
	120.00	118.92	99.10
	150.00	148.80	99.20
Recovery% \pm R.S.D			98.75 \pm 0.46
Invisiram [®] Tablet	12.00	11.88	99.00
	55.00	54.50	99.10
	120.00	118.52	98.77
	150.00	148.99	99.33
Recovery% \pm R.S.D			99.05 \pm 0.23

4. Conclusion

In the current study, we have described for the first time, the electrochemical determination of FAV with screen-printed electrode modified with MnO₂-rGO nanocomposite. The developed electrochemical platform showed outstanding electrocatalytic activity towards the sensitive determination of FAV with low values of detection and quantification limits along with wide linearity range. Furthermore, FAV was selectively determined using MnO₂-rGO/SPE platform in the presence of co-administered drugs with high recovery values. Based on the obtained results for the oxidation of FAV, the quantitative determination of FAV in pharmaceutical preparation and human fluids samples was achieved by a simple, rapid, selective and sensitive SWV technique. Furthermore, along with the excellent characteristics of high accuracy, sensitivity, and reproducibility, the developed sensor offers appropriate simple platform for the sensitive determination of FAV in the clinical work.

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Electronic Supplementary Information

Innovative electrochemical sensor for the precise determination of the new antiviral COVID-19 treatment Favipiravir in the presence of coadministered drugs

Mona A. Mohamed^{1,2§}, Ghada M. G. Eldin^{§1}, Sani M. Ismail², Nadia Zine², Abdelhamid Elaissari², Nicole Jaffrezic-Renault^{*2}, and Abdelhamid Errachid^{*2}

¹*Pharmaceutical Chemistry Department, National Organization for Drug Control and Research, Egyptian Drug Authority, Giza, Egypt*

²*Institut de Sciences Analytiques (ISA)-UMR 5280, Université Claude Bernard Lyon 1, 5 rue de la doua, 69100 Lyon, France*

§Equal first author contribution

S2. Experimental

S2.1. Apparatus

The Bio-logic SP 150 electrochemical workstation is used for analyzing the electrochemical properties of the samples. A one-compartment cell with a three-electrode set-up was connected to the electrochemical workstation through a C3-stand from BAS (USA). A platinum wire from BAS (USA) was employed as the auxiliary electrode. All the cell potentials were measured with respect to an Ag/AgCl (3.0 M NaCl) reference electrode from BAS (USA). A Cyberscan 500 digital (EUTECH Instruments, USA) pH-meter with a glass combination electrode served to carry out pH measurements. All the electrochemical experiments were performed at an ambient temperature of 25° C. Impedance spectra were recorded over a frequency range from 100 mHz to 100 kHz. Field Emission Scanning Electron Microscopy (FE-SEM) and energy-dispersive X-ray (EDX) measurements were carried out using a JSM-6700F scanning electron microscope (Japan Electro Company). FT-IR spectra were recorded on an IR-Affinity-1 Fourier Transform Infrared Spectrophotometer (Shimadzu, Japan). The crystalline phases were detected and identified using an X-ray diffractometer (XRD) on an X'Pert PRO MRD with a copper source at a scan rate (2θ) of 1°s^{-1} . Sigma Plot 10.0 was used for all statistical data. TEM images were performed by JEM-2100 (high-resolution transmission electron microscope) (HRTEM). Sigma Plot 10.0 was used for all statistical data.

2.2. Reagents and Solutions

All the chemicals were Sigma-Aldrich of analytical grade and were used without further purifications. A series of the Britton-Robinson (B.R.) universal buffers (pH 2.0–10.0) were prepared in deionized water and were used as supporting electrolytes. FAV, fingolimod, remdesivir, and hydroxychloroquine (HCQ) are provided by Rameda pharmaceutical company, Egypt.

Stock solution 1.0×10^{-3} M FAV was prepared by dissolving accurate weight in a little amount of methanol then it was quantitatively transferred to measuring flask. Methanol was added until the mark, and then it was sonicated for a while. Desired working solutions (1.0×10^{-5} – 1.0×10^{-3} M FAV) were prepared by appropriate dilution with methanol every day and the stock solution is used for one week only. Fresh human plasma was obtained from the blood bank (VACSERA, Cairo, Egypt). Screen-printed electrodes (SPE) were obtained from DropSens, Spain.

2.3.2. Synthesis of manganese oxide nanoparticles-reduced graphene composite (MnO_2 -rGO)

Graphene oxide (GO) was synthesized from graphite powder using the Hummers' method [1]. MnO_2 -rGR nanocomposites were prepared hydrothermally. In short, 0.092 g of GO were dispersed in 30 mL distilled water for 3 h with the aid of ultrasonication to obtain a complete homogeneous solution. Next, 2.51 g $Mn(NO_3)_2 \cdot 4H_2O$ was dissolved in 20.0 mL distilled water and added to the GO solution. The temperature of the solution was raised to 60 °C and its pH was adjusted to 10.0 using 4.0 M NaOH. After stirring for 2 h, the resulting suspension was transferred into 80 mL Teflon-lined stainless steel auto-clave. The volume was completed to 75 mL with ethanol. The autoclave was sealed and placed in an oven at 160 °C for 14 h for hydrothermal treatment. The autoclave was then allowed to cool at room temperature. The product was washed with deionized water several times and dried at 50 °C for 24 h.

2.4. Preparation of the used electrodes

Screen-printed electrodes (SPEs) form the basis of the MnO_2 -rGO sensor and allow the electrically wiring of the MnO_2 -rGO. MnO_2 -rGO/SPE modified electrodes were prepared by drop-casting 7.0 μ L of an aqueous solution containing 0.1 mg mL⁻¹ MnO_2 -rGO onto SPE with a micropipette. After 30 minutes, the ethanol evaporated (at ambient temperature) and the modified electrodes were

ready for use. The electroactive area of the electrodes was obtained by a previously reported method [2-4]. The electrochemical active area of the electrodes were found to correspond to: 0.082, 0.10, and 0.19 cm² for the bare SPE, GO/SPE, and MnO₂-rGO, respectively.

2.6. Analysis of real samples

Five tablets of “Invisiram tablets; 200 mg FAV/tablet” were weighed and the average mass per tablet was determined, and then grounded to a fine powder. A weighed portion equivalent to 1.0×10⁻³ M (FAV) was accurately transferred into a 100-mL volume calibrated flask containing 50 mL methanol. The content of the flask was sonicated for about 15 min and then filled up with methanol. Convenient more-dilute working solutions were then obtained by accurate dilution with methanol.

Plasma samples of three healthy volunteers were stored frozen until assay. Different aliquots of FAV were transferred separately into a series of 5-mL volumetric flasks spiked with 20 μL of fresh plasma samples using micropipette and the volume was completed to the mark with B.R buffer pH 7.0. The solution was transferred into the electrochemical cell to be analyzed without any further pretreatment.

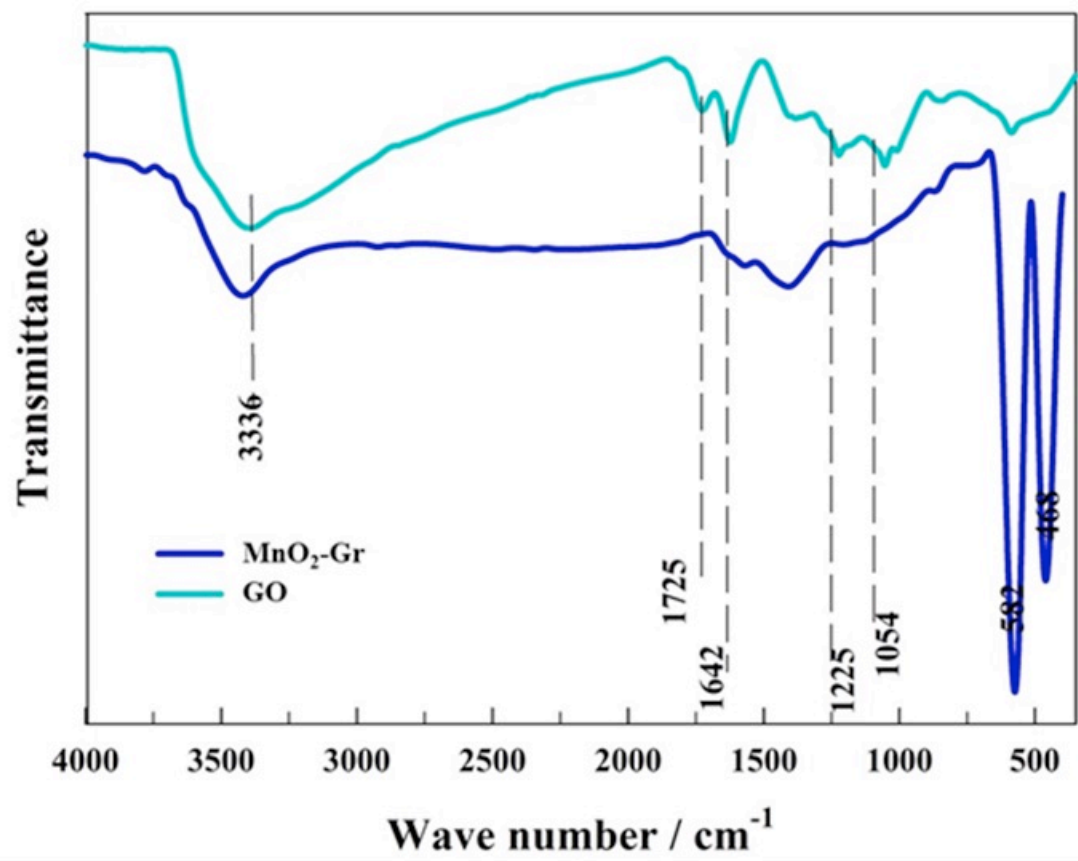


Fig. S1. FTIR spectra of GO and MnO₂-rGO

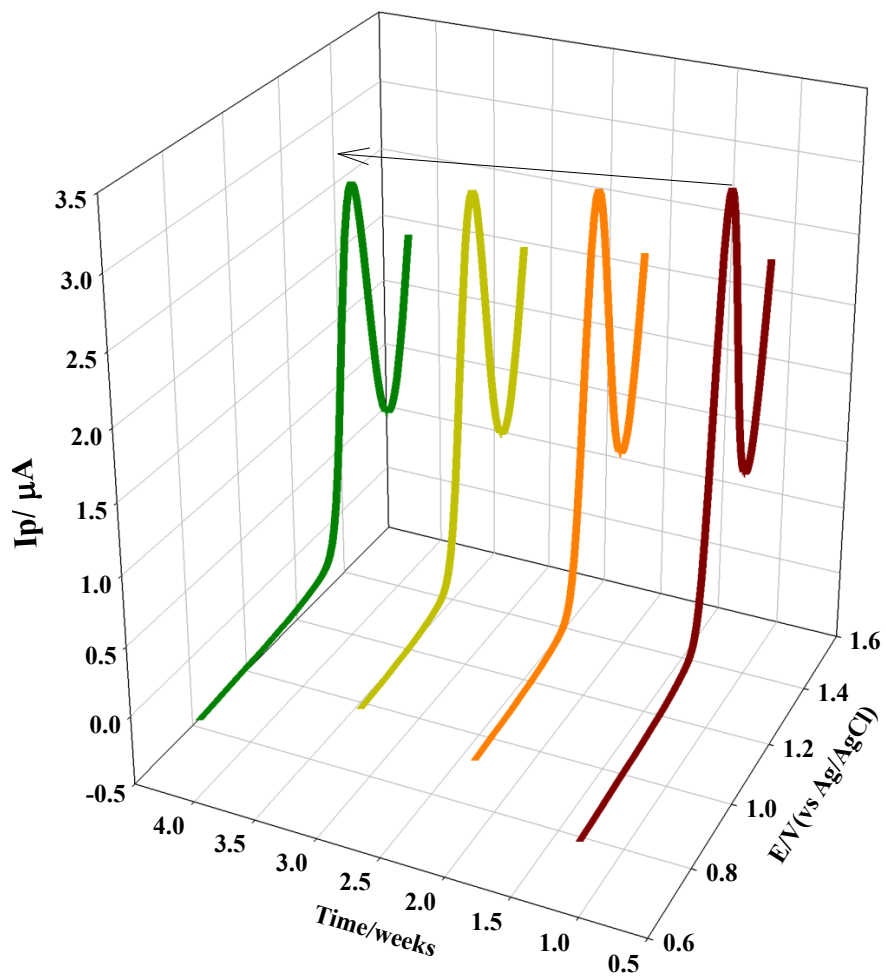


Figure S2. SWVs voltamograms of 1.0×10^{-5} M FAV using MnO_2 -rGO/SPE after 1- 4 weeks storage in B.R. buffer solution (pH= 7.0).

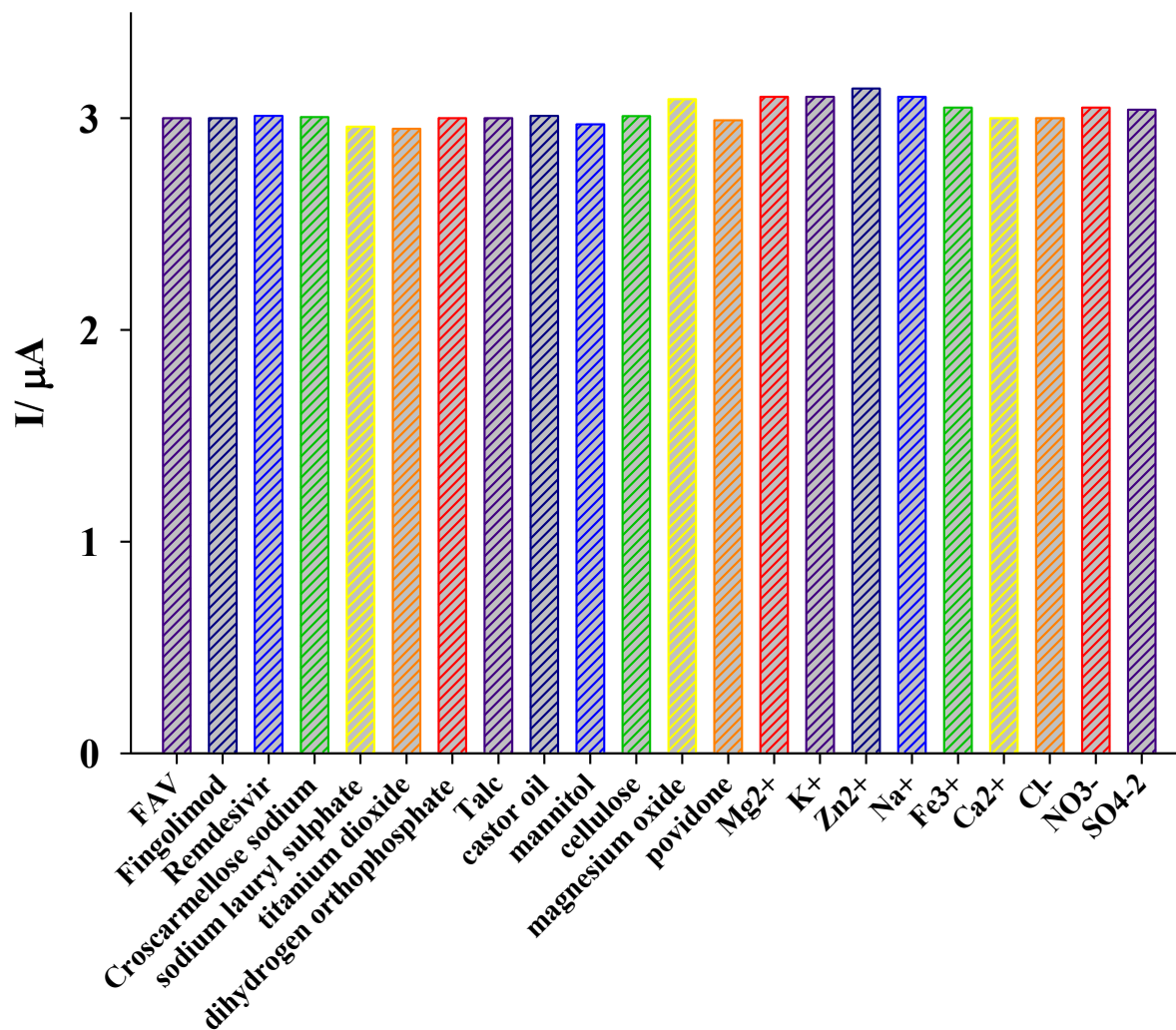


Figure S3. Effect of different interfering materials and ions 1.0×10^{-5} M FAV in a pH 7.0 B.R. buffer (0.04 M) solution.

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