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# A Novel Rutin Electrochemical Sensor Using Reduced Graphene Oxide/Magnetite/Silver Nanoparticle-Molecularly Imprinted Polymer Composite Modified Electrode

Shiru Yang<sup>1</sup>, Xin Yang<sup>1, 2, \*</sup>, Rong Tang<sup>1</sup>, Lizhi Xiong<sup>3</sup>, Yaqi Yang<sup>1</sup>, Yangjian Hu<sup>1</sup>, Chunmei Zhang<sup>1</sup>, Zijian Zhao<sup>2, \*</sup>

<sup>1</sup> Hunan Engineering Laboratory for Preparation Technology of Polyvinyl Alcohol Fiber Material, College of Chemistry and Materials Engineering, Huaihua University, Huaihua, 418000, P.R. China; <sup>2</sup> Key Laboratory of Research and Utilization of Ethnomedicinal Plant Resources of Hunan Province, College of Biological and Food Engineering, Huaihua University, Huaihua, 418000, P.R. China; <sup>3</sup> College of Materials and Chemistry Engineering, Hunan City University, Yiyang, 413000, P.R. China;

\*E-mail: <u>01yangxin@163.com (X. Yang); zjzhao72@163.com (Z.J. Zhao).</u>

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A novel rutin (RT) electrochemical sensor was fabricated by using reduced graphene oxide (rGO)/magnetite (Fe<sub>3</sub>O<sub>4</sub>)/silver nanoparticle (Ag)-molecularly imprinted polymer (MIP) composite to modify screen-printed electrode (SPE). The ternary composite of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag was prepared by the one-step solvothermal method. Subsequently, RT molecules were absorbed on the surface of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag due to the electrostatic force. Finally, the MIP was achieved by a polymerization reaction. The prepared composite was investigated by FTIR, XRD and VSM. The morphologies and electrochemical properties of different electrodes were characterized with FE-SEM, EIS, CV and DPV. Under the optimal conditions, the SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode exhibited highly sensitive and selective in determining of RT. The linear detection ranges (LDRs) were  $1.0 \times 10^{-2}$  to  $10.0 \ \mu M$  (*R*=0.9978) and 10.0 to  $3.0 \times 10^{3} \ \mu M$  (*R*=0.9965). The limit of detection (LOD) was as low as  $4.2 \times 10^{-3} \ \mu M$  (*S/N*=3). The proposed electrode was renewable, stable and reliable. It was used for determining RT in pharmaceutical samples with satisfactory results, confirming its promising application in routine RT analysis.

**Keywords:** Rutin; Reduced graphene oxide; Magnetite; Silver nanoparticles; Molecular imprinted polymer; Screen-printed electrode

# **1. INTRODUCTION**

Rutin (RT) is a natural flavonoid and has some biological activities [1]. Hence, developing analytical methods or instruments with high sensitivity and selectivity to monitor this species is of

great importance in pharmaceutics and clinics. Until now, several traditional analytical methods or instruments including HPLC [2], FIA [3], CE [4], electrochemiluminescence [5] and spectrophotometry [6] have been explored to RT determination. Although selective and sensitive determination of RT by these instruments can be accomplished, they are inherently expensive. Moreover, the time consuming and complicated operating processes for samples are included.

Since the structure of RT molecule contains several phenolic hydroxyl groups, it is obvious that RT can be oxidized on the electrode surface due to their electrochemical activities. Therefore, electrochemical methods have been successfully applied to detect RT and attracted considerable interest attribute to their portability, sensitivity, short analytical time as well as low cost [7-9]. Several electrochemical sensors have been fabricated to detect RT. For instance, Gholivand reported a film consisting of Cu-chitosan (CS)/multiwalled carbon nanotube (MWCNT) to modify glassy carbon electrode (GCE) for detecting RT [10]. Sun prepared the carbon ionic liquid electrode placed by graphene (Gr)-MnO<sub>2</sub> for the sensitive electrochemical determination of RT [11]. A RT electrochemical sensing platform was fabricated by Zhang based on nanorods of PEDOT grown on graphene oxide (GO) [12]. However, some factors limit the electrochemical detection of RT under physiological conditions. One is the interferences from other flavonoids and ascorbic acid (AA) as their oxidation potential overlaps with that of RT at the bare electrode. Another is that the concentration of RT is too low to detect in real pharmaceutical samples [13, 14]. To improve the measuring selectivity and sensitivity, an effective approach is to modify the electrode with selective and conductive materials.

Molecularly imprinted polymer (MIP) possesses binding sites complementary in shapes and sizes to recognize target molecules. It also possesses some advantages including chemical/physical stability, low cost, easy preparation and resistance of harsh environmental condition compare with natural biological receptors, which is considered as an ideal artificial recognition material to improve the measuring selectivity [15-17]. Based on these favorable properties, various MIPs modified electrodes have been widely used in determining of RT [18], brucine [19], propylparaben [20], glutathione [21]. However, most MIPs are organic materials, several drawbacks including slow mass transfer, poor binding kinetics and low binding capacity resulting from the use of them alone. Therefore, challenges still exist in fabricating an electrochemical sensor using MIP modified electrode.

Earlier papers revealed the use of MIP combines with conductive nanomaterials can overcome the above difficulties [22-24]. Various conductive nanomaterials combine to MIP have been recommended to enlarge the electrochemical signal, such as reduced graphene oxide (rGO), silver nanoparticle (Ag) and magnetite (Fe<sub>3</sub>O<sub>4</sub>). Zeng introduced the free radical polymerization and prepared a novel composite consisting of rGO and MIP. Making use of it as the recognition element to construct a 4-nitrophenol electrochemical sensor [22], Li reported a novel salbutamol electrochemical sensing platform based on MIP incorporated with Ag-N-rGO [23]. Zhang fabricated an amaranth magnetic molecularly imprinted electrochemical sensor (MIES) based on Fe<sub>3</sub>O<sub>4</sub>-rGO doped MIP membrane using magnetic field directed self-assembly [24]. Based on these advantages, we reasonably expect that the ternary composite of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag combines MIP hybrid composite could provide excellent electrochemical sensitivity and selectivity for the determination of RT.

This investigation aims to fabricate a novel electrochemical sensor using  $rGO/Fe_3O_4/Ag-MIP$  composite to modify screen-printed electrode (SPE) for the determination of RT. Attributing to the

synergistic effects originating from the combination of proposed particles, the modified electrode (SPE  $| rGO/Fe_3O_4/Ag-MIP \rangle$ ) exhibits improved sensitivity and selectivity. Meanwhile, the modified electrode's surface can be renewed quickly via an external magnetic field. Finally, the electrochemical sensor is employed to detect RT in medicinal tablets with excellent accuracies and recoveries.

## **2. EXPERIMENTAL**

## 2.1. Reagents and Apparatus

Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O, AgNO<sub>3</sub>, graphite, nafion solution, RT, acrylamide (AM), ethylene glycol dimethacrylate (EGDMA), 2, 2'-azobisisobutyronitrile (AIBN) and all other reagents with analytical reagent grade were obtained from sinopharm medicine holding co., ltd (Shanghai, China). The stock solutions of 0.1 M Na<sub>2</sub>HPO<sub>4</sub> and 0.1 M NaH<sub>2</sub>PO<sub>4</sub> were mixed with different proportion to prepare 0.1 M phosphate buffer solution (PBS) at various pH values. 18.2 M $\Omega$ ·cm doubly distilled water was used. The RT medicinal tablets were obtained nearby Huaihua University (2017.3).

The Shimadzu IR prestige-21 spectrometer and Rigaku Ultima IV X-ray powder diffractometer (XRD) were employed to record the Fourier transform infrared spectroscopy (FTIR) spectra and the crystallization degrees of the composite, respectively. The morphologies of different electrodes were carried out on the Zeiss Sigma HD field emission scanning electron microscope (FE-SEM). The magnetic measurement was performed on the Lakeshore-736 vibrating sample magnetometer (VSM). The DropSens corporation's unmodified or modified screen-printed electrode (SPE) connected to the CHI 660D electrochemical workstation (Shanghai Chenhua Instrument) was used to perform the electrochemical measurements. The SPE was integrated with a carbon working electrode, an Ag/AgCl reference electrode and a carbon auxiliary electrode.

## 2.2. Synthesis of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag

The modified Hummers method was applied to synthesize graphene oxide (GO) from graphite [25]. The obtained GO was cut into pieces and ground into powder for further use. In a typical procedure [26], 4 mmol of Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O and 100.0 mg of GO were dispersed in 60 mL of ethyleneglycol followed by adding of 1.2 mmol AgNO<sub>3</sub> and 45 mmol NaAc. The mixture formed a clear solution after ultrasonicated for 90 min. The resulted suspension was subsequent transferred into a Teflon-lined stainless steel autoclave. The sealed autoclave was put into a furnace, heated to 200 °C and maintained for 2.0 h, then taken out and cooled in the air. Finally, the black precipitates were collected with magnetically and thoroughly rinsed by absolute ethanol and doubly distilled water and dried at 50 °C for 24 h.

## 2.3. Synthesis of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP

RT, AIBN, EGDMA and AM were acted as template molecules, initiator, cross-linking agent and functional monomer in the polymerization reaction [27]. Briefly, 100.0 mg of dried rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag composite was dispersed in 50 mL of organic mixture solvent ( $V_{acetonitrile}$ : $V_{DMF}$ =3:1) by ultrasonication for about 30 min. Then, 0.2 mmol RT was dispersed into the mixture solvent with ultrasonic treatment for 15 min. Finally, 0.8 mmol AM, 20.0 mg AIBN and 4.0 mmol EGDMA were added into the mixture under stirring and purged with N<sub>2</sub>. The reaction was heated to 60 °C and maintained for 24 h. The obtained MIP was dried, ground into powders and treated in a ultrasonic cleaner with a organic mixture solvent ( $V_{methanol}$ :  $V_{acetic acid}$ =9:1) for 1.0 h to remove the RT template. The ultrasonic treatment was repeated for 5 times. Finally, the MIP was washed for 5 times by methanol to remove acetic acid residual and dried at 80 °C overnight. The procedures for rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP preparation are described in Fig. 1A. Without adding the RT template molecule, the blank non-imprinted polymer (rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP) was synthesized with the same polymerization procedures.

## 2.4. Preparation of the modified electrode

20.0 mg of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP composite was dispersed in 4.0 mL of *wt*. 0.5% nation solution to obtain a homogeneous suspension under vigorous ultrasonication. 10.0  $\mu$ L of the obtained suspension was deposited onto the working electrode and got dried under an infrared lamp (labeled as SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP). For comparison, 10.0  $\mu$ L of 5.0 mg·mL<sup>-1</sup> rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag and 10.0  $\mu$ L of 5.0 mg·mL<sup>-1</sup> rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag and 10.0  $\mu$ L of 5.0 mg·mL<sup>-1</sup> rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag and 10.0  $\mu$ L of and rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag and rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP modified SPE, respectively (labeled as SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag and SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP).

## 2.5. Analytical procedures

The SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode was inserted into a beaker containing 2.0 mL of pH=4.0 PBS and purged N<sub>2</sub> for 10 min. Subsequently, different concentrations of RT standard solutions were addition into PBS successively. The electrochemical performances of different electrodes and possible reduction mechanism of RT were investigated by cyclic voltammetry (CV) or differential pulse voltammetry (DPV). The CV measurements was carried out from 0 V to +0.8 V with a scan rate (*v*) of 0.1 V·s<sup>-1</sup>. In DPV measurements, the scan was performed from 0 V to +0.8 V at a *v* of 100 mV·s<sup>-1</sup> with the accumulation time and accumulation potential were 420 s and +0.1 V. The electrode's schematic diagram with possible reduction mechanism for RT is shown in Fig.1B. The RT medicinal tablets after pretreatment were used as the real samples and followed the procedures recommended as described. To identify the reliability of the method, the standard addition method was operated.



**Figure 1.** Schematic illustration of the syntheses of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP composite (A) and the diagram of the electrode with possible reduction mechanism of RT (B).

## **3. RESULTS AND DISCUSSION**

#### 3.1. Characterization of the rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP composite

Fig. 2 depicts FTIR spectra of GO, rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag, rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP and rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP. The spectrum of GO displays -OH (3400 cm<sup>-1</sup>), aromatic C=C (1620 cm<sup>-1</sup>), alkoxy (1060 cm<sup>-1</sup>), carboxyl C-O (1410 cm<sup>-1</sup>), epoxy C-O (1230 cm<sup>-1</sup>), and C=O (1730 cm<sup>-1</sup>) in Fig.2a [28]. However, as to rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag (Fig.2b), the skeletal vibration related to the Gr (1620 cm<sup>-1</sup>) is remained although most oxygen- containing groups decreases or disappears, which indicates the presence of rGO [29]. The absorbance band at 588 cm<sup>-1</sup> ascribes to Fe<sup>2+</sup>-O<sup>2-</sup> vibration and is consistent with the reported FTIR spectra for spinel Fe<sub>3</sub>O<sub>4</sub> [30]. In the rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP spectrum (Fig.2c), some absorption peaks range from 1000 cm<sup>-1</sup> to 2000 cm<sup>-1</sup> appear, which are attributed to the organic groups introduced from RT, AM and EDGMA in the MIP. Most characteristic absorption peaks of organic groups can be found in the spectrum of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP (Fig.2d).





Figure 2. FTIR spectra of GO (a), rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag (b), rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP (c) and rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP (d).

The XRD patterns of GO, rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag, rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP and rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP are shown in Fig. 3. As shown in Fig. 3a, the characteristic graphite peak  $(2\theta=26^{\circ})$  disappears after oxidation and is replaced by one well-defined peak at  $2\theta=10.8^{\circ}$  corresponding to GO. The diffraction peaks relate to both Fe<sub>3</sub>O<sub>4</sub> and Ag can be observed obviously in rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag composite (Fig. 3b). Six characteristic peaks for Fe<sub>3</sub>O<sub>4</sub> [2 $\theta$ =30.1° (220), 35.5° (311), 43.3° (400), 53.4° (422), 57.2° (511) and 62.5° (440)] are observed, which are in accordance with the face-centered cubic structure for the cubic phase Fe<sub>3</sub>O<sub>4</sub> in the standard XRD data (JCPDS No.19-0629) [31]. Four peaks locate at  $2\theta$ =77.7°, 64.6°, 44.4° and 38.2° indicate the (311), (220), (200) and (111) crystalline planes of Ag [26], respectively. It can be seen that no diffraction peaks arising from GO can be detected except the peaks assigned to Fe<sub>3</sub>O<sub>4</sub> and Ag, which indicates that GO is reduced into rGO effectively. In both rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP (Fig. 3c) and rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP (Fig. 3d) composite, no peaks relate to impurities are found.



Figure 3. XRD patterns of GO (a), rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag (b), rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP (c) and rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP (d).

The magnetic property of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP sample at room temperature is recorded by VSM using an applied field of -5000 Oe to 5000 Oe. The magnetic hysteresis loop shown in Fig. 4 indicates the ferromagnetic behavior of the composite. Due to the existence of Ag, rGO and MIP, the saturation magnetization (Ms) of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP composite (49.6 emu·g<sup>-1</sup>) is lower than that of Fe<sub>3</sub>O<sub>4</sub> (92 emu·g<sup>-1</sup>) [32]. The rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP composite displaying ferromagnetic behavior and can be rapidly magnetic separation with an external magnetic field (about 60 s), which is critical to apply them in magnetic electrochemical sensors.



**Figure 4.** Hysteresis loop of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP composite at room temperature. Inset: Photographic image of the behavior of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP composite in the magnetic field.

## 3.2. Characteristics of different electrodes

The flakes of graphite appear on the SPE (Fig.5a) [33]. Massive rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag nanoparticles seem to agglomerate via magnetic dipole-directed interaction (Fig.5b). The total sizes of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag nanoparticles are 15 nm to 20 nm. The morphologies of SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP and SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP electrodes are shown in Fig.5c and Fig.5d. There are great differences in the morphologies between the surfaces of two kinds modified electrodes. The rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP membrane is rough while the rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP membrane is homogeneous and smooth. The roughness of imprinted membrane on the SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode is beneficial for enhancing the response sensitivity and improving the rebinding efficiency of the sensor.





**Figure 5.** FE-SEM images of SPE (a), SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag (b), SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP (c) and SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP (d) electrodes.

## 3.3. Electrochemical performances of various electrodes

The interfacial properties of various electrodes are studied with electrochemical impedance spectroscopy (EIS) (Fig.6). The results were fitted to the Nyquist plots and the diameter of the semicircle part can correspond to the charge transfer resistance (Rct). The EIS of SPE shows a negligible Rct value with a very small semicircle diameter (Fig. 6a), implying that the characteristic of a diffusional limiting step of the  $[Fe(CN)_6]^{3-/4-}$  electrochemical process. The Rct of SPE | rGO/ Fe<sub>3</sub>O<sub>4</sub>/Ag electrode (Fig. 6b) increases a little, which is attributed to that the nafion used to fix nanomaterials can block the diffusion of  $[Fe(CN)_6]^{3-/4-}$  although rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag is highly electrical conductive. After SPE modified with the rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP before removing of the template, the Rct increases remarkably (Fig. 6c), revealing that the MIP is immobilized on the surface successfully. The nonconductive MIP on the electrode's surface forms the barrier and blocks the exchange of electron between the electrode and solution [34]. The Rct decreases after the template removed (Fig. 6d), revealing that the template is removed successfully and the cavities formed which accelerates the electron exchanges. The Rct locates between MIP before and after removing of the RT when immobilized SPE with rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP (Fig. 6e). The reason may be the immobilized NIP hinders the electron exchanges without the excess of RT may accelerate the electron exchanges.



**Figure 6.** EISs of 10 mmol·L<sup>-1</sup>  $[Fe(CN)_6]^{3-/4-}$  and 0.1 mol·L<sup>-1</sup> KCl at SPE (a), SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag (b), SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP before (c) or after elution (d) and SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP (e) electrodes.

The electrochemical behaviors recorded on different electrodes in 0.1 M pH=4.0 PBS containing 1.0  $\mu$ M RT are shown in Fig. 7. The electrochemical reactions of RT can be realized as a

pair of redox peaks appears on all electrodes. A pair of symmetrical peaks with the smallest redox peak currents appears on SPE (Fig. 7a). The redox peak currents increases remarkably on the SPE  $| rGO/Fe_3O_4/Ag$  electrode (Fig. 7b), indicating the reversibility of RT on the SPE  $| rGO/Fe_3O_4/Ag$  electrode increases.

The result can be ascribed to the presence of conductive rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag composite immobilized on the electrode, which provides porous structures for electrons exchanging of RT. Compared with SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag electrode (Fig. 7b), the SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode (Fig. 7c) exhibits higher redox peak current. That indicates that MIP has stronger absorbability toward the RT. However, the response of SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-NIP (Fig. 7d) electrode is lower than SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag electrode but still higher than SPE electrode. It may interpret that the electron hinder effect of NIP to current reduction is lower than the synergistic effect of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag composite to current amplification. Moreover, the redox peak current on the SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode increases with the increased RT concentration (Fig. 7e). The result reveals the potential application of SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode in RT analysis.



**Figure 7.** CVs of SPE (a), SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag (b), SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP (c), SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/ Ag-NIP (d) electrodes in presence of 1.0 μM RT and SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode added with 1.2 μM RT (e).

# 3.4. Optimization of the conditions for RT determination

# 3.4.1. The volume of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP solution

The volume of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP solution on the SPE is one of important parameters for electrochemical determination of RT. A series of modified electrodes were fabricated by dropping different volumes of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP solution. The redox peak currents of 1.0  $\mu$ M RT increased with the volume of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP solution modified onto the SPE reached to 10.0  $\mu$ L. Greater volumes of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP solution resulted in broaden voltammograms and decreased intensity peak currents. Therefore, 10.0  $\mu$ L of rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP solution is suitable.

### 3.4.2 The accumulation time and accumulation potential

The determination sensitivity can be improved by the accumulation operation as this can enrich the molecules of RT absorbed onto the surface of modified electrode. The effects of the accumulation time and accumulation potential on the detection of 1.0  $\mu$ M RT were examined in PBS. Both the anode (Ipa) and cathode (Ipc) peak currents of the RT increased quickly with the increased accumulation time at the first up to 420 s, indicating that an equilibrium to adsorption is achieved. Therefore, 420 s is suitable for RT analysis. Moreover, the Ipa and Ipc peak currents of RT increased gradually with accumulation potential increasing before +0.1 V. The redox peak currents declined a little with further increased accumulation potentials. Hence, +0.1 V is selected as an appropriate accumulation potential.

## *3.4.3. The v and pH*



**Figure 8.** CVs of 1.0  $\mu$ M RT with *v* of 0.10 (a), 0.08 (b), 0.06 (c), 0.04 (d), 0.02 (e) and 0.01(f) V · s<sup>-1</sup> at the SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode. Inset: the calibration plots of Ipa and Ipc *vs. v.* 

Fig. 8 shows the CVs of 1.0  $\mu$ M RT at SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode with different *v* range from 0.01 V·s<sup>-1</sup> to 0.1 V·s<sup>-1</sup>. The redox peak currents enhanced progressively along with the increases of *v*. The linear relationships of Ipa and Ipc peak currents with *v* are corresponding to the regression equations: Ipa( $\mu$ A)=0.4097+36.6823*v* (V·s<sup>-1</sup>) (*R*=0.9991), Ipc( $\mu$ A)=-0.1857-27.0821*v* (V·s<sup>-1</sup>) (*R*=0.9994), implying the system presents features corresponding to an adsorption- controlled process for RT [10]. According to the equation: Ipa=nFQv/4RT, n is calculated to be 2.12≈2, which suggests that the oxidation process of RT involved two electrons transfer, which is in agreement with the reference [35]. In addition, the redox peak potentials represent no obvious changes, suggesting that the reversibility for RT reaction on the modified electrode gets no influence from the change of *v*.

Fig. 9 shows CVs of SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode in PBS containing 1.0  $\mu$ M RT with different pH values. The biggest Ipa was found with pH=4.0. The peak potentials shifted negatively when the pH values increased, which displayed that protons participate in the electrochemical reaction [36]. A well linear relationship exists between pH and Epa with the increased pH values. The equation is Epa=-0.0487pH+0.5741(*R*=-0.9961). The slope of 48.7 mV·pH<sup>-1</sup> is nearby -59 mV·pH<sup>-1</sup> of the

Nernst system, showing an equal number of electrons and protons occurred during the electrochemistry reaction [1]. The mechanism of RT redox process is explained by a two-protons and two-electrons [10] and is shown in Fig.1B.



**Figure 9.** CVs of 1.0  $\mu$ M RT at SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode in PBS varying pH levels 3.0 (a), 4.0 (b), 5.0 (c), 6.0 (d), 7.0 (e) and 8.0 (f). Inset: the Epa *vs.* pH.

# 3.5. The detection of RT on the SPE $| rGO/Fe_3O_4/Ag$ -MIP electrode

The DPVs for the determination of RT on the SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode were recorded under optimal experimental conditions. The DPVs of the modified electrode toward RT at various concentrations in PBS are shown in Fig.10. Well-defined ipas are proportional to the concentrations of RT ( $C_{\text{RT}}$ ) in the linear detection ranges (LDRs) of  $1.0 \times 10^{-2}$  to  $10.0 \,\mu\text{M}$  and  $10.0 \,\text{to} 3.0 \times 10^{3} \,\mu\text{M}$ . The linearization equations are: ipa( $\mu$ A)=1.3444+1.0911 $C_{\text{RT}}(\mu\text{M})$  (R=0.9978) and ipa( $\mu$ A)=11.6403+0.0280 $C_{\text{RT}}(\mu\text{M})$  (R=0.9955), respectively. The limit of detection (LOD) is  $4.2 \times 10^{-3} \,\mu\text{M}$  (S/N=3).



**Figure 10.** DPVs of SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode in PBS containing 0 (a), 0.01 (b), 0.1 (c), 1.0 (d), 5.0 (e), 10.0 (f), 50.0 (g), 500.0 (h), 1000.0 (i), 1500.0 (j), 2000.0 (k), 2500.0 (l), 3000.0 (m) μM of RT. Insets: the calibration curves of ipa *vs.C*<sub>RT</sub>.

Compared with several RT electrochemical sensor reported previously (Tab.1), the modified electrode presented with excellent comprehensive performances. The surface of modified electrode can be renewed quickly with external magnetic fields in terms of the advantages of  $Fe_3O_4$  magnetic nanoparticle. It also shows the wide LDRs and low LOD. Those may be attributed to the synergistic effects resulted from the combination of those particles. Therefore, the SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode can be used for fabricating an RT electrochemical sensor.

Electrodes	Renewable	LDR(s)	LOD	References
Cu-CS/MWCNT GCE	Slow	0.05-100	0.01	[10]
GE CeO <sub>2</sub>	Slow	0.5-500.0	0.2	[13]
PAO <sup>a</sup> -Gr CILPE <sup>b</sup>	Slow	0.03-800	0.0083	[36]
$IL^{c}   CCE^{d}$	Slow	0.3-100	0.09	[37]
PtNPs <sup>e</sup> /rGO GCE	Slow	0.057-102.59	0.02	[38]
NiCo <sub>2</sub> O <sub>4</sub> /rGO GCE	Slow	0.1-150	0.01	[39]
GCE Gr	Slow	0.1-2.0	0.00232	[40]
SPE   rGO/Fe <sub>3</sub> O <sub>4</sub> /Ag-MIP	Quick	0.01-10.0, 10.0-3000	0.0042	This work

Table 1. Comparison of several RT electrochemical sensors (µM)

<sup>a</sup> Poly(acridine orange), <sup>b</sup> Carbon/ionic liquid paste electrode, <sup>c</sup> Ionic liquid, <sup>d</sup> Carbon ceramic electrode, <sup>e</sup> Pt nanoparticles

## 3.6. Stability, reproducibility and selectivity of the modified electrode

The SPE  $| rGO/Fe_3O_4/Ag$ -MIP electrode was stored at room temperature in drying conditions. The current response to 10.0  $\mu$ M RT kept about 90.5% of the initial value after six weeks, suggesting a long-range stability.

The reproducibility of the modified electrode was first valued at a RT concentration of 10.0  $\mu$ M with the same SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrode. For six successive assays, the *R.S.D.* was 2.63%. Six SPE | rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP electrodes fabricated independently were employed to determine 10.0  $\mu$ M of RT to check the electrode-to-electrode reproducibility. An acceptable reproducibility with *R.S.D.* of 3.14% was obtained.

Several substances that may interfere the responses of the SPE  $| rGO/Fe_3O_4/Ag - MIP$  electrode are evaluated. A relative error less than ±5% is defined as the selectivity of the modified electrode for 1.0 µM RT determination. No interference was observed with addition of 150-fold concentrations of Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Cd<sup>2+</sup>, Mn<sup>2+</sup>, Zn<sup>2+</sup>, NO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>2-</sup>, 100-fold concentrations of glucose, urea, serine, valine, histidine, asparagine and glycine, 30-fold concentrations of uric acid (UA), AA, dopamine, phenol, hydroquinone and morin. The results demonstrate a good selectivity.

#### 3.7. Application on medicinal tablets

The proposed electrode was employed to determine RT in medicinal tablets to test the reliability of this method. One RT medicinal tablet (labeled amount: 20 mg) was weighed and grinded

to a fine powder then extracted with 20 mL ethanol in the ultrasonic bath for 10 min. After filtration, the solution was diluted into 50 mL with the supporting electrolyte. Five parallel determinations were performed and the results are shown in Table 2. The standard RT solution was added into solution and the recoveries were detected after each determination. The detected contents in the medicine tablets were in good accordance to the labeled value. The recoveries were evaluated between 96.5% and 103.2%. The results indicate that the present method possesses good precision and accuracy for determining of RT in real sample.

Samples	Detected/mM	Added/mM	Found/mM	<i>R.S.D.</i> /%	Recovery/%
1	0.631	1.000	1.663	3.00	103.2
2	0.652	1.000	1.667	3.51	101.5
3	0.660	1.000	1.672	2.84	101.2
4	0.653	1.000	1.640	4.41	98.7
5	0.615	1.000	1.580	4.12	96.5

**Table 2.** Determination results of RT in medicinal tablets (n=5)

# 4. CONCLUSIONS

In conclusion, a highly sensitive and selective sensing platform based on disposable electrode for RT determination using rGO/Fe<sub>3</sub>O<sub>4</sub>/Ag-MIP composite modified SPE was fabricated. Attributing to the synergistic effect resulted from the combination of hybrid composite, the proposed modified electrode shows the merits of wide LDRs, low LOD, fast renewable as well as disposable design. By measuring the medicinal tablets, the practical application of RT electrochemical sensor is evaluated. It exploits new ways for magnetic MIP based composite in drug and food residues determination. Moreover, this universal sensing platform can be explored to determine other drugs and foods.

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