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Highly Sensitive Electrochemical Sensor for Anticancer Drug by a Zirconia Nanoparticle-Decorated Reduced Graphene Oxide **Nanocomposite**

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Supporting Information

ABSTRACT: Because of their large surface area and conductivity, some inorganic materials have emerged as good candidates for the trace-level detection of pharmaceutical drugs. In the present work, we demonstrate the detection of an anticancer drug (regorafenib, REG) by using an electrochemical sensor based on a nanocomposite material. We synthesized a zirconia-nanoparticle-decorated reduced graphene oxide composite (ZrO₂/rGO) using a one-pot hydrothermal method. Reduction of the graphene oxide supports of the Zr²⁺ ions with hydrazine hydrate helped in preventing the agglomeration of the zirconia nanoparticles and in obtaining an excellent electrocatalytic response of the nanostructure ZrO₂/rGO-based electrochemical sensor. Structural and morphological characterization of the nanostructure ZrO₂/rGO was performed using various analytical methods. A novel regorafenib (REG) electrochemical sensor was fabricated by immobilizing the asprepared nanostructure ZrO₂/rGO on to a glassy carbon electrode (GCE). The resulting ZrO₂/rGO/GCE could be used for the rapid and selective determination of



REG in the presence of ascorbic acid and uric acid. The $ZrO_2/rGO/GCE$ showed a linear response for the REG analysis in the dynamic range 11-343 nM, with a remarkable lower detection limit and limit of quantifications of 17 and 59 nM, respectively. The newly developed sensor was used for the accurate determination of REG in both serum samples and pharmaceutical formulations, with satisfactory results.

INTRODUCTION

Regorafenib [4-(4-(3-(4-chloro-3-(trifluromethyl) phenyl)-3flurophenoxy)-N-ethylpicolinamide] (BAY 73-4506) is an orally bioavailable multikinase inhibitor (MKI), which also obstructs multiple tumor pathways, inhibiting targets in the receptors of the vascular endothelial growth factor 1-3 (VEGF 1-3), fibroblast growth factor, and platelet-derived growth factor, including the mutant oncogenic kinesis c-KIT, RET, and B-RAF.^{1,2} This MKI generates dynamic metabolites, which could become agglomerated, particularly in elderly, malnourished patients or in patients treated for hepatocellular carcinoma, as in the case of other MKIs.³ Oral drugs present high protein binding and poor bioavailability and are effectively metabolized by CYP3A4 and UGT1A9 in the liver.^{4,5} Regorafenib (REG), being orally administrated, may lead to drug interactions and major toxicities that may lead to early termination of the treatment and thus diminish its chances of success. It is important to maintain the benefits of these treatments, particularly in the elderly or in patients treated for metastatic colon cancer and gastrointestinal tumors, which is approved by FDA.⁶⁻¹¹ The most serious adverse reaction was drug-induced hepatotoxicity, and a black box warning has been indicated by the US-FDA.¹² Thus, the detection of this anticancer active drug is extensively important and a universal challenge. Some of the sophisticated analytical methods such as high-performance liquid chromatography (HPLC),¹³ liquid chromatography-mass spectrometry (LC-MS),14-19 and spectrophotometry²⁰ are used for the detection of REG in urine, plasma, and other biological samples. However, the aforementioned methods are highly expensive, time-consuming, difficult procedures and require skilled personnel for the specimen, which restricts their particle application. To mitigate

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Figure 1. TEM, HRTEM, and SAED images of (a-c) pristine ZrO2 and (d-f) ZrO2/rGO nanocomposite.

these issues, as revealed earlier, much effort has been made to develop novel substituted methods. In this concern, the electrochemical technique is one of the best methods due to its easy operation, spontaneous detection, excellent sensitivity, inexpensiveness, simple pretreatment procedure, and short analysis time for monitoring of bioelectroactive molecules and pharmaceutical drugs. However, for the detection of bioelectroactive molecules, these electrochemical methods have some analytical complications like high overpotential requirement, the reversible process at the bare and carbon paste electrode, GCE, and by-products that may be deposited on the electrode surface, which decrease its activity. Nevertheless, a familiar approach to triumph over these issues is electrodes' surface modification with various materials, like polymers, $^{21-23}_{24-27}$ carbon materials, and metal-oxide nanoparticles. $^{24-27}$

For the last few decades, graphene oxide (GO) and reduced graphene oxide (rGO) have received significant interest owing to their excellent properties in electrochemical applications, such as good electric conductivity, large surface area, high chemical activity, and wide electrochemical window.^{28,29} Moreover, chemically, rGO is established as a promising supporting material for the uniform distribution of metal-oxide NPs.^{30–34}

In recent years, various metal-oxide-doped graphene oxide composites have been widely used in electrochemical devices and electrocatalysis. Metal oxides, particularly, transition-metal oxides have various physicochemical properties, such as morphological structure, oxygen stoichiometry, good electrochemical conductivity, and interfacial microenvironment of the reaction. Among the transition-metal oxides, zirconium oxide nanoparticles (ZrO₂ NPs) show excellent electrochemical properties, including nontoxicity, thermal stability, wide band gap, and good electrical and surface properties and are one of the most abundant metals.^{35,36} A critical issue in utilizing bare ZrO₂ nanoparticles is that they tend to aggregate and form large clusters during their synthesis.^{37,38} In this connection, rGO is an excellent material to mitigate the agglomeration of ZrO₂ NPs and subsequently enhance the electrochemical properties. Therefore, researchers have been giving dedicated extensive efforts to synthesize and explore ZrO₂ decorated on rGO sheets, for example, Pt/ZrO₂-RGO/GCE for significant enhancement of the catechol and hydroquinone oxidation,³ ZrO₂/rGO-based biosensor for detection of the oral cancer drug,⁴⁰ and Meth/ZrO₂/rGO-based immunosensor.⁴

To the best of our knowledge, this is the first example of electrochemical REG sensing in human blood serum and pharmaceutical formulations using $ZrO_2/rGO/GCE$. In this work, we tried to validate such a voltammetric sensor for the detection of REG. The prepared $ZrO_2/rGO/GCE$ can resolve overlapping signals from REG, uric acid (UA), and ascorbic acid (AA). In addition, the present work showed that this sensor possesses an excellent linear dynamic range (LDR) and limit of detection (LOD) for the novel REG determination (Scheme 1).

RESULTS AND DISCUSSION

Characterization of the ZrO₂/rGO Nanocomposite. Transmission electron microscopy (TEM), high-resolution TEM (HRTEM), and selected area electron diffraction (SAED) analyses were conducted to examine the morphology and structure of the synthesized nanocomposite. The TEM images of the pristine ZrO2, GO, and ZrO2/rGO nanocomposite are shown in Figure 1. Pristine ZrO₂ nanoparticles (Figure 1a) are nearly spherical with a uniform size of size of 6-9 nm, which is in good agreement with the calculated values based on the powder X-ray diffraction (PXRD) result. Moreover, the displaced-lattice spacing of 0.291 nm, determined from the HRTEM images (blue circles in Figure 1b) is consistent with the (111) plane of ZrO_2 . The pristine GO nanosheets are highly wrinkled, and the ZrO₂ nanoparticles (blue circles) are well decorated and uniformly distributed on the surface of the wrinkled rGO (Figure 1d-f). The SAED patterns for ZrO₂ (Figure 1c) and the ZrO₂/rGO nanocomposite shown in Figure 1f (inset) illustrate the crystalline dots instead of amorphous rings, which indicate the polycrystalline nature of the ZrO₂ nanoparticles and nanocomposite. To further confirm the formation of the nanocomposition, energy dispersive X-ray spectroscopy (EDX) analysis was employed. The presence of carbon, zirconium, and oxygen elements confirms the presence of ZrO₂ on to the GO surface (Figure S3).

The phase purities of the as-synthesized GO and the $ZrO_2/$ rGO nanocomposite were examined using PXRD. Figure S4a shows a diffraction peak at $2\theta = 10.7^{\circ}$, corresponding to the (001) planes of GO. The peaks of the ZrO₂/rGO nanocomposite, in Figure S4b, show the existence of both tetragonal and monoclinic mixed phases, which coincide with the standard cards (JCPDS card nos. 49-1642 and 37-1484, respectively) and also show that the peak at $2\theta = 10.7^{\circ}$, in Figure S4a, has shifted to 22.4° (002), indicating that GO has been reduced after treating it with the ZrO₂ nanoparticles. In addition, the particle size of the ZrO2 nanoparticles was calculated using the Debye–Scherrer equation $(D = 0.89\lambda/\beta)$ $\cos \theta$), where *D*, λ , β , and θ are the average particle size, wavelength of the Cu K $\!\alpha$ irradiation, intensity at the full width at half-maximum of the diffraction peak, and diffraction angle of the (111). The average particle size of pristine ZrO_2 is about 7 nm, in good agreement with the TEM result. These results clearly confirm the formation of the ZrO₂/rGO nanocomposite.

The Fourier transform infrared (FT-IR) spectra of the GO and ZrO_2/rGO samples were recorded at wave numbers 500–4000 cm⁻¹. Pristine GO has a large number of surface functional groups, as shown in Figure S5a, such as O–H, C=O, C=C, and C–O, which are confirmed by the IR bands at 3320, 1700, 1604, and 1009 cm⁻¹, respectively. In the ZrO_2/rGO sample shown in Figure S5b, the hydroxyl, carbonyl and epoxide functional groups had disappeared and also overall peak intensities decreased significantly, which confirm reduction of pristine GO leads to the formation of ZrO_2/rGO nanocomposite.

The chemical composition was further confirmed by X-ray photoelectron spectrometry (XPS). The wide-survey scan spectrum of the ZrO₂-doped rGO nanocomposite (ZrO₂/ rGO) is shown in Figure S6. The major peaks at 182.5, 284.9, and 530.2 eV are attributed to Zr 4p, C 1s, and O 1s, respectively. In addition, the peaks at 27, 333, and 433 eV attributed to Zr 4p, Zr 3p, and Zr 3s, respectively. The deconvolution spectrum of the Zr 3d peak, Figure S6 (inset), shows binding energies at 182.4 and 184.9 eV attributed to Zr $3d_{5/2}$ and Zr $3d_{3/2}$, respectively, which can be assigned to the Zr(IV) oxidation state. On the basis of these results, we confirmed that ZrO₂ is well embedded into the wrinkled rGO.

Electrochemical Behavior of REG. Figure 2 shows the cyclic voltammograms for the electrocatalytic oxidation of REG on the bare and modified GCE electrodes, recorded in the supporting electrolyte solution (phosphate buffered saline (PBS) 0.1 M, pH 7.0) in the presence of 0.01 mM REG at 50 $mV s^{-1}$. The voltammograms recorded on the bare GCE in the absence of REG did not show any redox peaks (Figure 2a), indicating that no faradic reactions occurred on the surface of the unmodified GCE electrode. Figure 2b shows that the addition of 0.01 mM REG to the supporting electrolyte solution results in the GCE exhibiting a lowest sensitivity reversible couple peak of high separation; $\Delta E_{\rm p}$ = 208 mV, which suggests a slow electron transfer. Figure 2c,d shows the recognizable electrochemical response of the ZrO₂/GCE and ZrO₂/rGO/GCE, respectively, during the oxidation of 0.01 mM REG, which is interpreted as a result of the enhanced sensitivity, electrode surface area, and improvement of the electrochemical activity of the GO support with ZrO₂ NPs. Figure 2d shows the ZrO₂/rGO/GCE and reveals well-defined reversible couple peaks at about $E_{\rm pa}$ = 275 mV and $E_{\rm pc}$ = 306 mV, attributed to the high catalytic effect during the oxidation



Figure 2. Cyclic voltammograms recorded in 0.1 M PBS, pH 7.0, at the scan rate of 100 mV s⁻¹ (a) blank and bare GCE electrode without REG, (b) bare GCE in the presence of 0.01 mM REG, (c) ZrO_2 -modified GC electrode in the presence of 0.01 mM REG, and (d) $ZrO_2/rGO/GCE$ in the presence of 0.01 mM REG.

of 0.1 mM REG. The $\text{ZrO}_2/\text{rGO}/\text{GCE}$ remarkably improved the reversible couple peaks and it should be emphasized that the peak-to-peak separation, that is $\Delta E_{\rm p}$, decreased to 31 mV. Finally, the results confirmed that the prepared $\text{ZrO}_2/\text{rGO}/$ GCE significantly improves the electrocatalytic ability to oxidize REG.

Effect of pH. The significant effects of the electrolyte pH on the determination of REG by electrocatalysis of ZrO₂/ rGO/GCE were studied for both current and potential. Figure 3 shows the effect of different pH values, in the range 5.5-8.0, investigated by differential pulse voltammetry (DPV) in a 0.01 mM REG solution and the relationship between I_{pa} and E_{pa} (anionic peak current and potential, respectively) with the buffer pH. Figure 3b shows that the anodic peak current of the REG electro-oxidation increases until the pH value becomes 7.0 and then decreases until the end of the experiment (pH 8.0). For this reason, the electrolytic solution with pH 7.0 was chosen for the complete electrocatalytic study. Figure 3b also shows that the formal REG potential shifts toward lower values with the increase in the supporting electrolytic solution. A better correlation coefficient was obtained for the pH vs $E_{\rm pa}$ which was confirmed by a slope of 0.053 09 V/pH (R^2 ⁺= 0.9621), in the range 5.5-8.0. According to the linear

regression analysis, the slope of the dE_p/dpH being close to the theoretical value of 0.059 V/pH indicates that the irreversible couple peaks involved the transfer of the same number of electrons and protons,⁴² in agreement with literature data.⁴³ The ZrO₂/rGO/GCE responds to the oxidation of REG according to the mechanism presented in Scheme 2. Thus, REG-keto oxidizes to REG-enol (the amide derivative) after an exchange of one electron and one proton via ZrO₂/rGO nanocomposite.³⁶

Influence of the Scan Rate. Figure 4a shows the influence of the scan rate, from 10 to 100 mV s⁻¹, on the cyclic voltammetry (CV) peak potential and current of 0.01 mM REG in the presence of 0.1 M PBS, pH 7.0, at the $ZrO_2/rGO/GCE$. The REG reversible couple peak current increases gradually with an increase in the scan rate. In addition, the REG oxidation and reduction peak currents (I_{pa} and I_{pc}) showed good linear correlation coefficients (R^2) as a function of the square root of the scan rates of the anodic and cathodic peaks with 10–100 mV s⁻¹ changing scan rates (Figure 4b), obeying the following linear regression equations

$$I_{pa} (\mu A) = 2.365 + 0.000035 \,\vartheta^{1/2} (mV^{1/2} s^{-1/2})$$

; (R² = 0.9923, n = 10)
$$I_{pc} (\mu A) = 3.588 + 0.000049 \,\vartheta^{1/2} (mV^{1/2} s^{-1/2})$$

; (R² = 0.9138, n = 10)

These results indicate that the electrochemical reactions of REG on the $ZrO_2/rGO/GCE$ are diffusion-controlled processes. The estimation of the REG electrochemical parameters, at different scan rates, was made with Laviron's eqs 1-3.⁴⁴

$$E_{\rm pa} = E^0 + \frac{2.3RT}{(1 - \alpha)nF} \log\vartheta$$
⁽¹⁾

$$E_{\rm pc} = E^0 - \frac{2.3RT}{\alpha nF} \log \vartheta \tag{2}$$

$$\log k_{\rm s} = \alpha \, \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log\left(\frac{RT}{nF\vartheta}\right)$$
$$\propto \frac{(1 - \alpha)nF\Delta E_{\rm p}}{2.3RT} \tag{3}$$



Figure 3. (a) DPV voltammograms obtained with the $ZrO_2/rGO/GCE$ in an electrolyte solution at different pH values, with 0.01 mM REG. (b) Calibration plot of the anodic peak current (curve-I) and the anodic peak potential (curve-II) vs the pH of the 0.1 M PBS solution, during the electro-oxidation of 0.01 mM REG, at a scan rate of 100 mV s⁻¹.

Scheme 2. Electrochemical Redox Process of REG by ZrO₂/rGO/GCE



Figure 4. (a) Cyclic voltammograms recorded at the $ZrO_2/rGO/GCE$ in the electrolyte solution at different scan rates from 10 to 100 mV s⁻¹. (b) Calibration plot of the anodic and cathode peak currents versus the square root of the scan rate, during the electro-oxidation of 0.01 mM REG in the presence of 0.1 M PBS, pH 7.0.



Figure 5. (a) Differential pulse voltammograms recorded at the $ZrO_2/rGO/GCE$ over an REG concentration of 11–343 nM in 0.1 M PBS at pH 7.0. (b) Linear calibration plot of the anodic peak current versus REG concentration.

where α is the electron transfer coefficient (0.76), *n* is the number of electrons, *F* is the Faraday constant (96 485 C mol⁻¹), *R* and *T* are the universal gas constant and temperature (K), respectively, and k_s is the standard heterogeneous rate constant (1.18) determining the slowest step of the REG electrochemical oxidation.

Analytical Performance of $ZrO_2/rGO/GCE$ for REG Detection. The $ZrO_2/rGO/GCE$ was tested by differential pulse voltammetry (DPV), to investigate the sensitivity of its response to various REG concentrations in the linear dynamic range of 11–343 nM REG in 0.1 M PBS, pH 7.0, at pulse height 60, pulse width 10, and scan rate 100 mV s⁻¹, as shown in Figure Sa. The intensity of the anodic peak current increased with the REG concentration. Figure 5b shows the linear dynamic-range plotting of the anodic peak current (I_{pa}) versus the REG concentrations and its linear regression equation I_{pa} =

9.4911(REG) + 3.434, (R^2 = 0.9963). The limits of detection and quantification can be calculated according to eqs 4 and $5^{45,46}$

$$LOD = 3 \times SD/B \tag{4}$$

$$LOQ = 10 \times SD/B \tag{5}$$

where SD is the standard deviation and *B* is the slope of the calibration plot. From the calibration plot, a detection limit of 11 nM and quantification limit of 59 nM were calculated, on the basis of S/N = 3 (signal to noise). These results confirmed that the $ZrO_2/rGO/GCE$ is a promising platform for the electrochemical determination of ultratrace of REG concentration.

Simultaneous Detection of REG, AA, and UA by ZrO₂/ rGO/GCE. The main objective of this study was sensing of REG, AA, and UA in a mixture. REG, AA, and UA mixture solution with different concentrations in 0.1 M PBS with pH 7 was prepared. DPV result for the simultaneous detection of REG, AA, and UA is presented in Figure 6. The oxidation peak



Figure 6. DPVs recorded on the $ZrO_2/rGO/GCE$ during simultaneous determination of 0.32–0.66 μ M REG, 0.58–1.26 μ M AA, and 0.08–0.52 μ M UA in 0.1 M PBS, pH 7.0. Insets: plots of the anodic peak currents against concentrations of REG, AA, and UA.

current increased synchronously on increasing the concentration of REG, AA, and UA. The DPV signal shows the linear relationship between the oxidation peak current and REG concentrations (Figure 6 inset) with LDR 0.32–0.66 μ M and the linear regression equation as follows: I_{pa} (μ A) = 15.46 C_{REG} + 0.5232 (μ M) (R^2 = 0.9943). The oxidation current increased parallel AA and UA concentrations with LDRs 0.58–1.26 and 0.08–0.52 μ M, respectively, and the linear regression equations as follows: I_{pa} (μ A) = 3.92 C_{AA} + 2.417 (μ M) (R^2 = 0.9749) and I_{pa} (μ A) = 15.85 C_{UA} + 4.97 (μ M) (R^2 = 0.975). This result indicated that the proposed electrochemical sensor enables the synergetic and sensitive detection of REG in the presence of AA and UA without significant interference from each other.

Effect of Interference Compounds. The effect of interferences for the determination of REG, AA, and UA mixture solution was investigated in 0.1 M PBS (pH 7.0) electrolyte solution in the presence of a possible interfering factor such as metal ions (Ca^{2+} , Mg^{2+} , and Zn^{2+}), glutathione, folic acid, or L-cysteine. The observed results are summarized in Table S1. No significant signal intensity change (less than 5% difference from their original signal intensity) was observed in the presence of interfering ions and molecules. The results indicate that the designed $ZrO_2/rGO/GCE$ displays good ability for simultaneous sensing of AA, UA, and REG in a matrix mixture, without any interference of the abovementioned species.

Real-Sample Analysis. The real-sample monitoring of the performance of the $ZrO_2/rGO/GCE$ was validated by the DPV determination of REG in human blood serum samples. To determine the accuracy of the results, 1.0 mL human blood serum samples were diluted 50 times with PBS to prevent the matrix effects of analytical determinations. The human blood serum samples were centrifuged before the measurements; the results are summarized in Table 1. The recovery rates for the different volumes of the samples ranged between 96.5 and 101.9% and the relative standard deviations were in the range 0.3–2.4%, showing the accuracy and efficiency of the

Table 1. Real-Sample Analysis of REG Using the Proposed Method by Triplicate (n = 3) Readings

samples	spiked sample (mM)	found (mM)	recovery (%)	RSD (%) ±SE
pharmaceutical	0.010	0.0098	98	2.54 ± 0.05
formulation	0.050	0.0509	101.8	1.98 ± 0.08
	0.100	0.0990	99	1.32 ± 0.06
blood serum	0.010	0.0098	98	2.42 ± 0.11
	0.050	0.0486	97.2	1.65 ± 0.07
	0.100	0.1026	102.6	1.09 ± 0.05

constructed electrochemical sensor. Therefore, the $\rm ZrO_2/$ rGO/GCE can be applied to real bioclinical samples.

Comparison with Other Established Methods. The sensitivity of the developed electroanalytical method was compared to that of some of the chromatography and spectrophotometry methods. Recently, Fujita et al., reported one method using high-performance liquid chromatography (HPLC) for simultaneous quantitative determination of REG in the human plasma and achieved LOQ of 10 ng mL⁻¹. In addition, Erp et al., reported liquid chromatography tandem mass spectrometry (LC-MS/MS) can achieve LOQ of 100 ng $mL^{-1}\ {\rm for}\ REG.$ The other spectroscopic method can analyze REG with an LOQ of 290 ng mL⁻¹. Compared with the reported methods, the presented method shows a better limit of detection and limit of quantification limit (Table 2). Due to its low cost, simplicity, high sensitivity, and rapid analysis time, the presented method has advantages over the other analytical methods.

CONCLUSIONS

Inorganic metal-oxide nanoparticles have been enormously employed as electrode material for a developed efficient electrochemical sensor. A simple one-pot hydrothermal synthesis of ZrO₂/rGO nanocomposite was successfully synthesized. The pristine ZrO₂ nanoparticles are about 7 nm and uniformly dispersed on the reduced graphene oxide sheet. Due to reduced graphene oxide support, the ZrO_2 metal-oxide nanoparticles illustrate excellent electrocatalytic performance toward REG anticancer drug. The developed ZrO₂/rGO nanocomposite was characterized by TEM, EDX, PXRD, FT-IR, and XPS measurements. TEM images clearly show the zirconia nanoparticles have uniform size and uniform distribution on the surface of rGO. The inorganic nanocomposite-based electrochemical sensor was successfully applied first time for the detection of REG anticancer drug. The fabricated ZrO₂/rGO nanocomposite exhibited an excellent electrocatalytic activity toward REG, with a linear dynamic range of 11-343 nM and the detection limit as low as 17 nM toward the detection of REG drug. The ZrO₂/rGO/ GCE was applicable for the joint determination of REG and a commonly reported interference AA and UA at pH 7.0. The calculated LOD/LOQ values by the present electrochemical method are better than those from chromatography and spectrophotometry methods. This electrochemical sensor shows promise for future exploration of rapid detection of other anticancer drugs in human blood serum and pharmaceutical formulation.

EXPERIMENTAL SECTION

Modification of the Glassy Carbon Electrode. Preparation of the $ZrO_2/rGO/GCE$: prior to use, the bare GCE

Tabl	le 2.	Comparison	ı of	the	Electroana	lytica	l Method	with	1 Reported	Analytica	l Technique	es
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method	$LDR (ng mL^{-1})$	$LOD (ng mL^{-1})$	$LOQ (ng mL^{-1})$	refs
HPLC	10-10 000		10.0	13
LC-MS/MS	25-25 000		25.0	14
LC-MS/MS	100-4000		100	15
spectrophotometric method	500-25 000	110	290	20
electroanalytical method	1.5–107 (11–343 nM)	5.00 (17 nM)	18.5 (59 nM)	present work

was polished with alumina powder (1, 0.3, and 0.05 μ m) and washed with an ethanol solution, followed by Millipore water under ultrasonication. For the preparation of the electrochemical sensor, 3 mg of the prepared ZrO₂/rGO was dispersed in 3 mL of Millipore water with 0.3 mL of Nafion solution and then ultrasonicated for 30 min until a uniformly dispersed ink was obtained. The ZrO₂/rGO ink (7 μ L) was drop cast onto the clean GCE surface and allowed to dry for 15 min at room temperature. The ZrO₂/GCE was fabricated in a similar manner.

Reproducibility and Stability of the Modified Electrode. Reproducibility and stability are key elements for electrode performance. To evaluate the reproducibility of the ZrO₂/rGO/GCE, we made five sensing electrodes and used them to investigate their CV current response on 1 μ M REG in phosphate buffered saline (PBS), pH 7.0, as shown in Figure S1a. The calibrated histograms in Figure S1b show a relatively standard deviation (RSD) of 0.54%. The repeatability of the modified electrochemical sensor values was obtained for the detection of 1 μ M REG in presence of the supporting electrolyte, PBS (pH 7.0). The ZrO₂/rGO/GCE average voltammetric response for seven successive determinations was 3.34% (Figure S2a). Moreover, the stability of the $ZrO_2/rGO/$ GCE was verified by the daily detection for 7 weeks of 1 μ M REG solution in presence of the supporting PBS electrolyte (pH 7.0). After each test of stability, the electrode was washed with deionized water, dried under an argon stream, and kept in empty glass tubes at room temperature. The electrochemical oxidation of the 1 μ M REG solution, in presence of the supporting PBS electrolyte (pH 7.0), using the ZrO₂/rGO/ GCE diminished by about 9.6% of their initial response during the 7 weeks, as shown in Figure S2b. Hence, the proposed method and the modified electrochemical sensor determined REG with higher reproducibility and stability than the $ZrO_2/$ GCE sensor.

Preparation of Real Samples for Analysis. A powdered Stivarga tablet (Nexus Lifecare Pvt. Ltd., Mumbai, India) containing 40 mg of REG was dissolved by ultrasonication in 25 mL of 0.1 M PBS buffer solution at pH 7.0. This solution was filtered and quantitatively diluted with buffer solution to get 0.1 mM REG solution that was used for the analyses. Fresh human blood serum samples were collected from healthy volunteers (S. V. University Health Center, S. V. University, Tirupati, India). Approximately, 2.0 mL of human blood serum was diluted with 100 mL of 0.1 M PBS, at pH 7.0, and the solution thus prepared was used for analysis, without any further treatment.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsome-ga.8b02129.

Cyclic voltammogram, EDS data, FT-IR, materials and methods, XPS analysis, XRD, and interferences of some foreign species (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Author Contributions

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

AA, ascorbic acid; CV, cyclic voltammetry; DPV, differential pulse voltammetry; EDX, energy dispersive X-ray spectroscopy; FT-IR, Fourier transform infrared; GCE, glassy carbon electrode; HRTEM, high-resolution transmission electron microscopy; LOD, lower detection limit; LOQ, limit of quantifications; REG, regorafenib; rGO, reduced graphene oxide; SAED, selected area electron diffraction pattern; TEM, transmission electron microscopy; UA, uric acid; XPS, X-ray photoelectron spectrometry; XRD, X-ray diffraction; ZrO₂ NPs, zirconium oxide nanoparticles

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