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Electrochemical studies of regorafenib in non-aqueous media

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Abstract: A novel tyrosine kinase inhibitor, regorafenib was electrochemically studied using a GC disc electrode in non-aqueous media. A well-resolved, irreversible, diffusion-controlled oxidation peak was obtained at 1.55 V in acetonitrile solution containing 0.1 M $TBAClO_4$. Experimental conditions such as scan rate, indicate that two electrons play a role in the electrochemical oxidation of regorafenib. The recommended method was successfully applied to the determination of regorafenib in drug capsules.

Keywords: Regorafenib; cyclic voltammetry; electrochemical oxidation.

1. Introduction

Regorafenib (Stivarga, Figure 1) is an orally administered inhibitor of protein kinases that plays a role in the maintenance of angionesis (e.g. VEGFR1-3 and TIE2), oncagenesis (e.g. c-kit, Ret, wild-type and V-600-mutated BRAF) and tumor microenvironment (e.g. PDGFR and fibroblast growth factor receptor).[1-3] Disclosure of the biotransformation pathways and metabolic activity of regorafenib is important because of oral administration. Regorafenib is metabolized by oxidative and conjugative biotransformation in the liver to give the first N-oxide metabolite M-2 in humans.[4-6] It is recently approved for the treatment of metastatic colorectal cancer (mCRC) in patients who have previously received all standard systemic anticancer treatments. In addition, regorafenib is a small-molecular tyrosine kinase inhibitor (TKI) exhibiting recovery in progression-free survival and overall survival in heavily pretreated patients with mCRC.[7] Therefore, regorafenib may offer a new treatment option to CRC patients.

Chromatografic methods have been reported for the measurement of regorafenib in biological samples[8-10]. These methods are time consuming and they require complex extraction and purification steps. Therefore, it was felt useful to develop electrochemical method for its determination. Recently, electrochemical methods have been well developed owing to their low cost, high sensitivity and short analysis time. Electroanalytical methods could be further applied to the detection of reaction mechanisms.

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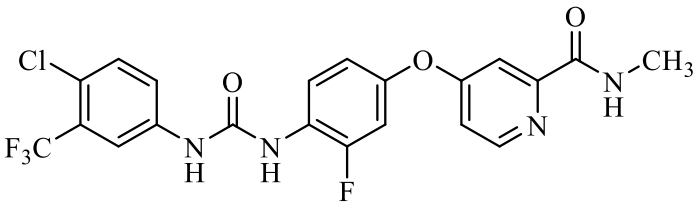


Figure 1. Chemical structure of regorafenib (4-(4-(3-(4-Chloro-3-(trifluoromethyl)phenyl)ureido)-3-fluorophenoxy)-N-methylpicolinamide).

In this study the electrochemical properties of regorafenib was first investigated. Accordingly, electrochemical analysis of regorafenib was conducted in acetonitrile solution at glassy carbon (GC) electrode using cyclic voltammetry and chronocoulometry methods.

2. Experimental

2.1. Materials

Regorafenib was obtained from pharmacies. Acetonitrile (HPLC grade) was purified by drying with calcium hydride, followed by distillation from phosphorus pentoxide. It was kept over molecular sieves (3 Å, Merck) in order to eliminate its water content as much as possible. Tetrabutylammonium perchlorate ($TBAClO_4$, Aldrich) was used after recrystallization.

2.2. Electrochemical instrumentation

Electrochemical measurements were carried out using a Bioanalytical Systems BAS-100B electrochemical workstation (Bioanalytical System Inc., Lafayette, IL, USA). All electrochemical experiments were performed with a three-electrode configuration. The working electrode was a glassy carbon (GC, BAS Model MF-2012, area = 0.072 cm^2) electrode. Platinum wire (BAS Model MW-1032) and $Ag/AgCl/KCl_{(sat)}$ (BAS Model MF-2078) electrodes were used as counter and reference electrodes, respectively. The working electrode was successively polished with 1.0, 0.3 and 0.05 µm alumina slurries (Buehler) on microcloth pads (Buehler) and then washed with water and sonicated for 10 min in acetonitrile. The electrode potentials were quoted versus $Ag/AgCl/KCl_{(sat)}$ reference electrode at room temperature. During electrochemical experiments, the electrolyte solutions were deoxygenated by foaming with high purity nitrogen gas.

The surface area of the GC electrode was calculated using 1.0 mM $K_3Fe(CN)_6$ solution containing 0.1 M KCl as a probe at different scan rates (0.1-1 V s^{-1}). For simple redox events, the Randles–Sevcik formula was used:

$$I_p = 2{,}687 \times 10^5 n^{3/2} A D^{1/2} C v^{1/2} \qquad (1)$$

where I_p refers to the anodic peak current (A), n is the number of electrons transferred, A is the surface area of the electrode (cm^2), D is the diffusion coefficient (7.6×10^{-6} cm^2 s^{-1}), C is the concentration (mol cm^{-3}) and υ is the scan rate (V s^{-1}). The surface area of the electrode was calculated to be 0.072 cm^2 from the slope of the plot of I_p as a function of $\upsilon^{1/2}$.

The solution of regorafenib was prepared in 0.1 M $TBAClO_4$/acetonitrile to a concentration of 4.7 µg/mL

3. Results and discussion

The electrochemical behavior of regorafenib was investigated at the GC disc electrode in anhydrous acetonitrile solution containing 0.1 M $TBAClO_4$ as the supporting electrolyte by using cyclic voltammetry (CV) (Figure 2). As shown in Figure 2, the cyclic voltammogram of regorafenib shows irreversible anodic peak at 1.55 V vs. Ag/AgCl/KCl (3.0 M). Upon reversing the potential scan, no reduction peak corresponding to these oxidation wave is observed indicating the irreversible nature of the electrode reaction. However, oxidation or reduction peak was not observed in background solution.

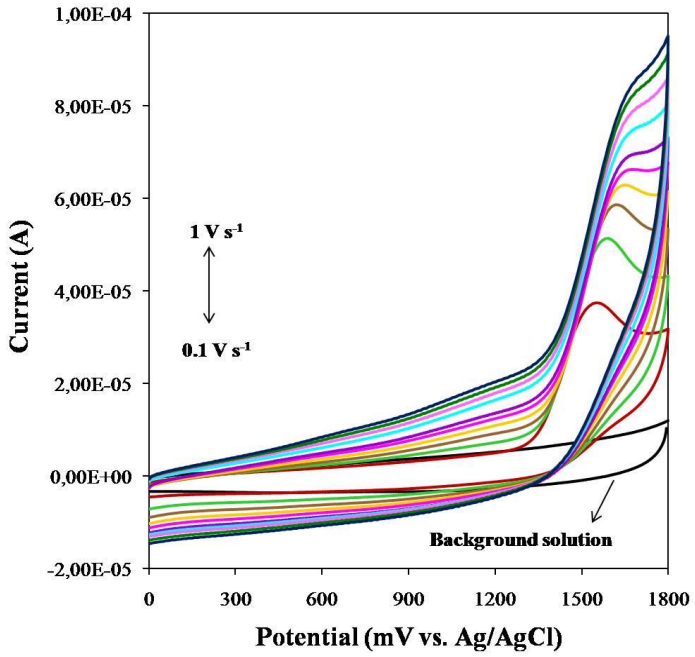


Figure 2. Cyclic voltammograms recorded at different scan rates in acetonitrile solution containing 1 mM regorafenib and 0.1 M $TBAClO_4$ at GC disc electrode. The scan rate is systematically varied from 0.1 to 1 V s^{-1}.

For detailed examine the electrochemical oxidation behaviour of regorafenib on GC electrodes, a series of the cyclic voltammograms were recorded at different scan rates (0.1-1.0 V s^{-1}) in acetonitrile solution containing 1 mM regorafenib and 0.1 M $TBAClO_4$ (Figure 2). The figure 2 depicts the peak current increases with increasing scan rate. In addition, anodic peak potentials shift to more positive potentials as the scan rate increases. The plot of anodic peak current (I_{pa}) against the square root of the scan rate ($\nu^{1/2}$) is linear (Figure 3a) and also the current function ($I_{pa}/\nu^{1/2}$) decrease with increasing $\nu^{1/2}$ (Figure 3b). These observations indicate that the oxidation of regorafenib at the GC was diffusion-controlled and electron transfer is followed by a chemical reaction.

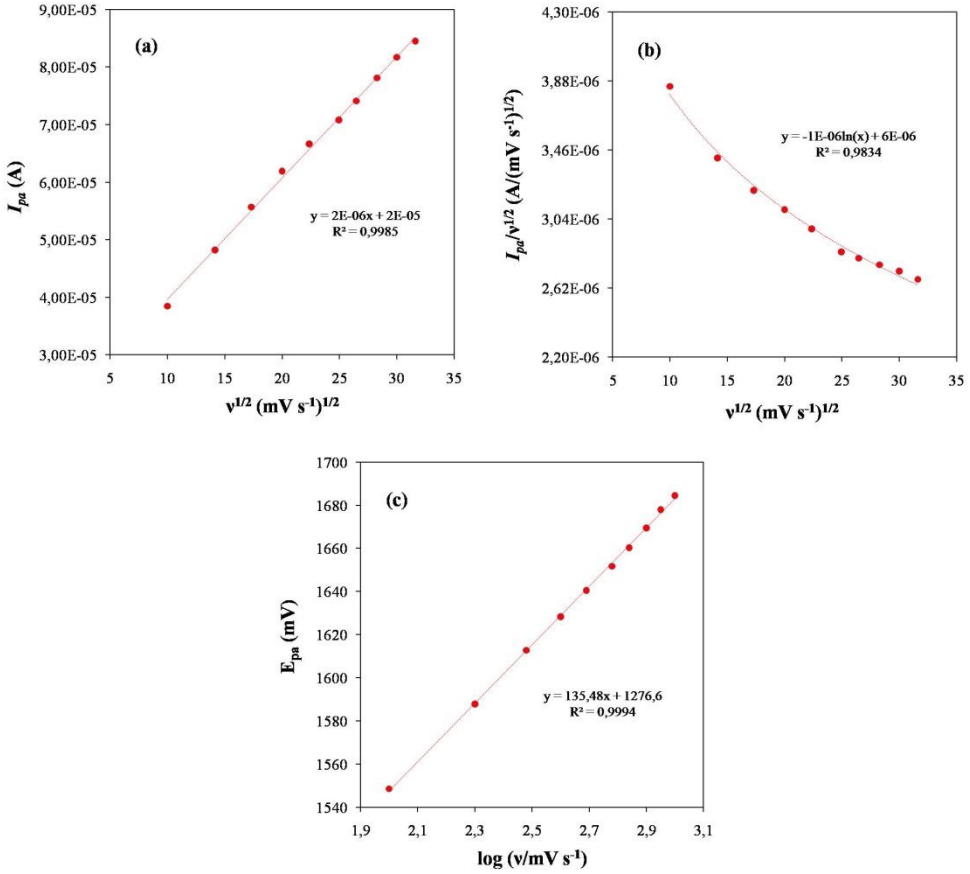


Figure 3. According to the results of cyclic voltammogram recorded at different scanning rates in acetonitrile solution containing 1 mM regorafenib and 0.1 M $TBAClO_4$; (a) Plot of peak current against square root of scan rate for oxidation peak at 1.55 V. (b) Plot of the current function ($I_{pc}/v^{1/2}$) against square root of the scan rate ($v^{1/2}$) for the oxidation peak. (c) Plot of peak potential (E_{pa}) against logarithm of scan rate for the oxidation peak.

In order to find the rate-determining step during the oxidation process of regorafenib, the electron transfer coefficient (α) was calculated from cyclic voltammograms using the variation of anodic peak potential (E_{pa}) with logarithm of scan rate (log v) according to Eq. 2,

$$E_{pa} = E^o + \frac{RT}{[(1-\alpha)n_aF]\left[0.78+\ln(D^{\frac{1}{2}}K_S^{-1}\right]} - 0.5\ln RT/[(1-\alpha)n_aF + \frac{RT}{[(1-\alpha)n_aF]}/21n\nu \qquad (2)$$

This equation is a general expression for the case of surface confined electroactive species with a concentration small enough derived by Laviron.[11-12]

According to this equation, αn_a can be determined by the variation of peak potential and scan rate. In this equation, *R* is the gas constant (8.314 J K^{-1} mol^{-1}); T is the room temperature (298 K); F is the Faraday constant (96500 C mol^{-1}) and n_a is the number of electrons transferred in the rate determining step. Figure 3c shows the variation of E_{pa} as a function of log v. From the slope of the graph, α value was calculated as 0.22. This result shows that the electron transfer process is a speed-determining step. On the other hand, the chemical step follows a rapid charge transfer reaction.

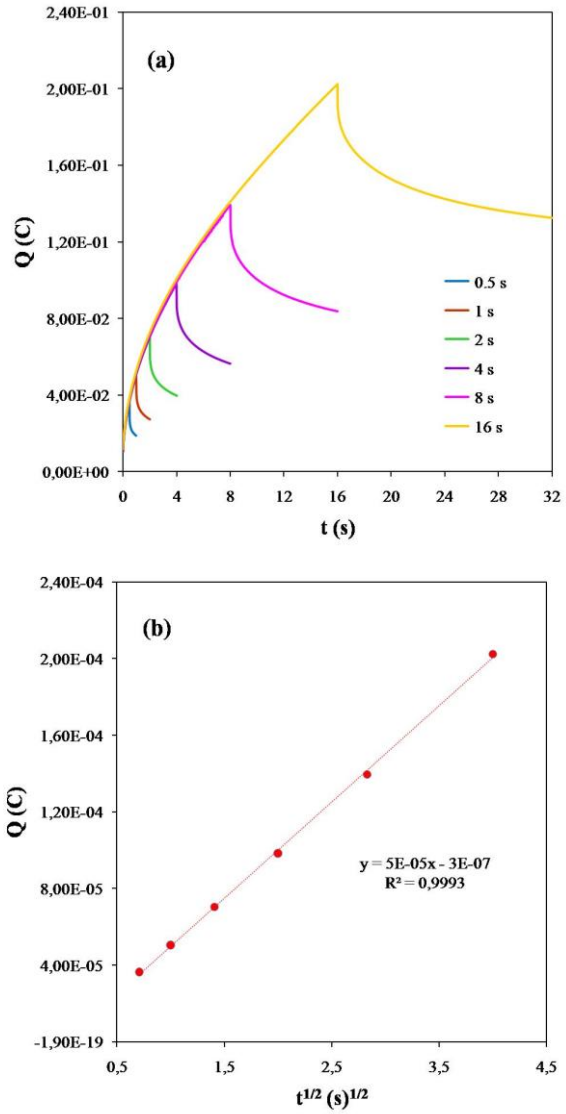


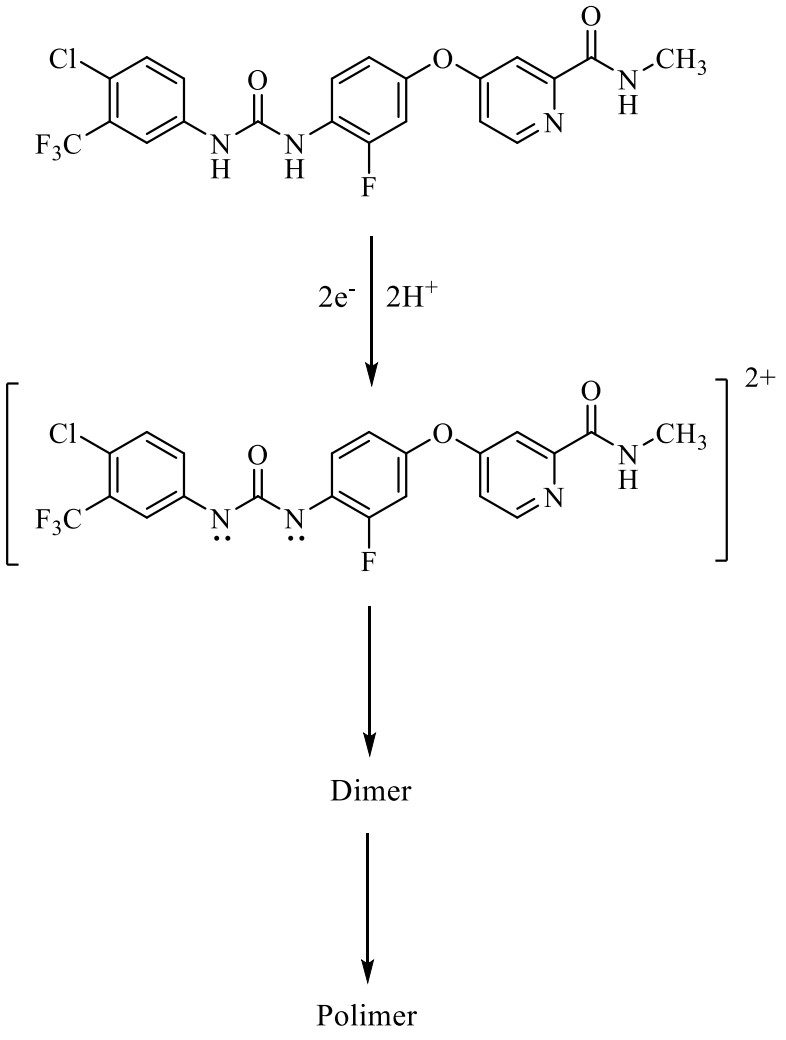
Figure 4 (a) Chronocoulograms recorded in acetonitrile containing 1 mM regorafenib and 0.1 M $TBAClO_4$ at different time fields. The potential was increased an initial step from 0.0 V to 1.5 V. (b) The plots of the total charge (Q) versus $t^{1/2}$ for regorafenib at the same conditions. The potential was increased an initial step from 0.0 V to 1.5 V (•).

Double potential step chronocoulometry measurements were also performed to determine the number of electrons transferred (n) during the electrochemical oxidation of regorafenib. Measurements were operated by applying potential steps from 0.0 V to 1.5 V vs. Ag/AgCl/KCl (3.0 M) in the time field 0.5-16 s. Figure 4a shows chronocoulometry curves recorded by stepping the applied potential from 0.0 V to 1.5 V. As shown in figure, the total charge (Q) step by step increases with time (t). In addition, the Q values for applied step potential are linearly proportional with $t^{1/2}$ (Figure 4b). The n value was described by the integrated Cottrell equation, known as the Anson equation [13] (Eq.3),

$$Q = \frac{2nFAC_0D^{1/2}}{\pi^{1/2}} t^{1/2} \quad (3)$$

where Q is the charge (C); n is the number of electrons transferred; F is Faraday's constant (96500 C mol^{-1}); A is the area of the working electrode; C_0 is the bulk concentration of regorafenib (1×10^{-6} mol cm^{-3}) and D is diffusion coefficient (taken to be 1.0×10^{-5} cm^2 s^{-1}). From the slope of the linear plot of Q vs. $t^{1/2}$, the value of n was calculated as 2.2 for oxidation of regorafenib at glassy carbon electrode.

The results demonstrate that regorafenib can be involved in two-electron oxidation process. A plausible mechanism is shown in Scheme 1. Therefore, it can be concluded that regorafenib lost two protons and two electrons in the oxidation process to form free radicals. After the first electron transfer, the oxidation mechanism involves a radical coupling following a proton loss and rearrangement steps. The intermediate undergoes rearrangement as a result of deprotonation and causes dimers. Also, these dimers are oxidized at a slightly more anodic potential from the oxidation step and form oligomeric species and polymers.



Scheme 1. Plausible mechanism for the electrooxidation of regorafenib at a GC

4. Conclusion

The electrochemical behavior of regorafenib has been studied at a GC in non-aqueous media by cyclic voltammetry and chronocoulometry methods. Regorafenib undergoes a two-electron change through a completely diffusion-controlled current process. The developed methods were successfully applied for the determination of regorafenib in drug capsules.

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