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Original Article

Electrochemical Determination of Calcium Dobesilate on Graphene Glassy Carbon Modified Electrode

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Abstract

Graphene modified glassy carbon electrodes have been developed for the determination of calcium dobesilate in pharmaceutical formulations. The prepared graphene and CNTs were used as a conductive substrate for electrochemical study of calcium dobesilate. The direct electrochemistry of calcium dobesilate showed a reversible cyclic voltammogram with a formal potential of 133 mV (vs. Ag/AgCl) for graphene modified electrodes in 0.1 M phosphate buffer. The linear concentration range of the sensor is 30-120 ng/mL for graphene modified electrode, respectively. The lifetime of biosensor is more than 2 weeks. The proposed modified electrode provides a new promising and alternative way to detect calcium dobesilate.

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Introduction

Calcium dobesilate (2, 5-dihydroxybenzenesulfonate) is a drug with antioxidant, anti-radical and angioprotective properties that was used as vasoprotective agent. Calcium dobesilate has been used for diabetic retinopathy (DR) and chronic venous insufficiency since 1997 and 1998 that was mentioned in the European Pharmacopoeia and the British Pharmacopoeia, respectively [1].

Calcium dobesilate has many different dosage forms that high-performance liquid chromatography (HLPC) [2], potentiometric titration [3], flow-injection biamperometric method [4], spectrophotometry [5] and chemiluminescence [6, 7] was used to detect it in pharmaceutical formulation and human biological fluids [8]. Because of need for rapid detection with low cost and more sensitivity, many nanomaterials were employed. Graphene has unique properties in nano science field. It has high surface area, high electrical conductivity, wide potential windows, fairly inert electrochemistry and good electrocatalytic activity for many redox reactions, low cost and strong mechanical strength.

In our study we developed new, fast and low cost electrochemical sensor to determination of calcium dobesilate in capsule. Graphene was used as a substrate modifier on glassy carbon electrode for facilitation of electron transferring. The prepared graphene modified electrode can be detecting of low amount of calcium dobesilate by choronoamprometry method.

Material and Methods

Apparatus and procedure

Electrochemical experiments were performed with an Autolab potentiostat (PGSTAT 101). A working glassy carbon electrode with a diameter of 3 mm, a silver/silver chloride (Ag/AgCl) reference electrode, containing 3 M, KCl and a platinum rod auxiliary electrode were used from metrohm. All potentials were measured and reported versus the Ag/AgCl reference electrode. Cyclic voltammetry experiments were performed at 0.1 V/s. The amperometric experiments were carried out by applying the desired potential and allowing the transient current to reach the steady-state value prior to the addition of the analyte and the subsequent current monitoring.

Reagents

Graphite powder was purchased from Fisher (Chemical Scientific grade). Dihydrogen phosphate (KH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4), NaBH4, hydrochloric acid and hydrogen peroxide were purchased from Merck. Ultrapure water from a Millipore-MilliQ system was used for preparing all solutions. All the reagents were used as received, without further purification and all experiments were carried out at room temperature (25°C).

Graphene oxide and graphene synthesis

Graphene oxide (GrO) was synthesized from graphite using the Hummers method and reduce graphene (RGr) was obtained by reduction of GrO with NaBH4 according our previous study [9]. Briefly, graphite, sodium nitrate

and potassium permanganate were added to concentrated sulfuric acid. After heating at 35◦C for 30 min, the reaction mixture turned greenish and pasty. Then, the reaction was carefully quenched by the slow addition of water. The paste was kept at 100 ◦C for 15 min and turned brownish. After further dilution with water it was allowed to cool to 30◦C for 30 min, during which it turned yellow. Hydrogen peroxide was carefully added to form colorless soluble manganese sulfate. The resulting GrO was isolated while still warm by filtration and the yellow-brown filter cake was washed with warm 5% diluted hydrochloric acid and finally with water. The resulting stable and brownish GrO aqueous solution was reduced by 1:1 Gr/$NaBH_4$ mass ratio, at room temperature, overnight. The graphene black precipitate was filtrated and washed with water.

Electrode preparation

2 µl graphene (0.1 mg/ml) was dropped on the glassy carbon electrode. Then graphene modified glassy carbon electrode was immerged on calcium dobesilate solution in 0.1 M phosphate buffer solution (pH 5.8) at air saturated condition. CNTs modified glassy carbon electrode was used in same condition.

Results and Discussion

Characterization of RGr

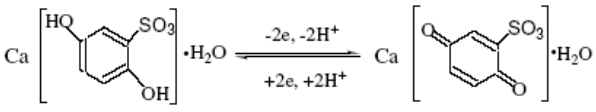
The SEM image of RGr is shown in Figure 1. As it obvious the sheet of graphene is formed successfully.

The electrochemical behavior of Calcium dobesilate on graphene modified glassy carbon electrode

Figure 2 shows the cyclic voltammograms (CVs) of calcium dobesilate on graphene modified glassy carbon electrode in air saturated PBS (0.1 M, pH 5.8). It is obvious that CNTs or graphene fixed on glassy carbone electrode do not show any response in this potential window. Therefore, these electrodes are appropriate for electrochemical study of calcium dobesilate. In presence of calcium dobesilate on graphene modified glassy carbon electrode, a couple of well defined reversible peak was observed with the anodic and cathodic peak potentials of 176 and 90 mV (vs. Ag/AgCl), respectively. The peak potential separation was determined as 86 mV. The formal potential (E0) of calcium dobesilate has been calculated as the average of cathodic and anodic peak potentials 133 mV (vs. Ag/AgCl).

Electrocatalytic activity of calcium dobesilate on graphene modified glassy carbon electrode

Direct electrochemistry of calcium dobesilate occurs according to Eq. 1. The electrocatalytic process is expressed as follows [10]:



Determination of calcium dobesilate concentration using graphene modified glassy carbon electrode is performed by choronoamprometry (Fig. 3 & 4).

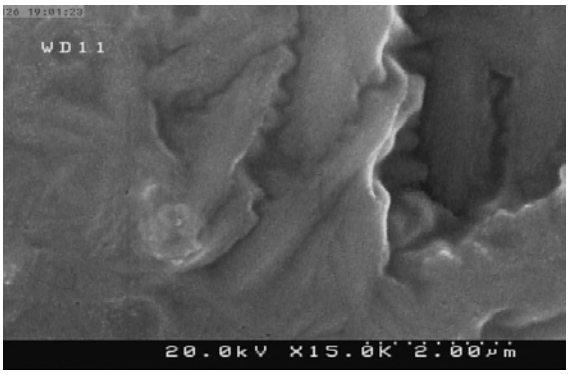


Figure 1. SEM images of RGr.

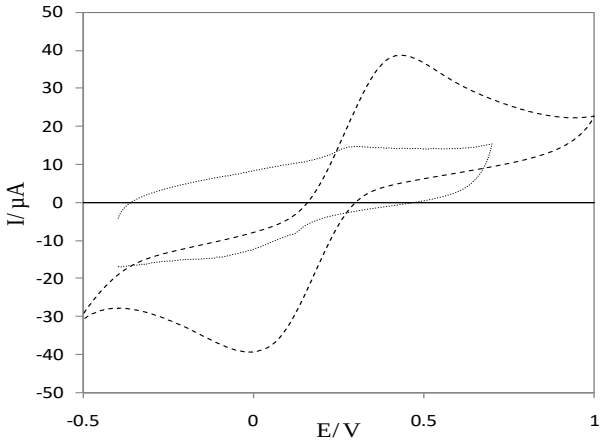


Figure 2. CVs of (—) bare RGr glassy carbon modified electrode, (….) Calcium dobesilate on CNTs glassy carbon modified electrode, (---) Calcium dobesilate on RGr glassy carbon modified electrode in 0.1 M PBS (pH 6.8). The scan rate was 100 mV/s at air-saturated conditions.

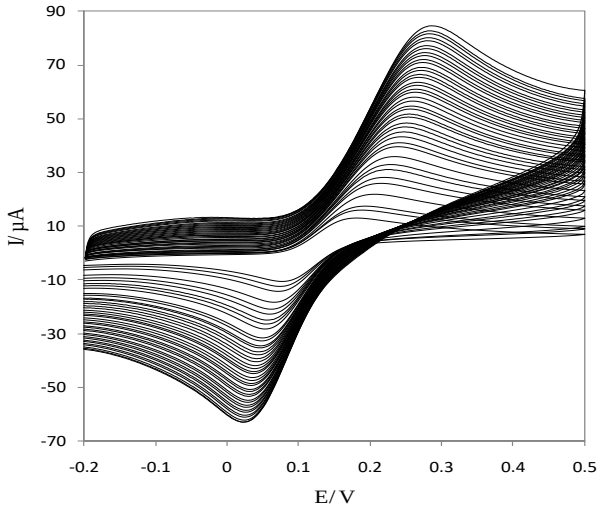


Figure 3. (A) CVs of Calcium dobesilate on RGr glassy carbon modified electrode in 0.1 M PBS (pH 6.8) at various scan rates of 5, 10, 20, 30, 50, 60, 70.80, 90, 100, 120, 150, 170, 200, 250 and 300 to 1000 mV/s from inner to outer, respectively.

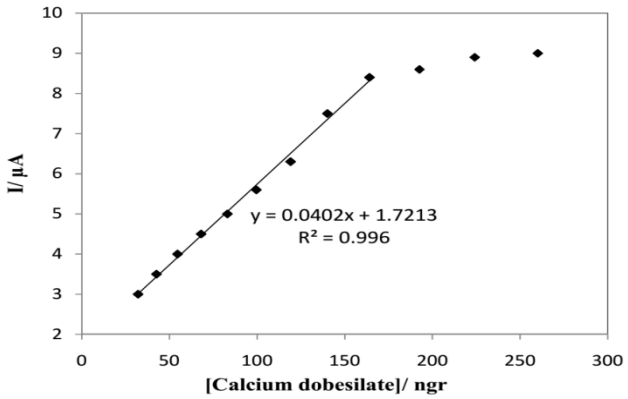


Figure 4. Calibration curve for the Calcium dobesilate on RGr glassy carbon modified electrode by choronoamprometry in 0.1 M PBS (pH 6.8) containing various concentrations of Calcium dobesilate at the air-saturated conditions. The Calcium dobesilate concentrations are from 30-270 ng/mL.

The current values linearly change with the concentration of 30-120 ngr/mL with a correlation coefficient (r) of 0.996 and with detection limit of 20 ngr at S/N=3.

Conclusion

We propose a new strategy to prepare a fast, easy and renewable biosensor by graphene immobilizing on glassy carbon electrode. Calcium dobesilate on graphene modified glassy carbon electrode exhibits a direct and reversible electrochemical reaction. In comparison with CNTs, graphene is very conductive for electron transferring of calcium dobesilate and so, it has a high current density for calcium dobesilate oxidation/reduction. The procedure is very simple, economical and does not require electrode polishing.

Acknowledgments

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