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# Highly sensitive and selective electrochemical sensor based on high-quality graphene/nafion nanocomposite for voltammetric determination of nebivolol

Engin Er[a], Hüseyin Çelikkan[b,*], Nevin Erk[a]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkey
[b] Department of Chemistry, Faculty of Science, Gazi University, Ankara, Turkey

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ABSTRACT

This research is concerned with the development of a facile, highly selective and sensitive graphene-based electrochemical nanosensing material for the detection of nebivolol (NBV). It is the first report that the electrochemical behaviour and quantitative analysis of NBV were investigated in detail on high-quality graphene/nafion nanocomposite modified electrode (GRE/NFN) using voltammetric techniques. The structure of high-quality graphene (GR) was characterized by Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analysis (TGA) and transmission electron microscopy (TEM). A broad peak at 1025 mV and well-defined peak at 1325 mV were observed by adsorptive stripping differential pulse voltammetry (AdsDPV) on GRE/NFN in the detection of NBV. Considering that the peak appeared at 1325 mV, GRE/NFN showed that NBV had a high electrocatalytic performance towards the NBV in the linear range from 0.5 to 24.0 μM with a low detection limit of 46 nM under the optimal experimental conditions. The GRE/NFN could be a promising material with high sensitivity and selectivity for the electrochemical sensing of NBV and can also be adopted to determine NBV in pharmaceutical and clinical samples.



## 1. Introduction

Beta blocker drugs are competitive antagonists of catecholamines in beta-adrenergic receptors in a wide range of tissues. Different types of adrenergic receptors are described under the actions and uses of sympathomimetics. Nebivolol (NBV), as shown in Scheme 1, is a competitive and highly selective β1-receptor antagonist with mild vasodilating properties, which has a beneficial effect due to its direct action on the endothelium with a possible nitric oxide release [1,2]. NBV is used clinically for the treatment of hypertension and chronic heart failure. The recommended dosage of NBV to relieve hypertension is 5 mg per day as it shows the best cardioselectivity at such dosage [2–4]. Therefore, the determination of the level of NBV has a great importance at a level of mg.

Graphene (GR), a two-dimensional (2D) carbon form with one-atom-thickness, has attracted great attention in many scientific fields due to its extraordinary properties such as a large surface area, excellent carrier mobility, with mechanical and thermal resistance [5–7]. Recently, it has been particularly used as an electrode material for the electrochemical sensing owing to its excellent electrical conductivity and enormous electro-active area [8–10]. Nafion (NFN), a perfluroinated sulphonated anionic copolymer, has been comprehensively employed as an electrode modifier agent with excellent properties such as its antifouling capacity, chemical inertness and high permeability to cations. The perfluorocarbon chains in NFN maintained the hydrophobicity of graphene and improved its dispersity. Also, the sulphonic groups prevented the stacking of graphene layers [11,12].

In recent years, graphene-based electrochemical nanosensors have been used particularly in the detection of bio-molecules and organic molecules, such as dopamine [13,14], uric acid and ascorbic acid [15,16], glucose [17], $H_2O_2$ [18] and DNA [19] using electrochemical techniques. Electrochemical techniques have also presented significant advantages such as high sensitivity, rapid response time, effectiveness and good-reproducibility for the detection of electroactive molecules [14,18,20]. It is a known fact that chemically modified electrodes have been proven to significantly enhance the electrocatalytic and electrochemical activity towards the mentioned above bio-molecules and organic molecules. However, the reports are limited related to the

* Corresponding author.
*E-mail address:* celikkan@gazi.edu.tr (H. Çelikkan).

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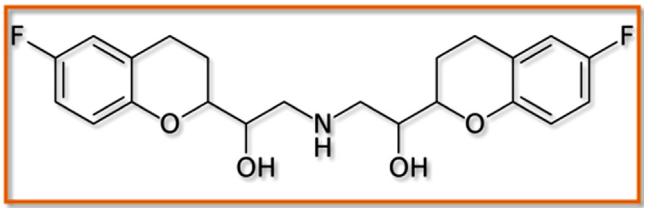


**Scheme 1.** Chemical structure of NBV.

electrochemical analysis of drug molecules using graphene-based modified electrode because graphene has only recently been discovered. Up to now, the literature reveals that several methods have been developed to detect NBV in pharmaceutical or human plasma using ultraviolet and fluorescence spectroscopy [21,22], high-performance liquid chromatography (HPLC) [23,24], liquid chromatography with tandem-mass spectroscopy (LC/MS-MS) [25,26]. However, there is only one study related to electrochemical detection of NBV [27]. Rahman et al. developed a drug sensor based on un-doped silver oxide nanoparticles on glassy carbon electrode using the *I–V* technique (two-electrode system) for the detection of NBV in pharmaceuticals. The authors claimed that proposed sensor shows good sensitivity and improved electrochemical *I–V* response towards NBV with its adsorption features on the surface of proposed sensor [27].

To the best of our knowledge, the literature contains no reported result about electrochemical behaviour or quantification of NBV in human plasma or pharmaceutical dosage form using voltammetric techniques with the modified electrode based on carbon-materials. The aim of this study was to develop a highly sensitive and selective electrochemical nanosensor based on high-quality graphene/nafion to detect NBV. In addition, graphene with its excellent properties such as high conductivity and large electroactive surface area make it suitable for the selection as a modifying material. In our study, the electrochemical behaviour of NBV was investigated in detail on the surface of the glassy carbon electrode (GCE) modified by high-quality graphene, and also the proposed sensor has been successfully applied to the determination of NBV in pharmaceutical tablets and biologic sample. The results revealed that graphene modified electrode demonstrated a high electrocatalytic activity towards the NBV.

## 2. Experimental

### 2.1. Chemicals and reagents

Graphite powder was purchased from Sigma–Aldrich Corp. All the chemicals used in graphene synthesis and electrochemical analysis were purchased from Sigma–Aldrich Corp. and were of analytical grade. All the solutions were prepared with ultra-pure water. NBV·HCl was supplied from Nobel Pharma Company. The Britton–Robinson buffer solution (BR) was prepared by the use of boric, phosphoric and acetic acid solutions, and its pH value was adjusted with 1.0 M NaOH and 1.0 M HCl. A stock solution of 1.0 mM NBV was dissolved in a methanol:water (1:1, v/v) mixture.

### 2.2. Apparatus and procedures

FT-IR spectra were recorded by IR-Affinity 1 with ATR attachment (Shimadzu Corp.). Thermal measurements were performed with a DTG-60H Thermogravimetry/Differential Thermal Analyzer (Shimadzu Corp.). TEM images were recorded using a Jeol JEM 1400 instrument at 120 kV. The whole electrochemical measurements were performed using METROHM-Autolab PGSTAT128N (Metrohm Autolab B.V., Netherlands) and IVIUM-CompactStat (Ivium Technologies, Netherlands) electrochemical analyzers. Graphene modified electrode was used as a working electrode. A Pt wire and Ag/AgCl electrode were used as the counter and reference electrodes, respectively. All experiments were carried out at room temperature (25 ± 2 °C).

### 2.3. Preparation of the high-quality graphene (GR) and graphene modified electrode (GRE/NFN)

High-quality graphene was produced by an effective chemical method, which consisted of two steps. First, GO was synthesized from graphite powder using Hummers' method [28]. Then, GO was reduced using phosphoric acid as previously reported by the authors [29]. Briefly, GO powder was added to the concentrated $H_3PO_4$ in a boiling flask and the mixture was reacted at 130 °C for 2 h under a reflux condenser. The reaction was terminated by keeping in the flask containing the mixture in a water/ice bath until it cooled. The resulting product was washed with ultra-pure water until it reached its supernatant pH. The final GR product was kept in a vacuum oven at 60 °C for a day for the characterization processes.

A 25 mL (1 mg ml$^{-1}$) GR solution containing nafion (0.25% m/v) was prepared to formation the GR/NFN nanocomposite for the electrochemical measurements. For the GR/NFN preparation, the nafion solution was added to obtain 0.25% (m/v) nafion concentration in the GR solution. Nafion is very suitable material to modify the GR, and the interaction between nafion and GR is occurred by way of oxygen functional groups situated on the surface of GR. The primary reason for preference of use of nafion in GR solution that nafion ensures the stability of GR in the solution form over time. For preparing the modified electrode, GCE with a diameter of 3 mm was polished with 0.05 μm alumina slurry on the polishing pad, was ultrasonically cleaned with ultra-pure water for 5 min and thoroughly washed with ultra-pure water. The GR/NFN solution (1 mg ml$^{-1}$) was dispersed until its uniform solution obtained. Then, 5 μL of finely dispersed GR/NFN solution was dropped onto the surface of GCE to obtain GRE/NFN and the modified electrode was dried at room temperature.

### 2.4. Voltammetric measurements

The modified electrodes were conditioned using cyclic voltammetry (CV) by scanning twenty times between the potential range of 0.8–1.6 V with the scan rate of 100 mV s$^{-1}$ in 0.1 M BR supporting electrolyte solution. This pre-treatment process was undertaken to ensure the sufficient stability of the electrode before the electrochemical analysis of NBV. The electrochemical behaviour and quantitative analysis of NBV were investigated using adsorptive stripping differential pulse voltammetry (AdsDPV) (step increment: 3 mV; amplitude: 50 mV; pulse period: 0.05 s). The content of NBV in the tablet and human plasma were analyzed by five times ($N$ = 5) using the standard addition method.

### 2.5. Preparation of samples for assay

#### 2.5.1. Pharmaceutical sample (tablet)

Ten VASOXEN® tablets (5 mg NBV or 5.45 mg NBV·HCl per tablet) were accurately weighed and finely grounded in a mortar. An adequate amount of prepared tablet powder, equivalent to a stock solution of 100 μM NBV, was weighed and transferred into a 50 mL calibrated flask. Then, the sample contained in the flask was sonicated to provide a complete dissolution of NBV in a methanol:water (1:1, v/v) mixture. The mixture was filtered through filter paper (whatman No: 42) and was suitably diluted