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# Electrochemical determination of nepafenac topically applied nonsteroidal anti-inflammatory drug using graphene nanoplatelets-carbon nanofibers modified glassy carbon electrode

Biljana Nigović*, Sandra Jurić, Ana Mornar

*University of Zagreb, Faculty of Pharmacy and Biochemistry, A. Kovacica 1, 10000 Zagreb, Croatia*

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ABSTRACT

Nepafenac is novel ophthalmic agents with a unique prodrug structure and superior corneal permeability to other currently available topical nonsteroidal anti-inflammatory drugs. A graphene nanoplatelets and carbon nanofibers modified glassy carbon electrode was developed and used as first electrochemical sensor for the determination of nepafenac. The effective combination of graphene and carbon nanofibers resulted in electrochemical sensor with large surface area, high sensitivity, good stability and selectivity. After method optimization, the square wave voltammograms, at a preconcentration time of 60 s, display two linear ranges of $4.0 \times 10^{-6}$–$1.5 \times 10^{-5}$ M and $2.5 \times 10^{-7}$–$4.0 \times 10^{-6}$ M for drug quantification with a detection limit of $6.3 \times 10^{-8}$ M. The modified electrode was employed for fast assay of nepafenac in ophthalmic solution with satisfactory results and rapid run time of 66 s in comparison with previously reported analytical methods.

## 1. Introduction

To control ocular inflammation after cataract surgery two types of drugs are used: corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs). The ophthalmic steroids may increase the risk for intraocular pressure elevation or infection. Therefore, NSAIDs are widely used for prevention and treatment of inflammation associated with cataract surgery as well as the management of postoperative pain, photophobia, intraocular pressure, hyperemia and pruritus without the adverse events that occur with corticosteroids [1]. However, topical NSAIDs are weak acids and hence, ionized in alkaline lachrymal fluid (pH of approximately 7.4) limiting corneal penetration. If the pH value of the NSAID preparations is adjusted to improve corneal permeability, the incidence of ocular irritation is also increased [2].

Nepafenac (NPF) is novel ophthalmic agents with a unique prodrug structure (Scheme 1) and superior corneal permeability to other currently available topical NSAIDs [3]. NPF is a benzoylbenzeneacetamide prodrug that is metabolized in vivo by intraocular hydrolases to its corresponding acid amfenac. The neutral and less polarized structure of prodrug facilitates penetration into the cornea where conversion occurs to the active form [4]. Therefore, NPF provides improved efficacy with activation in specific areas such as the ciliary body, choroid, retina, iris and cornea [3]. The rapid distribution of NPF into the anterior and posterior eye segments decreases surface accumulation and the associated adverse effects on the eye surface often observed with topical NSAIDs [5]. NPF ophthalmic suspensions, 0.1 i 0.3%, are approved in Europe and the United States to treat complication of cataract surgery. NPF as new-generation NSAID is also approved in Europe for the reduction in the risk of postoperative macular edema in diabetic patients [6].

Pharmaceutical analysis has become one of the most important stages in the therapeutic process that includes analytical investigations of active pharmaceutical ingredients and pharmaceutical dosage forms ensuring safety, efficacy and high quality of *medicines*. However, up to now, an official monograph for analysis of this drug in bulk form and pharmaceutical formulations has not been established in European pharmacopoeia and USP. At present, only few analytical methods have been developed for determination of NPF, such as HPLC with UV detection [7–9], stability indicating UHPLC method [10] and UV spectroscopy [9,11]. Meanwhile, electrochemical methods have proven to be very sensitive and useful for analysis of drugs owing to their simplicity, rapid response, low cost, green chemistry technology, miniaturization and possibility to on-line field monitoring [12,13]. In spite of that, to the best of our knowledge, there is no report on the determination of NPF molecule by electrochemical method.

In recent years, the modification of the electrode surface with various carbon nano-structures materials (e.g. carbon nanotubes, carbon nanoparticles, nanodiamonds and graphene) has been widely used to

* Corresponding author.
*E-mail address:* bnigovic@pharma.hr (B. Nigović).

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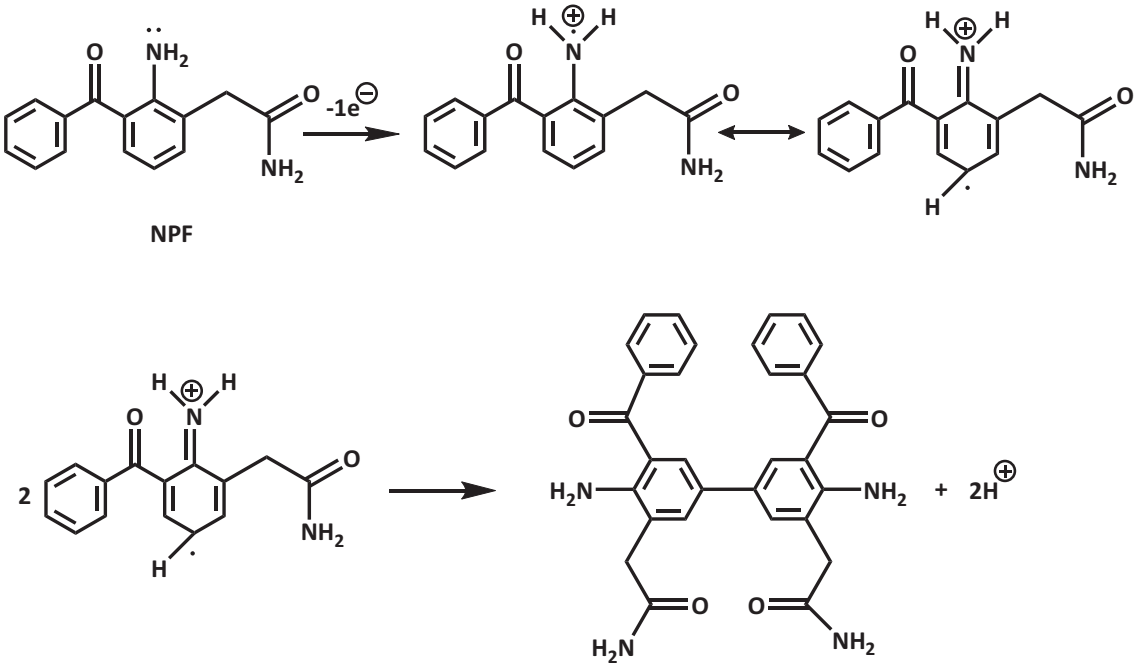


**Scheme 1.** Possible oxidation pathways of NPF at GNP-CNF/GCE.

improve the electrochemical responses of pharmaceuticals and biological compounds [14–16]. The application of graphene as a new member of this family has recently attracted a lot of attention in electrochemistry due to unique nanostructure and has become a vast area of research owing to its interesting physical and chemical properties [17]. Graphene modified electrodes present extraordinary advantages over conventional electrodes in electroanalytical applications due to their electrocatalytic effect, mass transport enhancement, increased conductivity, high porosity, highly effective surface area and wide potential windows. Graphene nanoplatelets (GNPs) comprised of short stacks of platelet-shaped graphene sheets have additionally improved mechanical properties such as stiffness, strength, and surface hardness due to their unique size and morphology [18]. On the other hand, carbon nanofibers (CNFs) are applied as promising nanostructured carbon materials in many fields, such as sensors, electrical devices, electrode materials for batteries and supercapacitors [19]. One of the most important properties of CNFs is their excellent electrical conductivity. In addition, CNFs possess more surface active groups, better mechanical stability and more active sites on the outer wall than carbon nanotubes [20]. CNFs are highly hydrophobic and tend to aggregate, but they can be dispersed in polymer matrix as the binder in electrochemical sensor development to achieve uniform and stable composite film [21]. Uniform dispersion of the CNFs inside polymer matrix is a key parameter which dictates the enhancement of electrical conductivity of the nanocomposites.

The aim of the present study is to investigate electrochemical behaviour of NPF utilizing graphene nanoplatelets and carbon nanofibers modified glassy carbon electrode (GNP-CNF/GCE) and to develop a simple voltammetric method for determination of NPF in pharmaceutical preparations without expensive and time-consuming pretreatments. We demonstrate that GNP-CNF/GCE leads to a strong interfacial accumulation of NPF molecules and hence, offers sensitive adsorptive stripping measurements near to the nM levels.

## 2. Experimental

### 2.1. Instruments

All electrochemical experiments were carried out using a μ-Autolab potentiostat (Eco Chemie, Utrecht, The Netherlands) controlled by GPES 4.9 software. A conventional three-electrode system was used for voltammetric measurements which consisted of a platinum wire as counter electrode, an Ag/AgCl/3 M KCl reference electrode, and a bare or modified GCE (3-mm diameter, Metrohm, Switzerland) as working electrode. Before preparation of the electrochemical sensor, the working electrode was polished with of 0.05 μm alumina slurry and then washed successively with ultrapure water, ethanol and water in an ultrasonic bath. Finally, the electrode was dried and ready for modification. Surface morphology studies have performed by a field emission scanning electron microscope Jeol JSM-7000F (Jeol Ltd., Tokyo, Japan).

### 2.2. Chemicals

NPF was supplied from Sigma-Aldrich (Steinheim, Germany). NPF ophthalmic suspension, containing 1 mg/mL active ingredient, was obtained from local pharmacy. GNPs (purity > 99.5%) with thickness between 2 and 18 nm and diameter from 4 to 12 μm and the CNFs (> 95%, outside diameter 200–600 nm, length 5–50 μm) were purchased from US Research Nanomaterials, Inc. (Houston, USA, http://www.us-nano.com). Nafion (5 wt% solution in a mixture of lower aliphatic alcohols and water), mannitol, disodium EDTA and benzalkonium chloride were obtained from Sigma-Aldrich (Steinheim, Germany). Other reagents were of analytical grade. Ultra pure water used for the preparation of standard solutions and buffers was obtained by a Milli-Q system (Millipore, Bradford, USA).

Stock solution of NPF ($1 \times 10^{-3}$ M) was prepared by dissolving in ethanol and stored under refrigeration. Standard solutions were prepared just before use by diluting the stock solutions with Britton-Robinson (BR) buffer solution (0.04 M in each of acetic, phosphoric and boric acids) adjusted to the desired pH with additions of a 0.2 M solution of NaOH.

### 2.3. Preparation of GNP-CNF/GCE

The GNPs-CNFs suspension was prepared by dispersing 1 mg of both carbon nanomaterials in 1 mL of 0.3% Nafion ethanol solution under ultrasonic agitation (Elmasonic S 30H, Elma, Germany) for 1 h at