Contents lists available at ScienceDirect

Electrochimica Acta

Nanostructured Alpha-NiCe Mixed Hydroxide for Highly Sensitive Amperometric Prednisone Sensors

Josué M. Gonçalves[a], Robson R. Guimarães[a], Bruno B.N.S. Brandão[a], Lucas P.H. Saravia[a], Pamela O. Rossini[a], Cícero V. Nunes Jr[a], Juliana S. Bernardes[b], Mauro Berttoti[a], Lucio Angnes[a], Koiti Araki[a],*

[a] Department of Fundamental Chemistry, Institute of Chemistry, University of Sao Paulo, Av. Prof. Lineu Prestes 748, Sao Paulo, SP, 05508-000, Brazil
[b] National Nanotechnology Laboratory (LNNano), National Center for Energy and Materials (CNPEM), Rua Giuseppe Máximo Scolfaro, 10.000, Campinas, SP 13083-970, Brazil

ABSTRACT

Nickel hydroxide nanoparticles with enhanced electrocatalytic activity and stability in the alpha phase were prepared by incorporation of 20 atom% of Ce(III) ions, that were shown to be oxidized to the catalytic active Ce(IV) species in the mixed hydroxide/oxide nanomaterial. Alpha-NiCe is constituted by smaller nanoparticles as compared with pure nickel hydroxide and can interact with prednisone, a widely prescribed medicine that is controlled by the International Anti-Doping Agency because of its use as anabolic agent. This compound is more or less easily hydroxilated by oxygen and then oxidized to the respective keto species, that was shown to be oxidized further by the modified electrodes upon loss of the alkyl side chain. Thus, a sensitive prednisone sensor was demonstrated exploring the electrocatalytic oxidation reaction instead of the reduction of prednisone to prednisolone, as previously reported in the literature. Sensitivity as high as $3.9 \times 10^{-2}\,A\,mol^{-1}\,L/cm^2$ and limit of detection as low as $8.4 \times 10^{-9}\,mol\,L^{-1}$ were achieved using flow injection analyses.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Corticosteroids are known to influence several biochemical and cellular events in the body, playing fundamental roles in human physiology. For example, prednisone (Scheme 1) is a synthetic corticosteroid readily available as tablets, capsules, injections, ointments and creams prescribed by doctors for the treatment of a wide variety of inflammatory diseases such as asthma, rheumatoid arthritis, allergies, headache and kidney diseases such as nephrotic syndrome. However, prednisone is just the precursor of the actual bioactive species prednisolone, which is generated in the liver upon reduction of the 11-oxo group by the enzyme 11-hydroxydehydrogenase. One of the most widely known aspects associated with prednisone is its unauthorized use as anabolic agent to enhance athletic performance. Accordingly, the International Olympic Committee and the International Anti-doping Agency have prohibited its use by professional athletes participating of official games [1].

Considering the high clinical relevance and the known abusive use, it is important to develop cheaper and more sensitive analytical methods to monitor the concentration of prednisone/prednisolone in body fluids as well as in pharmaceutical formulations. For example, several liquid chromatography (LC) and high performance liquid chromatography (HPLC) [2] based methods were developed by association with spectroscopic detection techniques such as in LC-MS [1,3–5], LC-UV [6] and LC-fluorescence [7]. Gas chromatography coupled with mass spectrometry (GC-MS), micellar capillary electrokinetic chromatography (MCEC) [8] and chemilluminescence, associated with flow injection methods [9] have also been used for that purpose. Nevertheless, all those techniques require quite laborious sample preparation procedures generally demanding toxic solvent(s) and time consuming extraction steps. Mazurek et al. [10] described a method for quantitative determination of prednisone in tablets by infrared attenuated total reflection and Raman spectroscopy. However, FTIR-ATR spectroscopy measurements have to be repeated several times to get sufficiently accurate data. There were also reports on the determination of prednisone and prednisolone by electrochemical methods such as polarography [11], but the waves are superimposed [12] hindering the analyses.

* Corresponding author.
  E-mail address: koiaraki@iq.usp.br (K. Araki).
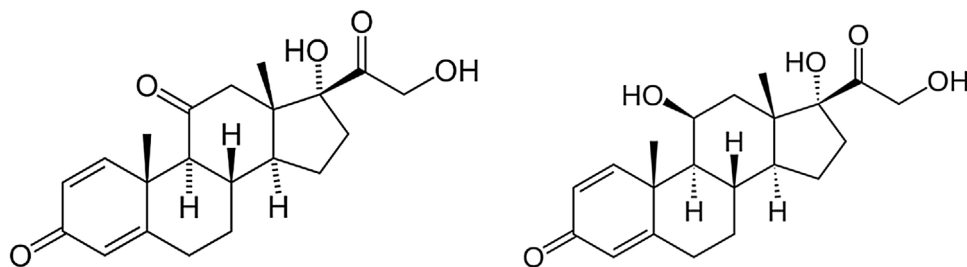
Scheme 1. Wire and frame structure of prednisone (left) and prednisolone (right).

More recently, simultaneous determination of both analytes in human body fluids and pharmaceutic formulations were successfully carried out [13] by square wave voltammetry using pyrolytic graphite electrodes (PGE) modified with single wall carbon nanotubes (SWNT). The modified electrodes exhibited good signals for reduction but only at quite negative potentials, respectively at -1.23 and -1.33 V *vs* Ag/AgCl (3.0 mol L^{-1} NaCl).

The use of nanomaterials for preparation of modified electrodes for electroanalytical purposes is a tendency that is gaining increasing momentum because new materials with excellent conductivity, chemical and mechanical stability, large surface area and electrocatalytic properties can be tailor made, translating in electrochemical sensors with enhanced response and sensitivity.

Nickel hydroxide is a material that has been explored for amperometric analyses of organic compounds and pharmaceutics [14], such as ascorbic acid [15], uric acid [15], dopamine [15,16], serotonin [16] and glucose [17–20], that has gained broad new perspectives when engineered as nanoparticles, particularly due to the possibility of stabilizing it in the more electrochemically active and responsive alpha phase, inhibiting its conversion to the thermodynamically more stable β-Ni(OH)$_2$ [21] by aging or redox cycling in strongly alkaline medium. In fact, recently the preparation and enhanced stability and electrochemical properties of nanostructured materials based on nickel and cobalt mixed hydroxides (α-Ni$_x$Co$_y$(OH)$_{2x+3y}$, where $x+y=1$) nanoparticles in the alpha phase, as well as their use for preparation of modified electrodes with improved performance for oxidation and quantification of isoniazide by cyclic voltammetry and chronoamperometry, were reported [22].

Considering that Ce^{IV} is a well-known catalyst for oxidation reactions as well as potential candidate as stabilizing agent of nickel hydroxide in the alpha crystalline phase, one decided to generate a mixed hydroxide material that can be electrochemically activated generating both, Ni^{III}(OOH) and Ce^{IV}O$_2$ species, thus encompassing synergic catalytic effects for oxidation of hydrogen peroxide and organic molecules. In fact, nanostructured CeO$_2$ has also attracted much attention for the development of electrochemical sensors and biosensors due to its biocompatibility, high chemical stability, and excellent electric conductivity [23–25]. Yagati et al. reported an enzymatic biosensor for H$_2$O$_2$ based on nanostructured CeO$_2$ films deposited on ITO electrodes [26]. Jha and co-workers prepared a nonenzymatic electrochemical H$_2$O$_2$ sensor based on 3D porous CeO$_2$/rGO xerogel composite [27]. More recently, CeO$_2$ nanoplates were bound on the surface of β-Ni(OH)$_2$ nanosheets generating a composite which self-assemble *in situ* forming three-dimensional hierarchical structures suitable for preparation of nonenzymatic H$_2$O$_2$ sensors [23]. Thus, herein described is a nanostructured material exhibiting enhanced stability and electrocatalytic activity for oxidation reactions, thanks to the incorporation of Ce(III) ions into the nickel hydroxide matrix, as demonstrated by the high sensitivity and wide dynamic range for amperometric quantification of prednisone in pharmaceutical products. The new nanomaterial was carefully characterized by spectroscopic and electrochemical methods, including impedance spectroscopy as well as transmission (TEM) and scanning transmission electron microscopy (STEM).

2. Experimental

2.1. Syntheses

All reagents and solvents were of analytical grade and used as received. Anhydrous glycerin, isopropyl alcohol and potassium hydroxide were purchased from Synth Brasil. Nickel(II) acetate tetrahydrate and cerium(III) acetate tetrahydrate were purchased from Sigma-Aldrich, and *n*-butyl alcohol acquired from Vetec.

Sol suspensions of pure nickel hydroxide and mixed nickel and cerium hydroxide nanoparticles were prepared in the stabilized alpha crystalline phase by dissolving 4.8 mmol of the metal acetates in 25 mL of glycerin and adding stoichiometric amount of KOH in *n*-butanol solution, at room temperature, according to a previously described procedure [14,28]. Samples containing only α-Ni(OH)$_2$ and α-Ni$_x$Ce$_y$(OH)$_{2x+3y}$, where x = 0.8 and y = 0.2, named α-NiCe, suspensions were prepared accordingly starting from Ni(II) acetate and a mixed Ni(II) acetate and Ce(III) acetate solution in glycerin. Ce(OH)$_3$ was prepared in similar way just replacing nickel(II) acetate by cerium(III) acetate.

2.2. Characterization

The samples were characterized by X-ray diffractometry (XRD) in a Bruker D8 Phaser equipment with a Cu Kα source ($\lambda = 1.5418$ Å, 30 kV, 15 mA, step = 0.05°) and air-scatter screens to avoid diffuse scattering at lower angles, in the 2θ range from 5 to 70° and 3 to 20°. The concentration of α-Ni(OH)$_2$ and α-Ni$_{0.8}$Ce$_{0.2}$(OH)$_{2x+3y}$ sol was determined by total X-ray fluorescence spectroscopy (TXRF) in a Bruker PICOFOX S2 spectrometer. Chemical surface analyses of nanocomposites were carried out by X-ray photoelectron spectroscopy (XPS), using a K-Alpha X-ray photoelectron spectrometer (Thermo Fisher Scientific, UK) equipped with hemispherical electron analyzer and monochromatic Al Kα (1486.6 eV) radiation source. Survey (full-range) and high-resolution spectra for Ce and Ni were acquired using pass energy of 200 and 50 eV, respectively, and data analyses carried out using the Thermo Avantage Software (Version 5.921). The XPS results presented in this work correspond to an average of at least three independent measurements performed on different regions of each sample.

Transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) images of nanocomposites were obtained in a JEOL JEM-2100 FEG equipment, at accelerating voltage of 200 kV. Samples were prepared on copper grids (Ted Pella) by dispersing 3 μL of a nanoparticle suspension diluted in DI-water.

Infrared spectra were recorded on a Bruker ALPHA FTIR spectrophotometer using samples dispersed in KBr pellets. Raman spectra were recorded in a WITec 300R Alpha confocal microscope