Methylparaben quantification via electrochemical sensor based on reduced graphene oxide decorated with ruthenium nanoparticles

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A B S T R A C T

MePa quantification was performed through an electroanalytical method, in which the presence of rGO/RuNPs on GCE surface allowed the increase of the charge transfer rate in the oxidation process of the target molecule. The characterization of the composite material was carried out by TEM, in which the presence of RuNPs with an average diameter of 3.1 ± 0.5 nm dispersed on the surface of rGO was observed.

The electroanalytical method developed showed a linear response range of 5.00×10^{-7}–3.00×10^{-6} mol L^{-1} and LOD of 2.40×10^{-7} mol L^{-1}. The results were statistically compared with the data obtained by the official method (HPLC) and shown to be equivalent, which allows discussion upon the use of electroanalytical methods of alternative forms of official analysis methods in cosmetic samples.

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1. Introduction

Parabens are widely used as conservatives in cosmetic formulations, pharmaceuticals and food. These compounds are synthetic esters of p-hydroxybenzoic acid, wherein the methyl and propylparaben, Fig. 1, are more frequently used in commercial applications.

The parabens have low toxicity and are used in products to prevent microbial and fungal attack. They have a certain solubility in water (approximately 2.5 g L^{-1}), which gradually increases with increasing temperature and, therefore, they are easily found in aquatic environments. However, recent researches had characterized these compounds as hazardous to the human health, since they present characteristics of endocrine disruptors, which can be associated with the length of their alkyl chain [1]. Moreover, studies have reported the occurrence of traces of parabens in breast tumors [2], in tissues of marine organisms and human urine [3,4] and in tissues of birds and fish [1].

According to the regulatory agencies, the Cosmetic Directive in Europe and ANVISA in Brazil, the maximum allowable concentration of such compounds in cosmetic formulations in the final product is 0.8% (g/g) for a mixture of esters and 0.4% (g/g) for single ester [5,6].

Justified by the supposed estrogenic effect of these compounds, studies have been developed using highly sensitive techniques which can identify parabens at trace levels. The standard technique for the detection of these compounds is high-performance liquid chromatography (HPLC). The drawback of such chromatographic technique is related to the demand of skilled labor, long time for analysis and high-cost equipment. In order to overcome such limitations, alternative methods have been proposed for the detection of emerging compounds. Among them, the most common methodologies employ electrochemical methods [7–9], reducing the response time and allowing the use of portable equipment and, consequently, *in situ* analysis.

Electrochemical methods cover numerous techniques coupled with sensitive and easy-to-use devices that can detect analytes as effectively as chromatography. The astonishing development of new materials (mainly the nano-scaled ones) observed in recent years is generating an entirely new field of nanostructured sensors, which are increasingly used for pharmaceutical, environmental and biological samples quantification [10–12]. To increase the analytical signal, which depends on the electron transfer capabilities of a particular electrode, numerous strategies have been developed.

Graphene oxide is a dimensional nanomaterial that has layers of self-assembled carbon atoms arranged in a hexagonal lattice [13]. Regarded as the most promising catalyst in numerous applications [14], graphene oxide has unique characteristics as excellent optical, thermal and magnetic properties, high conductivity, large surface area and low production cost, which make this nanomaterial one

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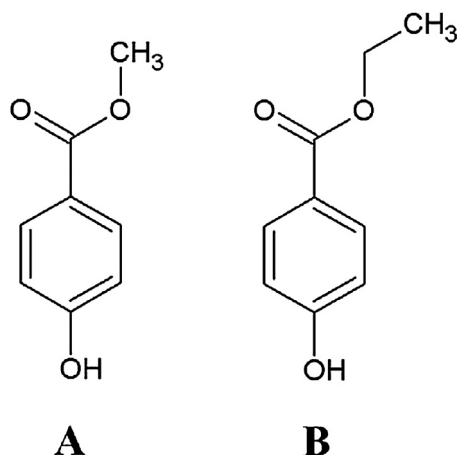 *E-mail address:* camiladm@usp.br (C.D. Mendonça).

Fig. 1. Molecular structure of (A) methylparaben and (B) propylparaben.

of the most studied in recent years. Graphene oxide can be reduced either chemically or electrochemically to generate a new and much poorer surface in oxygenated species attached to the main structure and that is known as reduced graphene oxide (rGO) [15].

Studies have shown that transition metal nanoparticles attached to graphene oxide or to reduced graphene oxide surfaces increase the conductivity and provide an effective gate for electron transfer, thus improving their catalytic properties and providing a new nanocomposite suitable to be used in many fields [14–17].

In the work herein, the results of electroanalytical determination of MePa using the reduced graphene oxide (rGO) electrode modified with ruthenium nanoparticles (RuNPs) in cosmetic samples are presented.

The verification of the use of the electroanalytical technique (DPV) as an alternative to HPLC is also highlighted in this work. The accuracy of the MePa concentration measurements performed by DPV is statistically compared with HPLC using the methodology proposed by Miller-Miller [18], in which the linear correlation between data obtained for recovery tests performed by both techniques indicates equivalences in the adopted response range.

2. Experimental

2.1. Chemicals and solutions

The aqueous solutions were prepared with water purified in a Barnstead Nanopure System (Thermo Scientific, USA), with resistivity $\geq 18\,M\Omega\,cm^{-1}$. All reagents used were of analytical grade and were used without further purification.

Methylparaben (MePa), N,N-dimethylformamide (DMF), monosodium phosphate and disodium phosphate were obtained from Sigma-Aldrich. Ethanol and methanol (HPLC grade) were obtained from Synth and J.T. Baker-Panreac, respectively.

The supporting electrolyte was a phosphate buffer solution ($0.1\,mol\,L^{-1}$), which was used in the preparation of working standard solutions by diluting the stock solution $1 \times 10^{-3}\,mol\,L^{-1}$ MePa.

Stock solutions of MePa ($6.57 \times 10^{-3}\,mol\,L^{-1}$) were prepared in methanol for the chromatographic analysis and stored in a refrigerator at $4\,°C$. In the chromatographic analysis, standard working solutions were prepared from the stock solution diluted with methanol: water-acetic acid (1%) (65:35, v/v).

2.2. Apparatus and procedures

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments were performed by using a model PGSTAT30 Autolab electrochemical system (Eco Chemie, Utrecht, Netherlands), coupled with an electrochemical cell with glassy carbon electrode (GCE) as the working electrode; Ag/AgCl ($3\,mol\,L^{-1}$ KCl) as the reference electrode; and a platinum foil as the counter electrode.

The HPLC-UV analysis was performed using a LC-10 series Chromagraph (Shimadzu), equipped with a C18 column ($25\,cm \times 4.6\,mm$ ID, $5\,\mu m$ particle) and a C18 pre-column ($2\,cm \times 4\,mm$ ID, $5\,\mu m$ particle), both from Sigma/Aldrich (Ascentis® Supelco), and UV detector (model SPD − 10AV).

2.3. Synthesis of graphene oxide

Graphene oxide (GO) was prepared according to the modified Hummers method [19,20]. Typically, 2 g of graphite and 2 g of $NaNO_3$ were mixed with 90 mL of H_2SO_4 (98%) in a 500-mL flask. The mixture was then stirred for 30 min in an ice bath, followed by the addition of 12 g of $KMnO_4$ under vigorous stirring. The reaction mixture was then held under refrigeration at $4\,°C$ for 24 h. Next, the mixture was stirred at $35\,°C$ followed by the addition of 90 mL of H_2O, also under vigorous stirring. After that, the reaction temperature was rapidly increased to $98\,°C$ and a further 90 mL of H_2O was added, followed by 60 mL of 30% H_2O_2 aqueous solution. The reaction mixture was again held under refrigeration at $4\,°C$ for 24 h. The obtained GO solution was centrifuged for 30 min at 8000 rpm and washed several times with 5% HCl and distilled water until the pH of the filtrate was neutral. Finally, the GO obtained was lyophilized for 24 h.

2.4. Synthesis of reduced graphene oxide with ruthenium nanoparticles nanocomposite (rGO/RuNPs)

The nanocomposite material was prepared by dispersing 100 mg GO in 100 mL double-distilled water by ultrasound treatment during 40 min. Subsequently, 0.5 mL of $RuCl_3$ ($2.5 \times 10^{-3}\,mol\,L^{-1}$) was added in the reaction flask. The mixture was heated up to boiling under continuous magnetic stirring, and then $375\,\mu L$ of 1% (w/v) sodium citrate solution were added. The mixture was boiled for 5 min and then 50 mg $NaBH_4$ in 2.5 mL water were added. The mixture was stirred under heating for other 30 min, then cooled to room temperature and filtered. The resulting solid was repeatedly washed with water and ethanol, dried under vacuum at room temperature and kept dry until use.

To evaluate the synergistic effect of rGO and RuNPs, the chemical synthesis of rGO was also performed as above, but without adding $RuCl_3$. The obtained rGO was analyzed by Raman spectroscopy and the resultant spectra (not included) showed the G and D bands, whose profiles are in full agreement with previously reported ones for such material [21–23].

The composite material rGO/RuNPs was suspended in DMF (1 mg/mL) and the formed suspension was dripped ($9\,\mu L$) onto the surface of the CGE. After drying the material at room temperature, the electrode was ready for use.

2.5. Preparation and analysis of cosmetic samples

A deodorant cream purchased in the local market with a known amount of MePa ($2.89 \times 10^{-3}\,mol\,L^{-1}$) was used for quantification of the substance through DPV and HPLC-UV. The stock solution was prepared by weighing a mass equivalent to about $8.54 \times 10^{-3}\,mol\,L^{-1}$ MePa and by diluting it in methanol on an ultrasonic bath for 15 min until complete homogenization, followed by centrifugation at 9000 rpm over 20 min. The supernatant was filtered through a filter membrane of $0.45\,\mu m$ (RC) porosity. This solution was diluted to known concentrations and used in both methods.