



# Carbon-nanotube amperometric sensor for selective determination of 4-chloroaniline in commercial chlorhexidine solutions



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## ABSTRACT

This work reports a carbon-nanotube amperometric sensor for the selective determination of 4-chloroaniline (4-CLA), the major degradation product found in commercial chlorhexidine solutions. The harmful 4-CLA is detected on a multi-walled carbon nanotube (MWCNT)-modified electrode, free from the interference of chlorhexidine and other substances present in such samples, with a detection limit of 50 nmol L<sup>-1</sup>. MWCNTs of two different dimensions were evaluated for 4-CLA detection and a 3-fold increase in sensitivity using both modified electrodes was obtained in comparison with the bare electrode. The association of batch-injection analysis with the amperometric detection provided high precision (1.2%) and sample throughput (130 h<sup>-1</sup>). Analyses of samples by capillary electrophoresis-tandem mass spectrometry (CE-MS/MS) attested the accuracy of the proposed method. Mouth rinse and skin disinfectant samples presented 4-CLA concentrations in the range between 4 and 235 μmol L<sup>-1</sup>.

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## 1. Introduction

Chlorhexidine (CLX), hexamethylenebis[5-(4-chlorophenyl)biguanide], and its salts (chlorhexidine digluconate or diacetate) have been widely used as an antiseptic agent in pharmaceutical products, such as contact lens solutions and mouth rinses for routine dental plaque control, disinfectants of skin and hands, and cosmetics [1,2]. However, a recent study has demonstrated the formation 4-chloroaniline (4-CLA) as one of the major degradation products of CLX after hydrolysis in acidic or basic conditions [3]. Due to its high toxicity and according to the current recommendation for the control of genotoxic impurities [4], the determination of 4-CLA in CLX solutions is of key importance to monitor the safety of healthcare and medicinal products that contains CLX. The United State Pharmacopeia limit for 4-CLA in chlorhexidine solutions is 3 ppm, in other words, the shelf-life of chlorhexidine solutions is limited by the appearance of 3 ppm (23.5 μmol L<sup>-1</sup>) [5].

Chlorinated anilines have been found as metabolites of a number of herbicides in soil [6] and are common water pollutants. Chlorinated anilines may be considerably less biodegradable and more

toxic than their precursors [7,8], and the potential environmental impact of this class of compounds is underlined. These aromatic amines are highly toxic because they can react easily in the blood to convert hemoglobin into methemoglobin, thereby preventing oxygen uptake [9].

To date, different methods were reported for the determination of 4-CLA, such as a gas chromatographic method applied for the analysis of surgical products and disinfectants based on CLX or triclocarban solutions [10], but most of the methods are high-performance liquid-chromatography (HPLC) methods. Some HPLC methods reported preconcentration steps to increase the detection limits during the sample preparation procedure [11–14]. The use of UV detectors was predominant for 4-CLA determinations in HPLC method [11–13,15,16]; however, fluorescence [14] and electrochemical detectors [11,17,18] were also reported for this aim. There are a few studies in the literature reporting the electrochemical determination of 4-CLA (without the association of a separation technique as HPLC). The amperometric detection of 4-CLA using a glassy-carbon electrode (GCE) at 1100 mV (vs Ag|AgCl|saturated KCl electrode) was associated with HPLC [17,18]. The electrochemical oxidation of 4-CLA was investigated on gold electrodes and GCE [19–21].

Carbon nanotubes (CNTs) continue to receive considerable attention in the development of electrochemical sensors due to their electrocatalytic properties [22–26]. The electrode

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modification with CNTs results in high sensitivity, low detection limits, reduction of over potentials, and high resistance to electrode surface fouling [22–26]. Edge-plane-like sites located at the ends of tubes and in the defect areas of the CNTs have been claimed as the responsible sites for their electrocatalytic activity [25,26]. Multi-walled CNTs (MWCNTs) consist of several concentric tubes of graphite inside one other. The effects of the chemical treatment and dimensions of MWCNTs on the electrochemistry of  $\text{H}_2\text{O}_2$  were investigated [27]. This study revealed that MWCNTs needs a small amount of oxygenated functional groups to facilitate the electron transfer to  $\text{H}_2\text{O}_2$  and a higher degree of oxidation (incorporation of oxygen content) was obtained in longer MWCNTs, which resulted in higher currents. However, the electrodes modified with shorter MWCNTs ( $15\text{--}45\text{ nm} \times 1\text{--}5\text{ }\mu\text{m}$ ) presented faster electron transfer (indicated by lower  $\Delta E_p$ ) [27]. On the other hand, the electrochemistry of phenolic compounds (dihydroxybenzene isomers, including dopamine, catechol, hydroquinone and *tert*-butylhydroquinone) was significantly affected by the size of MWCNTs [28]. GCE modified MWCNTs of shorter dimensions ( $6\text{--}9\text{ nm} \times 1\text{ }\mu\text{m}$ ) presented superior performance than bare GCE and GCE modified with MWCNTs of higher dimensions, and these results were correlated with the increase in defects on MWCNTs monitored by Raman spectroscopy and X-ray diffraction measurements [28].

In this work, the use of a MWCNT-modified electrode associated with batch-injection analysis is demonstrated for the amperometric determination of 4-CLA in commercial CLX solutions. Two different MWCNT sizes (diameter  $\times$  length,  $D \times L$ ,  $100\text{--}170\text{ nm} \times 5\text{--}9\text{ }\mu\text{m}$  and  $6\text{--}9\text{ nm} \times 5\text{ }\mu\text{m}$ , which corresponds to larger diameter, LD, and shorter diameter, SD-MWCNTs, respectively) were evaluated and compared with the unmodified glassy-carbon electrode (GCE) towards 4-CLA detection. It is important to emphasize that CLX undergoes electrochemical oxidation on GCE as previously demonstrated [29], thus the conditions of 4-CLA detection was selected in such a way that the interference of CLX was negligible. The results for different samples obtained by the proposed amperometric method were compared with those obtained by capillary electrophoresis-tandem mass spectrometry (CE-MS/MS).

## 2. Materials and methods

### 2.1. Reagents and samples

All reagents were of analytical grade and were used without further purification. Solutions were prepared with deionized water (Direct-Q3, Millipore, Bedford, MA, USA) with a resistivity no less than  $18.2\text{ M}\Omega\text{ cm}$ . Analytical grade phosphoric acid (85% m/v) was purchased from Reagen (Rio de Janeiro, Brazil), perchloric acid (70% m/v) from Vetec (Rio de Janeiro, Brazil), sodium hydroxide from Dinâmica (Diadema, Brazil), boric acid from QM (Cotia, Brazil), acetic acid glacial from Carlo Erba (Milan, Italy), chlorhexidine acetate (>98%) from Acros Organics (Geel, Belgium) and 4-chloroaniline from Sigma–Aldrich (São Paulo, Brazil) were used without further purification. Stock solutions of 4-CLA and CLX were freshly prepared just before the experiments by dilution in water. The Britton–Robinson (BR) buffer solution was composed by a mixture of  $0.04\text{ mol L}^{-1}$  acetic acid, boric acid, and phosphoric acid and the different pH values were adjusted with sodium hydroxide.

The background electrolyte (BGE) was  $0.030\text{ mol L}^{-1}$  acetic acid, pH 3.5, and was prepared on a weekly basis. Standard working solutions were prepared daily by appropriate dilution of different aliquots of the stock solutions in BGE, using 1,7-diaminoheptane (>99%) from Sigma–Aldrich (São Paulo, Brazil) as an internal stan-

dard. The sheath liquid for the electrospray ionization (ESI) source was prepared by diluting 30 times the BGE with methanol/water 9:1 (v/v), and its flow rate was  $6\text{ }\mu\text{L min}^{-1}$ .

Commercial samples of mouth rinses and skin disinfectants were obtained from local drug and hospital stores. For each analysis, the liquid samples were diluted in electrolyte prior to voltammetric analysis. The skin disinfectant sample contained 2% (m/v) chlorhexidine, alkyl dimethyl amine oxide, glycerin, ethanol, and hydroxyethylcellulose. Mouth rinse samples contained 0.12% (m/v) chlorhexidine, glycerin, sorbitol, alcohol, hydrogenated castor oil, citric acid, menthol, sodium cyclamate, and sodium saccharin. Chlorhexidine digluconate solution (20% m/v aqueous solution) from Alfa Aesar (Shrewsbury, USA) kept in an amber flask was also analyzed.

### 2.2. Functionalization of MWCNTs

Two multi-walled carbon nanotubes (MWCNTs) with different dimensions were used in this work, both purchased from Aldrich (Milwaukee, WI, USA). The multi-walled carbon nanotubes of smaller diameter (SD-MWCNT) and large diameter (LD-MWCNT) have dimensions of  $6\text{--}9\text{ nm}$  in outer diameter and  $5\text{ }\mu\text{m}$  in length (relative purity >95% wt.) and  $110\text{--}170\text{ nm}$  in outer diameter and  $5\text{--}9\text{ }\mu\text{m}$  in length (relative purity >90% wt.), respectively. An amount of 1 g of raw MWCNTs was first treated with a 3:1 (v/v) mixture of concentrated  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$  acids (1000 mL). This mixture was then sonicated for 3 h at  $40\text{ }^\circ\text{C}$  in an ultrasonic bath to introduce carboxylic acid groups on the MWCNT surface. After cooling to room temperature, the carboxylated MWCNTs (MWCNT-COOH) were added dropwise to 3000 mL of cold deionized water and then vacuum-filtered through a  $0.05\text{ mm}$  pore size filter paper. The filtrate was then washed with deionized water until pH 7.0. The carbon nanotubes were then dried in a vacuum oven at  $80\text{ }^\circ\text{C}$  for 8 h [30].

### 2.3. Working electrode preparation

Prior to modification, the GCE surface was polished with  $0.3\text{ }\mu\text{m}$  alumina powder suspension on a felt-polishing pad and then copiously rinsing with deionized water. When performing measurements using bare GCE, the electrode was placed immediately in the electrochemical cell after polishing.

Suspension of carbon nanotubes was prepared by adding MWCNTs (5 mg) to 5 mL of dimethylformamide and agitating the mixture using an ultrasonic bath for 30 min and a high-frequency sonicating tip for 5 min. To obtain a uniform layer of MWCNTs on the GCE surface,  $10\text{ }\mu\text{L}$  of suspension ( $0.1\text{ mg mL}^{-1}$ ) was placed on the surface of the GCE and the solvent was evaporated by exposure at  $50\text{ }^\circ\text{C}$  for 30 min. The electrochemical surface areas of the bare GCE, LD-MWCNT and SD-MWCNT calculated were 5.3, 7.0 and  $8.1\text{ mm}^2$ , respectively, using cyclic voltammetry of  $5/5\text{ mmol L}^{-1}$   $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$  and the Randles–Sevcik equation [31]. The resulting films were clearly visible to the naked eye. The modified electrode was rinsed sequentially with deionized water and working electrolyte before being placed in the cell. Electrode activation was performed by cycling in background electrolyte until reproducible scans were obtained. Three cycles were typically found to be sufficient to obtain reproducible scans.

### 2.4. Instrumentation

All electrochemical measurements were performed using a PGSTAT 128N (Eco Chemie, Utrecht, Netherlands) controlled by GPES 4.9.007 software (General Purpose Electrochemical System). The working, counter, and reference electrodes were a multi-walled carbon nanotube (MWCNT) modified glassy-carbon disk

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