Sensors and Actuators B 240 (2017) 417–425

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Chlorhexidine digluconate on chitosan-magnetic iron oxide nanoparticles modified electrode: Electroanalysis and mechanistic insights by computational simulations

Camila P. Sousa[a,*], Raissa C. de Oliveira[a], Tiago. M. Freire[a], Pierre B.A. Fechine[a], Michele A. Salvador[b], Paula Homem-de-Mello[b], Simone Morais[c], Pedro de Lima-Neto[a], Adriana N. Correia[a]

[a] *Departamento de Química Analítica e Físico-Química, Centro de Ciências, Universidade Federal do Ceará, Bloco 940, Campus do Pici, 60440-900, Fortaleza, CE, Brazil*

[b] *Centro de Ciências Naturais e Humanas, Universidade Federal do ABC, Av. dos Estados, 5001, Bloco B, sala 1017, 09210-580, Santo André, SP, Brazil*

[c] *REQUIMTE–LAQV, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, R. Dr. António Bernardino de Almeida 431, 4200-072, Porto, Portugal*

ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form 18 August 2016
Accepted 31 August 2016
Available online 1 September 2016

Keywords:
Chlorhexidine
Magnetic nanoparticles
Chitosan
Computational simulations
Pharmaceuticals

ABSTRACT

In this work, an electrochemical sensor based on modification of a glassy carbon electrode (GCE) with magnetic iron oxide nanoparticles (Fe_3O_4) and chitosan (CS) was developed to quantify chlorhexidine digluconate (CHD), a worldwide used antiseptic. Cyclic voltammetric and electrochemical impedance spectroscopy assays showed that the permeable Fe_3O_4/CS film on the GCE surface improved the conductivity and facilitated electron transfer. CHD response at GCE/Fe_3O_4/CS corresponds to an irreversible anodic diffusion-controlled process (at about 1.13 V in 0.04 mol L^{-1} Britton-Robinson buffer, pH 4.0) involving the transfer of two electrons and an equal number of proton. Quantum mechanics and Monte Carlo simulations were performed to give insights on the CHD oxidation process. The modification with Fe_3O_4/CS induced alterations in CHD geometry that led to degeneration of the highest occupied molecular orbitals (HOMO and HOMO−1), facilitating the oxidation process because both sides of the molecule contribute to these orbitals. It facilitated also, at least thermodynamically, the first electron lost. The oxidation of CHD probably conducts to the formation of two *p*-chloroaniline and, possibly, two biguanidine molecules. GCE/Fe_3O_4/CS exhibited suitable electroanalytical characteristics in terms of sensitivity (8.78 ± 0.58 A mol^{-1} L), linearity range (2.10×10^{-8}–2.09×10^{-7} mol L^{-1}), detection limit (5.7×10^{-9} mol L^{-1}; 0.005 mg kg^{-1}), intra-day repeatability (2.8% RSD), and reproducibility (4.6% RSD). To evaluate the accuracy and applicability of the proposed electroanalytical methodology, two CHD commercial formulations were analysed and the attained results were in agreement with those attained by the chromatographic reference method. Overall, the developed GCE/Fe_3O_4/CS exhibits appropriate performance and relevant advantages for CHD electroanalysis in commercial products.

1. Introduction

Chlorhexidine digluconate (CHD) is an antiseptic worldwide used in clinical practice and veterinary because it is effective against Gram-positive and Gram-negative microorganisms (although in a lesser extent) [1,2]. CHD (Fig. 1) is composed by two (*p*-chlorophenyl)biguanide bonded by an hexane chain. Despite the widespread use of CHD incorporated in mouthwash formulations, toothpaste, disinfectant solutions, soap, eye drops, gel, spray, and gum, etc. [1], the stability of this molecule is limited by the appearance of its primary degradation product *p*-chloroaniline that is quickly absorbed and metabolized being hemotoxic, as well as carcinogenic [3]. Therefore, pharmacopoeias limit the content of *p*-chloroaniline (e.g. 3 mg L^{-1} for mouthwashes), which restricts the usage of CHD at high concentrations [3]. The official method for quantifying CHD is high-performance liquid chromatography

---

* Corresponding author.
E-mail address: pinheiro.cs@gmail.com (C.P. Sousa).

http://dx.doi.org/10.1016/j.snb.2016.08.181
0925-4005/

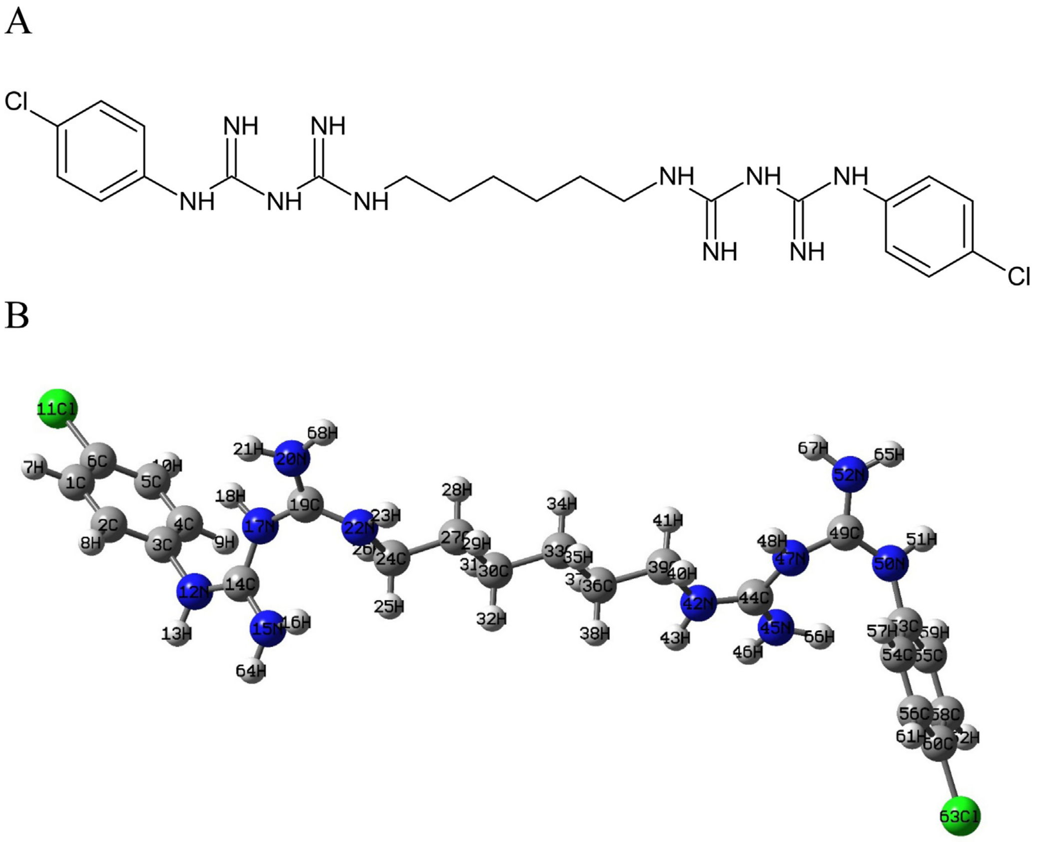

Fig. 1. (A) Chemical structure of chlorhexidine and (B) Optimized geometry of chlorhexidine at pH 4 (H_4CHD).

(HPLC) with UV detection [1], however other methods are also described using gas chromatography [4], fluorescence spectrophotometry [5], and capillary electrophoresis [6]. In addition to these traditional and laborious methods, some electroanalytical methods have been proposed [7–11]. Out of the scarce (five) previous works [7–11], only one was not based on mercury and consisted on amperometric determination by glassy carbon electrode (GCE) using a flow-injection analysis (FIA) system [7]. Mercury is one of the most toxic heavy metals in the environment [12] and consequently it has been banned from analytical laboratories; concerned analysts have been involved in developing more environmentally friendly electroanalytical procedures that follow the principles of green chemistry. Still, no previous study explored the application of modified electrodes so far. Furthermore, the reported electroanalytical methods [7–11] were severely affected by strong adsorption of CHD redox products on the electrode surface. Modified electrodes have been proved to deliver targeted information in a fast, simple, and low-cost fashion allowing control of final product quality, reduction of wastes and process cycle time, as well as replacement of costly and slow laboratory testing without the need for intensive sample preparation and destruction. Moreover, due to the significant input of nanosciences and nanotechnology, recent years have faced stimulating developments in the modification of electrode surfaces. Magnetic particles of nanometric dimensions, in particular iron oxide nanoparticles (Fe_3O_4), are particularly attractive due to their superparamagnetic properties but their uses are limited by their tendency to aggregate. Thus, chitosan (CS), a polyaminosaccharide, has been exploited as a stabilizing agent. Composites of these materials (Fe_3O_4-CS) [13,14] are becoming highly relevant for development of electrochemical detection platforms due to their inherent features such as biocompatibility, superparamagnetic properties, film-forming ability, good adhesion, appropriate mechanical strength, cost-effectiveness, and simplicity of preparation [15–21]. Recently, several type of biosensors (enzymatic and genosensors) that integrated Fe_3O_4-CS in their construction have been reported proving the valuable characteristics of this composite nanoparticles [19–21].

Thus, in this work, the advantages of combining Fe_3O_4 NPs and CS were explored to develop and optimize a rapid, simple, accurate and low-cost sensitive electrochemical approach for CHD analysis based on a modified electrode (GCE/Fe_3O_4/CS). Also, computational (quantum mechanics and Monte Carlo) simulations were performed to give mechanistic insights on the oxidation process of CHD at the modified electrode since this information was inexistent. Furthermore, the analytical performance of GCE/Fe_3O_4/CS was assessed in two worldwide used commercial products and compared with the reference HPLC method.

2. Experimental section

2.1. Chemicals

CHD solution (20% in ultrapure water), CS low molecular weight solution (20-300 cP, 1% w/v in 1% acetic acid), sodium dihydrogen phosphate dehydrate (99%), phosphoric acid (85%) and methanol gradient grade for HPLC ($\geq$99.9%) were purchased from Sigma-Aldrich (Steinheim, Germany). For synthesis of Fe_3O_4 NPs, ferric chloride hexahydrate (97%) and glacial acetic acid (99.7%) were purchased from Vetec (Brazil); ferrous sulphate heptahydrate (99%) from Dinâmica (Brazil). All other chemicals were of analytical grade. Britton-Robinson buffer (BR, 0.04 mol L^{-1}) was prepared by mixing 0.04 mol L^{-1} of phosphoric, acetic and boric acid; the pH was adjusted to the desired value by adding suitable amounts of