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Article

Electrochemical Oxidation of Chlorhexidine and its Amperometric Determination by Flow-Injection Analysis

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A oxidação electroquímica de clorexidina é investigada pela primeira vez e sua determinação amperométrica usando um sistema de análise por injeção em fluxo (FIA) é demonstrada. Um pico de oxidação bem definido foi observado próximo de 1,3 V em uma solução 0,1 mol L^{-1} de $HClO_4$ em eletrodo de carbono vítreo. O mecanismo da oxidação eletroquímica de clorexidina é dependente do pH e envolve a transferência de um único elétron, possivelmente através da formação de cátion radical. Os parâmetros do sistema FIA foram otimizados e um limite de detecção de 0,3 μmol L^{-1} foi obtido. O método proposto foi aplicado em enxaguantes bucais e desinfetantes de pele e comparados com cromatografia líquida de alta eficiência.

The electrochemical oxidation of chlorhexidine is investigated for the first time and its amperometric determination using a flow-injection analysis (FIA) system is demonstrated. An oxidation peak was observed at around 1.3 V in a 0.1 mol L^{-1} $HClO_4$ solution at a glassy-carbon electrode. The mechanism of the electrochemical oxidation of chlorhexidine is pH-dependent and involves a single-electron transfer possibly via radical cation formation. The FIA parameters were optimized and a detection limit of 0.3 μmol L^{-1} was obtained. The proposed method was applied in mouth rinses and skin disinfectants samples and compared with high-performance liquid chromatography.

Keywords: chlorhexidine oxidation, bare electrode, organochloride, antiseptic

Introduction

Chlorhexidine, hexamethylenebis[5-(4-chlorophenyl) biguanide], and its salts (e.g. chlorhexidine digluconate or diacetate) are antibacterial agents widely used in aqueous personal products such as contact lens solutions, mouth rinse, toothpastes, and as skin disinfectant in surgical hand scrubs in several concentrations. The use of chlorhexidine as a chemical antiplaque agent in mouth rinse solutions or in gel form has been widely reported.[1-3] However, a recent study has demonstrated that chlorhexidine undergoes hydrolytic degradation and its main degradation product is p-chloroaniline, which is hematotoxic and carcinogenic.[4] Therefore, the analytical control of chlorhexidine especially in mouth rinse solutions may indicate if degradation processes have started by detecting the consumption of chlorhexidine.

Several analytical methods have been developed for chlorhexidine determination using spectrophotometry,[5-7] high-performance liquid chromatography[8-10] and capillary electrophoresis.[11] Typically these analytical methods require time-consuming and laborious sample treatments such as solvent extraction, liquid-liquid extractions, excipient precipitation, and sample clean up steps, which can increase irreproducibility. On the other side, electrochemical analysis is an alternative technique which provides simple, fast, and sensitive determinations and frequently does not require laborious sample treatment steps. Electroanalytical methods were developed for chlorhexidine determinations based on its electrochemical reduction on mercury or mercury-film electrodes exploiting very negative potentials (from –1.5 to –1.9 V *vs.* SCE).[12-15]

This article reports on the electrochemical oxidation of chlorhexidine at a bare glassy-carbon electrode (GCE) and its electrochemical determination in a flow-injection-analysis (FIA) system coupled to amperometric detection based on the analyte oxidation.

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Experimental

Reagents and samples

The solutions were prepared using deionized water (Direct-Q3, Millipore, Bedford, MA, USA) with a resistivity of no less than 18 MΩ cm. Phosphoric acid (85% m/v) from Reagen (Rio de Janeiro, Brazil), nitric acid from Synth (Diadema, Brazil), sodium hydroxide from Dinâmica (Diadema, Brazil), acetic acid from Vetec (Rio de Janeiro, Brazil), boric acid from QM (Cotia, Brazil), and chlorhexidine acetate from Acros Organics (Geel, Belgium) (purity > 98%) from Sigma-Aldrich (St. Louis, MO, USA) were used without further purification. Stock solutions of chlorhexidine were freshly prepared just before the experiments by dilution in water. The Britton-Robinson (BR) buffer solution was composed by a mixture of 0.1 mol L^{-1} acetic acid, boric acid, and phosphoric acid and its different pH were adjusted with sodium hydroxide. 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 voltammetric analysis. Beyond chlorhexidine (0.12% m/v), the mouth rinse samples contained glycerin, sorbitol, alcohol, hydrogenated castor oil, citric acid, menthol, sodium cyclamate, and sodium sacacharyn. The skin disinfectant sample contained 2% m/v chlorhexidine, alkyl dimethal amine oxide, glycerin, ethanol, and hydroxyethylcelulose.

Electrochemical measurements

All electrochemical measurements were performed using a μ-Autolab Type III (Eco Chemie, Utrecht, Netherlands). The working, counter, and reference electrodes were a glassy carbon disk (∅ = 1.6 mm, ALS, Japan), a platinum wire, and a miniaturized Ag/AgCl (saturated KCl) electrode,[16] respectively. Constant-potential amperometric flow measurements were performed by using a home-made electrochemical wall-jet cell in the three electrodes configuration.[17,18] A single-line flow system was employed using 1.0-mm (i.d.) polyethylene tubing. The injection of standard or sample solutions was carried out by filling loops (polyethylene tubing) of varied volume (from 100 to 300 μL), which was connected to the single-line flow by a FIA valve. A syringe was used to fill the injection loop with sample or standard solutions by producing a negative pressure. The solutions were propelled by a peristaltic pump. Cyclic voltammograms were recorded at 50 mV s^{-1}. Square-wave voltammetry parameters were 8 mV step, 50 mV amplitude, and 50 Hz frequency. All electrochemical measurements were performed at room temperature, in the presence of dissolved oxygen.

High-performance liquid chromatography (HPLC) analysis

The HPLC measurements were performed using a Shimadzu LC-10 VP equipped with an UV-vis detector (SPD-10AV), a LC column (Lychrispher 100 A8 RP18-C18, 250 mm x 4.6 mm, 5 mm), a degasser (DGU-20A5), a manual injector (20 mL) and a pump (LC-10AD-VP). The mobile phase was composed of methanol and water (70:30, v/v). The detector was fixed at 260 nm. The flow rate was 0.8 mL min^{-1}.

Results and Discussion

The electrochemical oxidation of chlorhexidine at GCE was investigated in a 0.1 mol L^{-1} $HClO_4$ solution and in 0.1 mol L^{-1} BR buffer solutions (from pH 2.0 to 8.0). Figure 1A highlights the cyclic voltammogram of chlorhexidine oxidation at GCE in 0.1 mol L^{-1} $HClO_4$

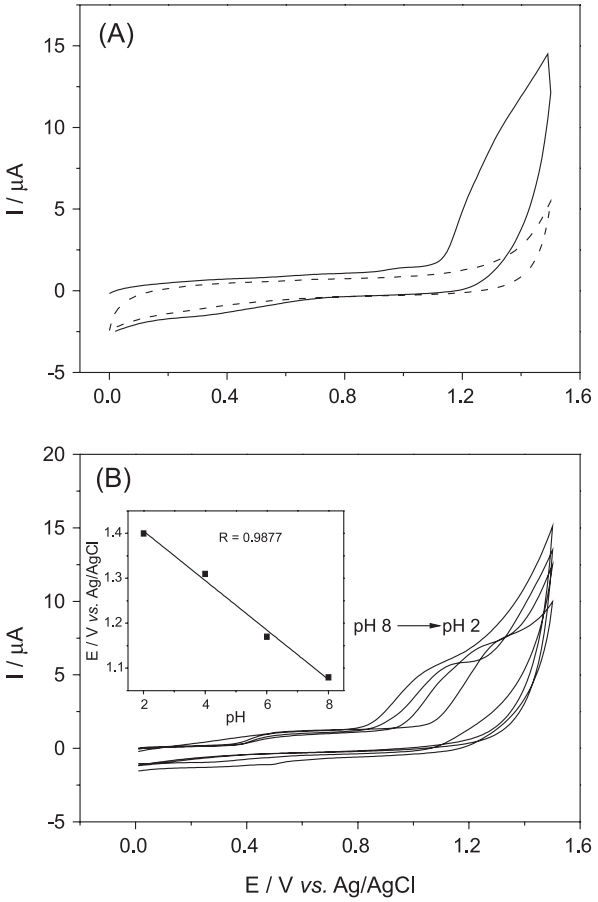
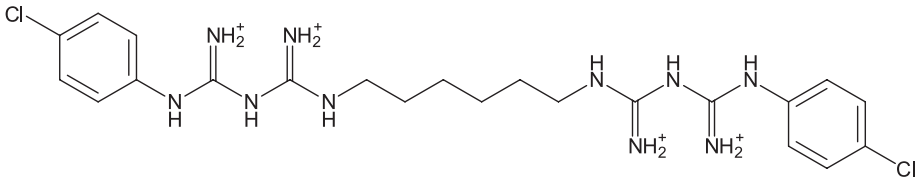


Figure 1. (A) Cyclic voltammograms of 1 mmol L^{-1} chlorhexidine in 0.1 mol L^{-1} $HClO_4$ and (B) in 0.1 mol L^{-1} BR buffer solutions of different pH values (2.0; 4.0; 6.0; and 8.0). Inset in (B) is the plot of potential *vs.* pH. Scan rate: 50 mV s^{-1}.



Scheme 1. Chemical structure of the predominant species of chlorhexidine in acidic medium.

whilst Figure 1B presents the cyclic voltammogram of chlorhexidine in BR buffer solutions. Inset of Figure 1B is the plot of potential *vs.* pH.

An irreversible oxidation peak was observed at 1.3 V using a bare GCE in 0.1 mol L^{-1} $HClO_4$, with oxidation starting at ca. 1.1 V. Similarly, an irreversible oxidation peak was observed in the voltammograms performed in all BR buffer solutions; however, a shift in the oxidation peak was observed. The voltammetric responses in BR buffer of different pH values provided strong evidence that the mechanism of the electrochemical oxidation of chlorhexidine is pH-dependent. As long as the pH was increased, a shift in the peak potential towards less positive potential values was observed. The plot of peak potential *vs.* pH values presented a slope of 55 mV per pH unit (inset of Figure 1B), which indicates that the same number of protons and electrons is involved in the electro-oxidation process. Using square-wave voltammetry,[19] the number of electrons was calculated in one-electron (considering $\alpha = 0.5$ for an irreversible system). The pKa of chlorhexidine is 10.8, indicating that this compound primarily exists in the protonated form (see Scheme 1). Then, one proton from one of the four protonated amino groups can be released on the electrochemical oxidation of chlorhexidine and so the loss of one electron may be reasonably inferred. Therefore, the process involves a single-electron transfer possibly via radical cation formation at the deprotonated amino group. Similarly, mechanisms involving the formation of radical cation have been demonstrated for the electrochemical oxidation of chloroaniline and aromatic amines.[20,21]

The second and third cyclic voltammetric scans (not shown) presented similar profiles of the blank scan as long as the pH was increased (especially at pH 6 and 8), which indicates a strong adsorption process at the GCE. Considering the development of an electroanalytical method for chlorhexidine determination, the best choice is the use of acid electrolytes in order to reduce adsorption processes. Amperometric measurements in BR buffer solutions at different pH values (2 to 6) confirmed adsorption processes in such a way that the relative standard deviation (RSD) for consecutive injections of 5 μmol L^{-1} chlorhexidine was higher than 20% in all cases (constant decrease in current for repetitive injections). Therefore, a 0.1 mol L^{-1} $HClO_4$ solution was used for further measurements. Using data from cyclic voltammetric studies carried out in 0.1 mol L^{-1} $HClO_4$, the plot of the oxidation peak current of chlorhexidine (at 1.3 V) *vs.* the square root of scan rate (v) (from 10 to 300 mV s^{-1}) is linear (I (A) = $-1.18 \times 10^{-7} + 2.57 \times 10^{-7}$ $v^{1/2}$ (V s^{-1}); r = 0.998) which suggests that the oxidation process is controlled by diffusion under these conditions.

A hydrodynamic voltammogram for chlorhexidine based on amperometric measurements in the FIA system is presented in Figure 2.

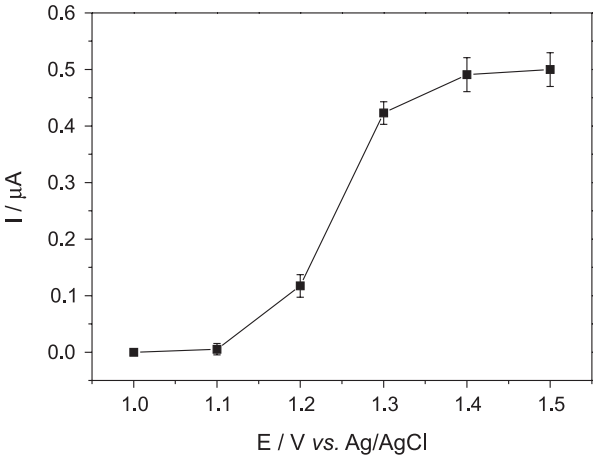


Figure 2. Hydrodynamic voltammogram obtained by plotting the peak current values (average of 3 injections) for 10 μmol L^{-1} chlorhexidine as function of applied potentials. Electrolyte: 0.1 mol L^{-1} $HClO_4$.

An oxidation current for chlorhexidine was observed at potentials higher than 1.2 V. The potential of 1.3 V was selected for further amperometric measurements, which presented lower standard deviation and significant current increase. FIA parameters were evaluated in order to obtain the highest signal for chlorexidine oxidation. Figure 3 presents the variation of (A) injected volume and (B) flow rate.

A slight higher current and lower standard deviation (n = 3) was observed for an injection volume of 200 μL of 10 μmol L^{-1} chlorhexidine in the FIA system (Figure 3A), which was thus selected for further amperometric recordings. The flow rate of the FIA system (Figure 3B) was evaluated keeping constant the injection volume of 200 μL of 10 μmol L^{-1} chlorhexidine. It was observed a linear increase of current for flow rate from 1.0 to 3.0 mL min^{-1}

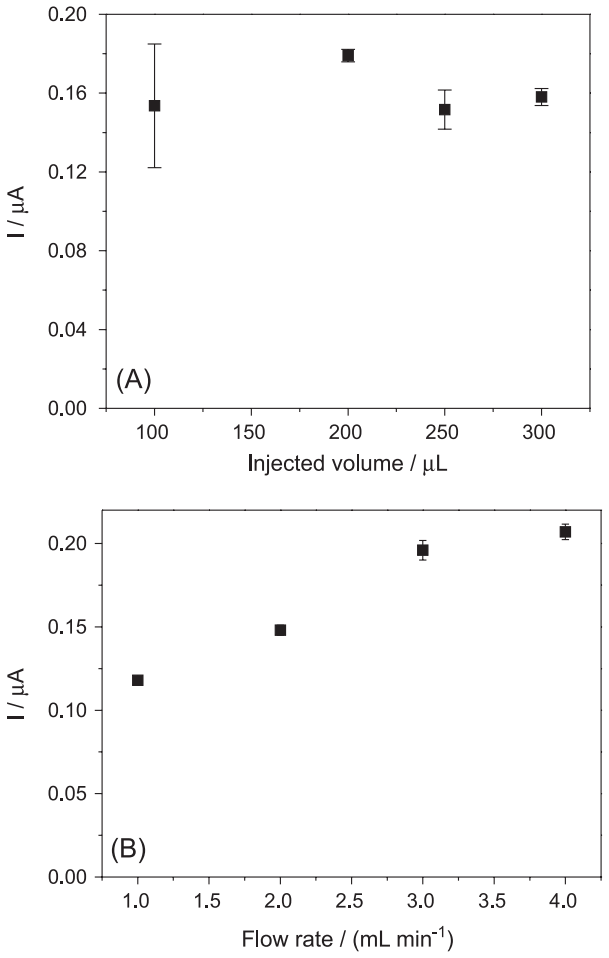


Figure 3. Optimization of FIA parameters: variation of (A) injected volume (100, 200, 250 and 300 μL) and of (B) flow rate (1.0, 2.0, 3.0 and 4.0 mL min^{-1}) based on triplicate injections of 10 μmol L^{-1} chlorhexidine. Electrolyte: 0.1 mol L^{-1} $HClO_4$.

and a plateau was reached at 4.0 ml min^{-1}. The flow rate of 3.0 mL min^{-1} was selected for further amperometric measurements.

The linear working range was evaluated using the optimized FIA conditions. A linear behaviour with a good correlation coefficient was verified from 1 to 10 μmol L^{-1} chlorhexidine. The limit of detection (LOD) for chlorhexidine determination was calculated in accordance to IUPAC (LOD = 3sB/S, in which sB is the standard deviation of baseline noise and S is the slope of the analytical curve, 0.029 μA $μmol^{-1}$ L). Using the slope of the calibration curve (inset of Figure 4), the detection limit was estimated in 0.3 μmol L^{-1}. The RSD for 10 repetitive measurements of 5 μmol L^{-1} chlorhexidine was 5.1%.

The optimized FIA method with amperometric detection was applied for chlorhexidine determination in commercial samples of mouth rinses and skin disinfectants. For comparison, the samples were also analyzed by HPLC. All results are presented in Table 1. The amperometric response for injections of standard solutions of chlorhexidine (analytical curve) and samples (after adequate dilution) is presented in Figure 4.

Table 1. Chlorhexidine concentration (m/v) in commercial samples analyzed by the proposed FIA method and by HPLC (n = 3)

Samples	Label / %	FIA / %	HPLC / %
A	0.12	0.12 ± 0.01	0.12 ± 0.01
B	0.12	0.12 ± 0.01	0.10 ± 0.01
C	2.00	1.96 ± 0.09	1.83 ± 0.09

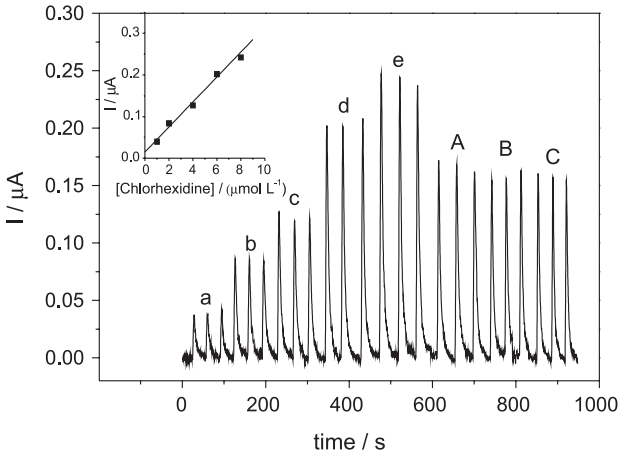


Figure 4. FIA amperometric responses of the GCE for triplicate injections of (a) 1; (b) 2; (c) 4; (d) 6; and (e) 8 μmol L^{-1} chlorhexidine standard solutions and (A–C) commercial samples. Inset: corresponding calibration curve (R = 0.994). Electrolyte: 0.1 mol L^{-1} $HClO_4$; injected volume: 200 μL; flow rate: 3.0 mL min^{-1}.

The results obtained by the proposed FIA method were in agreement with those obtained by HPLC at the 95% confidence level (the calculated *t*-values from the paired Student's *t*-test were smaller than the critical value, 2.78, for n = 3), attesting the accuracy of the proposed method. Other compounds (described in experimental section) contained in the different commercial samples did not interfere on the voltammetric determination of chlorhexidine. Additionally, recovery tests were performed using sample B spiked with a known amount of chlorhexidine (half concentration of its labeled value). Recovery values of 97 ± 1% (n = 3) were obtained which also attests the accuracy of the proposed method and absence of interference from sample matrix.

Table 2 presents a comparison of the analytical characteristics between the proposed FIA method and other electroanalytical methods using mercury-based electrodes. The proposed method presents low detection limit, and its main advantages over previous reported methods are the use of mercury-free sensor and high analytical frequency (40 h^{-1}).

Table 2. Comparison of analytical characteristics between the proposed method and other electroanalytical methods using mercury-based electrodes (n = 3)

Technique Detection potential / V	Sensitivity / (μA μmol^{-1} L)	Analytical range / (μmol L^{-1})	LOD / (μmol L^{-1})	Ref.
DPP –1.6 V (*vs.* Ag/AgCl)	n. m.	3-60	0.5	12
DPAdSV –1.53 V (*vs.* Ag/AgCl)	n. m.	0.22-1.11	0.055	13
SWV –1.54 V (*vs.* SCE)	0.2	0.5-0.20	n. m.	14
DPV –1.9 V (*vs.* SCE)	0.65	10-79	1.5	15
Amperometry +1.3 V (*vs.* Ag/AgCl)	0.029	1-10	0.3	This work

DPP: differential-pulse polarography; DPAdSV: differential-pulse adsorptive stripping voltammetry; DPV: differential-pulse voltammetry; SCE: saturated calomel electrode; n. m.: not mentioned.

Conclusions

We have shown for the first time the electrochemical oxidation of chlorhexidine using a GCE. Based on this oxidation process, a FIA method with amperometric detection was developed for chlorhexidine determination in different commercial samples. The proposed method is highly-sensitive, free of interferences from sample matrices, and accurate (confirmed by comparative determinations using HPLC). Moreover, it is only necessary the use of a bare GCE (oxidation of chlorhexidine) instead of mercury-based electrodes (employed for reduction of chlorhexidine), which have been avoided due to the metal toxicity.

Acknowledgements

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