

## Research Article

# Scanning Electrochemical Microscopy as a Tool for the Characterization of Dental Erosion

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When the tooth is exposed to acidic environments, an irreversible loss of dental hard tissue occurs in a process called dental erosion. In this work, the scanning electrochemical microscopy (SECM) was used to probe the consumption of protons at the vicinity of a tooth surface with a platinum microelectrode fixed at  $-0.5$  (V) versus Ag/AgCl/KCl<sub>(sat)</sub>. SECM approach curves were recorded to assess the extent of diffusion in the solution close to the tooth substrate. SECM images clearly demonstrated that the acid erosion process is very fast at solution pH values in the range between 3 and 4.

## 1. Introduction

The last decades have witnessed major advances in dental medicine and oral health. One of these breakthroughs was the improvement in oral hygiene and the practice of healthier diets, which are the main factors contributing to greater longevity of the dentition. However, more people are consuming high-acid food and drinks, hence exposing their teeth to the risk of erosion. These acid substances slowly soften the tooth surface, making it more susceptible to abrasion and contributing to the loss of the chemical structure. Erosion is the loss of tooth enamel, the hard, protective coating of the tooth, composed of impure calcium hydroxyapatite,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  [1]. The process is caused by acid attack that does not involve bacteria and results in tooth wear of dentin hypersensitivity, loss of form and color of the tooth, which may require restorative complex interventions [2]. When the enamel is worn away, the dentine underneath is exposed, which may lead to pain and sensitivity.

The deterioration of the tooth structure has been investigated by several methods. Xu et al. showed changes in the microstructure of dentin and its composition by using

electrochemical impedance spectroscopy and other surface analysis techniques assisted in the chemical and structural characterization of the investigated material [3]; Cheng et al. used scanning electron microscopy and atomic force microscopy aiming to improve the understanding of the erosion process and obtained qualitative and quantitative information on the morphology and structure of enamel before and after exposure to acid solutions [4]. Atomic force microscopy was used by Quartarone et al. in an in vitro study, where the surface roughness of teeth samples kept in contact with acid drinks was evaluated and the results showed deep holes in the structure of enamel [5]. Heurich et al. using confocal laser scanning microscopy, a technique widely used to quantitatively analyze enamel surfaces eroded by acidic solution, obtained quickly and accurately single 3D image topographic differences between an eroded enamel surface and a given reference area [6].

Scanning Electrochemical Microscopy (SECM) is an attractive technique to get localized information about the nature and properties of a sample surface [7–11]. The probe is a microelectrode electrically biased, and the current is monitored as the sensor is scanned precisely in the  $x$ ,  $y$ , and  $z$  directions. The technique can be used, for instance, to obtain

images that show consumption of an electroactive species in a microscopic scale, such as in the investigation of oxygen consumption by a cyclic-tetrameric copper(II) complex immobilized on the surface of a glassy carbon electrode [12]. SECM has already been used to probe localized fluxes of electroactive species diffusing through dentinal tubules [13–16]. Accordingly, the aim of this paper is to demonstrate that SECM can be used to provide interesting in situ information on the chemical dissolution of bovine dental enamel by acidic solutions with low pH values. The high-resolution capability of the technique offers the possibility for monitoring different products against enamel and dental erosion in a rapid and simple experiment.

## 2. Experimental

**2.1. Materials.** All reagents were of analytical grade and supplied by Merck (Darmstadt, Germany). Deionized water processed through a Nanopure Infinity purification system ( $18\text{ M}\Omega\text{ cm}^{-1}$ ) (Barnstead, Dubuque, IA, USA) was used to prepare the solutions.

**2.2. Electrochemical Measurements.** An Autolab PGSTAT 30 (Eco Chemie) bipotentiostat with data acquisition software made available by the manufacturer (GPES 4.8 version) was used for electrochemical measurements. A platinum disc microelectrode was used as working electrode, and a homemade Ag/AgCl (saturated KCl) and a platinum wire were used as reference and counter electrodes, respectively.

**2.3. Microelectrode Fabrication.** A cleaned  $25\text{ }\mu\text{m}$  diameter platinum fiber (Alfa Aesar, Massachusetts, USA) was connected to an Ni/Cr wire with silver ink conductive paint (Joint Metal Comércio LTDA, São Paulo, Brazil), inserted into a glass capillary and vacuum-sealed with the P-97 Flaming/Brown Micropipette Puller (Sutter Instrument Company, USA). In order to ensure reproducible measurements, the microelectrode surface was polished with sandpaper (no. 400) and alumina slurry ( $1\text{ }\mu\text{m}$ , Alfa Aesar, Massachusetts, USA). Then, the microelectrode surface was rinsed with water and sonicated for 5 minutes in distilled water. The radius of the microelectrode was determined by measuring the steady-state current in a  $10\text{ mmol L}^{-1}\text{ K}_3\text{Fe}(\text{CN})_6$  solution containing  $0.1\text{ mol L}^{-1}\text{ KCl}$  as supporting electrolyte [17].

**2.4. SECM Experiments.** Scanning Electrochemical Microscopy (SECM) experiments were performed using a Sensolytics Base SECM (Sensolytics, Bochum, Germany) instrument with option High-Res. The SECM tip was a  $25\text{ }\mu\text{m}$  diameter platinum disc microelectrode ( $RG = rg/a = 10$ , where  $rg$  is the radius of the electrode plus surrounding insulator and  $a$  is the radius of the disc microelectrode). A homemade Ag/AgCl (saturated KCl) and a platinum wire were used as reference and counter electrodes, respectively. The tip positioning in the  $z$ -axis was accomplished by using oxygen as redox mediator in experiments, where the microelectrode was polarized at  $-0.8\text{ (V)}$  versus Ag/AgCl/KCl<sub>(sat)</sub>

[18]. The steady-state current was monitored by moving the tip from the bulk solution toward the substrate surface at a constant scan rate of  $2.5\text{ }\mu\text{m s}^{-1}$ . A touch of the tip to the insulating surface could be usually detected by a sharp decrease of the current because of the hindering effect (negative feedback). The approach curve and SECM images involving the evaluation of localized pH changes were obtained by measuring the diffusion-limiting current for reduction of  $\text{H}^+$  with the microelectrode biased at  $-0.5\text{ (V)}$  versus Ag/AgCl/KCl<sub>(sat)</sub>. Current data displayed either in the approach curves or SECM images were normalized for the diffusion-limiting current determined experimentally with the tip located in the bulk solution. In order to avoid the loss of activity of the platinum microelectrode tip during the acquisition of SECM images, its surface was cleaned by an electrochemical procedure. Accordingly, the tip had its potential biased at  $0.2\text{ (V)}$  versus Ag/AgCl/KCl<sub>(sat)</sub> for 10 s between each experiment [12].

**2.5. Teeth Samples Preparation.** Bovine mandibular incisor teeth were extracted without visible fractures, and the soft tissue was removed by using a periodontal instrument. The samples were subjected to prophylaxis with pumice paste and water and stored in a disinfectant solution (0.5% chloramine T) at  $4^\circ\text{C}$  to prevent unwanted bacterial growth.  $5 \times 5 \times 5\text{ mm}$  cross-sections of the teeth were obtained by using a cutting machine (Extec Labcut 1010). The enamel surface was polished using sandpaper (nos. 220, 320, and 600), followed by a  $1\text{ }\mu\text{m}$  alumina paste (Alfa Aesar, Massachusetts, USA), and then cleaned by ultrasonication to remove residues from polishing. The samples were washed and stored in deionized water at room temperature prior to SECM experiments.

## 3. Results and Discussion

**3.1. Microelectrode Response to  $\text{H}^+$ .** The monitoring of pH changes at the vicinity of the tooth during acid erosion was carried out by using a platinum microelectrode. Preliminary experiments to assess the behavior of the probe were performed by recording cyclic voltammograms in  $0.1\text{ mol L}^{-1}\text{ KCl}$  solutions containing  $\text{H}^+$  at concentrations varying from  $0.05$  to  $0.8\text{ mmol L}^{-1}$ . In this paper, for the sake of simplicity we shall assume that protons concentration is the same as their activity; hence, pH is a measurement of the  $\text{H}^+$  concentration. Figure 1 shows a typical voltammogram recorded at  $20\text{ mV s}^{-1}$ , a characteristic sigmoidal wave both on the forward and the backward scan being obtained [19]. The limiting current was linear in  $\text{H}^+$  concentration according to the following equation:  $I(\text{nA}) = -4.1 + 0.14 [\text{H}_2\text{SO}_4]\text{ mmol L}^{-1}$ ,  $r = 0.9968$ .

**3.2. Concentration Profiles.** The protons consumption at the teeth surface was first investigated by performing a SECM approach curve experiment in a solution of  $\text{pH} = 3.4$ . The tip was biased at  $-0.5\text{ (V)}$  versus Ag/AgCl/KCl<sub>(sat)</sub>, where the reduction of solvated protons is mass-transport controlled. As the tip was moved close to an insulating surface

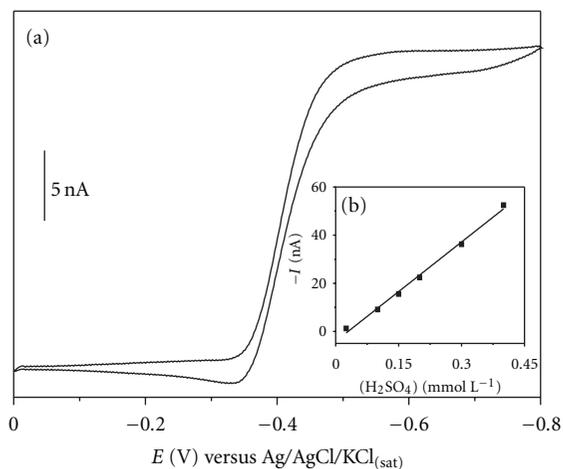
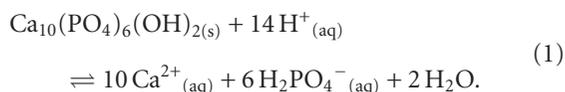


FIGURE 1: Cyclic voltammogram recorded with a platinum microelectrode in a  $0.2 \text{ mmol L}^{-1} \text{ H}_2\text{SO}_4 + 0.1 \text{ mol L}^{-1} \text{ KCl}$  solution ( $\text{pH} = 3.4$ ) (a) and calibration plot (b) Scan rate =  $20 \text{ mV s}^{-1}$ .

(curve A in Figure 2), the steady-state current decreased because of hindered diffusion. In this case, the negative feedback effect is expected to appear only when the distance between substrate and tip is around a few  $L$  units ( $L =$  ratio between tip-substrate distance and microelectrode radius) [20]. No hysteresis between forward and back approaches was noticed, hence, this experiment was not time dependent. On the other hand, a clear difference is noticed when the experiment is repeated using a tooth sample as substrate (curve B in Figure 2). In this case, the current decrease starts at regions very far from the substrate surface (around  $2000 \mu\text{m}$ ), which is an indication of the chemical consumption of protons and the development of a concentration gradient. The process involving the enamel dissolution is time dependent, which explains the significant hysteresis observed in the reverse scan. Figure 3 shows a schematic picture of the processes occurring at the tip microelectrode (proton sensing) and the tooth surface (enamel acid erosion).

**3.3. SECM Images.** The current at the probe is proportional to the concentration of  $\text{H}^+$  in solution. The local proton concentration may change in the gap between the positioned microelectrode and the tooth surface owing to the acid erosion of the enamel, according to the following equation:



Scanning in a rastered pattern was performed at a constant substrate-tip separation above the tooth sample ( $10 \mu\text{m}$ ). All the images presented in this paper relate to a  $300 \mu\text{m} \times 300 \mu\text{m}$  area of the substrate. The SECM images in Figure 4 correspond to a 2D representation of the local tip current. Since the tooth surface is flat, the current changes indicate variations in the proton concentration at this point in the space. A previous experiment was performed by scanning the tip over an insulating substrate

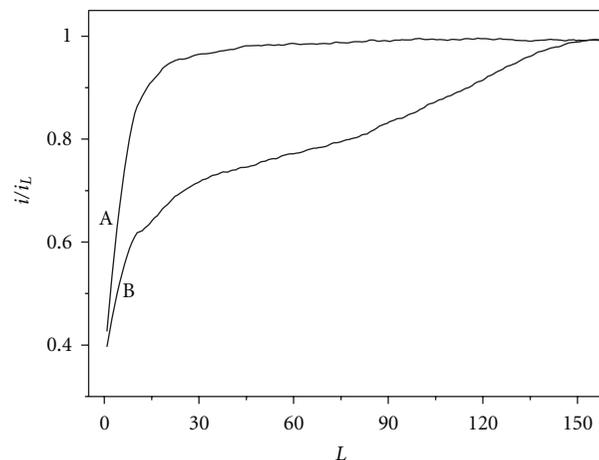


FIGURE 2: Approach curves obtained with a platinum microelectrode ( $r = 12.5 \mu\text{m}$ ) in a  $0.2 \text{ mmol L}^{-1} \text{ H}_2\text{SO}_4 + 0.1 \text{ mol L}^{-1} \text{ KCl}$  solution ( $\text{pH} = 3.4$ ) for an insulating substrate (A) and a teeth sample (B),  $L =$  ratio between tip-substrate distance and microelectrode radius. Scan rate =  $10 \mu\text{m s}^{-1}$ .

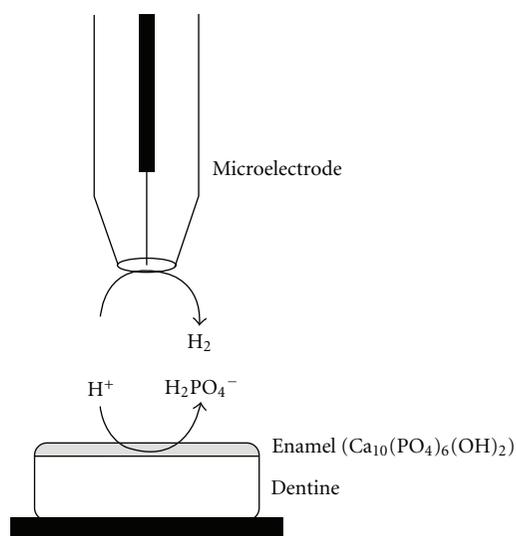


FIGURE 3: Schematic representation of the SECM experiment.

that is inert towards  $\text{H}^+$  reaction. The obtained image (a) reveals no significant change, which indicates the good and reproducible response of the probe and the flatness of the surface and also that no convective effects caused by the tip movement or solution stirring during the scan are noticed.

A noticeable difference is seen when the same experiment was repeated using the tooth as substrate (image (b)). By comparing images (a) and (b) in Figure 4, one can conclude that the results observed in this study clearly demonstrate the protons consumption due to the enamel erosion and that the remarkable current changes are not a consequence of convection due to tip displacement. By taking into account the time required for obtaining one image (around 5 minutes) at the experimental conditions used in this work (scan rate =  $20 \mu\text{m s}^{-1}$ ), it is possible to conclude that the

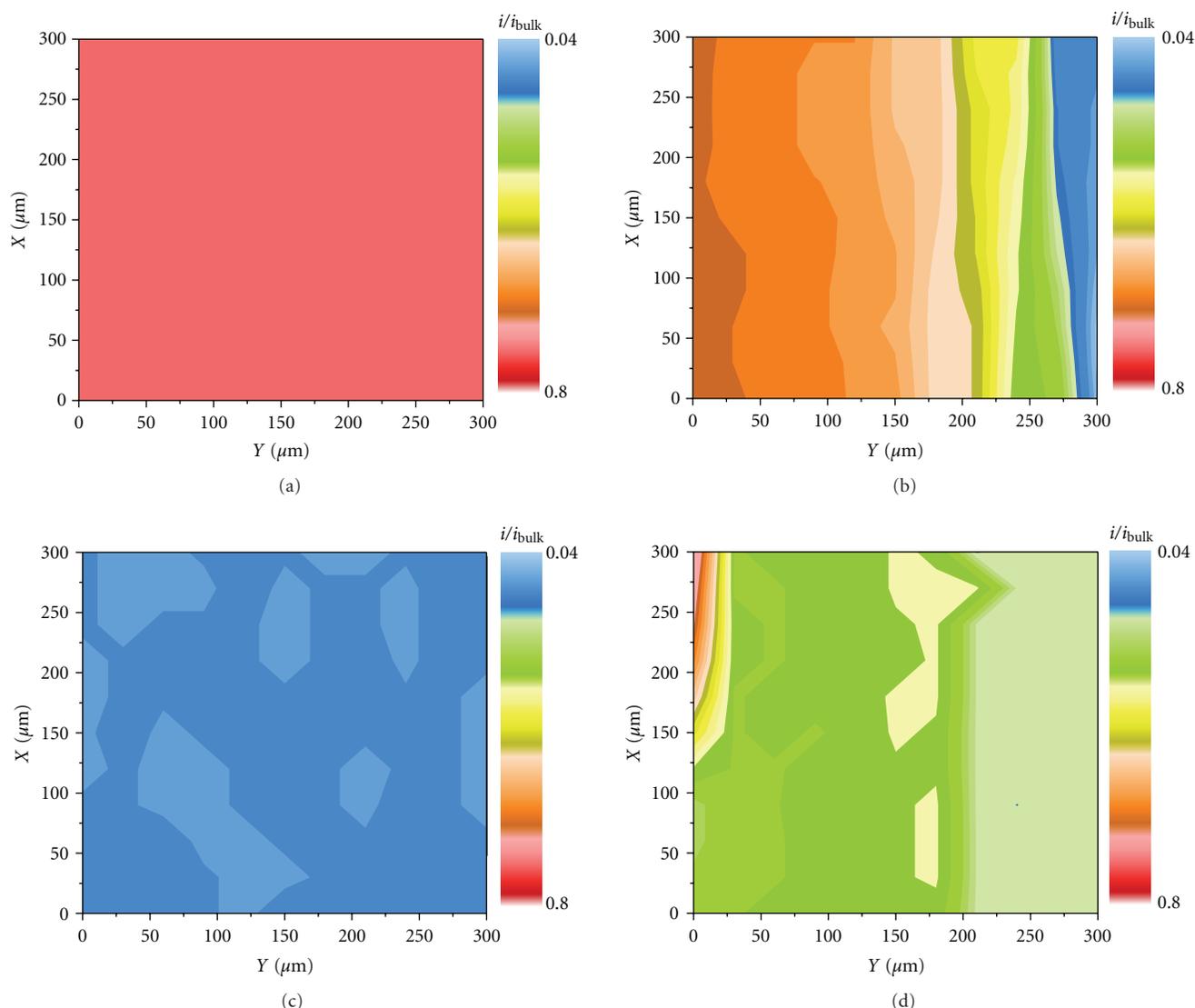


FIGURE 4: SECM 2D images obtained with a platinum microelectrode ( $r = 12.5 \mu\text{m}$ ) in a  $0.2 \text{ mmol L}^{-1} \text{ H}_2\text{SO}_4 + 0.1 \text{ mol L}^{-1} \text{ KCl}$  solution ( $\text{pH} = 3.4$ ) for an insulating substrate (a) and a tooth sample at different times: 0 minutes (b), 60 minutes (c), and 70 minutes (d). Image (d) was recorded after solution stirring. Scan rate =  $20 \mu\text{m s}^{-1}$ .

erosion process is fast. This is in agreement with results of kinetic studies on the chemical dissolution of calcium hydroxyapatite at pH values in the range 3.7 to 6.5 [21] and acid-induced dissolution experiments using SECM [22].

A new image (c) was obtained after one hour, and no significant change during the scan was observed. On the other hand, a different situation was noticed when the solution was stirred for homogenization prior to the acquisition of a new image (d). In the beginning of this experiment the current is similar to the one obtained in the bulk solution, but its decay is very fast probably because of the removal of the chemical resistant enamel outer layer [6, 23]. A final experiment (image not shown) was carried out using an acetate buffer solution of the same  $\text{pH} = 3.4$ . The current values were almost the same during the whole scan, indicating that the pH is maintained at a constant value because protons are efficiently supplied by the buffer.

## 4. Conclusions

The dissolution rate of enamel during acid erosion is dependent on some chemical parameters such as pH and calcium and phosphate-containing salts concentration [23]. From the results shown in this work, one can suggest that the enamel dissolution is a relatively fast process that causes the proton diffusion layer to extend to a large distance into the bulk solution.

The dissolution mechanism is controlled by intrinsic interfacial phenomena, and it is well known that in the presence of some ions, such as fluoride, the rate of erosion of calcium hydroxyapatite is greatly reduced. Moreover, SECM can be easily employed as a powerful tool to investigate the complex chemical reactions responsible for acid erosion as well as in the elucidation of the mechanism of protection by inhibiting ions. Another possibility to prevent dental

erosive demineralization is the use of TiF<sub>4</sub> or NaF varnishes, whose protective effect is attributed to the formation of acid-resistant blocking layers [24, 25]. The enamel erosion has been typically monitored by ex-situ contact profilometry [26, 27] and the effectiveness of such treatments can be also easily assessed through a series of simple and fast SECM experiments.

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