Determination of Paracetamol in Presence of Ascorbic Acid in Pharmaceutical Products by Scanning Electrochemical Microscopy

Alex S. Lima, *Gabriel N. Meloni*, *Mauro Bertotti**

Instituto de Química – USP, São Paulo, Brazil
*e-mail: mbertott@iq.usp.br

Received: January 30, 2013
Accepted: March 11, 2013
Published online: May 9, 2013

Abstract
The selective amperometric determination of paracetamol in pharmaceutical formulations containing ascorbate was achieved by removing the interfering species in the diffusion layer created between a platinum substrate and a disc microelectrode in a Scanning Electrochemical Microscopy (SECM) configuration, while the target analyte was kept unconsumed. After complete depletion of ascorbate, paracetamol was detected at the SECM tip in a free-interference solution zone. The influence of the substrate potential and the gap distance on the efficiency of ascorbate removal was systematically examined. The effectiveness of the device towards the determination of paracetamol in pharmaceutical samples was evaluated and under optimal conditions the results obtained agreed well with the labeled value.

Keywords: Ascorbic acid, Paracetamol, Scanning electrochemical microscopy, Microelectrodes

DOI: 10.1002/elan.201300055

1 Introduction

Acetaminophen, also known as paracetamol (PCT), is a commonly used antipyretic and analgesic agent. When used at recommended doses this medicament is safe, but larger ingestions can make it toxic [1], making its determination in pharmaceutical compounds very important [2]. Several techniques have been used for the determination of PCT such as spectrophotometry [3], electrochemistry [4], liquid chromatography [5], electrophoresis chip [6], chemiluminescence [7], Raman spectroscopy [8] and titrimetry [9]. However, some developed methods have disadvantages associated with high costs, long analysis time or need for sample treatment, making them unsuitable for routine analysis. Electroanalytical methods can overcome these drawbacks, as they are simple, have low cost, are available for miniaturization and can potentially be applied to real-time in vivo determinations [10].

Electroanalytical methods are well suited for PCT analysis because the molecule can be typically oxidized at potentials around 0.5 V vs. Ag/AgCl (sat. KCl), avoiding problems with the majority of possible interfering species [11]. However, the oxidation potential of ascorbic acid (AA), which is widely found in biological fluids and some pharmaceutical preparations, is rather close to the corresponding one of PCT, consequently AA is a major interfering species in the determination of PCT. This problem can be alleviated by the functionalization of the electrode surface, and different modifiers and electrochemical pretreatments of electrodic surfaces have been reported in the literature for PCT analysis [2,12].

Electrochemical cells with multiple electrode configurations can be devised with the attempt to enhance the selectivity of analytical determinations. For instance, we have already shown that by using a flow-through cell, i.e., the solution reaches firstly an upstream electrode, where the interfering species is completely depleted, and then a downstream electrode, where the target substance is detected, glucose and ascorbic acid can be determined at certain experimental conditions [13]. Closed-spaced electrodes in thin layer electrochemical cells, where one of the electrodes is used as a prereactor to remove the interfering species, have also been reported in the literature [14]. A similar approach involves the use of a microelectrode and a larger counter electrode arranged face to face at a distance that can be precisely adjusted by means of a positioning mechanical system, such as in Scanning Electrochemical Microscopy [15]. This approach has already been reported in the selective determination of hydrogen peroxide, produced in an enzymatic reaction between glucose and glucose oxidase, after complete removal of ascorbate [16].

The aim of the present work was the development of a rapid and sensitive method for the determination of PCT in the presence of AA by SECM. This was accomplished by complete electrolysis of AA in the diffusion layer of a platinum surface, hence PCT was detected at this interference-free microenvironment using a SECM tip. The influence of some experimental parameters on

the extent of the AA removal, such as the distance between the SECM tip and the substrate and electrolysis time were examined. The analytical usefulness of the proposed electrochemical methodology was demonstrated by determining PCT in a pharmaceutical formulation containing both PCT and AA.

2 Experimental

2.1 Chemicals

All the solid reagents were of analytical grade and were used without further purification. Acetic acid, sodium acetate, potassium ferrocyanide, potassium chloride, ascorbic acid, and paracetamol were obtained from Merck (Darmstadt, Germany). The supporting electrolyte used in all experiments was a 0.1 mol L^{-1} acetate buffer solution with pH 4.1.

2.2 Apparatus and Methods

SECM experiments were performed using a Sensolytics SECM (Sensolytics, Bochum, Germany) instrument with High-Res option. The SECM tip was a Pt disk-shaped microelectrode with 20 μm diameter ($RG = rg/a$ was approximately 10, where rg is the radius of the microelectrode along with the surrounding insulator, and a is the radius of the disk-shaped microelectrode), which was fabricated using a P-97 Flaming/Brown Micropipette Puller (Sutter Instrument Company, USA). The tip was approached to the platinum surface while monitoring the reduction current in a 15 mmol L^{-1} potassium ferrocyanide + 0.1 mol L^{-1} KCl solution until a positive feedback response was observed, as described in the literature [17]. Unless otherwise stated, measurements were carried out at a fixed z-position at an initial distance of 20 μm between the SECM tip and a platinum surface, which was used as substrate.

The amperometric experiments were performed by connecting the electrochemical cell to an Autolab PGSTAT 30 (Eco Chemie) bipotentiostat with data acquisition software made available by the manufacturer (GPES 4.8 version). The experiments were carried out in a in a home-made flow electrochemical cell using a Ag/AgCl (saturated KCl) electrode and a platinum wire as the reference and counter electrodes, respectively. The working electrodes were a platinum electrode ($d = 4$ mm) and a platinum microelectrode, as the substrate and tip, respectively.

2.3 Sample Preparation

A tablet sample of a commercial drug brand containing both AA and PCT (containing 40 mg of AA and 500 mg PCT) was ground into a fine powder. A given amount of the powder was weighed and dissolved in acetate buffer. The sample solution was transferred to a 100-mL flask and diluted with acetate buffer. Appropriate amounts of this diluted solution were transferred to the electrochemical cell for the determination of PCT. The standard additions were carried out in separate containers. Each container received the same amount of sample solution, different amounts of standard solution and their volumes were adjusted to the same final value by adding buffer solution. The prepared solutions were then added to the electrochemical cell through a set of holes in its wall, thus providing a slow laminar flow and preventing the movement of the tip. The solutions were added in the cell from the less concentrated one to the more concentrated one

3 Results and Discussion

3.1 SECM Studies

High selectivity is usually required to distinguish the desired signal from electrochemical interferences, as in the amperometric determination of paracetamol in the presence of ascorbate in pharmaceutical products. This can be achieved by using SECM with the gap between the substrate electrode and the tip microelectrode being designed to be very close to restrain the solution diffusing from the outside boundary layer into the inside layer. By applying a suitable potential on the substrate, the concentration of the oxidation-favored species present in the sample is significantly reduced and the signal at the tip is almost entirely due to the electron-transfer process involving the target analyte.

A preliminary experiment was carried out in a supporting electrolyte solution containing only AA. Approaching curves obtained with potassium ferrocyanide as a redox mediator were used to position the platinum microelectrode (SECM tip) at a fixed height (20 μm from the platinum substrate). After positioning the microelectrode, the flow electrochemical cell was washed with distilled water and filled with a 1 mmol L^{-1} AA + acetate buffer solution. The cell washing was done using a very low flow rate, therefore the SECM tip was not disturbed during this step. Then, experiments were carried out to achieve the optimal potential applied to the platinum substrate where ascorbate would be completely depleted over the microelectrode tip-substrate gap. Accordingly, the dependence of the microelectrode current response on substrate potential was examined in the range 0.10 to 0.80 V vs. $Ag/AgCl_{(Sat.\ KCl)}$ to AA and 0.30 to 0.80 V vs. $Ag/AgCl_{(Sat.\ KCl)}$ to PCT. In these experiments, the SECM tip was polarized at 0.80 V vs. $Ag/AgCl_{(Sat.\ KCl)}$ for monitoring ascorbate concentration changes.

Figure 1 a shows significant changes at the SECM tip with varying the substrate potential. A sharp current decrease was noticed when the potential applied to the substrate was 0.30 V, with a less significant change being observed at around 0.50 V. The current monitored at the microelectrode remained constant and almost zero for potentials more positive than 0.60 V. The decrease in the current monitored at the SECM tip was a consequence of the AA oxidation at the platinum substrate.

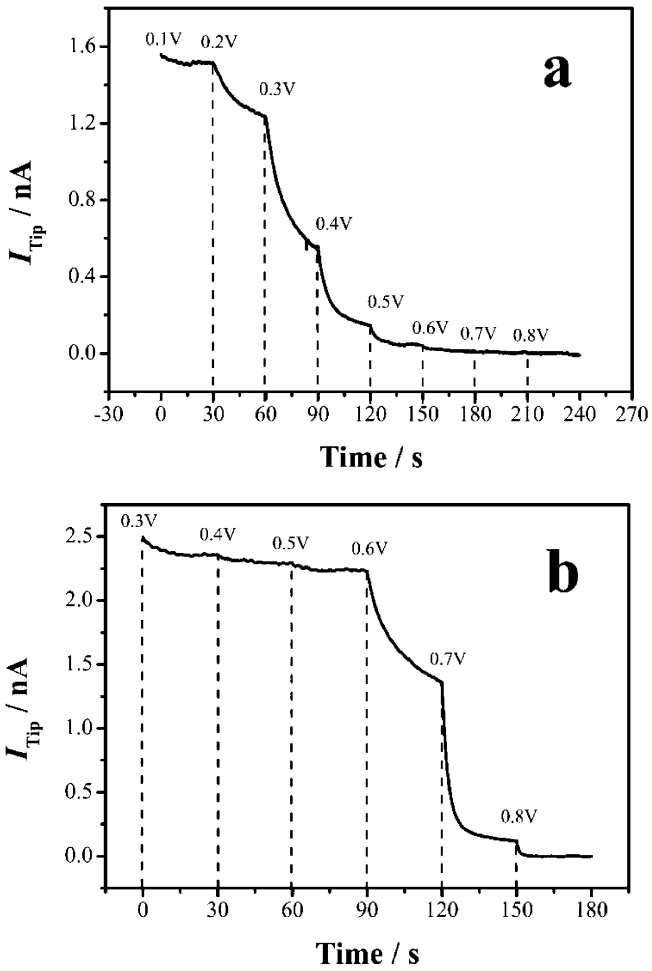

Fig. 1. Tip current dependence on substrate potential. (a) 1 mmol L^{-1} AA + 0.1 mol L^{-1} acetate buffer solution; (b) 1 mmol L^{-1} PCT + 0.1 mol L^{-1} acetate buffer solution. E_{Tip} = 0.80 V. Tip-substrate distance = 20 μm.

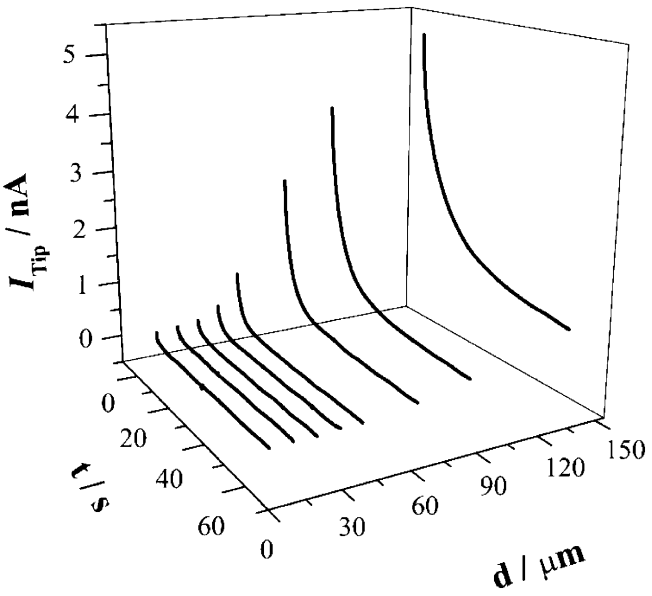

Fig. 2. Tip current dependence on tip-substrate distance and electrolysis time of AA at the substrate (E = 0.50 V) for experiments performed in a 1 mmol L^{-1} AA + 0.1 mol L^{-1} acetate buffer solution. E_{Tip} = 0.80 V.

The experiments were repeated in the supporting electrolyte solution containing only 1 mmol L^{-1} PCT and, i.e., the potential was varied at the substrate and remained fixed at the tip at a condition where the oxidation of PCT was mass-transport controlled (0.80 V). Similar trends were noticed, as shown in Figure 1b. However, in this case changes in the current at the tip started to occur only when the substrate potential was set at 0.60 V, less significant changes being observed at around 0.80 V. This is attributable to the anodic process of PCT being more difficult to take place at the substrate, in comparison with AA.

The slight difference in the oxidation potential of AA and PCT was used to selectively monitor PCT at the SECM tip after complete electrolysis of AA in the gap between tip and substrate. The potential chosen for the removal of the interfering species was 0.50 V, a condition where AA is oxidized at a significant rate at the substrate, whereas PCT is not electroactive.

In order to investigate the effect of the electrolysis time and the tip-substrate distance on the current detected at the SECM tip, experiments were performed in a 1 mmol L^{-1} AA + 0.1 mol L^{-1} acetate buffer solution with the SECM tip polarized at 0.80 V. Figure 2 shows a plot of dependence of current monitored at the microelectrode on time at different tip-substrate distances.

As seen on Figure 2, the tip current decreased to zero in a very short time window when experiments were performed at tip-substrate distances smaller than 50 μm. The decrease in faradaic current at the SECM tip is a consequence of the fast depletion of AA at the vicinity of the microelectrode surface, i.e., the system behaves as a thin-layer electrochemical cell. At larger tip-substrate distances, the influence of time on current is noticed, because of the formation of a growing diffusion layer in the solution gap between the substrate and the microelectrode. Accordingly, all further experiments were performed by sampling the current at the tip at 40 s, were a steady state zero current for AA oxidation was observed for all tip-substrate distances smaller than 50 μm.

The influence of the tip-substrate distance on the current measured at the tip was systematically investigated. The experiments were performed in a 1 mmol L^{-1} AA + 0.1 mol L^{-1} acetate buffer solution with the SECM tip polarized at 0.80 V. Figure 3 shows a plot of current monitored at the microelectrode at different tip-substrate distances. Results were obtained at two different experimental conditions, i.e., substrate nonpolarized (open circuit) and polarized at 0.50 V. When experiments were carried out with the unbiased substrate, a typical SECM approaching curve was obtained, lower current values being recorded as the substrate-tip distance decreases, because the AA diffusion to the tip surface is inhibited and the blocking effect of the substrate takes place. A different

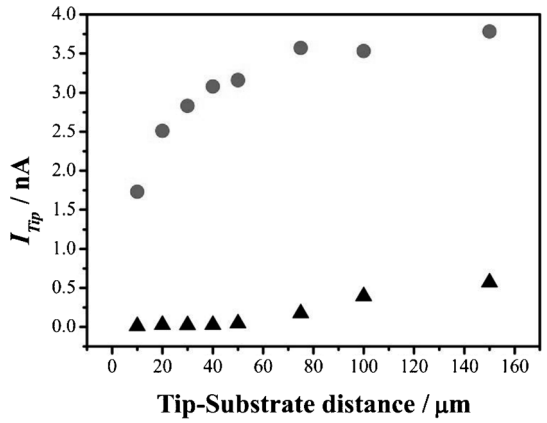

Fig. 3. Influence of tip-substrate distance on tip current for experiments performed with the substrate in two different conditions: biased at 0.50 V (▲) and unbiased (●). Electrolysis time = 40 s. $E_{Tip} = 0.80$ V.

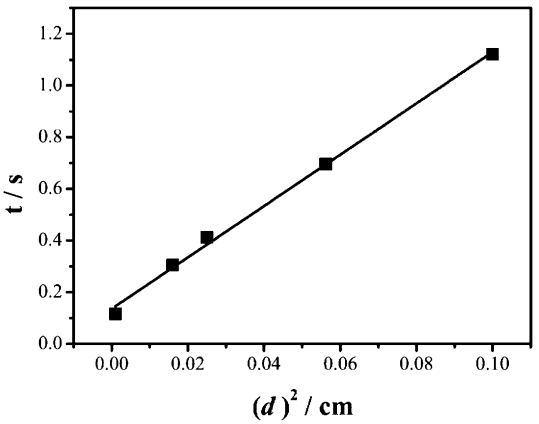

Fig. 4. Transit time as a function of the tip-substrate distance in experiments carried out in a 1 mmol L^{-1} AA + 0.1 mol L^{-1} acetate buffer solution.

profile was obtained when the substrate was biased at 0.50 V. In this case, current values at the tip were significantly lower and the influence of tip-substrate distance was less pronounced. These results indicate that AA is almost entirely oxidized at the layer confined between the tip and the substrate, hence the efficiency of the interference-removing step is very high at a relatively large tip-substrate distance. Taking these results into account, further experiments were performed at a tip-substrate distance of 20 μm, a condition where AA is entirely oxidized at the tip-substrate gap in a relatively short time.

The time taken for the diffusion layer formed after substrate polarization to reach the tip (transit time) can give information on the diffusion coefficient of the diffusion species, allowing the reliability of the proposed methodology to be assessed. This was accomplished by following the current at the tip, which was held at a constant potential where ascorbic acid undergoes diffusion controlled oxidation (0.80 V). The experiment was performed in a solution containing 1 mmol L^{-1} AA + 0.1 mol L^{-1} acetate buffer and the substrate was maintained at open-circuit. Then, the substrate potential was set at a value suitable for the anodic oxidation of AA and the influence of this process was monitored at the tip. Because of the growing of the diffusion layer, a current decrease was noticed at the tip a few moments later, yielding information on the transit time value. Taking into account that the small volume comprising the tip and that substrate behaves as a thin layer electrochemical cell [18], the transit time by diffusion between the tip and substrate is

$$d = K\ (Dt_d)^{1/2} \quad (1)$$

where D is the diffusion coefficient, t_d is the transit time and K is a constant dependent on the geometry of the space through which the electroactive species diffuses. K value was determined experimentally by calibration with a probe of known diffusion coefficient (ferricyanide). Figure 4 shows a plot of the transit time as a function of the square root of the tip-substrate distance. The straight line demonstrates that equation 1 prevails. From the slope of the straight line, the diffusion coefficient was found to be $(6.28 \pm 0.03) \times 10^{-6}$ $cm^2 s^{-1}$ which compares reasonably with values reported in the literature, 6.6×10^{-6} $cm^2 s^{-1}$ [19].

3.2 Analytical Application

A solution prepared by dissolving a tablet sample in acetate buffer was placed in the electrochemical cell and the current at the tip was measured before and after standard additions of known amounts of a PCT solution using the proposed protocol. Figure 5 shows the analytical curve and the value for PCT concentration in the sample was found to be 450 mg per tablet ($n = 3$), which is in good agreement with the value on the label, 500 mg per tablet.

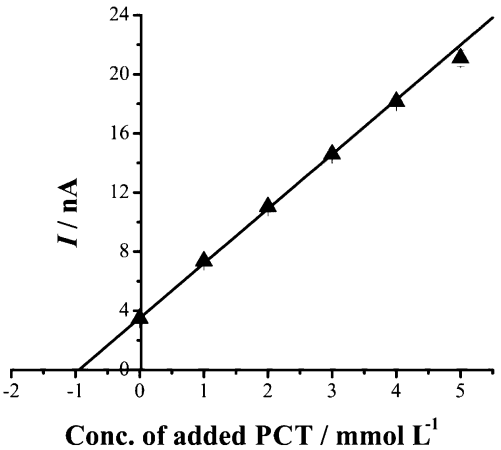

Fig. 5. Analytical curve obtained by standard addition of known amounts of PCT to a sample solution. Substrate potential: 0.50 V. $E_{Tip} = 0.80$ V.

4 Conclusions

General methods used to eliminate the influence of interfering species include removal by using appropriate membranes or modification of the electrode surface with redox mediators to gain selectivity. In the present paper we reported our efforts on the development of a new tool and such an electrochemical depletion approach provides a reproducible and easily controllable method to improve the practical performance of electrochemical sensors. The determination of paracetamol in the presence of ascorbate was carried out to demonstrate the usefulness of the proposed method, as a model system, but we envisage the use of the device in much more complex matrices like biological (cell culture, fluids, tissues), wastewaters and environmental samples, in which the redox potential of possible electroactive interfering species is close to the potential of the target analyte. In such chemical systems the choice of the substrate potential is critical, because an important requirement is the quantitative electrolysis of the interfering species. This would demand a potential where a fraction of the target analyte is consumed, but, in this case, the analytical determination would still be feasible, even though with less sensitivity owing to the partial consumption of the analyte.

In conclusion, we tried to report and characterize systematically a new concept, and its analytical applicability. Further efforts will be directed towards the fabrication of a screen-printed microelectrode located at an optimized position in the diffusion layer of a substrate electrode, making this approach promising for field analytical applications. To this end, the theoretical simulation of the electrolytic consumption of the interfering species at the vicinity of the substrate is required for the assessment of the tip-substrate separation in a condition where the analyte can be selectively detected in as close to real time as possible.

Acknowledgements

FAPESP, *CNPq*, *CAPES* for financial support and Prof. Dr. *Tiago L. Ferreira* for the helpful discussions.

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