

## Caffeine Determination at a Carbon Fiber Ultramicroelectrodes by Fast-Scan Cyclic Voltammetry

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A possibilidade de determinação eletroanalítica de cafeína com ultramicroeletrodo de fibra de carbono (CF-UME), utilizando voltametria de varredura rápida, foi avaliada. Os CF-UMEs foram submetidos a pré-tratamentos eletroquímicos. Parâmetros como o número de ciclos para cálculo da média, velocidade de varredura, intervalo de potencial e o tipo de pré-tratamento superficial foram otimizados. Sob condições otimizadas, foi possível alcançar intervalo linear de resposta entre 10,0 e 200  $\mu\text{mol L}^{-1}$ , obtendo limites de detecção de 3,33  $\mu\text{mol L}^{-1}$ . O método foi aplicado à determinação de cafeína em amostras comerciais de fármacos, com erros entre 1,0 e 3,5% em relação ao valor do rótulo e valores de adição e recuperação de 97-114%.

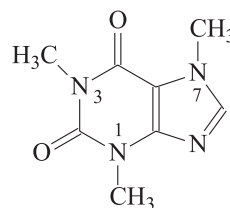
Caffeine determination using a fast-scan voltammetric procedure at a carbon fiber ultramicroelectrode (CF-UME) is described. The CF-UME was submitted to electrochemical pretreatment. Parameters such as number of acquisition cycles, scan rate, potential window, and the electrochemical surface pretreatment were optimized. Using the optimized conditions, it was possible to achieve a LDR from 10.0 up to 200  $\mu\text{mol L}^{-1}$ , with a LOD of 3.33  $\mu\text{mol L}^{-1}$ . The method has been applied in the determination of caffeine in commercial samples, with errors of 1.0-3.5% in relation to the label values and recoveries of 97-114% within the linear range.

**Keywords:** caffeine, carbon fiber ultramicroelectrode, fast scan voltammetric determinations

### Introduction

Caffeine (1,3,7-trimethylxanthine, Figure 1) is a methylxanthine derivative that is widely ingested by drinking coffee, cola-beverages, pharmaceutical formulations, energetic drinks and tea, acting as a diuretic and stimulant of the central nervous and cardiovascular systems.<sup>1</sup> Caffeine is considered to be a risk factor for cardiovascular diseases and may affect behavior by causing depression and hyperactivity. All these considerations have led to an increased interest in the development of reliable methods for the evaluation and the quantification of caffeine in food, pharmaceutical products<sup>2-5</sup> and of course, biological samples.<sup>6-8</sup>

Chromatographic methods for purine determination including caffeine are summarized in a review<sup>9</sup> and, some



**Figure 1.** Caffeine structure, highlighting the positions 1, 3 and 7.

methods of the caffeine analysis were presented by de-Maria and Moreira.<sup>10</sup>

Although the advantages of chromatographic procedure in the analysis of caffeine and other purines, electrochemical procedures can represent an interesting alternative for *in vivo* or biological fluid analysis with low waste generation and lower analysis costs.

Some recent electrochemical methods and the respective analytical parameters used in caffeine analysis are summarized in a Table 1, including references.<sup>11-18</sup>

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Previously reported<sup>19-23</sup> electrochemical methods based on stripping of Cu(II) complexes and adsorptive stripping of purines from Hg<sup>24</sup> had also been presented. The performance of rough pyrolytic graphite and carbon fiber ultramicroelectrodes in amperometric determination of some purines, including caffeine, in relation to the surface activation can also be found.<sup>25,26</sup>

Unique properties of ultramicroelectrodes make them very attractive in electroanalytical measurements that require high spatial and temporal resolution.<sup>27</sup> At ultramicroelectrodes, under typical slow scan rates of voltammetry, signal-to-noise ratios (S/N) can increase up to one decade because of efficient mass transport to the electrode resulting from edge effects. In addition, because of the small dimensions and low IR drop, fast scan voltammetric procedures<sup>28-33</sup> and measurements in highly resistive media are possible at UMEs.<sup>34</sup>

According to Wightman and Wipf,<sup>31</sup> fast scan voltammetry is useful in preventing accompanying chemical reactions, understanding mechanism and kinetics of heterogeneous electron transfer, reduction in time of analysis, faster response to potential changes, minimizing in the size of devices for *in vivo* analysis, use of non-expensive instrumentation, among others.

The background current observed in fast scan voltammetry (FSV) in a blank solution is due to several processes, including double-layer charging, redox reactions of the surface functional groups, such as quinones, and redox reactions of impurities in the electrolyte solution. Thus, the magnitude of the background current may be more than two orders greater than that of the oxidation current of an analyte. Consequently, FSV requires a stable background current to produce a reliable background that can be used in background subtraction procedures. The formation of oxides<sup>35</sup> and the roughness of the electrode surface can change during a voltammetric determination,

and these changes can produce significant modifications in the background current.

In this work, highly active carbon fiber ultramicroelectrodes (CF-UME) were produced, to act as sensors in sensitive determinations of caffeine under physiological conditions. The main challenge in this work was to prepare an electrode capable of operating under fast scan rates in a relatively high anodic potential, in which caffeine is oxidized, allowing the background subtraction. The main advantages of using the CF-UME in this case are the possibility of sensor miniaturizing as well as the speed of the measurements, for future *in vivo* or biological fluid analysis of caffeine.

In this sense any comparison with arrays and/or conventional sized electrode is difficult once they are not suitable for *in vivo* determinations, although it is well known that square-wave voltammetry in conventional sized carbon electrodes led to  $\mu\text{mol L}^{-1}$  limit of detection level, despite strong adsorption is noted in purine analysis.<sup>25</sup> On the other hand UME-arrays permit to reach lower limits of detection, but are not always suitable for *in vivo* analysis.

## Experimental

### Reagents and solutions

All chemicals were of analytical reagent grade. Monobasic sodium phosphate monohydrate (Mallinckrodt), anhydrous dibasic sodium phosphate (Mallinckrodt), potassium chloride (Mallinckrodt), potassium ferricyanide (Merck) and caffeine (Sigma) were used as received.

Stock caffeine solutions in the desired concentrations were prepared daily just before use in 70.0 mmol L<sup>-1</sup> phosphate buffer (pH 7.4). Potassium ferricyanide  $5.0 \times 10^{-3}$  mol L<sup>-1</sup> was prepared in KCl 0.50 mol L<sup>-1</sup>. All the determinations were performed at room temperature.

**Table 1.** Some electrochemical methods and their respective parameters

Authors	Electrode	Method	Electrolyte	Linear range / (mol L <sup>-1</sup> )	LOD / (mol L <sup>-1</sup> )
Aklilu <i>et al.</i> <sup>14</sup>	MCPE <sup>a</sup>	SWV	Phosphate buffer, pH 6	0 to $0.5 \times 10^{-3}$	$0.3 \times 10^{-6}$
Alizadeh <i>et al.</i> <sup>16</sup>	MIP-CP <sup>b</sup>	DPV	Phosphate buffer, pH 7	$6.0 \times 10^{-8}$ to $2.6 \times 10^{-5}$	$1.5 \times 10^{-8}$
Lourenção <i>et al.</i> <sup>11</sup>	BDD <sup>c</sup>	DPV	Acetate buffer, pH 4.5	$3.0 \times 10^{-7}$ to $9.1 \times 10^{-5}$	$1.4 \times 10^{-7}$
Lourenção <i>et al.</i> <sup>12</sup>	BDD-cp <sup>d</sup>	DPV	0.1 mol L <sup>-1</sup> H <sub>2</sub> SO <sub>4</sub>	$9.7 \times 10^{-6}$ to $1.1 \times 10^{-4}$	$7.0 \times 10^{-6}$
Ly <i>et al.</i> <sup>18</sup>	GPE <sup>e</sup>	SWV	Phosphate buffer, pH 9	0 to $2.6 \times 10^{-3}$	$4.7 \times 10^{-5}$
Martínez-Huitle <i>et al.</i> <sup>17</sup>	N-BBD <sup>f</sup>	DPV	Phosphate buffer, pH 7	$2.0 \times 10^{-7}$ to $1.2 \times 10^{-5}$	$1.0 \times 10^{-7}$
Sanghavi and Srivastava <sup>15</sup>	MCNT-PE <sup>g</sup>	AdsDPV	Phosphate buffer, pH 7	$2.3 \times 10^{-7}$ to $6.6 \times 10^{-5}$	$8.8 \times 10^{-8}$
Yang <i>et al.</i> <sup>13</sup>	N-MWNTs <sup>h</sup>	DPV	0.01 mol L <sup>-1</sup> H <sub>2</sub> SO <sub>4</sub>	$6.0 \times 10^{-7}$ to $4.0 \times 10^{-4}$	$2.3 \times 10^{-7}$

<sup>a</sup>MCPE: 1,4-benzoquinone modified carbon paste electrode; <sup>b</sup>MIP-CP: molecularly imprinted polymer carbon paste electrode; <sup>c</sup>BDD: boron-doped diamond electrode; <sup>d</sup>BDD-cp: boron-doped diamond cathodically pre-treatment electrode; <sup>e</sup>GPE: graphite pencil electrode; <sup>f</sup>N-BDD: nafion-modified boron-doped diamond electrode; <sup>g</sup>MCNT-PE: *in situ* surfactant-modified carbon nanotube paste electrode; <sup>h</sup>N-MWNTs: nafion/multi-wall nanotubes composite film-modified electrode.

## Electrodes

A saturated calomel electrode (SCE) was used as a reference electrode, and a carbon fiber (7  $\mu\text{m}$  diameter; CTA, Brazil) was used as the working electrode.

Fabrication of carbon fiber ultramicroelectrodes (CF-UME) followed a previously described procedure.<sup>36</sup> Briefly, carbon fiber was first connected to a copper wire with silver epoxy (EPO-TEK 410E; Epoxy technology, USA). After the silver epoxy was cured for 24 h, the carbon fiber with the copper wire set was sealed in a micropipette tip with a polyurethane resin (Poliquil, Brazil). The CF-UME was left overnight at room temperature. After curing, the tip of the electrode was sanded off in a polishing wheel (Arotec, Brazil) using 600-grit silicon carbide paper (Arotec, Brazil), and finally the surface was gently polished in the polishing wheel with 0.1  $\mu\text{m}$  particle size  $\gamma$ -alumina suspension (Arotec, Brazil). Before use, the polished electrodes were sonicated<sup>37,38</sup> in isopropyl alcohol and in doubly distilled water during 5 min in each solvent. The response of the polished electrodes was tested by cyclic voltammetry with 5.0  $\text{mmol L}^{-1}$  potassium ferricyanide in 0.5  $\text{mol L}^{-1}$  KCl solution at 50  $\text{mV s}^{-1}$ .

## Electrochemical pretreatment of the CF-UME

In this work two electrochemical pretreatments were used and selected on the basis of the results in the FSV procedure. One was an adaptation of a mild procedure proposed by Hernández and co-workers.<sup>36,39</sup>

Procedure adapted from Hernandez and co-workers<sup>36,39</sup> proposal (H)

In this case the CF-UME was submitted to 120 cycles of potential between 0.0 and +1.65V (*vs.* SCE) at 200  $\text{mV s}^{-1}$  in 70  $\text{mmol L}^{-1}$  phosphate buffer pH 7.4.

Procedure adapted from the Brajter-Toth and co-workers<sup>34</sup> proposal (BT)

In this case the CF-UME was submitted to 4000 cycles of potential between -1.0 and +1.65 V or other desired positive potential (*vs.* SCE) at 10  $\text{V s}^{-1}$  in 70  $\text{mmol L}^{-1}$  phosphate buffer pH 7.4.

## Instrumental

The instrumental for FSV was a Potentiostat/Galvanostat PGSTAT30 from AUTOLAB (Eco Chemie) equipped with a Scan-Gen and an ADC-750 modulus for high scan and low current acquisition respectively. A two-electrode configuration cell was used inside a homemade Faraday

cage during the FSV measurements in order to minimize the environmental noise. It was not used any kind of electronic filter in all measurements.

For caffeine determinations, a chosen number of scans were recorded under a set of fixed experimental conditions such as scan rate, potential window and number of acquisition scans. The measured currents were averaged and stored.

Before each measurement, background currents were recorded in the supporting electrolyte without analyte, under exactly the same experimental conditions used in the analytical determinations of caffeine. These currents were stored, averaged and used latter for digital background subtraction from the caffeine voltammograms with the help of a personal computer.

The equipment used presents a certain compromise between the number of cycles and the scan rate. In the present case, if one acquire 190 cycles the maximum scan rate admitted is limited to 100  $\text{V s}^{-1}$  in the potential window -1.0 to +1.65 V, due to the number of points to be stored in the buffer memory and the response time.

## Comparative chromatographic method

Chromatographic determinations were performed in a Shimadzu LC-10AD UP equipped with a SPD-M10A UP UV detector. The chromatographic conditions were C-18 column (5 m, 6  $\times$  250 mm), detection wavelength 275 nm, water:methanol:acetic acid (69:28:3) mobile phase, flowing at 1.0  $\text{mL min}^{-1}$  in room temperature.

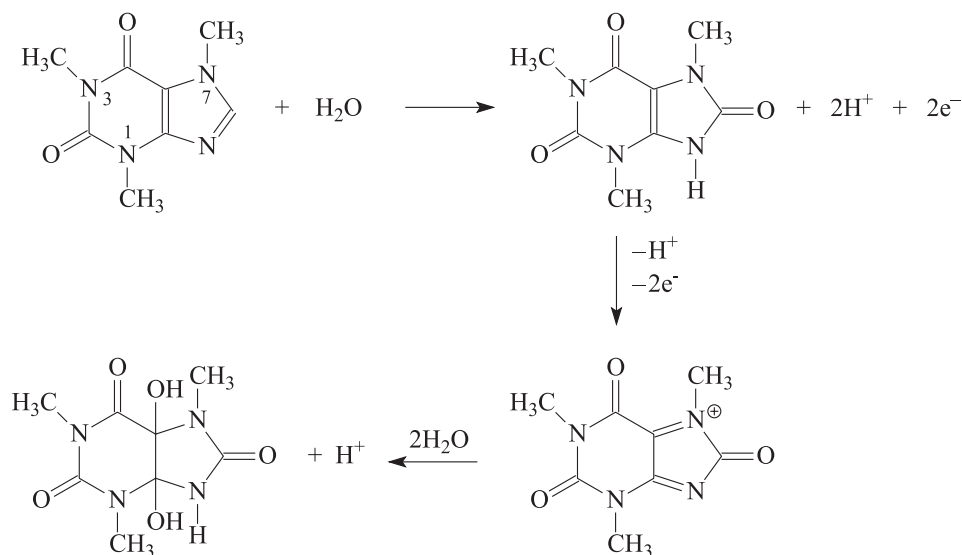
## Results and Discussion

According to Hansen and Dryhurst<sup>40</sup> and Spătaru,<sup>41</sup> in cyclic voltammetry, caffeine presents a single oxidation peak at approximately +1.5 V (*vs.* SCE), due to an oxidation mechanism similar to xanthine, however without formation of uric acid since the nitrogen atoms at position 3 and 7 (Figure 1) are substituted. The oxidation occurs via a 4 electrons process resulting in a substituted uric acid (IV) that is not electroactive. The mechanism proposed by the authors is represented in the reaction Scheme 1.

In order to establish a procedure for the determination of caffeine using a CF-UME at fast scan rate, several experimental parameters were optimized, as described below.

## Evaluation of the pretreatment procedures performance

The electrochemical pretreatment of the CF-UME surface was necessary in order to reach a stable and



**Scheme 1.** Mechanism of caffeine oxidation, proposed by Hansen and Dryhurst,<sup>40</sup> and Spătaru.<sup>41</sup>

reproducible response. This is the basis of the background subtraction procedure.<sup>42</sup>

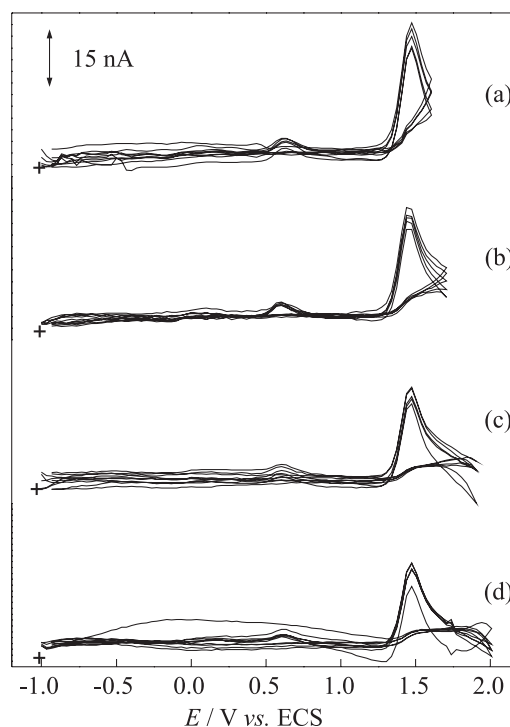
According to McCreery and Cline<sup>43</sup> the electrochemical pretreatments are the easiest to perform in the CF-UME surface. However, although there are several different kinds of activation/stabilization procedures proposed in the literature, there is no hitherto general procedure to be used and a specific treatment should be used/optimized in each case.

The main challenge here is the relatively high anodic potential in which the caffeine oxidation peak is observed, in which the electrode must be stable and present a reproducible surface that allows one to have trustable background subtraction.

For instance two pretreatments were chosen to evaluate what was the best for this specific case. The best results were obtained with the BT procedure, which gave most reproducible results after the background subtraction. This is in agreement with previous results obtained for other purines with this procedure.<sup>27</sup>

#### Evaluation of the best potential window for the electrochemical pretreatment and measurements

In relation to the original BT procedure, only the positive extreme potential was changed and evaluated since caffeine oxidizes irreversibly at potentials > +1.4 V (*vs.* SCE), used as the upper limiting potential in the original procedure. Figure 2 presents the background subtracted voltammograms averaged after 190 cycles for 500 μmol L<sup>-1</sup> caffeine at CF-UMEs electrochemically pretreated by the BT procedure, using different anodic limiting potentials.



**Figure 2.** Effect of the potential window used in the pre-treatment of the CF-UME in the background subtracted voltammograms of 500 μmol L<sup>-1</sup> caffeine in phosphate buffer pH 7.4, using scan rate  $\nu = 100 \text{ V s}^{-1}$  and average of 190 cycles: (a) -1.0 to +1.60 V, (b) -1.0 to +1.7 V, (c) -1.0 to +1.9 V, and (d) -1.0 to +2.0 V.

Considering that at +1.5 V (*vs.* SCE) the peak is not complete, the potential window was extended up +1.6, +1.9 and +2.0 V (*vs.* SCE), resulting in a complete caffeine oxidation peak. However there is a limit for this extension since at +1.9 and +2.0 V (*vs.* SCE) as upper limits, the response becomes less reproducible compromising the background subtraction.

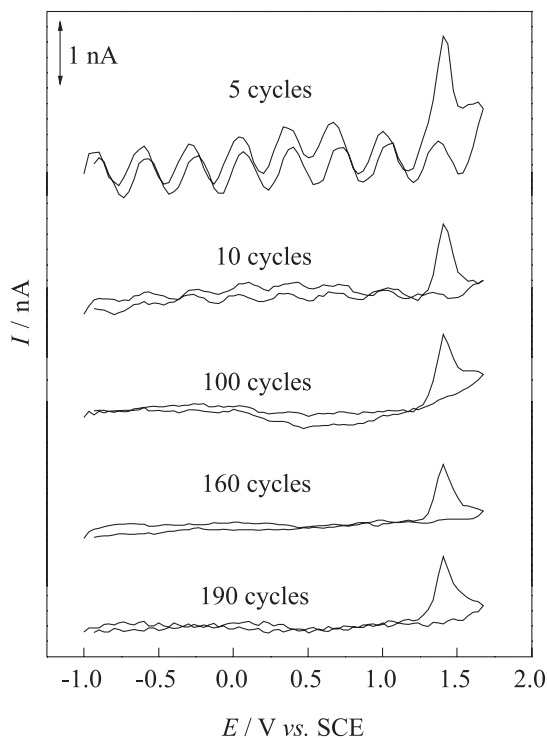
This could be related to an additional treatment of the electrode surface when submitted to higher limiting potentials during the measurements at  $E > +1.7$  V (vs. SCE). The continuous surface change causes a non stable response as previously reported.<sup>35</sup>

Thus a  $-1.0$  to  $+1.65$  V (vs. SCE) potential window was chosen for further studies. Under this potential window, a well defined response could be found, without affecting the background subtraction.

#### Number of acquisition cycles and scan rate optimization

The number of cycles is an important feature in the background subtraction procedure, since few cycles result in highly noisy voltammogram while a larger number of cycles makes smaller the analytical frequency. Figure 3 presents how the number of cycles influences the peak signal for caffeine determination ( $50 \mu\text{mol L}^{-1}$ ) at a CF-UME pretreated by the BT procedure at  $100 \text{ V s}^{-1}$ , in the  $-1.0$  to  $+1.65$  V (vs. SCE) potential window.

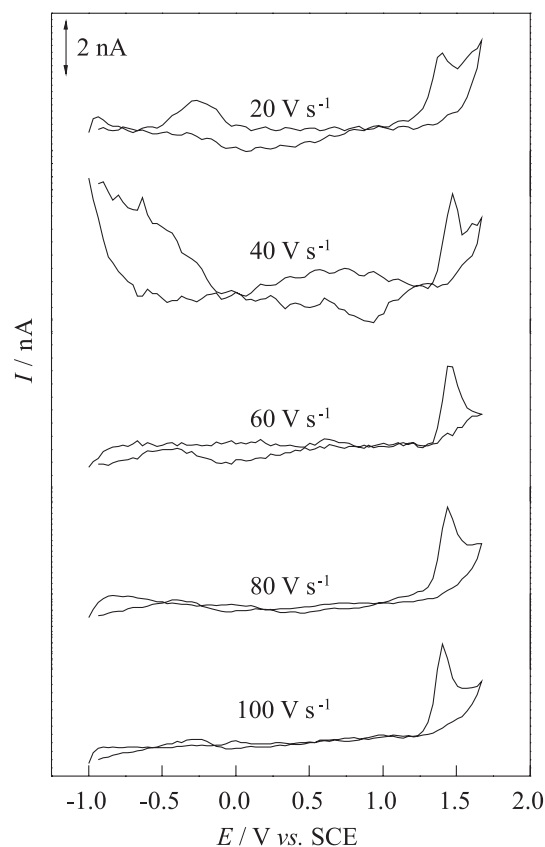
This test led us to conclude that 190 cycles is enough to have a well defined signal with a relatively high sensitivity in relation to the other number of cycles investigated. It is important to point out that at the configuration available in the equipment used in this work, 190 cycles represent the upper limit of measurements that can be stored.



**Figure 3.** Effect of the number of acquisition cycles for the better signal averaging. Caffeine concentration of  $50 \mu\text{mol L}^{-1}$  in phosphate buffer pH 7.4,  $\nu = 100 \text{ V s}^{-1}$ , potential window of  $-1.0$  to  $+1.65$  V.

These results are in agreement with the discussion of Hsueh *et al.*,<sup>37</sup> showing that the definition of the number of acquisition cycles for the average is fundamental in obtaining good results using the background subtraction procedure.

The effect of the scan rate in the background-subtracted voltammograms of caffeine is presented in Figure 4. It is possible to observe that as higher the scan rate better definition of the voltammograms can be achieved. According to some authors the higher scan rates prevents the fouling of the electrode surface. However, as in the optimization of the number of cycles,  $100 \text{ V s}^{-1}$  is the upper limit for the instrument used in this work under the configuration used and the described conditions.



**Figure 4.** Effect of scan rate on the voltammograms of  $50 \mu\text{mol L}^{-1}$  caffeine in phosphate buffer pH 7.4, potential window of  $-1.0$  to  $+1.65$  V.

#### Caffeine's analytical curve using FSV and the optimized parameters

Once established the experimental and instrumental parameters for data acquisition, within the instrumental limitations (Table 2), an analytical curve was obtained for caffeine between  $10$  and  $200 \mu\text{mol L}^{-1}$ , which was linear in the concentration range evaluated, obeying the equation 1:

$$I = -0.105 \times 10^{-9} A + 94.86 \times 10^{-3} A \text{ mol}^{-1} L \quad (1)$$

(n = 6, R = 0.9981)

with a limit of detection of  $3.33 \mu\text{mol L}^{-1}$ , considering the equation 2

$$\text{LOD} = 3 \text{ sd blank/slope} \quad (2)$$

in which sd is the standard deviation of the blank signal and slope is the angular coefficient of the analytical curve, according to Miller and Miller.<sup>44</sup>

**Table 2.** Optimized set of experimental parameters for the determination of caffeine using the pre-treated CF-UME in a fast scan cyclic voltammetry procedure

Parameters	Studied range	Optimum value
Pre treatment	H and BT	BT
Anodic potential limit / V	1.6-2.0	+ 1.65
Number of the cycles	5-190	190
Scan rate / (V s <sup>-1</sup> )	20-100	100

#### Commercial samples analysis: standard addition procedure

These studies were performed to verify the possibility of using CF-UME for caffeine determination. As probe samples, pharmaceutical commercial tablets were proposed. According to the Brazilian Pharmacopoea<sup>45</sup> twenty tablets of each sample were grinded and a selected amount equivalent to one tablet (average tablet mass) was dissolved and filtered in order to eliminate insoluble excipients.

Initially a recovery test was performed with one of the samples, Cafiaspirina<sup>®</sup> (acetylsalicylic acid 650 mg + caffeine 65 mg), in order to evaluate the performance of the developed procedure in the probe samples. The results are presented in Table 3. For each addition of caffeine five determinations were taken.

Recoveries between 97 and 114% were obtained. Considering the concentration level of caffeine in the sample and that the analysis was made directly in the matrix, without need of sample treatment, the results could be considered satisfactory and appropriate for the sample analysis.

**Table 3.** Results obtained for standard addition

Sample	Caffeine <i>per</i> tablet / mg			E <sub>1</sub>   <sup>c</sup> / %	E <sub>2</sub>   <sup>d</sup> / %
	Label	CF-UME <sup>a</sup>	HPLC <sup>b</sup>		
Doril <sup>®</sup>	30	29.65 ± 0.01	29.68 ± 0.02	1.17	0.10
Dorflex <sup>®</sup>	50	50.72 ± 0.01	50.41 ± 0.06	1.44	0.61
Cafiaspirina <sup>®</sup>	65	62.7 ± 0.4	63.2 ± 0.4	3.54	0.79

<sup>a</sup>n = 4; <sup>b</sup>n = 3; <sup>c</sup>E<sub>1</sub> = [(CF-UME - label)/label] × 100; <sup>d</sup>E<sub>2</sub> = [(CF-UME - HPLC)/HPLC] × 100.

**Table 3.** Results for recovery experiments for Cafiaspirina<sup>®</sup> sample

Additions	[Caf] / (μmol L <sup>-1</sup> )		Recovery <sup>a</sup> / %
	Add	Found	
1	12.47	14 ± 2	110 ± 14
2	24.87	28 ± 5	114 ± 21
3	37.22	36 ± 3	97 ± 9
4	61.97	62 ± 6	99 ± 10

<sup>a</sup>n = 5.

Since Ohmic drop has not been compensated in the experiments, one should expect that differences could be observed when analyzing standard solutions and real samples. However, as in present work the standard addition approach and a 70.0 mmol L<sup>-1</sup> phosphate buffer supporting electrolyte were used in all the determinations, this effect was probably minimized.

Thus, three pharmaceutical samples were analyzed using both the proposed fast scan voltammetric procedure at the CF-UME and a comparative chromatographic procedure. The results are presented in Table 4.

The results of standard addition suggested that the proposed procedure was appropriated for caffeine determination in commercial pharmaceutical samples with relative errors lower than 3.54 and 0.79% (in modulus) when compared with manufacturer's claimed values and the reference HPLC procedure respectively.

According to the *t*-Student test the results from the CF-UME voltammetric method and those from the chromatographic procedure agreed in the 95% confidence level for Doril<sup>®</sup> and Cafiaspirina<sup>®</sup>, while for Dorflex<sup>®</sup> they agreed in 99.9%.

It is also important to point out that Cafiaspirina<sup>®</sup> and Doril<sup>®</sup> samples contain acetylsalicylic acid in concentration 10 times higher than caffeine, while the Dorflex<sup>®</sup> tablet also contains dipyrone (300 mg), orphenadrine citrate (35 mg) and caffeine (50 mg).<sup>46</sup> With the results obtained, one can assert that interference from the other active principles present in the tablets in the analysis is negligible, using the standard addition approach.

As the proposal is a future use of the electrode for *in vivo* determinations possible interferences from other

ingredients present in more complex matrix should be considered in further studies. However, as caffeine oxidizes in a relatively high potential it is preserved from interferences in its own peak. Problems can be caused by adsorbed species or even oxidation/reduction products of these ingredients.

## Conclusions

The BT adapted pretreatment of the carbon fiber ultramicroelectrodes produces on CF-UME, a reproducible surface that allowed the background subtraction procedure even at higher anodic potential, which is sensitive in caffeine determinations under physiological conditions. High sensitivity of the new method of caffeine determinations that is shown here may facilitate the use of CF-UMEs with FSV technique, in the determination of caffeine in biological samples and point to the potentiality for *in vivo* analysis in the future. Although similar limits of detection have been found with other voltammetric techniques, it should be pointed out that using the CF-UME in this case allow sensor miniaturizing and faster measurements, without interference from other pharmaceuticals.

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