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Article

Simultaneous Voltammetric Determination of Acetaminophen and Caffeine at a Graphite and Polyurethane Screen-Printed Composite Electrode

Thalita R. Saciloto, Priscila Cervini, Éder T. G. Cavalheiro*

Departamento de Química e Física Molecular, Instituto de Química de São Carlos, Universidade de São Paulo, CP 676, 13560-970 São Carlos-SP, Brazil

Um seletivo método eletroquímico foi desenvolvido para determinação individual ou simultânea de paracetamol e cafeína em tampão fosfato pH 6,0, utilizando um eletrodo compósito impresso descartável à base de grafite e poliuretana (EIGPU), em Voltametria de Pulso Diferencial (DPV). Os picos de oxidação do paracetamol e cafeína aparecem em 0,3 V e 1,3 V (*vs.* pseudo-Ag/AgCl), respectivamente, mostrando a possibilidade de determinação simultânea de ambos analitos utilizando o EIGPU, além da determinação individual dos mesmos. A curva analítica da determinação simultânea mostrou uma resposta linear para as duas substâncias. O paracetamol apresentou uma região linear entre 1,00 - 40,0 μmol L^{-1} com limite de detecção de 0,84 μmol L^{-1} e a região linear da cafeína foi entre 4,00 - 200 μmol L^{-1}, com limite de detecção de 1,6 μmol L^{-1}. O método proposto foi aplicado na determinação simultânea de paracetamol e cafeína em três formulações farmacêuticas, com resultados que concordaram com HPLC ao nível de confiança de 95% (Teste *t*-Student).

A selective electrochemical method was developed for the individual or simultaneous determination of acetaminophen and caffeine in phosphate buffer pH 6.0 on graphite and polyurethane screen-printed composite electrode (EIGPU) using Differential Pulse Voltammetry (DPV). The oxidation peaks of acetaminophen and the caffeine appeared at 0.3 V and 1.3 V (*vs.* pseudo-Ag/AgCl), respectively, showing the possibility of simultaneous determination of both analytes, at the EIGPU, besides the individual determination. Analytical curves for the simultaneous determination showed a linear response for both compounds. The acetaminophen presented a linear region in the concentration range 1.00 - 40.0 μmol L^{-1} with detection limit of 0.84 μmol L^{-1}, and the caffeine presented a linear region, in the concentration range 4.00 - 200 μmol L^{-1} with detection limit of 1.6 μmol L^{-1}. The proposed method was applied in the simultaneous determination of acetaminophen and caffeine in three pharmaceutical formulations, with results similar to those obtained using a HPLC method, at 95% confidence level (Student *t*-Test).

Keywords: acetaminophen, screen printed electrode, graphite-polyurethane composite

Introduction

Screen-printed electrodes (SPEs), which are used as economical electrochemical substrates, have gone through improvements over the past few decades with respect to both their format and their printing materials. These electrodes can be easily replaced between each analysis, eliminating the need for electrode surface regeneration.[1,2]

Besides, SPEs are characterized by simplicity of use, low cost and good reproducibility of each unit, with special attention to convenience associated with this type of electrode,[3,4] sometimes leading to more interesting devices than conventional electrodes.[5,6] Thus, these interesting features have allowed their marketing as disposable electrodes.

Finally, several studies have shown that the use of SPE in electroanalysis ensures adequate sensitivity, selectivity, linearity, reproducibility and robustness for development of electroanalytical methodologies.[3,7]

Recently an extensive review of the various applications and developments of screen-printing electrodes was presented by Meng Li *et al.*[8] In that paper it is possible to note the electrochemical applications of SPEs in environmental analysis, including the determination of organic compounds, heavy metals and gas pollutants.

*e-mail: cavalheiro@iqsc.usp.br

Previously, electrochemical techniques have been implemented for the estimation of acetaminophen[9-12] and caffeine[13-16] when present individually. Moreover, other instrumental techniques are also used for the individual determination of these two substances, such as, spectrophotometry,[17-19] chemiluminescence,[20,21] colorimetry,[22] capillary electrophoresis,[23] amperometric biosensors,[24] for acetaminophen, and various methods for analyzing caffeine have been developed, including spectrophotometric[25] and chromatographic.[26-28]

The most commonly reported methods for the simultaneous determination of acetaminophen and caffeine are amperometric,[29] spectrofotometric,[30-34] chromatography,[35-42] fluorescence,[43,44] but these methods require a long time for extracting and purifying the active principles before analysis.

Although, the American Pharmacopoeia recommends liquid chromatography as the official method[45] for the quantification of acetaminophen and caffeine in pharmaceutical products, voltammetric methods can be an interesting alternative for the simultaneous determination of such pharmaceuticals requiring low cost instrumentation and generating lower amount of waste.[29,46,47]

Usually, for acetaminophen and caffeine, limits of detection (LOD) at 0.49 μmol L^{-1} and 0.035 μmol L^{-1}, respectively, were described in a simultaneous determination using a boron-doped diamond electrode,[46] and 0.0258 μmol L^{-1} and 0.083 μmol L^{-1}, respectively, using an *in situ* surfactant-modified multiwalled carbon nanotube paste electrode.[47]

Thus, the present work describes the use of a screen printed electrode based on a graphite and polyurethane composite (EIGPU), and the demonstration of its analytical potentiality as an electroanalytical sensor, in the selective and sensitive determination of acetaminophen and caffeine by Differential Pulse Voltammetry (DPV) individually as well as simultaneously.

The main advantages of using the polyurethane resin had already been presented.[48] Briefly it is hydrophobic preventing swelling during analysis in aqueous media, it is bi-component making easy to add modifiers, has a low cost, easy to prepare and made from a vegetable oil derivative, a renewable raw material source.

In fact although the vegetable oil PU and screen printed electrodes have already been used, the novelty here is the preparation of printed electrodes using the PU-graphite (GPU) composite and its application to a classical model. Thus, this new form for using the GPU composite, once it has been used before in the determination of many analytes as a conventional working electrode,[49-52] but in the present approach the composite was used to construct the printed device as well as the electrical contacts of the SPE.

Experimental

Reagents and solutions

Solutions were prepared with water purified in a Barnstead™ EasyPure® RoDi (Thermoscientific, model D13321) system with resistivity ≥ than 18 MΩ cm.

The acetaminophen used was of analytical grade reagent (Sigma-Aldrich) and the caffeine purchased in local magistral drugstore in USP grade, both used without further purification. Stock 5.00 mmol L^{-1} acetaminophen and caffeine in 0.100 mol L^{-1} phosphate buffer pH 6.0 solutions were prepared freshly and adequately diluted, separately in order to obtain the working solutions. The pharmaceutical samples were Tylenol DC® (Janssen-Cilag Farmacêutica Ltda., Brazil), Excedrin® (Novartis Biociências S.A., Brazil) e Maxidrin® (Kley Hertz S.A., Brazil), purchased in local drugstores.

Apparatus

Voltammetric experiments were performed using an AUTO-LAB PGSTAT-30 (Ecochemie, The Netherlands) potentiostat/galvanostat coupled to a personal computer and controlled with a GPES 4.9 software.

The screen printed electrode (Figure 1) based on a 60% graphite-polyurethane composite (EIGPU) was used as working electrode (Φ = 3 mm) as well as in the electrical contacts of the SPE. The electrical resistance of the working electrode was measured as 2.03 ± 0.03 kΩ as determined in a mercury pool against a platinum wire and a bench multimeter (Minipa, MDM-8045).

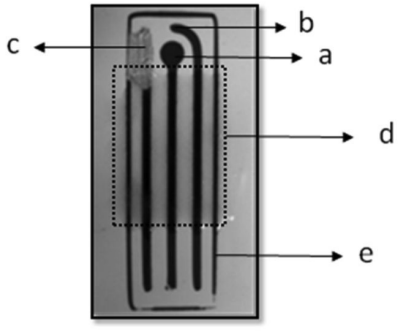

Figure 1. Design of the EIGPU with the components: working electrode (a), auxiliary electrode (b), silver glue (c), insulanting (d) and electrical contact (e).

The auxiliary electrode was also made with the 60% (graphite-PU, m/m) composite. The reference electrode

was a pseudo-Ag/AgCl, silver epoxy. All measurements were performed at room temperature.

Although the electrodes are inexpensive enough to be disposable, one single electrode was used for each set of measurements or pharmaceutical sample analysis. The repeatability of the electrode response was verified by measuring its response in an 1.3×10^{-4} mol L^{-1} acetaminophen solution in 0.100 mol L^{-1} phosphate buffer pH 6.0; successively for 10 cyclic voltammograms in the range −0.4/0.8 V (*vs.* pseudo-Ag/AgCl).[53] The reproducibility was also evaluated, using electrodes treated exactly in the same way, in a 5 mmol L^{-1} $[Fe(CN)_6^{3-}]$ in 0.5 mol L^{-1} KCl solution. The currents were 18.0 μA, 19.1 μA and 16.8 μA resulting in an average current of 17.9 μA ± 0.11, for the three electrodes.

The measurements of scanning electronic microscopy (SEM) were performed using Zeiss DSM 940-A equipment operated to 5 kV in different magnification.

Preparation of the screen printed electrodes

The EIGPU was prepared as previously described.[54] Briefly the manufacturing process of screen printed electrode consists essentially of forcing the ink, formed by a mixture of the GPU composite with the solvent to pass through a mask to be deposited on a PVC plate, 3.0 mm thick.

Finally, the set of imprints that make up the printed electrode is partly covered by a layer of pure polyurethane resin, acting as an insulation to define the area of electrical contact at one end. At the other end there was another uncoated portion to define the active area allowing the electrodes to be exposed. To one of the imprints it was attached a silver epoxy strip (Conductive Silver Epoxy Kit, Electron Microscopy Sciences, USA) to serve as a pseudo-reference electrode.

Procedures for pharmaceutical formulations analysis

According to the Brazilian Pharmacopoeia recommendations[55] twenty tablets were weighed and powdered. Accurately weighed portions of powder equivalents to 500 and 65.0 mg for acetaminophen and caffeine, respectively, for Tylenol DC®, Excedrin® and Maxidrin® according to the label, were dissolved in 100 mL of 0.100 mol L^{-1} phosphate buffer pH 6.0 to obtain solutions correspondent to 3.0×10^{-3} mol L^{-1} of acetaminophen and 7.5×10^{-3} mol L^{-1} of caffeine. The solutions were submitted to ultrasound for 15 minutes in order to complete the dissolution.

The solutions of the samples of Tylenol DC® and Excedrin® were dissolved in 10 mg of activated charcoal for 5 minutes at 40 ºC and then filtered to remove the dye present in the commercial formulation to prevent interference during the analysis. According to the recovery results it looks like the other excipients do not severely interference in the simultaneous determination of both pharmaceuticals once DPV results agreed with those from HPLC.

Comparison method

The comparison method was realized according to the American Pharmacopeia recommendations (USP XXXII).[45] Chromatographic determinations were performed in a Schimadzu LC-10AD UP HPLC system equipped with a SPD-10A UP UV detector, LC-6AD pump (610) and software Class-VP. The chromatographic conditions were C-18 (15 cm x 4.6 mm, 5 μm), detector wavelength 225 nm, acetonitrile mobile phase, flowing at 0.8 mL min^{-1}, at room temperature.

Optimization of the parameters in DPV

The electrochemical behavior of acetaminophen and caffeine on the EIGPU was studied by differential pulse voltammetry (DPV).

In the case of simultaneous voltammetric analysis of acetaminophen and caffeine, due the caffeine is present in lower concentration in the pharmaceutical formulations when compared to the concentration of acetaminophen (65:500 mg), the oxidation peak is less intense and consequently more difficult to detect. Based on this, and considering that the two analytes will be determined simultaneously, optimizations of parameters for caffeine determinations were performed first.

It is also important to note that the caffeine has a relatively high oxidation potential in carbon-based electrodes,[15] which represents significant challenge for performance testing of a new electrode type.

As already explain, for each set of measurements or pharmaceutical analysis described bellow one single electrode was used. The repeatability test revealed that the mean peak current of 10 cyclic voltammograms of a 1.3×10^{-4} mol L^{-1} acetaminophen in 0.100 mol L^{-1} phosphate buffer pH 6.0 was 2.04 ± 0.09 μA.[53]

Results and Discussion

Figure 2 presents the scanning electronic microscopy (SEM) of one working electrode in order to evaluate the recovering of the support by the graphite-PU ink under the conditions used in the electrode preparation. It is possible

to observe that the recovery of the supporting surface is homogeneous, uniform and without defects or exposure of the PVC support.

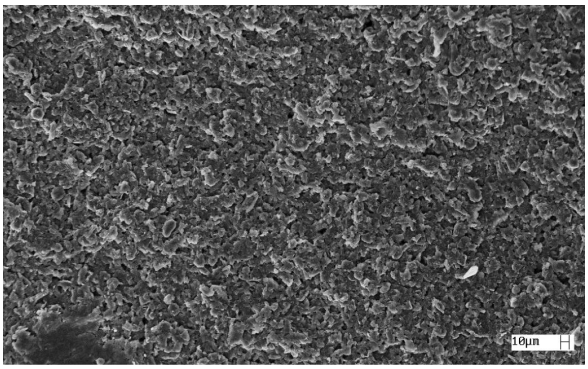

Figure 2. SEM image of the working electrode surface of a EIGPU, under a 500x magnification.

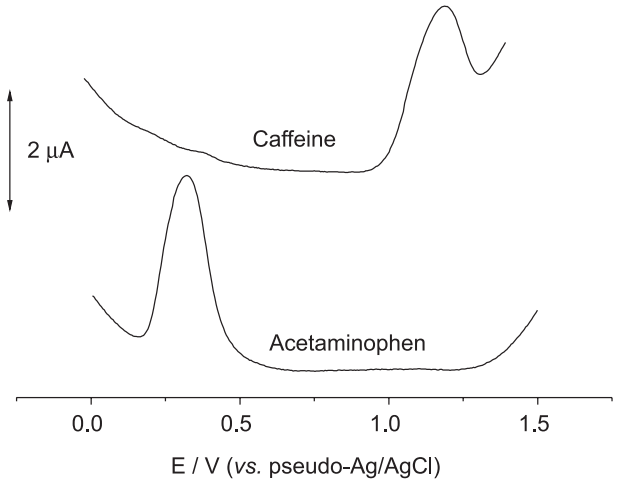

Figure 3. Differential pulse voltammetric curves obtained for 1.0×10^{-4} mol L^{-1} acetaminophen and caffeine, in 0.100 mol L^{-1} phosphate buffer pH 6.0, scan rate 25 mV s^{-1} and pulse amplitude 75 mV.

Effect of the scan rate and pulse amplitude

First, the effect of the scan rate on the caffeine DPV signal was evaluated between 5 and 100 mV s^{-1}. Best results were obtained at 25 mV s^{-1}, for the peak current intensity and resolution. The same study was performed for acetaminophen with reasonable results up to 70 mV s^{-1}. However, due to the sensitivity required by caffeine, a scan rate of 25 mV s^{-1} was chosen for further studies.

The effect of pulse amplitude was also performed for caffeine and acetaminophen, being possible to observe, in both cases, an increase of the peak current up to 100 mV. However, this pulse amplitude causes distortion in the peak shape, which is not suitable for quantitative purposes. Thus, in view of the need for higher sensibility for caffeine in simultaneous determinations, pulse amplitude of 75 mV was selected for these studies.

Effect of the hydrogen ion concentration

The phosphate buffer was chosen based on previous results,[53] and considering its use in biological studies.

The effect of the hydrogen ion concentration was evaluated varying the pH in 0.100 mol L^{-1} phosphate buffer from 5.00 to 10.0. The voltammetric response of acetaminophen at EIGPU was evaluated, with better response in pH 6.0, which presented higher current intensity and better defined voltammetric profiles.

The oxidation peaks of acetaminophen and the caffeine appeared at 0.3 V and 1.3 V (*vs.* pseudo-Ag/AgCl), respectively (Figure 3). Although these voltammograms are taken from solutions of the individual drugs, they clearly demonstrate the possibility of simultaneous determination of these pharmaceuticals.

The electrochemical behavior of caffeine and acetaminophen are very well known, as described by Sanghavi *et al.*[47]

Briefly one can say the acetaminophen undergoes a reversible oxidation process at 0.3 V (*vs.* pseudo-Ag/AgCl) involving 2 electron transfers and two protons. By its turn caffeine presented an irreversible oxidation process at 1.3 V (*vs.* pseudo-Ag/AgCl) involving 4 electrons and four protons.[47]

Individual determination of acetaminophen and caffeine

After optimization of experimental parameters described above, analytical curves were obtained for EIGPU. The voltammetric measurements were performed using the same electrode, without renewing the surface between the successive determinations.

In both cases the peak currents were measured by extrapolating the base line and taking the current at potentials of maximum signal. Of course it is not easy to take such values at low concentrations.

Figure 4a presents the voltammograms obtained by DPV and the analytical curve for acetaminophen.

The investigated interval was between 1.00 and 200 µmol L^{-1} acetaminophen (Acet) in 0.100 mol L^{-1} phosphate buffer pH 6.0. The linear region was determined between 1.00 and 100 µmol L^{-1}, obeying the linear equation 1:

$$I_p = 0.025\ \mu A + 0.017\ \mu A\ \mu mol^{-1}\ L\ C_{Acet},$$
$$(n = 9, R = 0.9997) \quad (1)$$

The LOD determined as three times the standard deviation of the blank (S_d) divided by the angular coefficient of straight line (b)[56] was 1.2 µmol L^{-1}.

Figure 4b presents the voltammograms obtained by DPV and the analytical curve for caffeine (Caf).

The investigated interval was also between 1.00 and 200 μmol L^{-1} caffeine in 0.100 mol L^{-1} phosphate buffer pH 6.0. The linear region was determined between 4.00 and 180 μmol L^{-1}, obeying the linear equation 2:

$$I_p = -0.011\ \mu A + 0.011\ \mu A\ \mu mol^{-1}\ L\ C_{Caf}, \quad (n = 8, R = 0.9996) \qquad (2)$$

The limit of detection was 2.7 μmol L^{-1}, determined as described above.

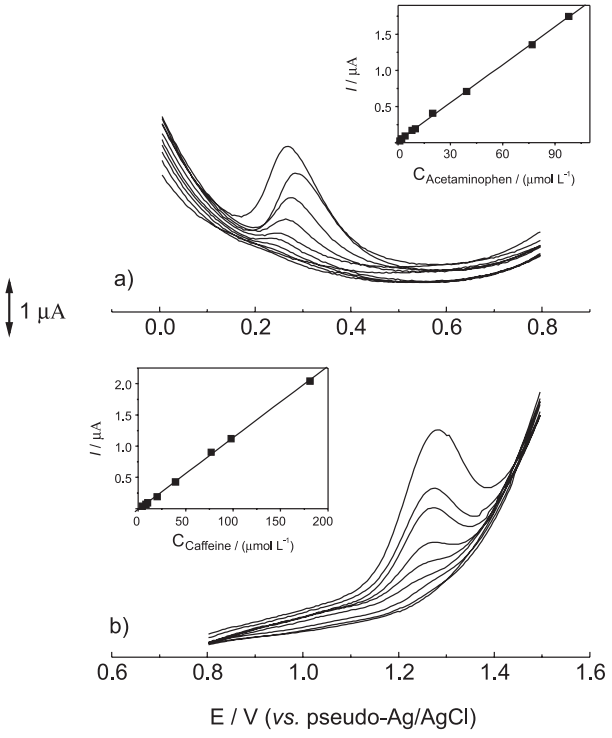

Figure 4. Differential pulse voltammetric curves obtained for a) acetaminophen and b) caffeine, in 0.100 mol L^{-1} phosphate buffer pH 6.0, at concentrations between 1.00 and 200 μmol L^{-1}, scan rate 25 mV s^{-1} and pulse amplitude 75 mV. In the inset, the analytical curve.

Simultaneous determination of acetaminophen and caffeine

DPV experiments were carried out for the simultaneous determination of paracetamol and caffeine in phosphate buffer pH 6.0, using parameters optimized previously at the EIGPU.

The DPV curves presented oxidation peaks at 0.3 V for acetaminophen and 1.3 V for caffeine (*vs.* pseudo-Ag/AgCl). The peak separation of about 1.0 V clearly allows the simultaneous determination of these compounds.

To further investigate the electrochemical solution when both substances are present in solution, DPV curves were obtained in the presence of a large excess of acetaminophen or caffeine in the 0.100 mol L^{-1} phosphate buffer pH 6.0, as presented in Figure 5 which includes the blank voltammograms.

The individual determination of acetaminophen in concentrations between 1.00 and 200 μmol L^{-1} it was accomplished in solutions containing caffeine fixed at 6.0×10^{-5} mol L^{-1} (Figure 5a). On the other side the determination of caffeine was performed in the same concentration of acetaminophen, between 1.00 and 200 μmol L^{-1}, in solutions containing acetaminophen at the fixed concentration of 6.0 x 10^{-5} mol L^{-1} (Figure 5b).

An examination of Figure 5a allows concluding that the oxidation peak current for acetaminophen increases regularly as its concentration is increased, in the presence of a fixed caffeine concentration. The caffeine peak current remains fairly constant, with $I_{p(CAF)} = 1.62\ \mu A \pm 0.2$ (n = 9).

Similarly, as shown in Figure 5b, the peak oxidation current for caffeine increases regularly as its concentration is increased in the presence of a fixed concentration of acetaminophen, whose oxidation peak current remains almost constant, with $I_{p(ACET)} = 2.17\ \mu A \pm 0.2$ (n = 11).

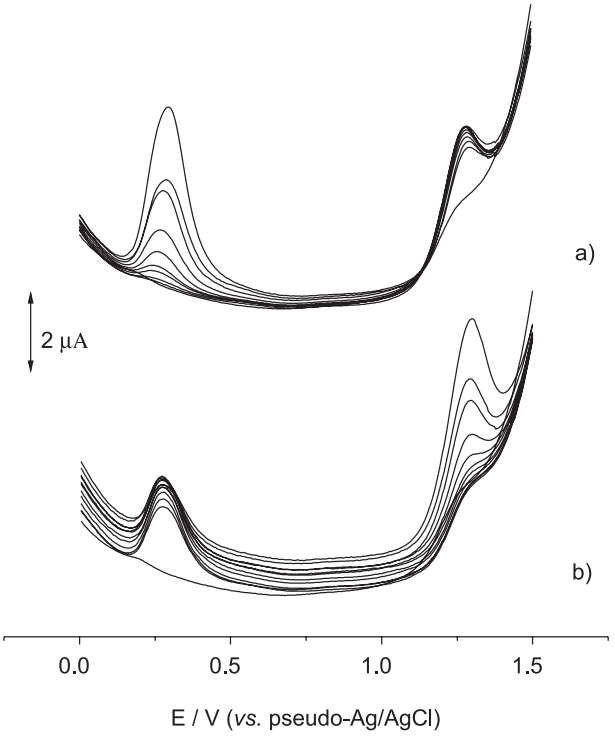

Figure 5. Differential pulse voltammograms obtained at the EIGPU for a) acetaminophen at concentrations between 1.00 and 200 μmol L^{-1} in the presence of 6.0 x 10^{-5} mol L^{-1} caffeine; b) caffeine at concentrations between 1.00 and 200 μmol L^{-1} in the presence of 6.0×10^{-5} mol L^{-1} acetaminophen. Scan rate 25 mV s^{-1} and pulse amplitude 75 mV.

It is possible to observe that any change in the caffeine signal occurs when acetaminophen concentration increases

(Figure 5a). However the opposite situation is seen when caffeine concentration is increased once the base line of acetaminophen is affected (Figure 5b).

This means, that it is necessary to take care with the base line during the quantitative simultaneous analysis of theses analytes. Such change can be a result of some interaction of caffeine (or its oxidation products) with the working electrode surface.

After this previous study, acetaminophen and caffeine were simultaneously determined by changing their concentrations at the same time. Figure 6 shows the DPV voltammograms obtained for solutions containing acetaminophen and caffeine in 0.100 mol L^{-1} phosphate buffer pH 6.0, when both had their concentrations varied.

The analytical curves for acetaminophen and for caffeine (inset in Figure 6) present linear response in the concentration range investigated.

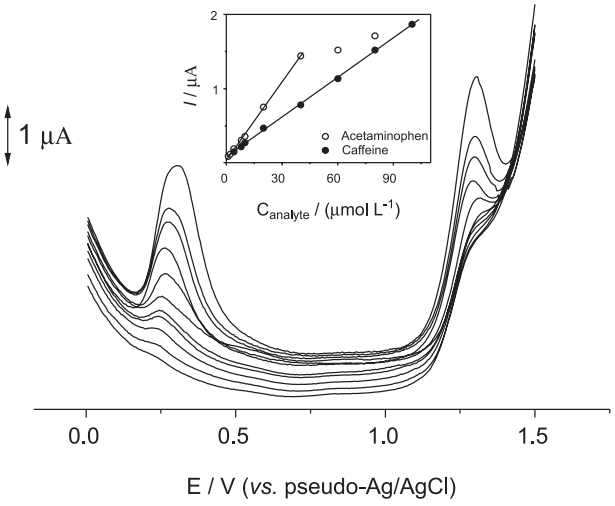

Figure 6. Differential pulse voltammetric curves obtained at the EIGPU for acetaminophen and caffeine at equal concentrations between 1.00 and 200 µmol L^{-1}, scan rate 25 mV s^{-1} and pulse amplitude 75 mV. In set the analytical curve.

The acetaminophen analytical curve presented a linear region in the concentration range 1.00 - 40.0 µmol L^{-1}, obeying the linear equation 3.

$$Ip = -0.038\ \mu A + 0.035\ \mu A\ \mu mol^{-1} L\ C_{APAP}, \quad (n = 7, R = 0.9993) \qquad (3)$$

The caffeine analytical curve presented a linear region in the concentration range 4.00 - 200 µmol L^{-1}, obeying the linear equation 4.

$$Ip = 0.084\ \mu A + 0.017\ \mu A\ \mu mol^{-1} L\ C_{CAF}, \quad (n = 8, R = 0.9997) \qquad (4)$$

The detection limits[56] were 0.84 µmol L^{-1} and 1.6 µmol L^{-1} for acetaminophen and caffeine, respectively.

The limit in the linear range for acetaminophen is probably due to the competition with caffeine (or its oxidation products) by the active sites of the electrode.

As presented above sub-micromol L^{-1} levels has previously been reached in the simultaneous electroanalytical determination of caffeine and acetaminophen.[46,47] Regarding the BDDE similar results were found for acetaminophen, while lower LODs were described for caffeine in both BDDE and surfactant-modified multiwalled carbon nanotube paste electrode. The last one also presented best results for acetaminophen. However the EIGPU is easier to prepare, disposable and non-modified, being suitable for screening procedures, field and pharmaceutical analysis of both drugs, including the mobile capabilities.

Pharmaceutical formulations analysis

Commercial pharmaceutical samples (tablets) containing both acetaminophen and caffeine were analyzed to simultaneously determine these pharmaceuticals in order to evaluate the validity of the proposed method.

The method of standard addition was used to quantify these substances in samples of DC Tylenol® (Janssen-Cilag Pharmaceuticals Ltd., Brazil), Excedrin® (Novartis Biosciences SA, Brazil) and Maxidrin® (Kley Hertz SA, Brazil).

Recovery experiments carried out to evaluate matrix effects after standard additions yielded an average recovery of 94.9% for acetaminophen and 90.9% for caffeine, indicating that there any important matrix interferences for the samples analyzed by the proposed DPV method.

Table 1 presents the values of the amounts of acetaminophen and caffeine simultaneously determined in the analysis of three pharmaceutical formulations.

The results obtained by DPV were compared with those obtained by HPLC recommended method[45] and agreed with the reference procedure within 95% confidence level, according to the t-Student test. These results are shown in Table 2.

The results show that there is not significant differences between the results obtained and tabulated suggesting that there no interference from the other concomitants in the commercial formulations, after removing the coloring species in Tylenol DC® and Excedrin®.

Conclusions

The EIGPU composite electrode can be used for the quantitative determination of acetaminophen and caffeine, individually or mixed in the sample.

Table 1. Recovery coefficients of acetaminophen (A) and caffeine (B) in three pharmaceutical formulations analyzed

Samples		Addition	Added / (μmol L^{-1})	Found / (μmol L^{-1})	Recovery / %
Tylenol DC®	A	1º	40.0	36.1	90.0
		2º	60.0	59.3	99.0
		3º	80.0	77.6	97.0
					Average ± sd 95.3 ± 4
	B	1º	100.0	87.7	88.0
		2º	150.0	141.0	94.0
		3º	200.0	182.5	91.0
					Average ± sd 91.0 ± 3
Excedrin®	A	1º	40.0	34.8	87.0
		2º	60.0	58.6	97.0
		3º	80.0	77.0	96.2
					Average ± sd 93.4 ± 5
	B	1º	100.0	85.7	86.0
		2º	150.0	140.0	93.4
		3º	200.0	184.8	92.4
					Average ± sd 90.6 ± 4
Maxidrin®	A	1º	40.0	38.1	95.3
		2º	60.0	57.3	95.6
		3º	80.0	78.1	97.6
					Average ± sd 96.1 ± 1
	B	1º	100.0	89.0	89.0
		2º	150.0	138.4	92.2
		3º	200.0	184.5	92.2
					Average ± sd 91.1 ± 1

sd: standard deviation; n = 3.

Table 2. Simultaneous determination of acetaminophen (A) and caffeine (B) in pharmaceutical formulations using EIGPU / DPV and HPLC

Samples	Substance	Labeled / mg	DPV[a,b]	HPLC[a,b]	E_1[c] / %	E_2[d] / %
Tylenol DC®	A	500	516 ± 1	514 ± 2	3.2	0.4
	B	65.0	67 ± 2	66 ± 2	3.0	1.5
Excedrin®	A	500	524 ± 2	518 ± 3	4.8	1.1
	B	65.0	66 ± 1	63 ± 2	1.5	4.7
Maxidrin®	A	500	520 ± 3	519 ± 3	4.0	0.2
	B	65.0	68 ± 2	68 ± 1	4.6	0.0

[a]result ± standard deviation; [b]n = 3; [c]DPV *vs.* Labeled (DPV – Labeled / Labeled) x 100%; [d]HPLC *vs.* DPV (DPV – HPLC / HPLC) x 100%.

Furthermore, the method involves much lower instrumental and analysis costs, and much lower amount of waste generation when compared with chromatographic procedures and although the electrodes are disposable, they can be used for each formulation without replacement. In the present work only three electrodes were used during the optimization and analysis steps.

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