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Voltammetric determination of some alkaloids and other compounds in pharmaceuticals and urine using an electrochemically activated glassy carbon electrode

Research Article

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Abstract: Electrochemical behaviour of some alkaloids, namely: caffeine, aminophylline, theophylline, codeine phosphate and papaverine hydrochloride, that are in solution in various combinations or in the presence of other compounds contained in pharmaceuticals or in real samples (urine) was investigated using cyclic voltammetry (CV), square-wave voltammetry (SWV) and differential pulse voltammetry (DPV) on electrochemically activated glassy carbon electrode.

The proposed electroanalytical methods were successfully applied in the simultaneous determination of these alkaloids in different combination or in the presence of other compounds. The great part of these combinations can be analyzed simultaneously because they practically do not interfere.

The electrochemical test methods attempted to detect the presence of alkaloids in urine samples collected from subjects who consumed coffee (caffeine), and from a patient under treatment with *Miofilin*® (aminophylline). Urine samples were determined after filtration, without prior dilution and after dilutiion with acetate buffer at pH 4.5. Best results were obtained using DPV performed on electrochemically activated glassy carbon electrode. Thus in samples taken from subjects who drink coffee the caffeine concentration detected was 6.21×10^{-7} mol L^{-1} in the first sample and 7.77×10^{-7} mol L^{-1} in the second sample, while aminophylline concentration detected was 1.15×10^{-7} mol L^{-1}.

Keywords: *Alkaloids • Electrochemically activated glassy carbon • Voltammetric methods • Pharmaceuticals*

1. Introduction

Caffeine (*1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione*), is a xanthine derivative naturally present in cocoa, coffee and tea and is added to many beverages. The presence of caffeine in coffee, beverages and tea acts as a diuretic and stimulant to the central nervous and cardiovascular systems [1,2]. Caffeine is used in analgesic pharmaceutical formulations.

Theophylline (*1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione*) is a methyl-derivative of xanthine, most commonly used in the treatment of the symptoms of obstructive pulmonary disease and bronchial asthma [3-5].

Aminophylline (*1,3-dimethyl-7H-purine-2, 6-dione 1, 2-diamine*) consists of theophylline with ethylenediamine in 2:1 ratio, used in the treatment of bronchial asthma, anaphylactic shock [6], and as a body fat reducer [7].

Codeine phosphate (*7,8-didehydro-4,5α-epoxy-3-methoxy-17methyl morphinan-6α-ol phosphate*) has for a long time been used as an effective analgesic and also as an agent against cough in pharmaceutical preparations [8-11]. Codeine has no narcotic properties but its analgesic properties are ten times weaker than that of morphine. Previous approaches, including gas chromatography, chemiluminescent and high performance liquid chromatography, have been reported for codeine determination [12-14]. However, these methods are time consuming or solvent-usage intensive.

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New pharmaceutical preparations that are being developed require fast, sensitive, simple, inexpensive, and a specific method for quantitative determination of codeine in oral formulations. *Shih et al.* report the determination of codeine using a nontronite clay-modified screen-printed electrode by both square-wave voltammetry and flow injection analysis [11]. Also the amperometric detection of codeine and its metabolite, morphine following capillary zone electrophoresis separation has been described. A carbon-disk electrode used as working electrode for codeine and morphine exhibited good response at 0.90 V (*versus* Ag/AgCl) [15]. *Pournaghi-Azar et al.* used the Prussian blue film modified-palladized aluminium electrode for determination of codeine [16].

Papaverine hydrochloride (*1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline hydrochloride*), is an alkaloid with antispastic activity. It acts on smooth muscle and is used to treat various cardiovascular disorders, gastrointestinal tract, and the gall bladder. In pharmaceutical formulations, papaverine is most often encountered in the form of hydrochloride. The methods most often used for determination of papaverine hydrochloride in pharmaceutical formulations and plasma include spectral, polarographic and chromatographic methods [17]. Ion selective electrodes for detection of papaverine hydrochloride and related derivatives have also been developed since they provide advantages of a simple operating design, reasonable selectivity and could be used in solutions with colour or are turbid [18]. The study of the electrochemical behaviour of alkaloids could help in understanding the complex mechanism of enzymatic reaction [19]. This is why there have been several studies aimed at developing reliable methods for the evaluation and quantification of those compounds, and several analytical methods have been proposed including UV-spectrometry [20], thin layer chromatography [21], capillary electrophoresis and gas chromatography [22-25], HPLC [11,14,19,26,27], mass spectroscopy [23], enzymatic methods [28,29] and electrochemical methods [17,18]. Generally, these methods are expensive, time-consuming and complicated [30,31]. In contrast, electrochemical methods have better features, such as simplicity, high sensitivity, and ease of use [32,33].

Glassy carbon electrodes have been used for the amperometric detection of caffeine, theophylline and theobromine in beverages, directly and after solid-phase extraction [34]. The electrochemical behaviour of caffeine on Nafion–Graphene modified glassy carbon electrode was investigated by cyclic voltammetry and differential pulse voltammetry with excellent performance [35]. Nafion/multi-wall carbon nanotubes composite film coated glassy carbon electrode were also developed for sensitive voltammetric determination of caffeine [36]. Martínez-Huitle *et al.* [37] used Nafion®-modified boron-doped diamond electrodes, while Alizadeh *et al*. [38] used a molecularly imprinted polymer as sensor for detecting caffeine. Differential-pulse voltammetry [39] and square-wave voltammetry [40,41] have also been employed for accurate determination of caffeine and other alkaloids in drug formulations. In the case of theophylline, electrochemical methods with a detection limit of about 10^{-7} mol L^{-1} have also been reported, including cobalt phthalocyanine modified carbon paste electrode [28], xanthine oxidase electrode [29], boron-doped diamond electrode [19], nafion/lead-ruthenium oxide pyrochlore chemically modified glassy carbon electrode [16] carbon nanotube modified glassy carbon electrode [18] and theophylline oxidase modified graphite electrode [3].

The use of carbon and platinum electrodes in the anodic determination of xanthines is rendered difficult by interference from oxidation background currents that are not precisely reproducible. This is because relatively large positive potentials (>1.5 V *vs*. Ag/AgCl) are required for reasonable sensitivity, in amperometry and voltammetry [19].

Ziyatdinova *et al*. [42] developed a voltammetric procedure for direct determination of papaverine in pharmaceuticals using graphite electrodes.

The study of electrochemical behaviour of some purine alkaloids (aminophylline, caffeine and theophylline), codeine phosphate and papaverine hydrochloride in various combinations, or in the presence of other compounds (paracetamol, acetylsalicylic acid, ascorbic acid, uric acid, phenobarbital or propyphenazone), contained in pharmaceutical forms or in real samples (urine), on electrochemically activated glassy carbon electrode was initiated in order to elaborate new electroanalytical methods for their detection, individually and simultaneously.

2. Experimental Procedure

2.1. Instrumentation

An AUTOLAB PGSTAT 30 potentiostat/galvanostat (Ecochemie, The Netherlands) equipped with specific soft GPES, was employed for electrode activation and other voltammetric determinations. For pH measurement a ChemCadet pH meter was used. All measurements were done at room temperature (20-23ºC).

Experiments were conducted in three electrode geometry, with a glassy carbon working electrode, a platinum wire auxiliary electrode, and Ag/AgCl (3 M KCl)

reference electrode. All measurements were carried out in a glass cell, containing 5.0 mL of solution, without stirring.

All the electrochemical experiments were repeated three times, and the values used and current intensity values reported represent the arithmetic mean of the obtained values in the experiments.

The detection limit was calculated as three times the standard deviation for the electrolyte divided by the slope of the analytical curve for each compound [43].

2.2. Materials and methods

Solid electrodes made from glassy carbon (d = 3 mm) in native form and electrochemically activated purchased from BAS Inc. (West Lafayette, USA) were used as working electrodes in electrochemical determination.

Deionised water was used for preparation of buffers and all other aqueous solutions. All chemicals were analytical grade, and used as received without further purification.

2.2.1. Electrochemical activation of the glassy carbon electrodes

The glassy carbon electrodes were first rinsed with deionised water, polished carefully with alumina powder having different particle size to a mirror finish surface. After rinsing with deionised water, the electrodes were cleaned in an ultrasonicating bath for five minutes in water, alcohol and again water. The electrochemical pre-treatment of glassy carbon electrode was performed by anodic oxidation at 1.8 V *vs*. Ag/AgCl, for 300 s in 0.1 M phosphate buffer solution at pH 5. The electrode was then cycled between 0 and 0.8 V with a scan rate of 0.1 V s^{-1} until a stable current potential curve was obtained (ten cycles). The activation procedure has to be repeated after each determination or the electrode can be used again, after continuous sweep for four cycles in potential range from 0 to 1.0 V in 0.1 M phosphate buffer solution at pH 7.4.

2.3. Procedure

Ten tablets or capsules of each of the pharmaceutical formulation analyzed were accurately weighed and finely powdered in a mortar. An adequate amount of the powder was weighed and transferred to a 25 mL calibrated flask and completed to volume with 0.2 mol L^{-1} acetate buffer pH 4.5. The standard addition method was used for analyzing the pharmaceutical samples.

The solution was transferred into the voltammetric cell and deaerated with pure nitrogen for 10 minutes to remove oxygen. The cyclic voltammograms were obtained by scanning the potential from 0 to 1.8 V at a scan rate of 0.1 V s^{-1}. The differential pulse voltammograms were obtained by scanning potential from 0 to 1.8 V at a scan rate of 0.04 V s^{-1} and pulse amplitude of 0.1 V. The square-wave voltammograms were obtained by scanning potential from 0 to 1.8 V with the frequency of 75 Hz, amplitude of 0.05 V and scan increment of 0.005 V.

3. Results and Discussion

3.1. Investigation of the electrochemical behaviour of the studied alkaloids

The electrochemical behaviour of some alkaloids, such as: caffeine, aminophylline, theophylline, codeine phosphate and papaverine hydrochloride in various combinations or in the presence of other compounds contained in pharmaceutical forms or in real samples (urine): paracetamol, acetylsalicylic acid, ascorbic acid, uric acid, phenobarbital or propyphenazone was investigated using cyclic voltammetry, square-wave voltammetry and differential pulse voltammetry on electrochemically activated glassy carbon electrode.

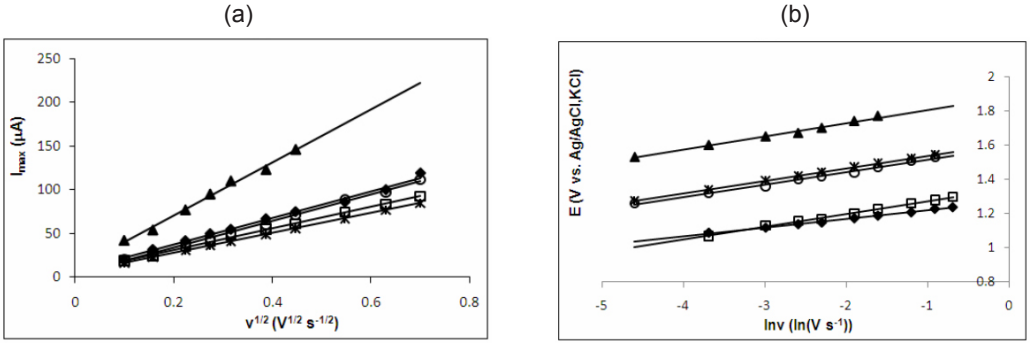


Figure 1. Linear variation of: (a) current peak intensity with the square root of the scan rate; (b) peak potential with the logarithm of the potential scan rate for 10^{-3} M aminophylline (□) theophylline (□), caffeine (▲), codeine phosphate (○) and papaverine hydrochloride (✖) solutions in acetate buffer at pH 4.5 (Working electrode electrochemically activated glassy carbon; reference electrode Ag/AgCl, (3 M KCl); auxiliary electrode Pt wire; 20°C).

3.1.1. Selection of the experimental conditions

The study of the influence of scan rate on the anodic peak current with electrochemically activated glassy carbon electrode was also carried out for the studied alkaloids within the range of 10 to 500 mV s^{-1}. It was found that the peak current increased linearly with the square root of scan rate, Fig. 1a with good correlation coefficients, indicating diffusion control. For aminophylline, the linear variation of the peak current with the square root of the scan rate is described by the equation: $I_p = 153.46\ v^{1/2} + 6.5471$ (μA), ($R^2 = 0.9925$), for caffeine: $I_p = 302.89 v^{1/2} + 9.9557$ (μA), ($R^2 = 0.9929$), for theophylline: $I_p = 123.37\ v^{1/2} + 6.9298$ (μA), ($R^2 = 0.9974$), for codeine phosphate: $I_p = 151.14\ v^{1/2} + 3.824$ (μA), ($R^2 = 0.9950$), and for papaverine hydrochloride the equation is: $I_p = 114.23\ v^{1/2} + 4.914$ (μA), ($R^2 = 0.9915$).

The peak potential varies linearly with the logarithm of scan rate, Fig. 1b and the equations describing this dependence have good correlation coefficients.

For aminophylline, the linear variation of the peak potential with the logarithm of the scan rate is described by the equation: $E_p = 0.0508\ \ln v + 1.2727$ (V), ($R^2 = 0.996$), for caffeine: $E_p = 0.0781\ \ln v + 1.8854$ (V), ($R^2 = 0.9914$), and for theophylline: $E_p = 0.0751\ \ln v + 1.3493$ (V), ($R^2 = 0.9965$), for codeine phosphate: $E_p = 0.0753\ \ln v + 1.5902$ (V), ($R^2 = 0.9914$), and for papaverine hydrochloride the equation is: $E_p = 0.0735\ \ln v + 1.6137$ (V), ($R^2 = 0.9906$).

For all studied alkaloids, the oxidation peak potential was observed to shift positively with increase in scan rate, which confirms irreversibility of the oxidation processes. The result indicates that the oxidation of the studied alkaloids at the electrochemically activated glassy carbon electrode is diffusion-controlled irreversible oxidation process. These results are also consistent with relatively small electron transfer rate constant [19]. Various supporting electrolytes for all compounds were investigated using cyclic voltammetry: phosphate buffer, Britton-Robinson buffer, acetate buffer, and $LiClO_4$ 0.1 M in EtOH. The best results were obtained with the 0.2 mol L^{-1} acetate buffer at pH 4.5. The experimental procedure involves a systematic study and optimization of the parameters that affect the SWV and DPV response. In Table 1 are presented the SWV and DPV parameters and their optimum values.

3.1.2. Linear range and detection limit

The relationships between the anodic peak currents and the concentration of studied compounds were examined by square-wave voltammetry and differential pulse voltammetry in acetate buffer at pH 4.5. The dependence of the voltammetric signal on the concentration is illustrated by the calibration curves obtained. In the case of aminophylline the results obtained by SWV and DPV are presented as an example in Figs. 2a and 2b.

The calibration curves for the studied alkaloids, paracetamol and acetylsalicylic acid, present good linear response in the concentration range 10^{-7} mol L^{-1} to 10^{-4} mol L^{-1}, and good correlation coefficients. Table 2 presents the corresponding equations, the detection limits and the results of the statistical analysis of the experimental data such as slopes, intercept, the correlation coefficients along with standard deviation of intercept (S_a) on the ordinate and

Table 1. SWV and DPV parameters and their optimum values

Voltammetric method	Parameters	Studied range	Optimum value
SWV	Frequency (Hz)	10 – 100	75
	Amplitude (mV)	10 – 100	50
	Scan increment (mV)	1 – 5	5
DPV	Modulation time (ms)	5 – 10	5
	Amplitude (mV)	10 – 150	100
	Scan rate (V s^{-1})	0.01 – 0.1	0.04

SWV = square-wave voltammetry; DPV = differential pulse voltammetry

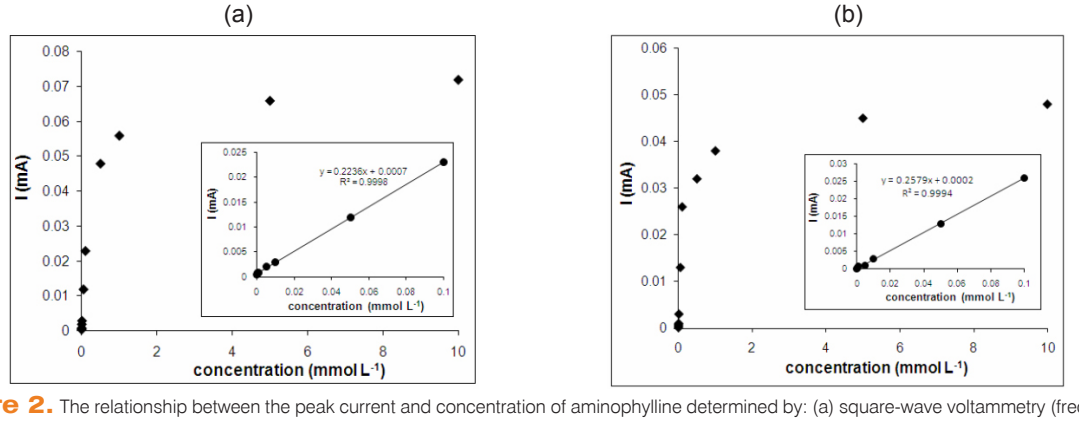


Figure 2. The relationship between the peak current and concentration of aminophylline determined by: (a) square-wave voltammetry (frequency 75 Hz; amplitude 50 mV; scan increment 5 mV; 20°C); (b) differential pulse voltammetry (scan rate 0.04 V s^{-1}; modulation amplitude 100 mV; modulation time 5 ms; 20°C) in 0.2 mol L^{-1} acetate buffer pH 4.5 on electrochemically activated glassy carbon electrode (reference electrode Ag/AgCl; auxiliary electrode Pt wire). Inset: The linear relationships between the peak current and concentration of aminophylline in the range 10^{-7} – 10^{-4} mol L^{-1}

Table 2. Calibration data and analytical parameters obtained by square-wave voltammetry and differential pulse voltammetry

Analytes	Electro chemical method	Equation for the calibration curves $I_p = a + b \cdot C$		R^2	Linear range (mol L^{-1})	LOD (mol L^{-1})	LOQ (mol L^{-1})	RSD%
		$a \pm S_a$	$b \pm S_b$					
Aminophylline	SWV	0.7±0.0019	223.61±0.72	0.9998	$10^{-7} - 10^{-4}$	2.5×10^{-8}	8.5×10^{-8}	0.7
	DPV	0.2±0.0079	257.84±0.63	0.9994	$10^{-7} - 10^{-4}$	9.2×10^{-8}	3.1×10^{-7}	0.5
Caffeine	SWV	0.4±0.0041	729.75±0.52	0.9986	$10^{-7} - 5\times10^{-5}$	1.7×10^{-8}	5.6×10^{-8}	2.8
	DPV	0.8±0.0022	317.25±0.85	0.9982	$10^{-7} - 10^{-4}$	2.0×10^{-8}	6.9×10^{-8}	1.4
Theophylline	SWV	0.5±0.0017	386.64±0.73	0.9963	$10^{-7} - 10^{-4}$	1.3×10^{-8}	4.3×10^{-8}	1.6
	DPV	0.9±0.0076	266.11±0.81	0.9977	$10^{-7} - 10^{-4}$	8.6×10^{-8}	2.9×10^{-7}	1.3
Codeine phosphate	SWV	0.9±0.0096	373.63±1.27	0.9995	$10^{-7} - 10^{-4}$	7.7×10^{-8}	2.5×10^{-7}	0.5
	DPV	0.7±0.0076	283.46±1.03	0.9996	$10^{-7} - 10^{-4}$	8.1×10^{-8}	2.7×10^{-7}	0.8
Papaverine hydrochloride	SWV	0.1±0.0014	150.10±0.77	0.9979	$10^{-7} - 10^{-4}$	2.7×10^{-8}	9.3×10^{-8}	1.7
	DPV	0.2±0.0008	78.31±1.01	0.9996	$10^{-7} - 10^{-4}$	3.0×10^{-8}	1.1×10^{-7}	2.2
Paracetamol	SWV	0.9±0.0007	106.92±0.96	0.9977	$10^{-7} - 5\times10^{-5}$	1.9×10^{-8}	6.5×10^{-8}	0.9
	DPV	0.4±0.0048	202.41±1.20	0.9977	$10^{-7} - 10^{-4}$	7.2×10^{-8}	2.4×10^{-7}	1.4
Acetylsalicylic acid	SWV	0.6±0.0008	216.71±0.85	0.9981	$10^{-7} - 10^{-4}$	1.2×10^{-8}	3.6×10^{-8}	1.1
	DPV	0.2±0.0008	70.24±0.69	0.9956	$10^{-7} - 10^{-4}$	3.4×10^{-8}	1.2×10^{-7}	0.8

I_p = the peak intensity of current (μA); a = intercept (μA) and b = slope of the calibration curves (μA L mmol^{-1}); S_a = the standard deviation of intercept (μA); and S_b = the standard deviation of slope (μA L mmol^{-1})

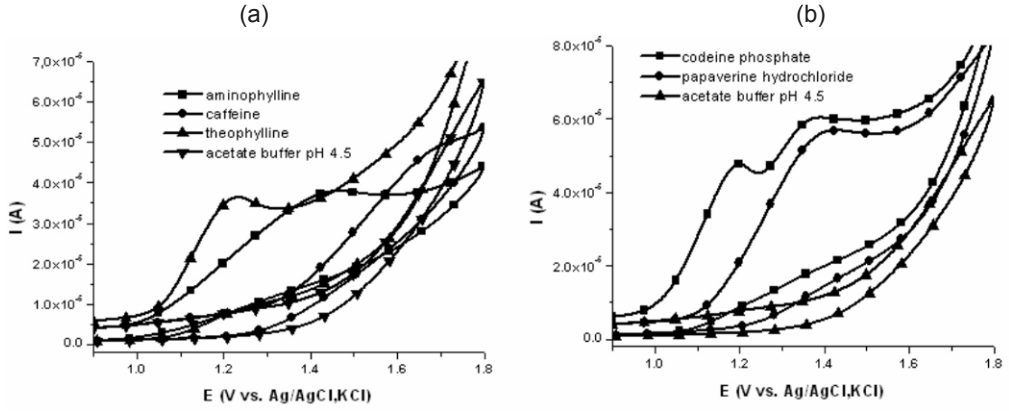


Figure 3. Cyclic voltammograms of 10^{-3} mol L^{-1}: (a) purine alkaloids (aminophylline, theophylline and caffeine); (b) codeine phosphate and papaverine hydrochloride solutions in 0.2 mol L^{-1} acetate buffer pH 4.5 on electrochemically activated glassy carbon electrode (reference electrode Ag/AgCl; auxiliary electrode Pt wire; scan rate 0.1 V s^{-1}; 20°C)

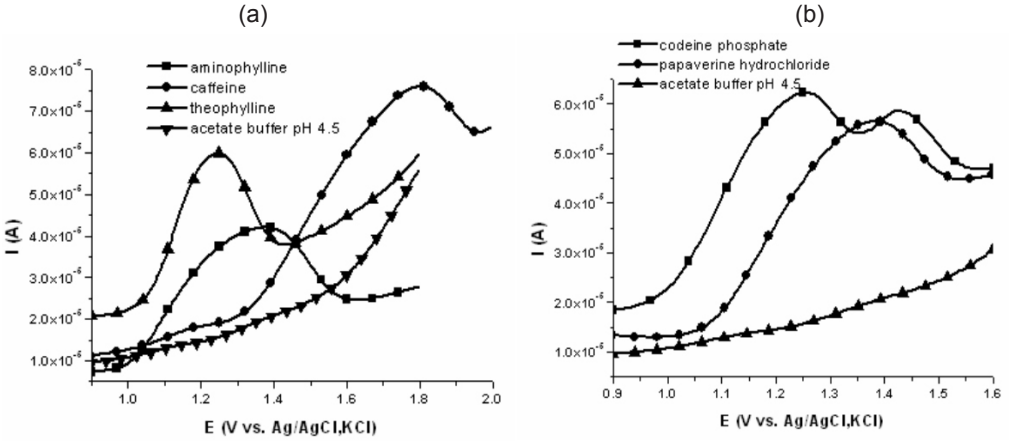


Figure 4. Square-wave voltammograms of 10^{-3} mol L^{-1}: (a) purine alkaloids (aminophylline, theophylline and caffeine)); (b) codeine phosphate and papaverine hydrochloride solution in 0.2 mol L^{-1} acetate buffer pH 4.5 on electrochemically activated glassy carbon electrode (reference electrode Ag/AgCl; auxiliary electrode Pt wire; frequency 75 Hz; amplitude 50 mV; scan increment 5 mV; 20°C)

the standard deviation of slope (S_b). The good linearity of the calibration graphs and the negligible scatter of the experimental points are clearly evident by the values of the correlation coefficients and standard deviation for all the studied compounds both in SWV and DPV experiments.

3.1.3. Sensitivity/detection limits and limits of quantitation

The LOD were calculated as tree time the standard deviations of intercept (S_a) divided by the slopes of the regression lines (b), while the LOQ were estimated as ten time the standard deviations of intercept (S_a) divided by the slopes of the regression lines (b) [44,45].

The calculated LOD and LOQ for the standard alkaloids solutions obtained both for DPV and SWV are presented in Table 2.

The repeatability was determined by successive measurements (n = 3) of 5×10^{-5} mol L^{-1} alkaloids solutions. Relative standard deviation values from 0.4 to 2.8% were obtained.

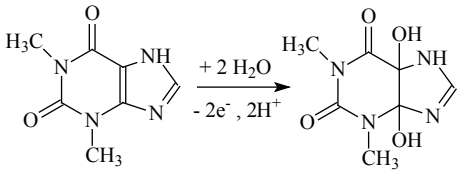
The reproducibility was evaluated by measuring the oxidation current values for similar fresh alkaloids solutions over a period of ten days. Differences of the oxidation current values from 0.9 to 5.5 %, compared to the first day were obtained, revealing good reproducibility. The measurements were done with fresh solutions prepared daily.

3.2. Individual determination of studied compounds

Cyclic voltammograms obtained for the studied alkaloids on electrochemically activated carbon electrode (Fig. 3), presented an irreversible oxidation peak at 1.25 V for theophylline, 1.4 V for papaverine hydrochloride, 1.45 V for aminophylline, 1.75 V for caffeine, while for codeine phosphate there are two irreversible oxidation peaks at 1.15 respective 1.375 V *vs*. Ag/AgCl.

In the case of square-wave voltammetry, (Fig. 4), the electrochemical behaviour is the same as that for cyclic voltammetry and the signals appear at 1.2 V for theophylline, 1.35 V for papaverine hydrochloride, 1.4 V for aminophylline, 1.8 V *vs*. Ag/AgCl for caffeine, while for codeine phosphate there are two irreversible oxidation peaks at 1.2 respective 1.4 V *vs*. Ag/AgCl.

The best results were obtained in the case of differential pulse voltammetry, (Fig. 5), because the signals are shifted to less positive values for all the studied substances, at 1.1 V for theophylline, 1.3 V for papaverine hydrochloride, 1.35 V for aminophylline, 1.5 V for caffeine, and at 1.1 respective 1.3 V *vs*. Ag/AgCl for codeine phosphate.



Scheme 1. The proposed mechanism for theophylline electrochemical oxidation in aqueous medium [46]

The theophylline electrochemical oxidation was studied by Zhu *et al.* [46], and a possible electrode reaction for theophylline electrochemical oxidation might be expressed as in Scheme 1.

We presume that electrochemical oxidation might have led to destruction of the π bond conjugate system in theophylline and a diol derivative of theophylline is formed. The same mechanism is valid in the case of aminophylline which is theophylline with ethylenediamine.

The electrochemical oxidation of caffeine has been studied by other authors [19,35,47], and the mechanism is shown in Scheme 2. The reaction process is an overall $4e^-$, $4H^+$ process. A $2e^-$, $2H^+$ oxidation of the C-8–N-9 bonds was performed to give the substituted uric acid. The electrochemical oxidation of substituted uric acid proceeds rapidly in a $2e^-$, $2H^+$ process to lead to an unstable diimine species which is then attacked by water molecules in a step-wise fashion to be converted into an imine-alcohol and then substituted uric acid-4,5 diol. The uric acid-4,5 diol compound produced is unstable and decomposes to various products depending on the solution pH [48,49].

Pournaghi-Azar *et al.* [16] studied the codeine electrochemical oxidation and presume that bis-codeine is the unique oxidation product. The mechanism is presented in Scheme 3.

3.3. Simultaneous determination of the studied compounds

Square-wave and differential pulse voltammetric experiments were carried out to further investigate the electrochemical behaviour when two substances are present in solution and to determine the influence of one to the other, in order to determine simultaneously different combinations of both compounds. The studied combinations were: caffeine and codeine phosphate, caffeine and paracetamol, caffeine and acetylsalicylic acid, caffeine and ascorbic acid, codeine phosphate and caffeine, codeine phosphate and paracetamol, codeine phosphate and acetylsalicylic acid, codeine phosphate and ascorbic acid.

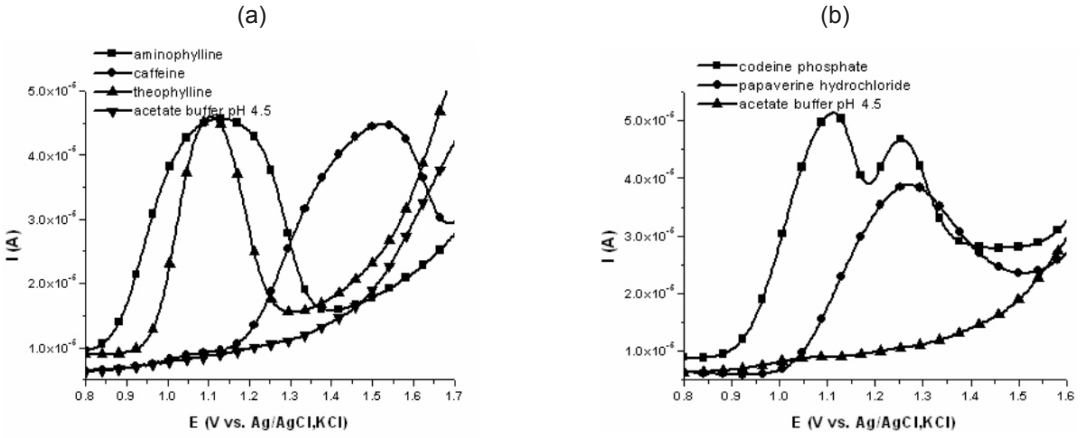
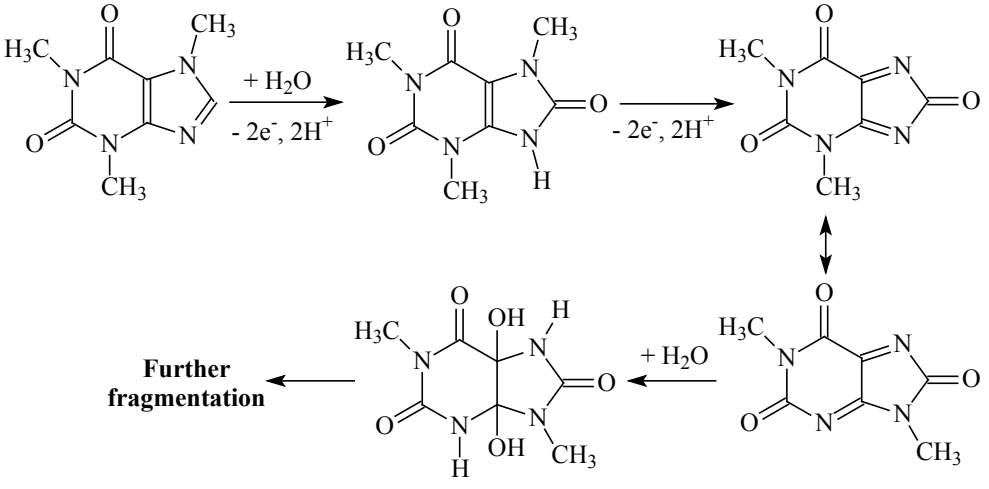
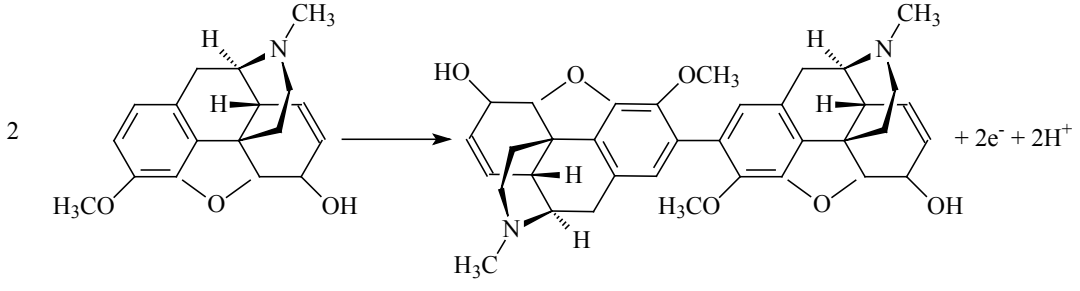


Figure 5. Differential pulse voltammetric curves of 10^{-3} mol L^{-1}: (a) purine alkaloids (aminophylline, theophylline and caffeine); (b) codeine phosphate and papaverine hydrochloride solutions in 0.2 mol L^{-1} acetate buffer pH 4.5 on electrochemically activated glassy carbon electrode (reference electrode Ag/AgCl; auxiliary electrode Pt wire; scan rate 0.04 V s^{-1}; amplitude 100 mV; modulation time 5 ms; 20°C)



Scheme 2. The proposed mechanism for caffeine electrochemical oxidation in aqueous medium



Scheme 3. The proposed mechanism for codeine electrochemical oxidation in aqueous medium [16]

From the data obtained on the 10^{-3} mol L^{-1} bicomponent solutions mentioned before it can be observed that the oxidation potentials are well separated in all cases and this clearly allows the simultaneous determination of the compounds. The best results were obtained with differential pulse voltammetry, Figs. 6 - 8.

An examination of Fig. 6a allows the conclusion that the peak oxidation current for codeine phosphate increases regularly with its concentration increasing at a fixed concentration of caffeine (its oxidation peak remains fairly constant). Similarly, Fig. 6b show that the peak oxidation current for the caffeine increases with the

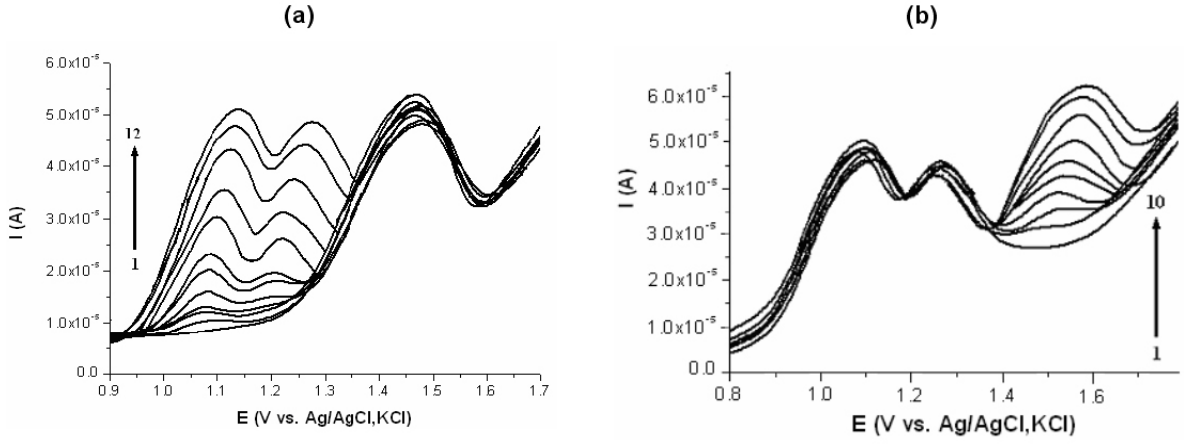


Figure 6. Differential pulse voltammograms on electrochemically activated glassy carbon electrode for (a) a 10^{-3} mol L^{-1} caffeine solution in the presence of different codeine phosphate concentrations: (1) 0, (2) 5×10^{-7}, (3) 10^{-6}, (4) 5×10^{-6}, (5) 10^{-5}, (6) 5×10^{-5}, (7) 7.5×10^{-5}, (8) 10^{-4}, (9) 2.5×10^{-4}, (10) 5×10^{-4}, (11) 7.5×10^{-4}, (12) 10^{-3} mol L^{-1}, in acetate buffer pH 4.5 and (b) a 10^{-3} mol L^{-1} codeine phosphate solution in the presence of different caffeine concentrations: (1) 0, 10^{-6} (2), (3) $5\cdot10^{-6}$, (4) 10^{-5}, (5) 5×10^{-5}, (6) 10^{-4}, (7) 2.5×10^{-4}, (8) 5×10^{-4}, (9) 7.5×10^{-4}, (10) 10^{-3} mol L^{-1}, in acetate buffer pH 4.5 (reference electrode Ag/AgCl; auxiliary electrode Pt wire; scan rate 0.04 V s^{-1}; amplitude 100 mV; modulation time 5 ms; 20°C)

increasing of its concentration at a fixed concentration of codeine phosphate. In the case of codeine phosphate it can be observed that even if the oxidation peaks are shifted to more positive potential values when the concentration increases, they remain well separated (Δ E = 0.25 V).

Figs. 7a and 7b presents the simultaneous determination of a 10^{-3} mol L^{-1} caffeine solution with different concentrations of paracetamol and acetylsalicylic acid, respectively. These two substances have the same behaviour as codeine phosphate for the simultaneous electrochemical determination in the presence of caffeine, but the oxidation peaks are more clearly separated in this case (Δ E = 0.9 V for paracetamol and Δ E = 0.5 V for acetylsalicylic acid).

The peak oxidation current for paracetamol and ascorbic acid are increasing with the increase of concentration at a fixed concentration of codeine phosphate (Fig. 8a, respective Fig. 8b).

After this study, the solutions containing two (Fig. 9) and three components (Fig. 10) having equal concentrations of 10^{-3} mol L^{-1} were determined and the results were compared with the data obtained in individual determination. The best results were obtained with SWV on electrochemically activated glassy carbon electrode.

3.4. Interferences

Various possible interferents were examined for their effect on the determination of alkaloids. Paracetamol, acetylsalicylic acid, ascorbic acid, uric acid, citric acid, phenobarbital, propyphenazone and sodium carbonate, all of which can be present in pharmaceutical formulation with the studied alkaloids, were tested and the obtained results were compared with that obtained using alkaloids standard solutions. Measurements of the peak currents for each solution were repeated three times and the average current values were used. If the presence of an interferent altered the average current signal of alkaloids derivatives by less than ± 5%, we considered that caused no interference [50]. The analyses of the obtained data allowed us to conclude that these compounds do not interfere or at least the interference is negligible, when are determined by cyclic, square-wave and differential pulse voltammetry on electrochemically activated glassy carbon electrode.

3.5. Application of voltammetric methods to pharmaceuticals containing alkaloids

Some commercial pharmaceutical formulations (tablets and capsules) containing different alkaloids were analyzed to determine these substances simultaneously in order to evaluate the validity of the proposed methods (cyclic voltammetry, differential pulse voltammetry and square-wave voltammetry on electrochemically activated glassy carbon electrode).

The best results were obtained with square-wave and differential pulse voltammetry, and the results are presented in Table 3.

The signals were confirmed to be due to the substances studied by enhancement of the peaks when standard correspondent solutions were added to the sample solutions.

As can be observed in Table 3, the amount of active substances found in the drug samples are fairly close to the labelled amounts. The recovery rates of the active substances when the pharmaceutical formulation contains only one active compound are between 98.9

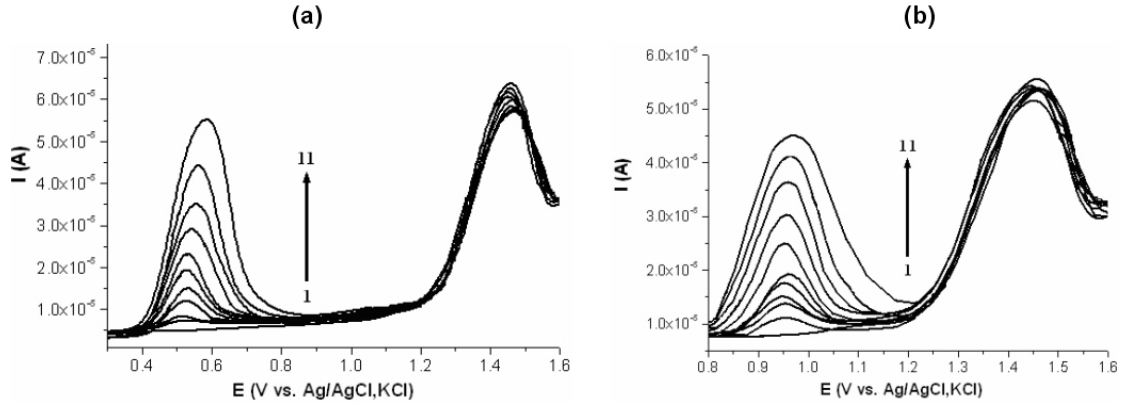


Figure 7. Differential pulse voltammograms on electrochemically activated glassy carbon electrode for (a) a 10^{-3} mol L^{-1} caffeine solution in the presence of different paracetamol concentrations: (1) 0, (2) 10^{-6}, (3) 5×10^{-6}, (4) 10^{-5}, (5) 5×10^{-5}, (6) 7.5×10^{-5}, (7) 10^{-4}, (8) 2.5×10^{-4}, (9) 5×10^{-4}, (10) 7.5×10^{-4}, (11) 10^{-3} mol L^{-1} in acetate buffer pH 4.5 and (b) a 10^{-3} mol L^{-1} caffeine solution in the presence of different acetylsalicylic acid concentrations: (1) 0, (2) 10^{-6}, (3) 5×10^{-6}, (4) 10^{-5}, (5) 5×10^{-5}, (6) 7.5×10^{-5}, (7) 10^{-4}, (8) 2.5×10^{-4}, (9) 5×10^{-4}, (10) 7.5×10^{-4}, (11) 10^{-3} mol L^{-1}, in acetate buffer pH 4.5 (reference electrode Ag/AgCl; auxiliary electrode Pt wire; scan rate 0.04 V s^{-1}; amplitude 100 mV; modulation time 5 ms; 20°C)

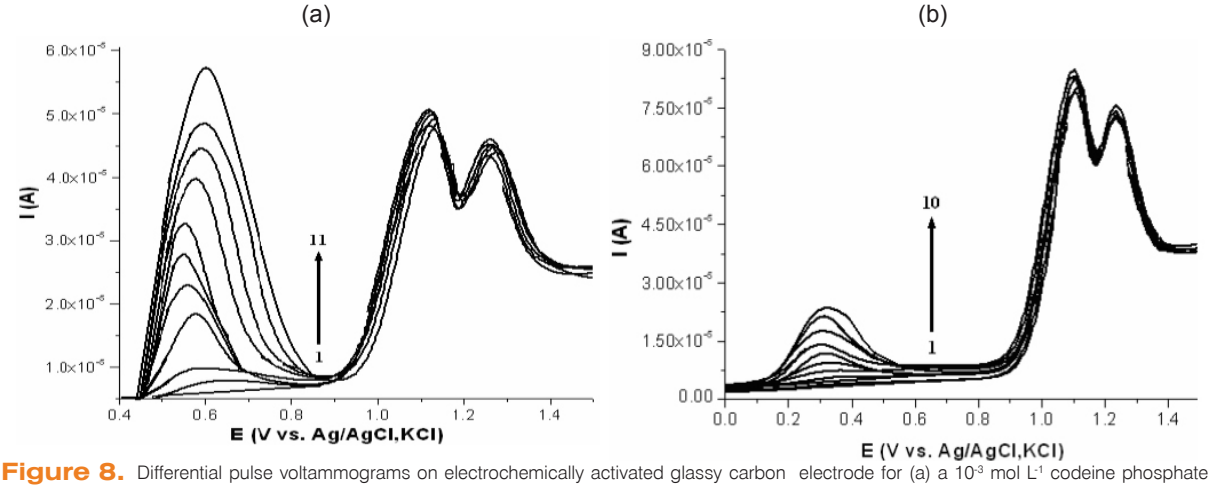


Figure 8. Differential pulse voltammograms on electrochemically activated glassy carbon electrode for (a) a 10^{-3} mol L^{-1} codeine phosphate solution in the presence of different paracetamol concentrations: (1) 0, (2) 10^{-6}, (3) 5×10^{-6}, (4) 10^{-5}, (5) 5×10^{-5}, (6) 7.5×10^{-5}, (7) 10^{-4}, (8) 2.5×10^{-4}, (9) 5×10^{-4}, (10) 7.5×10^{-4}, (11) 10^{-3} mol L^{-1}, in acetate buffer pH 4.5 and (b) a 10^{-3} mol L^{-1} codeine phosphate solution in the presence of different ascorbic acid concentrations: (1) 0, (2) 10^{-5}, (3) 2.5×10^{-5}, (4) 5×10^{-5}, (5) 7.5×10^{-5}, (6) 10^{-4}, (7) 2.5×10^{-4}, (8) 5×10^{-4}, (9) 7.5×10^{-4}, (10) 10^{-3} mol L^{-1} in acetate buffer pH 4.5 (reference electrode Ag/AgCl; auxiliary electrode Pt wire; scan rate 0.04 V s^{-1}; amplitude 100 mV; modulation time 5 ms; 20°C)

and 99.8% with SWV and between 99 and 99.8% with DPV, respectively (see as example the voltammograms obtained on *Miofilin®,* with aminophylline as the active substance, Fig. 11a). When the pharmaceutical formulations contains two and three active compounds the recovery rates are smaller, but it still can be considered to be good recoveries (between 93 and 99.7% with SWV and 94.6 to 99.6% with DPV). Fig. 11b presents the voltammograms obtained for *Saridon®,* with caffeine and paracetamol as the active substances. The relative standard deviations between 0.2 and 1.9% were obtained

The accuracies (expressed as relative difference between obtained and theoretical concentration, BIAS %) of the assay procedures were determined by analysing in the same day, three different samples of each compound and one different sample of each at three different occasions, respectively. The values of mean relative error are acceptable (Table 3); this shows the accuracy obtained by using these methods.

The precision of the proposed procedures was estimated by analyzing all the compounds in tablets assay solutions for three times in three successive days using SWV and DPV. The percentage recoveries based on the average of three separate determinations are given in Table 3. The results confirmed both the good precision of the proposed procedure and stability of the drug's solution.

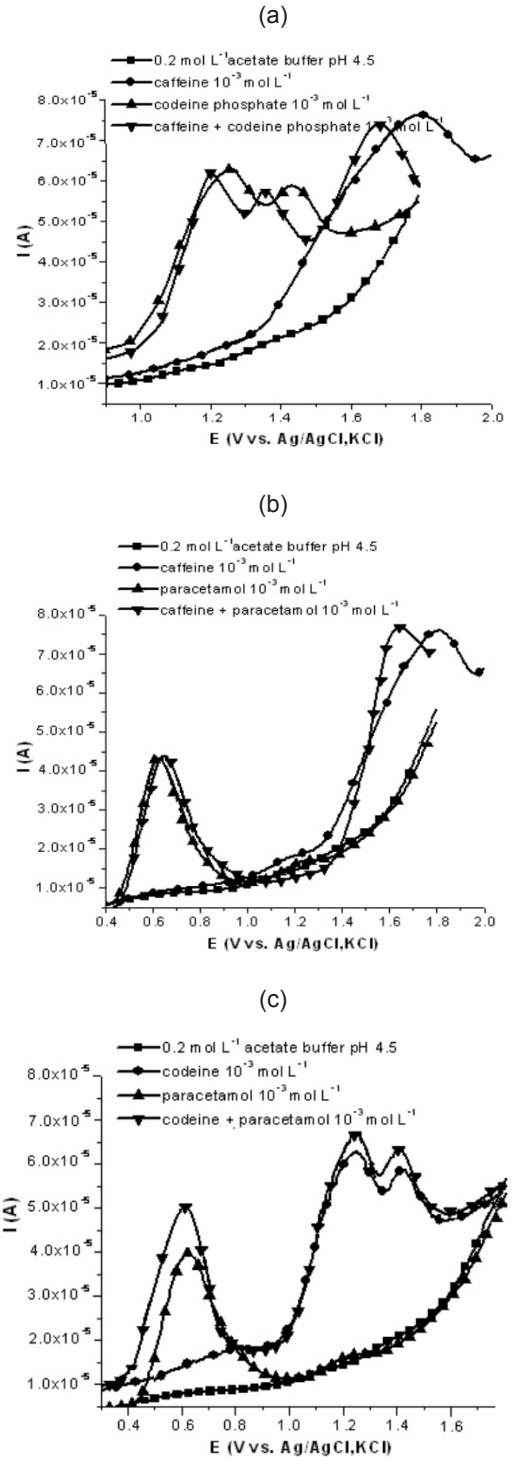


Figure 9. Square-wave voltammograms on electrochemically activated glassy carbon electrode for individual and simultaneous determination for (a) 10^{-3} mol L^{-1} caffeine and codeine phosphate solutions; (b) 10-3 mol L^{-1} caffeine and paracetamol solutions; (c) 10-3 mol L^{-1} codeine phosphate and paracetamol solutions in 0.2 mol•L^{-1} acetate buffer pH 4.5 (reference electrode Ag/AgCl; auxiliary electrode Pt wire; frequency 75 Hz; amplitude 50 mV; scan increment 5 mV; 20°C)

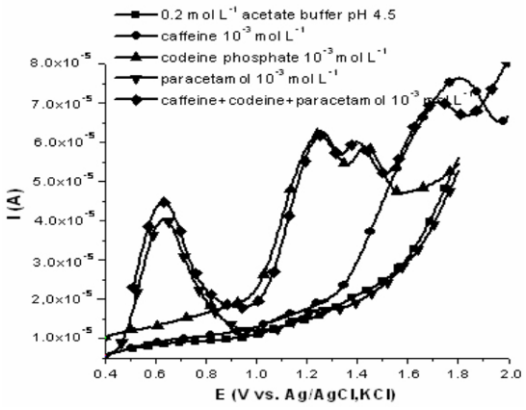


Figure 10. Square-wave voltammograms on electrochemically activated glassy carbon electrode for individual and simultaneous determination for 10^{-3} mol L^{-1} caffeine, codeine phosphate and paracetamol solutions in 0.2 mol L^{-1} acetate buffer pH 4.5 (reference electrode Ag/AgCl; auxiliary electrode Pt wire; frequency 75 Hz; amplitude 50 mV; scan increment 5 mV; 20°C)

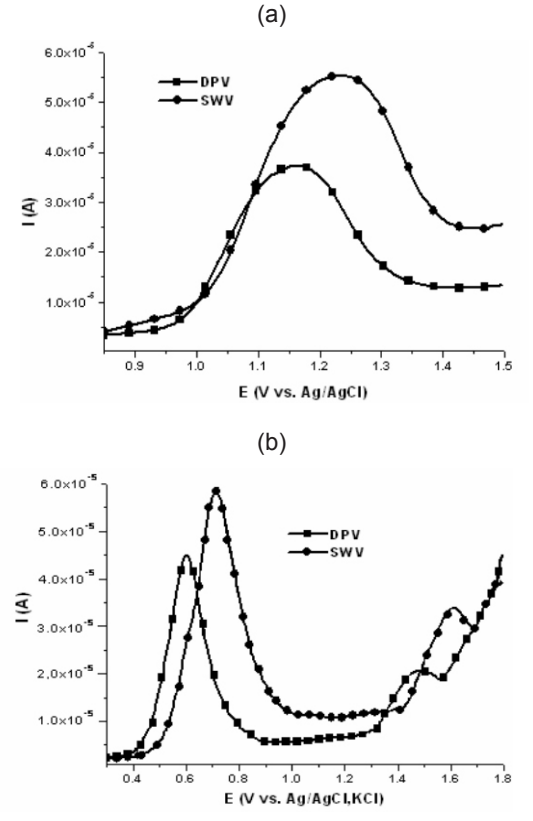


Figure 11. Square-wave (frequency 75 Hz; amplitude 50 mV; scan increment 5 mV) and differential pulse (scan rate 0.04 V s^{-1}; amplitude 100 mV; modulation time 5 ms) voltammograms obtained on electrochemically activated glassy carbon electrode for: (a) *Miofilin®* (aminophylline) and (b) *Saridon®* (paracetamol and caffeine) in 0.2 mol L^{-1} acetate buffer pH 4.5 (reference electrode Ag/AgCl; auxiliary electrode Pt wire; 20°C)

Table 3. Precision and accuracy obtained for pharmaceutical formulation (tablets and capsules)

Pharmaceutical formulation/ Producer	Active compound	mg/ tablet	Found (mg)		Recoveries ± RSD (%)		Accuracy (BIAS%)	
			SWV	DPV	SWV	DPV	SWV	DPV
Aminofilină 100 **Arena Group SA**	aminophylline	100	99.5	99.4	99.5±0.2	99.4±1.1	-0.5	-0.6
Miofilin 100 **Zentiva**	aminophylline	100	99.8	99.7	99.8±0.6	99.7±1.3	-0.2	-0.3
TheoSR200 **Glaxo Smith Kline**	theophylline	200	199	198.6	99.5±0.8	99.3±0.6	-0.5	-0.7
Teotard 350 **KRKA**	theophylline	350	347.6	347.2	99.3±1.8	99.2±1.4	-0.68	-0.8
Fosfat de codeină 15mg **Laropharm**	codeine phosphate	15	14.8	14.9	98.9±0.6	99.0±1.2	-1.33	-0.66
Papaverină clorhidrat,100mg **Laropharm**	papaverine hydrochloride	100	99.6	99.1	99.6±0.7	99.1±1.9	-0.4	-0.9
Papaverină clorhidrat,100mg **Arena Group SA**	papaverine hydrochloride	100	98.9	99.2	98.9±0.6	99.2±0.6	-1.1	-0.8
Sanador **Laropharm**	paracetamol	500	496.5	499	99.3±1.3	99.8±1.1	-0.7	-0.2
N-Antigripal **SC Meduman SA**	paracetamol	500	491.5	483.5	98.3±0.9	96.7±1.7	-1.7	-3.3
	caffeine	50	47.65	47.9	95.3±0.8	95.8±0.6	-4.7	-4.2
Paradoren 30/500 **Arena Group SA**	paracetamol	500	491.5	495.5	98.3±0.9	99.1±0.5	-1.7	-0.9
	codeine phosphate	30	29.2	29.5	97.3±0.2	98.4±1.2	-2.6	-1.66
Saridon **Bayer**	paracetamol	250	247	249	98.8±0.6	99.6±0.2	-1.2	-0.4
	caffeine	50	48.9	49.3	97.8±0.8	98.6±1.4	-2.2	-1.4
Calmant Forte **SC Remedia SRL**	paracetamol	400	386.4	391.2	96.6±1.7	97.8±1.1	-3.4	-2.2
	caffeine	25	23.8	24	95.2±0.5	96±0.7	-4.8	-4.0
	codeine phosphate	20	18.7	19	93.5±0.8	95±1.2	-6.5	-5.0
Calmogripin **SC Remedia SRL**	paracetamol	200	199.4	199.2	99.7±0.3	99.6±0.3	-0.3	-0.4
	caffeine	25	24.2	24.7	96.8±0.8	98.8±0.2	-3.2	-1.2
	codeine phosphate	10	9.6	9.59	96.0±0.5	95.9±1.1	-4.0	-4.1

SWV = square-wave voltammetry; DPV = differential pulse voltammetry

3.6. Real sample analysis (human urine)

The electrochemical test methods attempted to detect the presence of alkaloids in urine samples collected from individuals consuming coffee (the presence of caffeine), and from a patient under treatment with *Miofilin*® (the presence of aminophylline, which is the active substance in this pharmaceutical product).

Urine samples collected from subjects were determined after filtration, without prior dilution and after dilution with 0.2 mol L^{-1} acetate buffer to pH 4.5 with the aid of cyclic voltammetry, square-wave voltammetry and differential pulse voltammetry. The best results were obtained with differential pulse voltammetry on electrochemically activated glassy carbon electrode for

Table 4. Data obtained for human urine samples

Samples	Active compound	Peak current (μA)	Concentration (mol L^{-1})
Sample 1	caffeine	41	6.21×10^{-7}
Sample 2	caffeine	53	7.77×10^{-7}
Sample 3	aminophylline	6	1.15×10^{-7}

samples diluted with acetate buffer to pH 4.5.

For correct attribution of the oxidation peak to active substance as measured by the urine samples volumes of caffeine and aminophylline solutions were added one millilitre of solution, then another millilitre in order to check the influence of analyte concentration on the electrochemical response respectively. It is to be noted that in all examined cases, the intensity peaks which were attributed to the electrochemical oxidation process of caffeine (samples 1 and 2) and aminophylline (sample 3) are increasing when adding corresponding analyte solutions, which proves their correct allocation.

The caffeine and aminophylline concentrations determined from the current intensity values for the correspondent oxidation peaks are presented in Table 4.

4. Conclusions

Square-wave voltammetry and differential pulse voltammetry on electrochemically activated glassy carbon electrode can be used for the qualitative and quantitative determination of the studied alkaloids (aminophylline, caffeine, theophylline, and codeine phosphate and papaverine hydrochloride) and of other compounds contained in pharmaceutical formulations or in urine samples.

From the electrochemical determinations it can be observed that these compounds do not present significant interference when two or more are present in solution at the same time, so the proposed methods can be easier adopted for the simultaneous determination of alkaloids in real samples and pharmaceutical formulations. These electroanalytical methods have the advantages of being less time consuming and less expensive than classical analytical methods such as HPLC and GC-MS.

Since the response time for electrochemical determination is very fast, the other advantage of the proposed method is that all the measurements can be done immediately after the electrode activation.

Various commercial pharmaceutical forms containing aminophylline, caffeine, theophylline, codeine phosphate, papaverine hydrochloride or/and paracetamol as active compounds were analyzed by square-wave voltammetry and differential pulse voltammetry and yielded good average recoveries for all the tested substances (from 95.9 to 99.8%).

The electrochemical test methods attempted to detect the presence of alkaloids in urine samples (caffeine and aminophylline). Best results were obtained using DPV performed on electrochemically activated glassy carbon electrode. Thus the detected caffeine concentration is 6.21×10^{-7} mol L^{-1} and 7.77×10^{-7} mol L^{-1}, while aminophylline concentration detected is 1.15×10^{-7} mol L^{-1}.

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