DETERMINATION OF CAFFEINE AT UNMODIFIED CARBON PASTE ELECTRODE

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In this paper, electrochemical and electroanalytical characterization of caffeine has been carried out, for the first time, with unmodified carbon paste electrode and in combination with cyclic voltammetry and square-wave voltammetry as the measuring techniques of choice. After defining the proper conditions for the electrode oxidation in highly acidic media and setting the key experimental parameters accordingly, a simple method was subsequently tested on model analyses of real samples (energy and Cola drinks).

Introduction

Caffeine (3,7-dihydro-1,3,7-trimethyl-1*H*-purine-2,6-dione) is a derivative of xanthine and belongs among the family of natural alkaloids. This substance is well known for its effect upon the central nervous system and can be found in various food products, such as soft and energy drinks, coffee and tea. In other words,

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caffeine apparently represents the most common and most frequent alkaloid, accompanying the human population for centuries.

From an analytical point of view, the determination of caffeine in various food and pharmaceutical products is always a challenge, also due to its different concentration levels and occurrence in samples with very diverse matrices. Thus, there has been great effort to elaborate reliable procedures for its determination, which can be illustrated on a collection of separation methods employing (i) liquid chromatography with UV-detection [1], with a diode-array detector [2] or being coupled with MS-MS tandem [3]; (ii) gas chromatography with MS detection [4], or combined with the previous drop-to-drop solvent microextraction [5], as well as ultrasound-assisted extraction-dispersive liquid-liquid microextraction [6]. Another possible approach has then utilised (iii) Fourier transform infrared spectrometry (FT-IR), being applicable to determine caffeine in selected soft drinks and black tea leaves [7,8].

Concerning electroanalytical measurements, they are generally able to compete with other instrumental techniques thanks to inexpensive instrumentation and relatively simple analysis. However, in the case of caffeine, one should consider that its electrochemical characteristics involve potentially uneasy oxidation — for the corresponding reaction scheme, see Fig. 1 — taking place at considerably high positive potentials, even beyond +1.3 V vs. Ag/AgCl [9]. This requires a careful choice of the working electrode which has to offer sufficiently wide potential range and a low background current near the anodic potential limit in order to avoid a possible overlap of the oxidation signal of caffeine with that of the approaching electrolyte decomposition.
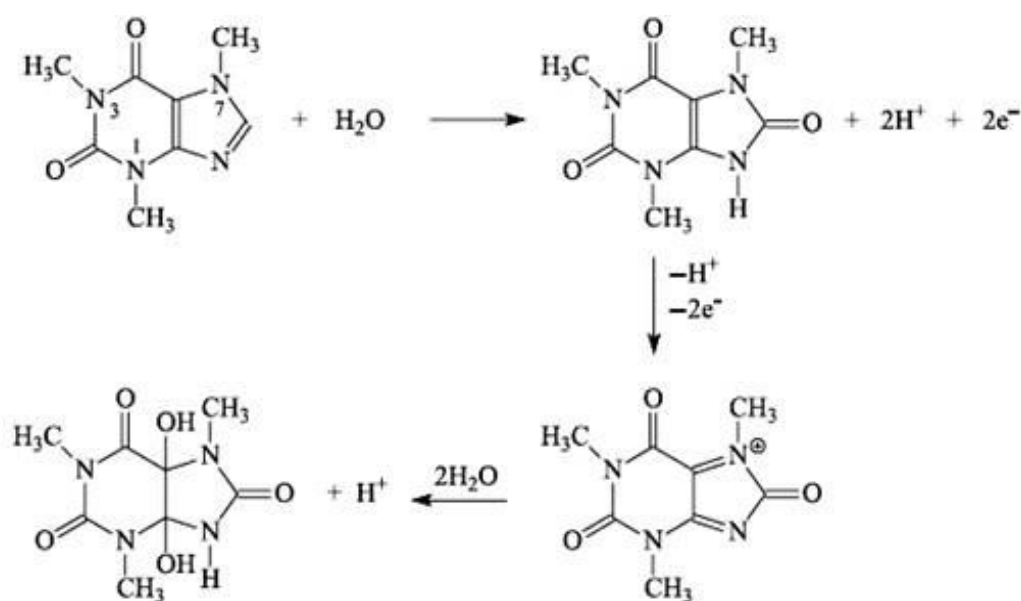

Fig. 1   Scheme of the two-step oxidation of caffeine with hydrolysis of the oxidation product (taken from Ref. [9])

For such conditions, the most reliable electrochemical detection of caffeine can be accomplished when using carbon-based electrodes [10], namely with (i) the glassy carbon electrode (GCE) used either in the bare configuration [11] or being pretreated with a suitable modifier like Nafion® [12]; (ii) graphite pencil electrode (GPeE, [13]); (iii) boron-doped diamond electrode (BDDE), again, as bare [14] or chemically modified [15]; and with some other ones, such as edge-plane pyrolytic graphite electrode (PyGE [16]), carbon fiber ultramicroelectrode (CFimE [17]), and carbon ceramic electrode (CCeE [18]).

Finally, solid-like carbon paste electrodes (CPEs [19,20]) have been shown particularly applicable to the determination of caffeine, which can be documented on a series of methods [21-25]; each one employing a chemically or a biologically modified carbon paste electrode (CMCPE or CP-biosensor, respectively). Mostly, the respective procedures have benefited from specific modifications of the carbon paste material [20], enabling to maintain the experimental conditions for effective oxidation of caffeine, occurring at more favourable — i.e., less positive — potentials.

When going through the individual methods with CPEs, CMCPEs and CP-biosensors, a highly selective determination of caffeine was described by Alizadeh *et al.* [21] introducing a voltammetric sensor with the incorporated molecularly imprinted (caffeine-selective) polymer applicable in analyses of urine and drug samples. A CPE containing 1,4-benzoquinone was then used by Ly *et al.* [22] for indirect determination of caffeine in coffee, utilising the suppression of 1,4-benzoquinone response in the presence of caffeine. The same research team then described an *in-vivo* monitoring with specially implanted neurosensor [23] that had combined the efficiency of thin bismuth layer with catalytic properties of carbon nanotubes; all being integrated in one configuration of the Bi/CNTs-CPE type. In this work, caffeine was determined simultaneously with catechol and a special diagnostic kit could then be implanted into fish or rat neuro-cells. Yet another work has come from Ly *et al.* [24], examining the new detection tool for caffeine based on CNTs and the immobilized DNA. As experimentally proved, their sensor could be implanted into a leaf skin or an animal brain cell and subsequently employed in real-time / *in-vivo* analysis. Last but not least, Sanghavi *et al.* [25] introduced a CPE modified with CNTs and additionally *in situ*, with surfactant *Triton X100*®; the resultant configuration being employed for separate or simultaneous determination of three analytes – caffeine, acetaminophen, and aspirin; each one and altogether as well detectable in urine, whole blood, and selected pharmaceutical samples.

Because none of the methods described above had dealt with measurements at unmodified carbon paste(s), this encouraged us to revise the direct oxidation of caffeine with CPE(s) typical for research work within our working group (see, e.g., Ref. [20] and Refs therein). In the following sections, an initial study of this kind is summarised via the most important observations and results. As the latter

seems to have some promise, it is already decided to continue in this investigation with some novel types of CPEs, having undergone recently the inevitable basic characterisation [26-28].

Experimental

Chemicals and Solutions

All chemicals used for the preparation of stock solutions were of analytical grade and purchased from Merck and Sigma-Aldrich, or Lachema (Brno, the Czech Republic). The stock solutions of H_2SO_4, HNO_3, $HClO_4$, and HCl were prepared as 1 M in concentration when using the respective chemicals and distilled water. For the proper experimentation, all the solutions were diluted as required. Water used throughout the work was obtained by double distillation of deionised water in a laboratory made distillation unit. All the measurements were carried out at ambient temperature (22 ± 1 °C).

Sample Preparation

Samples of energy drinks and soft cola drinks were degased in an ultrasonic bath for 15 minutes. From so prepared sample, a volume of 2 ml was taken for analysis otherwise performed in the supporting electrolyte of 20 ml as the total volume.

Methods and Procedures

Measuring Apparatus and Other Instrumentation

An electrochemical system EMSTAT (Palm Instrument BV, Holland) was used in combination with PS-Trace 3.0 software. This assembly was connected to an external electrode stand with the three-electrode cell incorporating the working electrode (CPE, see below), an Ag/AgCl/3M KCl as the reference, and a Pt-plate serving as auxiliary electrode. Stirring was accomplished with magnetic bar(s) rotated at ca. 400 rpm. Wherever needed, measurements of pH were made with an InoLab device (model "pH 720"; WT Werke, Germany) with the combined pH-electrode ("Sen Tix 41" type; the same manufacturer).

Preparation of the Working Electrode

Carbon Paste. The mixture of choice was prepared by thoroughly hand-mixing of 75 % (w/w) graphite powder ("CR-5"; Maziva Týn, the Czech Republic) and 25 % (w/w) paraffin oil (Merck), when using porcelain mortar with a pestle.
Carbon Paste Electrode. The homogenised paste was packed into a piston driven carbon paste holder of our own construction [29]. The carbon paste surface was renewed by extruding of ca. 0.5 mm of the carbon paste mixture out of the holder with subsequently smoothing with wet filter paper. Normally, this mechanical renewal was made before each new set of measurements; for instance, prior to analysis of each sample.

Electrochemical Measurements

Cyclic voltammetry (CV) with linear scan was used in the fundamental electerochemical studies; typically, within a potential range from +0.8 to +1.7 V vs. Ag/AgCl at a scan rate of 50 mV s^{-1}. Square-wave voltammetry (SWV) was the technique of choice for all quantitative measurements; usually, with the SWV-ramp set to the following conditions: initial potential, E_{init} = +0.8 V; final potential, E_{fin} = +1.8 V; pulse amplitude, ΔE_{SW} = 50 mV; frequency, f_{SW} = 50 Hz, and the step increment, i_{SW} = 5 mV. Before each voltammetric scan, a potentiostatic cleaning step had to be applied by setting a potential of –1.0 V vs. Ag/AgCl for 60 s.

Results and Discussion

pH-Dependence Studies. As with other organic compounds studied electrochemically, the electrode behavior in dependence of pH (of the supporting electrolyte) was examined first. The respective studies performed in a series of Britton-Robinson buffers with pH from 1.8 to 12.0 have shown that the best developed response could be obtained in the most acidic solution; see Fig. 2. Moreover, this oxidation signal could be somewhat improved in yet more acidic media as shown in measurements in diluted mineral acids; namely, H_2SO_4, HNO_3, $HClO_4$, and HCl. By evaluating the overall signal-to-noise characteristics, a solution of 0.5 M H_2SO_4 was finally found as the most suitable, representing the supporting electrolyte of choice in all subsequent measurements.

Concerning the oxidation of caffeine at higher pH, it was confirmed that the corresponding signals rapidly decreased with the increasing pH; moreover, the background of the unmodified CPE in such solutions had started to increase markedly, deteriorating principally the electrode performance under such conditions.
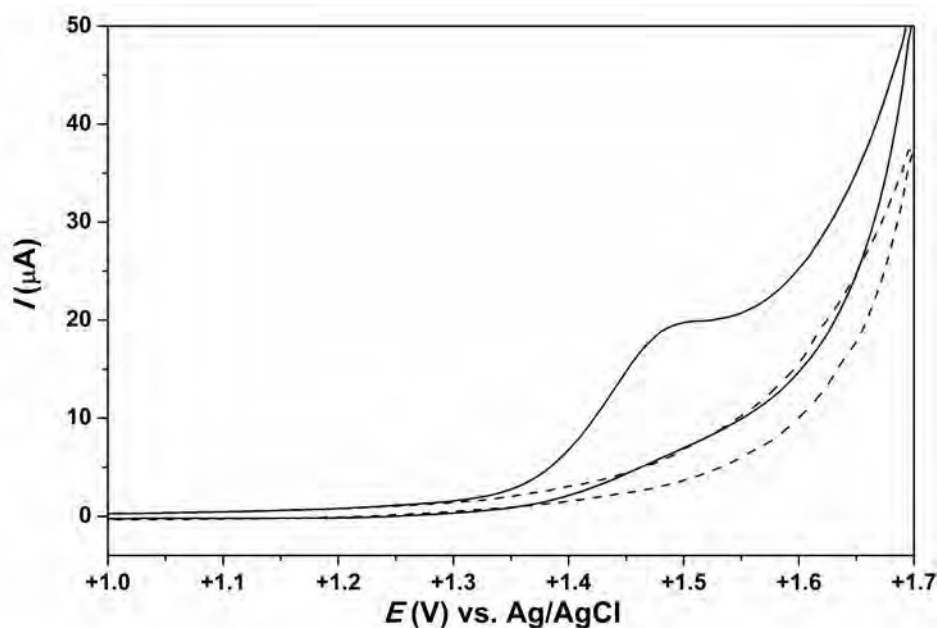
Fig. 2   Cyclic voltammetry of caffeine using CPE in an acidic medium (pH 1.8). Dashed line (- - -) blank, full lines (—) caffeine; c(caffeine) = 100 µmol l^{-1}

"I_P vs. $v^{1/2}$" Study. The nature of the electrode process of interest was examined next, studied again in the most acidic solution of Britton–Robinson buffer, pH 1.8. It was found that there was linear dependence between the peak height of caffeine and the square root of scan rate, confirming the dominance of the diffusion-controlled transport of caffeine towards the electrode surface.

Instrumental Parameters (of the SWV Ramp). The individual setting needed for the SWV mode has been taken from our previous study on the determination of nicotine [30] being very similar in nature; especially, with respect to the oxidation process and its signal.

Calibration Measurements. The following experiments were devoted to the calibration measurements in the concentration range down to the micromolar level. The resultant calibration curve illustrated in Fig. 3 was found pretty linear, corresponding to the regression data specified by the figure inset.

Model Analyses of Real Samples. Two typical samples with caffeine were selected: (i) energy drinks (in further text denoted as "En") and (ii) Cola-type drinks ("Col"), when the individual determinations were evaluated by means of the multiple standard additions method. Typical voltammograms already recorded in the SWV mode are depicted in Fig. 4 and the respective results summarized in Table I. (Note: Due to a test character of the quantitative analysis, all the determinations were performed as the single determinations only; i.e., $n = 1$).

When comparing the results with the values declared by manufacturer(s), it can be seen that all the analyses of energy-drink samples (En) have given satisfactory results although being slightly lower (of ca. 10 %) than the expected content.
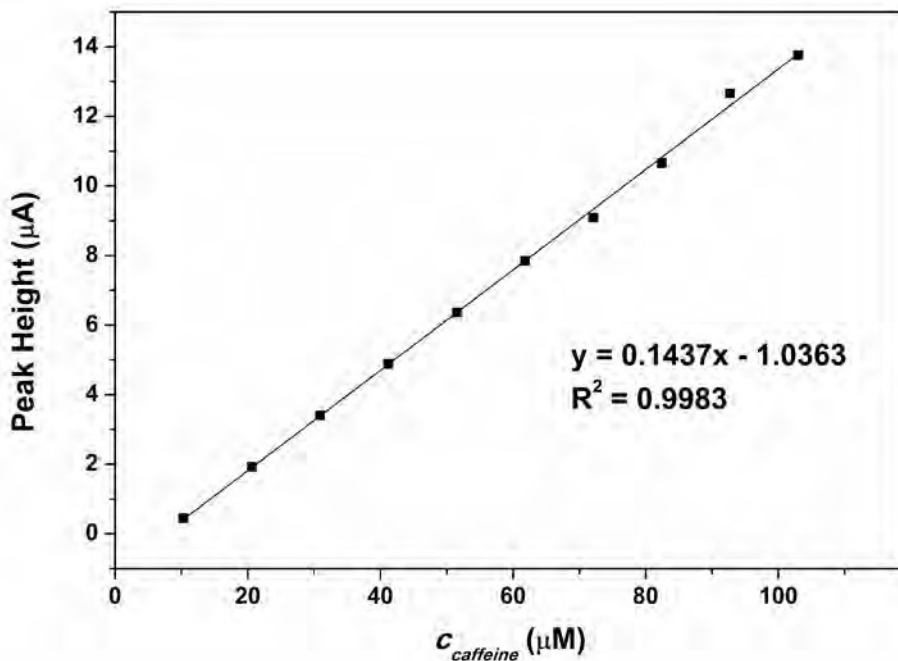
Fig. 3   Calibration dependence of SWV determination of caffeine at the low micromolar level with unmodified CPE in 0.5 M H_2SO_4. For more details, see Experimental
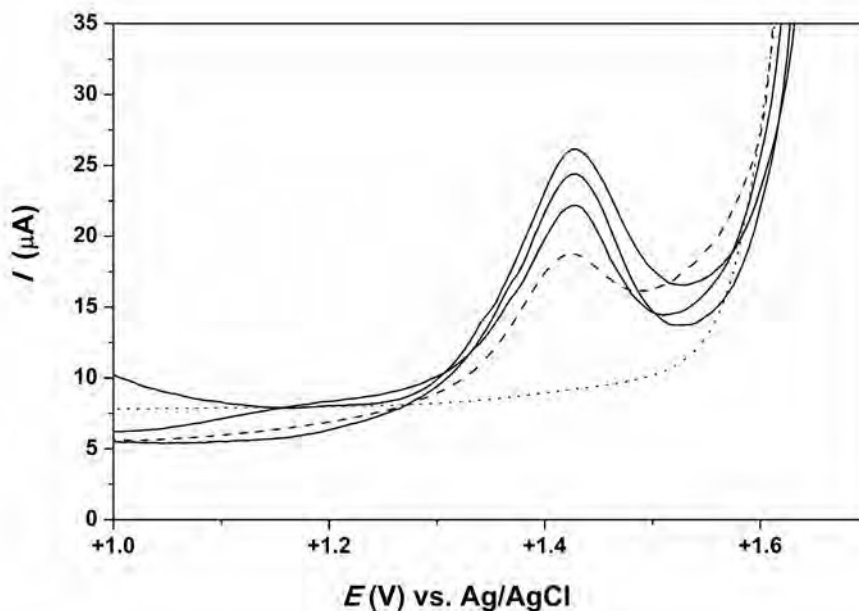

Fig. 4   Typical SW-voltammograms obtained by analysing the model sample of energy drink: dotted line ($\cdots$) baseline; dashed line (- - -) sample; full lines (—) standard additions (3 aliquots) with concentration c(std) = 51.5 μmol l^{-1}. For more details, see Experimental

Table I   Voltammetric analysis of model real samples. Survey of results

Sample	Declared, mg per 100 ml	Found, mg per 100 ml
En No. 1	32	26.9
En No. 2	32	25.5
En No. 3	30	25.4
En No. 4	32	26.2
En No. 5	32	28.9
En No. 6	32	29.5
Col No. 1	15	8.2
Col No. 2	15	6.1

In the case of Cola-type samples, however, the individual analyses led to significantly lower results (about a half of the declared content), which can perhaps be explained by some specific matrix effect and the presence of a substance hindering caffeine to be oxidized quantitatively. This suggested clearly that the whole method is still at the initial stage and some experimental conditions have to be further studied and appropriately reoptimised.

Conclusion

In this article, an unmodified carbon paste electrode was for the first time tested for electrochemical characterization of caffeine and its determination in real samples. An initial study of this kind and the series of model analyses have resulted in the following statements:

- Electrochemical oxidation of caffeine at unmodified CPE of typical composition (made of paraffin oil) takes place at rather high potential (of about +1.2 V vs. Ag/AgCl), requiring a highly acidic medium, with pH < 1.
- Despite these unfavourable conditions, the respective oxidation peak is diffusion-transport controlled, satisfactorily reproducible, and thus applicable as the analytical signal in quantitative analysis.
- In combination with sensitive SW-voltammetry, the (unmodified) CPE tested can be used for the determination of caffeine in energy and Cola drinks; nevertheless, the initial simple version of the method can be recommended only for analyses of orientation character, with higher relative error (of about ±10 %), while its more reliable adaptation would require still further studies and overall improvement of some experimental parameters.

Acknowledgement

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