Microchemical Journal 142 (2018) 297–304

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

A progressive electrochemical sensor for food quality control: Reliable determination of theobromine in chocolate products using a miniaturized boron-doped diamond electrode

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ARTICLE INFO

Keywords:
Dimethylxanthine alkaloid
Boron-doped diamond electrode
Electrochemical sensor
Voltammetric techniques
Real sample analysis

ABSTRACT

In the present paper, a miniaturized boron-doped diamond electrode (BDDE) is proposed as a progressive electrochemical sensor for simple, fast and reliable quantification of dimethylxanthine alkaloid, theobromine (TB). Using cyclic voltammetry, the studied xanthine provided one well-shaped, irreversible and diffusion-controlled oxidation peak at relatively high potentials (+1.2 V vs. Ag/AgCl/3 M KCl reference electrode) in the presence of 0.1 M H_2SO_4. After selection of suitable experimental conditions, the linear calibration curves for TB were obtained in the concentration range from 0.99 up to 54.5 μM with the sensitivity of 0.07 μA/μM providing both differential pulse (DPV) and square-wave voltammetric (SWV) techniques, respectively. The elaborated voltammetric protocol yielded low detection limits of 0.42 and 0.51 μM accompanied by adequate intra-day repeatability (relative standard deviation of 2.5 and 1.7%) using DPV and SWV procedure, respectively. The interference study revealed the reasonable selectivity when taking the target food samples into account. The practical applicability of the voltammetric protocol using a miniaturized BDDE was verified in the analysis of six commercially available brands of chocolate products with the determined mass percentages of TB ranging from 0.75 to 2.24% and from 0.69 to 2.15% using DPV and SWV procedure, respectively. The obtained results were also in a good agreement with those achieved by reference titration method with potentiometric indication. The progressive electrochemical sensor based on a miniaturized BDDE has appeared to be an attractive candidate for practical applications in food quality control. Besides, the proposed voltammetric protocol presents advantages when compared to others techniques (e.g. chromatography), concerning simplicity, cost, speed of analysis, waste generation (environmentally friendly) and samples pretreatment (only dilution in electrolyte solution prior to analysis).

1. Introduction

Theobromine (IUPAC name: 3,7-dimethylpurine-2,6-dione, here abbreviated as TB), also known as xantheose, is a dimethylxanthine alkaloid present in high amounts in cocoa, and thus in miscellaneous foods such as chocolate and related products [1]. Although chemically very similar to other methylxanthines such as caffeine and theophylline, TB stimulates the central nervous system to a lesser extent than these derivatives [2]. Ingestion of TB manifests health benefits, including protection of the enamel surface of teeth [3], cough suppression [4] and cardiovascular protection [5]. More recently, TB was found to inhibit uric acid crystallization, suggesting that it may be useful in the treatment of uric acid nephrolithiasis [6]. In addition, TB exerts adverse effects on animal physiology such as reduction in cattle milk yield, thymus atrophy in rats, retarded growth and lethargy in pigs [7].

In recent decades, nutrition research has focused on the investigation of various bioactive dietary compounds, widely found in many plant-based foods and beverages, in order to elucidate their beneficial properties to human health. In this respect, the popularity of chocolate products makes TB one of the most frequently determined analyte in

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https://doi.org/10.1016/j.microc.2018.07.007
Received 15 June 2018; Received in revised form 8 July 2018; Accepted 9 July 2018

food analysis. Besides, the safety and tolerability of TB are in conformity with the benefit and risk evaluation in clinical practice [8]. Therefore, due to the widespread consumption of TB and its potential physiological effects, both health professionals and consumers desire to know real contents of this alkaloid in various food products. In this viewpoint, it should be noted that food companies within the commercially available brands of chocolate products usually do not provide detailed nutrition information, especially on TB content. Based on the food relevance and medicinal objectives of TB, novel and advanced analytical protocols of high efficiency for the rigid food quality control of this dimethylxanthine alkaloid are still needed. Additionally, reliable analytical methods to support measurements made for compliance with nutritional and dietary supplement labelling laws are also needful by laboratories in the food testing and nutrition communities.

Although a great variety of analytical methods and procedures have been established and applied to the detection and determination of TB in various matrices, a fully automated and validated high performance liquid chromatography with ultraviolet detection (HPLC-UV) [9, 10] and mass spectrometry (HPLC-MS) [11, 12] are the most frequently used in past few years. Moreover, capillary electrophoresis [13] and spectrophotometry [14] have also been among the most recently developed analytical methods for TB sensing. Despite the indubitable accuracy, precision and sensitivity of all aforementioned methods, they possess some practical restrictions that should come into consideration in case of particular experiment design including relatively costly equipment, usage of toxic organic compounds (solvents) generating high amounts of waste as well as complex and time-consuming procedures for sample preparation which make them unsuitable for on-site and decentralized sensing. Thus, searching for new, rapid and low-cost analytical methods and protocols for simple and reliable determination of TB in various matrices is still actively pursued.

Meanwhile, electrochemical methods have proven to be comfortable and beneficial tool for trace determination of various substances owing to the simplicity, fast response, low cost, technology based on green chemistry, miniaturization and possibility to on-line field monitoring [15]. Moreover, these methods are less sensitive to matrix effects when compared to aforementioned analytical methods. Furthermore, the knowledge of the redox properties of a particular analyte could also be an important pharmaceutical tool since it can be relevant to understand its metabolic fate or in vivo redox processes and pharmacological activity [16]. Up to now, data from scientific literature has reflected a lack of reported papers dealing with electrochemical determination of TB. It is due to the fact that the most researchers in the area of electroanalytical chemistry have largely made effort on study of electrochemical behaviour and determination of other methylxanthines such as caffeine and theophylline [17, 19, 20]. With regards to TB, the pioneering work was introduced by Hansen and Dryhurst who explored electrochemical oxidation of caffeine and TB on stationary pyrolytic graphite electrode (PGE) with identification of their electrolysis products, however, without any analytical performance evaluation [21]. Recently, Peng et al. developed nanobiohybrid sensor based on glassy carbon electrode (GCE) modified by both carboxyl-functionalized multiwalled carbon nanotubes (fMWCNTs) and soluble biopolymer sodium salt of carboxymethylcellulose (CMC) for TB determination in green tea, chocolate and coffee samples. The limit of detection (LOD) was found to be 0.31 μM [22]. Vinjamuri et al. examined the selectivity of caffeine- and TB-imprinted polypyrrole electrodes using pulsed amperometric detection (PAD) enabling quantification up to equimolar amounts of these competitive analytes [23]. A hyphenated method based on HPLC in combination with amperometric determination (AD) for adenine, caffeine, theophylline and TB was presented by Meyer et al. The authors used GCE as working electrode in wall jet arrangement with a detection potential set on +1.4 V after elimination of matrix effects by application of solid-phase extraction (SPE) resulting in LOD of 2.5 ng for TB [24]. However, despite the adequate applications of conventional (graphite, GCE) [21, 24] and chemically modified electrodes [22, 23] for electrochemical study and/or sensing of TB, the electrochemists, material engineers and nanotechnologists are constantly forced to investigate and develop material platforms as electrode substrates for perspective and fool-proof electrochemical sensors and biosensors.

Boron-doped diamond (BDD) has attracted distinguished attention in scientific community in last decade as a potential candidate for next-generation and environmental-friendly electrochemical sensor platforms. It is due to the unique properties of this advanced electrode material such as wide working potential range (ca. 3.5 V), high electrical conductivity, low background current, good chemical and mechanical stability, enhanced signal-to-noise ratio and quasi-metallic/metallic conductivity according to the boron doping level in diamond structure [25–28]. Owing to these properties, the electrochemical sensors based on a BDD electrode (BDDE) render high sensitivity, low values of detection limits with reversible or quasi-reversible electron transfer kinetics as well as long-term stability [29]. Nowadays, many researchers have tried to develop and apply high-performance electrochemical sensors using a BDDE in a miniaturized scale in order to reduce and minimize the whole measurement set-up. In this respect, the commercial miniaturized electrochemical sensor based on BDD material has been recently applied in drug analysis by our working group [30]. As to TB sensing, Spătaru et al. firstly examined the electrochemical behaviour and determination of xanthine and its naturally occurring *N*-methyl derivatives (theophylline, caffeine and TB) on BDDE. The electrochemical activity of TB was observed in supporting electrolyte in the pH range from 2 up to 8 and the current response of TB appeared to be linear within the concentration range of 1–400 μM with the achieved sensitivity of 0.045 μA/μM. However, the real practise analysis of food products containing TB was not provided by this method [31].

In general, one of the main goals of modern analytical chemistry consists in an application of rapid high throughput methods and procedures at the small and miniaturized scale, accompanied by simultaneous reduction in the number of experiments/measurements, time of analysis, energy and reagent consumption. Considering electrochemical sensing of TB, as listed above, it can be concluded that there is a few published papers, mostly dealing with the use of conventional and/or chemically modified electrodes. Taking these facts into account, a progressive miniaturized electrochemical sensor based on a BDD working electrode is herein proposed for the first time for simple, rapid and reliable quantification of TB in chocolate food products. The presented voltammetric protocol includes the selection of suitable experimental conditions for electrochemical oxidation of TB on the miniaturized BDD sensor, assessment of nature of analyte electrode reaction, analytical performance evaluation using pulse voltammetric techniques, method validation and interference study. Likewise, the variety of commercial chocolate food products are considered to be simply and reliably analyzed with the results accomplished in a good agreement with those obtained by the reference titration method with potentiometric indication. In view of this fact, the developed voltammetric protocol could be considered as a sensitive, rapid and cost-effective alternative for TB sensing to other analytical methods and procedures so far reported in scientific literature.

2. Experimental

2.1. Chemicals

Analytical standard of TB (CAS No. 83-67-0, purity ≥ 98.5%) was bought from Sigma Aldrich (Slovakia). 10 mM stock solution of TB was prepared by dissolution of suitable amount of this standard in small volume of deionized water and filled up completely with deionized water to 100 mL in volumetric flask. This stock solution was constantly stored in refrigerator (when not used), without any consistency changes during a few weeks. Britton-Robinson (BR) buffers in pH range of 2–12