

Optimization of simultaneous voltammetric determination of pyridoxine and caffeine

Amir Shaaban Farag, Milan Sýs, and Karel Vytřas

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Studentská 573, 532 10 Pardubice, Czech Republic,
E-mail: st56093@student.upce.cz

Abstract

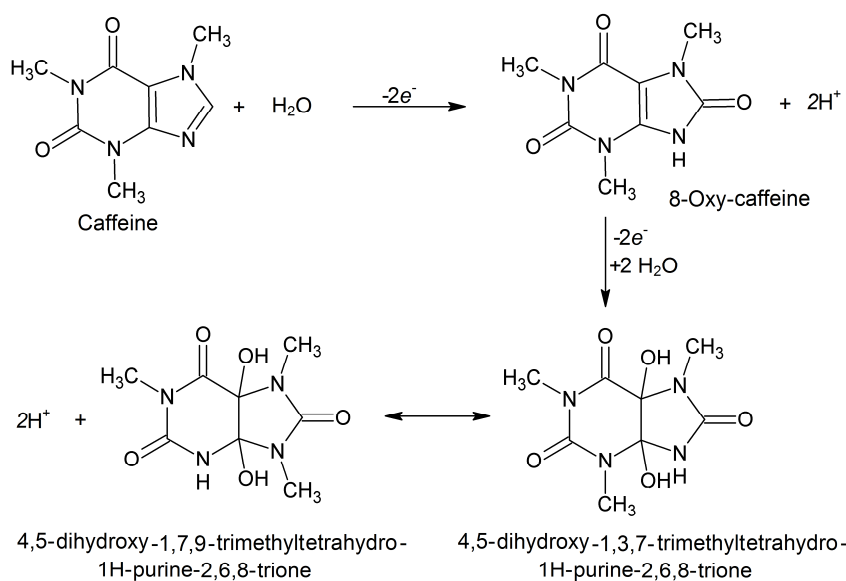
This electrochemical study is focused on finding the optimal working conditions necessary for simultaneous voltammetric determination of xanthine alkaloid caffeine and pyridoxine (vitamin B₆) dietary food supplement. Consequently, the aim is to develop a simple method which should be suitable for monitoring of these biologically active organic compounds in commercial energy drinks. This contribution should be understood as an initial study of the simultaneous determination of both substances. Their anodic oxidation at a glassy carbon electrode coated with a thin layer of Nafion[®] using differential pulse voltammetry in the Britton-Robinson pH 4.5 buffer as the supporting electrolyte has been optimal for the time being. Further, the addition of small amounts of selected surfactants (nonionic Triton X-100, anionic sodium dodecyl sulfate, cationic cetylpyridinium chloride, and 1,3-didecyl-2-methylimidazolium chloride) to the selected BRB solution was tested to improve the repeatability of the obtained results.

Key words: Carbon nanomaterials; Caffeine; Energy drinks; Glassy carbon electrode; Nafion[®]; Pyridoxine; Surfactant.

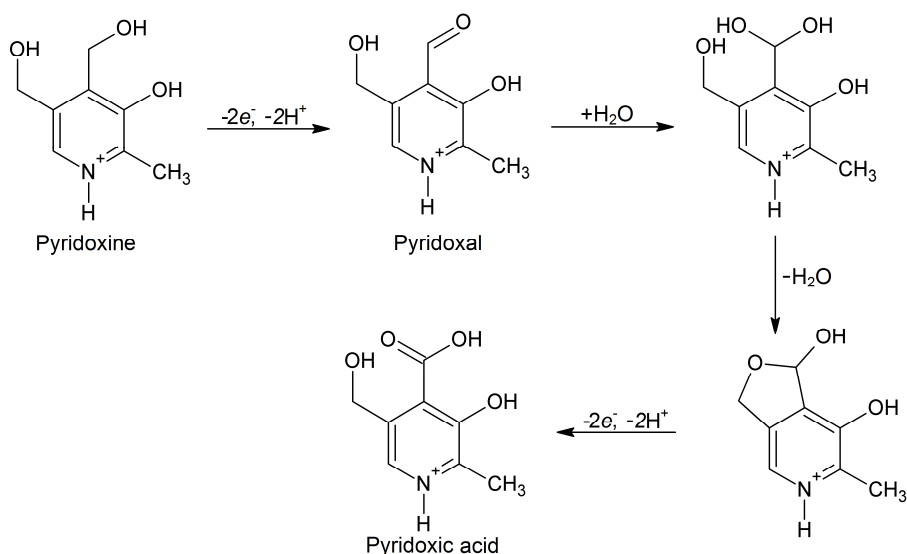
Introduction

Both pyridoxine (known as vitamin B₆, further VB₆) and caffeine (CA) are used as the main components of commercially attractive energy drinks. Other common ingredients include sugars such as glucose and sucrose, as well as taurine and cobalamin (vitamin B₁₂). The development of a simple simultaneous determination without the need for chromatographic techniques¹ can contribute to the routine management of food safety. Unmodified working electrodes usually do not provide sufficient sensitivity for use in the analysis of energy drinks, namely ~ 2 mg pyridoxine per 100 mL together with other electroactive species. One of these concomitants is caffeine, the concentration of which is nearly 32 mg per 100 mL. No wonder that finding the optimal conditions for their simultaneous determination was a major challenge within this study. To date, voltammetric approaches have been published^{2,3} only for the separate determination of either pyridoxine or caffeine in various energy drinks. The proposed electrochemical behavior of said analytes in pure aqueous electrolytes is shown in Schem. 1.

This study is to be understood as an initial step in the simultaneous determination of these substances. In optimization, ideal working conditions must be found guaranteeing a sufficient distance between the corresponding oxidation peaks (selectivity) on the one hand, as well as conditions favoring pyridoxine against caffeine (sensitivity) on the other. Here, the glassy carbon electrode (GCE) and its surface modifications were widely tested in 0.1 mol L⁻¹ Britton-Robinson buffers (BRB) with different pH values. The GCE surface was coated with a thin layer of various Nafion[®] (sulfonated fluoropolymer-copolymer) composites with carbon nanomaterials such as multi-walled nanotubes and single-layer graphene. Optimal conditions for the analysis of model samples mimicking the actual content of pyridoxine and caffeine were then chosen.



Scheme 1a. Proposed electrochemical oxidation pathway of caffeine.



Scheme 1b. Proposed electrochemical oxidation pathway of pyridoxine.

Experimental

Analytical standards of pyridoxine and caffeine, Nafion[®], surfactants such as sodium dodecyl sulfate (SDS), cetylpyridinium chloride (CPC), Triton X-100, 1,3-didecyl-2-methylimidazolium chloride (TEGO[®] trant A 100) were purchased from Sigma-Aldrich (Prague, Czech Republic). Carbon nanomaterials (CNTs), namely multi-wall carbon nanotubes (MWCNTs) of diameter 10-30 nm; length 5-15 μm ; specific surface area 40-300 $\text{m}^2 \text{g}^{-1}$ from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China) and single-layer graphene (resistivity $\leq 0.30 \Omega \text{ cm}$; specific surface area 400-1000 $\text{m}^2 \text{g}^{-1}$) from ACS Material, LLC (Medford, USA) were used as surface modifiers. Chemicals needed for preparation of 0.1 mol L^{-1} BRB were obtained from Lach-Ner (Neratovice, Czech Republic). Ultrapure water ($\rho = 18.3 \text{ M}\Omega \text{ cm}$) obtained by passing of the already deionized water again through a purification unit (Milli-Q system, Millipore) was used throughout the experimental work.

All electrochemical measurements were performed on conventional three-electrode arrangements, each containing one of the working electrodes tested, the reference (Ag/AgCl/3.0 mol L⁻¹ KCl) and the auxiliary electrodes (Pt-wire). Mentioned setup was connected to a potentiostat PGSTAT101 from Metrohm (Prague, Czech Republic) operating with Nova software. The modification of GCE surface was made by addition of 10 μ L dispersion of CNTs (2 mg mL⁻¹ in 1 % Nafion[®] neutralized by 8 % ammonia solution) and allowed to dry under laboratory conditions for 30 min. Appropriate dispersions were prepared using an ultrasonic bath for at least 10 min.

To add 1 mL of energy drink to 9 mL of the supporting electrolyte, concentrations of $\sim 10 \mu\text{mol L}^{-1}$ VB₆ and $\sim 150 \mu\text{mol L}^{-1}$ CA were calculated and therefore used as model ratio for optimization. Unmodified GCE, GCE covered by thin layer of Nafion[®], MWCNTs, graphene, and their various combinations were tested in BRB buffers containing different surfactants using anodic differential pulse voltammetry (DPV) at potential step (E_{step}) 5 mV, potential amplitude (E_{ampl}) 70 mV, and scan rate (v) 50 mV s⁻¹.

Results and Discussion

From chemical point of view, VB₆ (2-methylpyridin hydroxymethyl derivative) and CA (methylxanthine alkaloid) can be classified as nitrogen-containing heterocycles that are involved in the protonation reaction. It is well defined that in strongly acidic solutions, both are present as positively charged ions. Therefore, their anodic oxidation usually occurs at high potential values. Within this study, it was confirmed that their peak potentials (E_p) were indeed shifted to more negative stresses with rising pH supportive electrolyte values. An analogous dependence of the current response (I_p) on electrolyte pH values has been observed and therefore, optimal sensitivity can be achieved in the solution of strong inorganic acids^{2,3}. However, it was found that the corresponding anodic peaks overlapped at pH values below 3 (Fig. 1). The required distance between their oxidation peaks was achieved at pH 4.5.

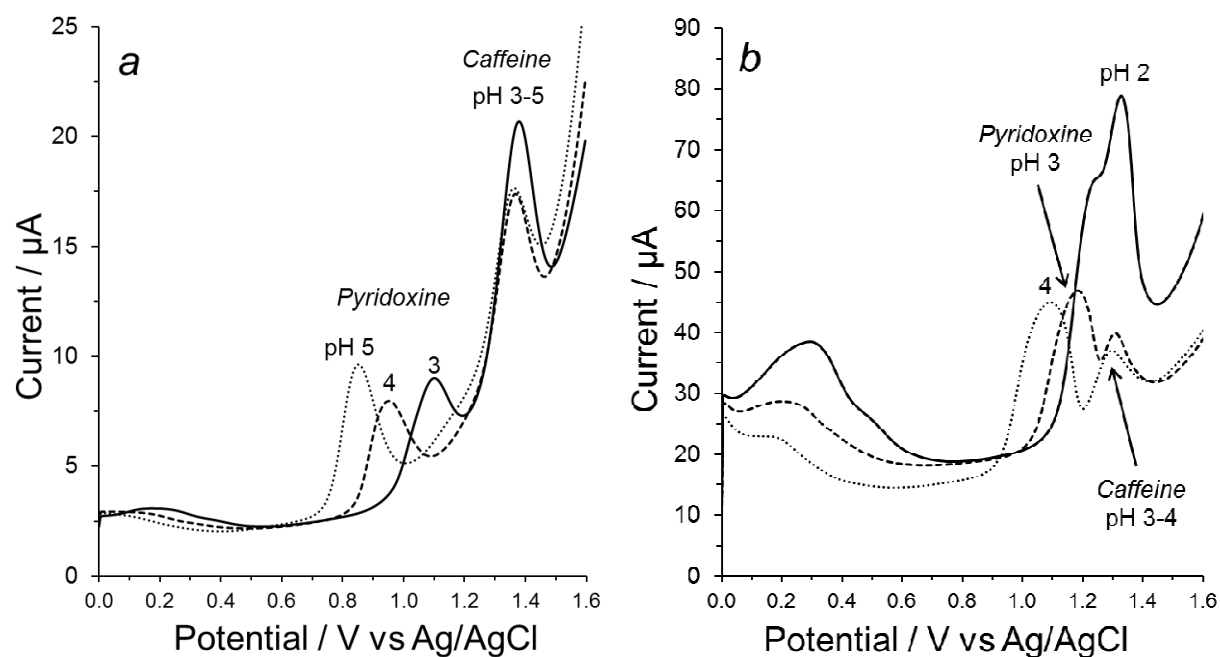


Fig. 1. Effect of 0.1 mol L⁻¹ BRBs pH values on the analytes separation, measured at GCE modified with Nafion[®] (a) and at GCE containing MWCNTs-Nafion composite (b).

Surface modification with perfluorinated resin Nafion[®] was used in several voltammetric methods directed to CA determination³⁻⁵. The reason why Nafion[®] is widely implemented can be found in its molecular structure, which contains a sulfonic function group. In weak acidic media, GCE surfaces coated with a thin layer of Nafion[®] or its composites with tested CNTs are negatively charged. In contrast, pyridoxine and caffeine are positively charged molecules. Due to the possible electrostatic interaction, higher current responses were obtained for modified electrodes than for bare GCE. The presence of Nafion[®] caused VB₆ to provide higher current response than CA at the same concentrations. An explanation can be found in existing steric hindrances. Most nitrogen atoms in the CA molecule are linked to methyl groups, but pyridoxine has a different structure. Moreover, it is necessary to mention that VB₆ is smaller molecule and it is thus easier to get to the electrode surface.

In the case of CNTs, it was found that composite material from unoxidized graphene and Nafion[®] did not provide reproducible results. Highest peak current values were obtained for composite of MWCNTs and Nafion[®]. Unfortunately, insufficient repeatability with relative standard deviations (RSD) of ~13 % for VB₆ and ~14 % for CA (five analyses of 100 μmol L⁻¹ solutions) were calculated. However, this problem was solved by adding surfactants to the supporting electrolyte. Different types of surfactants were tested. The ~2.9 % RSD for VB₆ and ~9.4 % for CA was achieved as optimum when 40 μmol L⁻¹ Triton X-100 was used. It is important to note that sufficient sensitivity and repeatability (2.7 % RSD for VB₆ and 1.1 % RSD for CA) at GCE coated with only a thin layer of Nafion[®] without the presence of any surfactant was observed. Therefore, further electroanalytical studies with the latter type of working electrode are planned.

Conclusions

On the basis of the results obtained, it can be stated that the simultaneous determination of pyridoxine and caffeine in energy drinks (testing on a model sample) can be carried out using anodic differential pulse voltammetry at Nafion[®] modified glassy carbon electrode. It seems that it is enough to find the optimal conditions of the aforementioned pulse voltammetric technique and the developed simple electroanalytical method can be used for real sample analyses.

Acknowledgment

The support received from the Faculty of Chemical Technology, University of Pardubice (project No. SGS-2018-001) is gratefully acknowledged.

References

1. Sather K., Vernig T.: *Concor. J. Anal. Chem.* 2, 84 (2011).
2. Brunetti B., Desimoni E.: *J. Food Compos. Anal.* 33, 155 (2014).
3. Švorc L., Tomčík P., Svítková J., Rievaj M., Bustin D.: *Food Chem.* 135, 1198 (2012).
4. Martínez-Huitle C.A., Fernandes N.S., Ferro S., De Battisti A., Quiroz M.A.: *Diam. Relat. Mater.* 19, 1188 (2010).
5. Schrenk M.J., Villigam R.E., Torrence N.J., Brancato S. J., Minter S. D.: *J. Membrane Sci.* 205, 3 (2002).