

New Approaches to The Voltammetric Determination of Amphiphilic Electrochemically Active Substances

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ABSTRACT

This contribution deals with new approaches to the voltammetric determination of amphiphilic electrochemically active substances. A new sensor that can be used to determine this group of frequently investigated compounds in various matrices was developed within the scope of our research. It is based on a glassy carbon electrode on the surface of which an asolectin (phospholipid) multilayer is formed. In the first step, the analyte is accumulated into this surface layer and then determined using differential pulse or direct current voltammetry.

Capsaicinoids can be considered as a model group of amphiphilic compounds and methods for their determination using the above-mentioned sensor were developed and tested by our research group. The results achieved using the phospholipid modified and bare electrode were compared with those obtained by the direct voltammetric method and HPLC/UV. The results were used to objectively evaluate the pungency of chili peppers.

Additionally, a mass spectrometer hyphenated with an electrochemical cell was used to elucidate the electrochemical reactions of capsaicinoids and identify their main oxidation products.

Keywords: Amphiphilic, molecule, Capsaicinoids, Electrochemistry, Modified, surface, Sensor, Voltammetry

INTRODUCTION

Voltammetry is one of the traditional and very reliable (electro) analytical techniques applicable to the analysis of various biologically active compounds (BACs), important from the environmental, agricultural, or medical point of view, their metabolites and analogs. They can exhibit lipophilic or hydrophilic properties or both of them. The latter are referred to as amphiphilic and our attention was paid to the possibilities of their determination within our research. Capsaicinoids (CPDs), which are responsible for the pungency of chili papers, can be assigned to this group. Dihydrocapsaicin (*N*-(4-hydroxy-3-methoxybenzyl)-8-methylnonanamide, CAS 19408-84-5, DHC) and capsaicin ((6*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide, CAS 404-86-4, CA), Scheme 1, represent 90% and more of the total CPDs content. Objective determination of pungency of chili peppers belongs to frequently solved tasks in food and medicinal analysis.

In this manuscript, the application of *ex-situ* extractive stripping voltammetry (ExSV) utilizing a phospholipid modified glassy carbon electrode is described.

METHODOLOGY

The glassy carbon electrode (GCE) (BASi, USA) used was modified by asolectin (Fluka, Switzerland) (containing about 25 % of lecithin). Britton-Robinson buffer (BRB) solution (pH 2) was used as the supporting electrolyte. Autolab PGSTAT128N (MetrohmAutolab, The Netherlands) was used for voltammetric measurements of all model CPDs samples as well as chili pepper samples (Madras chili; Goat Horn Pepper; Habanero yellow; Naga Jolokia; and Carolina Reaper).

The phospholipid modified GCE (PLMGCE) was prepared from a bare, in methanol, hexane, and water sonicated and on alumina polished GCE. Finally, it was electrochemically activated in an aqueous NaCl solution, rinsed with water, and then dried. After that, asolectin solution in hexane was dropped onto the GCE disc and immersed in the NaCl solution [1,2].

HPLC measurements were performed on the DionexUltiMate 3000 HPLC equipped with UV-VIS detector (Thermo Fisher Scientific, USA). On-line EC/MS experiments were realized by Potentiostat ADLCl (Laboratorní přístroje, Czech Republic) with a porous graphite electrode, NE-1002X syringe pump (New Era Pump Systems, USA), and Agilent 1100 Series LC/MSD Trap (Agilent Technologies, USA) with electrospray ionization (ESI).

RESULTS

In our experiments, 3 methods were developed and tested [2]. A two-compartment electrochemical cell, consisting of a large volume compartment (LVC) and a small volume compartment (SVC), was used for all direct current (DCV) and differential pulse (DPV) voltammetric measurements. The sample was inserted into the inner SVC and the BRB (pH 2) was poured into the outer LVC.:

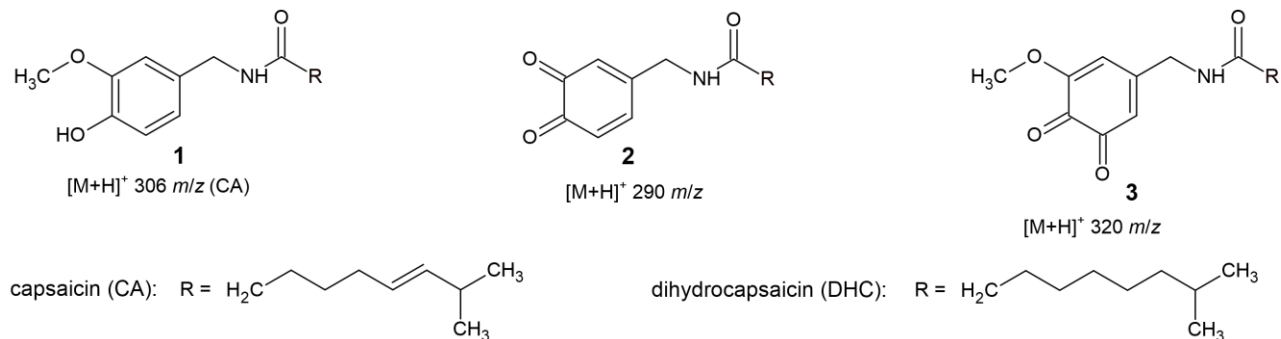
Direct method: In ethanol macerated, sonicated, and filtrated chili peppers samples containing CPDs were mixed with ethanol and BRB pH 2 and placed into the SVC and analyzed and on a bare GCE ($E_{in} = +0.1$ V, $E_{fin} = +1.0$ V, scan rate 20 mV s⁻¹).

Transfer Method on the Unmodified Electrode: CPD samples were prepared in the same way as in the case of the method “A”, but a bare and polished GCE was exposed to the CPD solutions for a defined time. The measurement was realized on the rinsed GCE after transferring it into blank BRB pH 2. The GCE must be polished after each measurement.

Transfer Method on the Modified Electrode: The PLMGCE was exposed to the analyzed CPD extract, then rinsed with water. The following steps of the procedure were analogous to method B). PLM cleaning was performed by washing in distilled water.

These three independent methods were used for the determination of DHC, CA, and their mixtures in their model samples. The achieved results were compared with those of HPLC with UV detection (Table 1) [2].

CPDs were oxidized on the porous GE in flow-through coulometric cell on-line hyphenated to a mass spectrometer to confirm the proposed reaction pathway and identify reaction products. The results are summarized in Scheme 1. The main oxidation product is quinone (product 2). In addition, product 3 was also identified in the electrolyzed solution in lower yields.



Scheme 1. Mechanism of electrochemical reactions of CA and DHC in acidic aqueous-methanolic media. Values of m/z are indicated for protonated molecules of the CA and its reaction products detected in the ESI+ mode of the EC/MS experiment [2].

Table 1. Comparison of determination of CA, DHC, and their mixture in model sample Susin direct DPV on GCE (method A), transfer ExSDPV on PLMGCE (method C), and HPLC with UV detection [2].

Method	Analyte	Slope [$\mu\text{A L mg}^{-1}$]	Intercept [μA]	r	LOD* [mg L^{-1}]	LDR [mg L^{-1}]
ExSDPV-PLMGCE	CA	0.00742±0.00019	0.0279±0.0059	0.9995	2.1	3.0-60.0
ExSDPV-PLMGCE	DHC	0.0118±0.0044	***	0.980	9.3	9.3-60.0
ExSDPV-PLMGCE	CA+DHC(6:4)	0.0082±0.0010	***	0.9992	8.3	8.3-60.0
DPV-GCE	CA	0.100±0.025	***	0.991	8.5	8.5-30.0
DPV-GCE	DHC	0.073±0.048	***	0.977	13	13.0-30.0
DPV-GCE	CA+DHC(6:4)	0.089±0.073	***	0.981	13	7.3-15.0
		Slope [$\text{L mg}^{-1}\text{mAU min}$]	Intercept [mAU min]	r	LOD* [mg L^{-1}]	LDR [mg L^{-1}]
HPLC/UV	CA	0.5256±0.0084	***	0.9999	2.7	3.0-150.0
HPLC/UV	DHC	0.511±0.011	***	0.9999	2.7	3.0-150.0
HPLC/UV	CA+DHC(6:4)	0.520±0.019	***	0.998	2.7	3.0-150.0

* Calculated using IUPAC direct signal method; ** Omitted on the level of significance $\alpha = 0.05$

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